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Pathogen transmission at the domestic-wildlife interface: A growing challenge that requires integrated solutions

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Pathogen transmission at the domestic-wildlife interface: A growing challenge that requires integrated solutions

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Editorial: Pathogen transmission at the domestic-wildlife interface: a growing challenge that requires integrated solutions

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Editorial on the Research Topic
Pathogen transmission at the domestic-wildlife interface: a growing challenge that requires integrated solutions

1 Introduction

Wildlife has coexisted with domestic animals in dynamic systems over thousands of years. Domestic-wildlife interfaces are intricate, encompassing physical spaces where wild and domestic species overlap and potentially interact, posing risks of pathogen transmission. The nature of this interface has changed over time and across landscapes, leading to continuous emergence of different conflicts. In addition, human processes that alter ecosystems have led to more interconnected interfaces and increased opportunities for the emergence and spread of shared pathogens (1).

The main goal of this Research Topic was to promote integrative research at domestic-wildlife interfaces globally to characterize and better understand specific eco-epidemiological drivers of pathogen transmission. This knowledge is essential to support subsequent strategies and interventions for disease management and control.

2 Organization of the Research Topic and new findings

The fourteen manuscripts comprising this Research Topic of scientific articles cover diverse aspects of domestic-wildlife interfaces. Systematic reviews, original research, case reports, and perspective articles contributed to a deeper knowledge of these interfaces and the eco-epidemiological drivers of pathogen transmission. The majority of contributions

focused on domestic-wild mammals (57.1%), and animal tuberculosis (TB), with avian interfaces also explored (28.6%), notably investigating highly pathogenic avian influenza (HPAI) virus H5N1. This breakdown by taxa group and pathogen was similar to literature reviews on these interfaces performed over the last decade (2, 3). A major difference in our Research Topic is the contribution of articles on African swine fever (ASF), reflecting the increased interest in domestic-wild suid interfaces around the world. Disease epidemiology (35.7%) and control (35.7%) were primary areas of investigation, followed by surveillance (14.3%) and predictive modeling (14.3%).

Thompson et al. reviewed the historical perspective of the World Organization for Animal Health (WOAH) on wildlife, and its role in defining the wildlife compartment of this interface and contextualizing the wildlife health framework of WOAH. They articulated a WOAH-led One Health approach with cross-sectoral collaboration to address challenges and assist in preserving wildlife population health and biodiversity conservation. However, communication gaps between the health and environmental sectors, and scarce resources for wildlife health surveillance in many countries, hinders international information sharing and limits availability of epidemiological data on wildlife. This often leads scientists and managers to rely on indirect inference. Hayes et al. performed a scoping review of the scientific literature to illustrate different methodological approaches explored to precisely infer epidemiological outcomes at this complex and dynamic interface. They included a total of 56 research articles published during 2001-2023 with the main focus on mathematical modeling of drivers of disease transmission between domestic and wild hosts. Strengthening wildlife disease surveillance efforts globally requires interdisciplinary collaboration and integration of diverse datasets. By embracing transparency, integrating the One Health approach, and leveraging advanced modeling techniques, the global community may enhance wildlife disease surveillance and mitigate associated risks.

Among domestic-wild mammal interfaces, the relationship among wild boar (*Sus scrofa*) and domestic pigs was highlighted. The interplay between them and disease transmission is a focal point in epidemiological research across different regions. In Corsica (France), Dupon et al. merged different approaches including social sciences, epidemiology, animal husbandry, and geography to estimate the risks of interaction between domestic pigs and wild boar based on pig production practices. They discussed how the information obtained could inform control efforts of shared porcine diseases in extensive farming, not only in Corsica but also at larger territorial scales. In the United States (US), Brown et al. described the state of the knowledge available on ASF, which poses a significant threat to the domestic-wildlife interface and global food security. The authors aimed to prepare the policy context for an integrated and coordinated response against a potential ASF outbreak. Free-ranging or feral suids constituting invasive populations in the US are mostly hybrids of domestic and wild lineages (4), which adds some disease management differences and hinder this response. These animals underscore the need for a holistic approach, considering sociological factors with the same urgency and determination as has been given to the surveillance aspects. In Eastern Poland, the risk factors related to transmission dynamics of ASF virus at the wild boar-domestic pig interface were investigated by Pepin et al. between 2014 and 2019. Results showed that while risk factors related to pig ASF cases did not predict disease detection in wild boar, multiple risk factors for wild boar were able to predict case detection in domestic animals. In addition, they showed that spill over from wild boar to domestic pigs might be more frequent than the reverse, but that the structure of surveillance systems hindered this quantification, highlighting the importance of investigating the movement patterns of both swine species to better understanding transmission routes at this interface. In an experimental study conducted in Spain, Kosowska et al. assessed the potential transmission of an attenuated ASF virus isolate (vaccine candidate) between infectious wild boar and directly exposed naïve domestic pigs, examining the transmission of this viral strain, clinical signs and the level of interaction between *Suidae* species. Authors found that wild boar were successfully protected, did not transmit the virus to susceptible pigs and survived the challenge with the virulent ASF virus isolate during the experiment, without showing ASF-compatible signs or associated viremia. This observation suggests that the presence of wild boar infected with an attenuated virus in ASF-affected areas may reduce the spreading of virulent isolates and virus introduction into the domestic pig husbandry. Altogether, these outcomes may help decision-making related to targeted control actions against ASF in field conditions. These studies collectively underscore the importance of understanding disease dynamics at the domestic-wildlife interface. By combining interdisciplinary methodologies and spatial analyses, researchers aim to enhance our epidemiological knowledge and disease management, ultimately safeguarding and securing animal and human health.

Similarly, TB is another key disease at the domestic-wildlife interface, with humans also included in this complex multi-host system. In Nepal, transmission of *Mycobacterium tuberculosis* complex between elephants and humans was evidenced by Man Rajbhandari et al. They sequenced the whole genome of the strains isolated from two deceased Asian elephants (*Elephas maximus*) and one human. The elephant-derived isolates were closely related to human-derived isolates previously described in the same country, supporting the presence of zooanthroponosis or bidirectional transmission. This highlighted the need for a One Health approach for TB prevention and control at this interface, because it is a serious threat not only to humans and livestock, but also to wildlife species critical to biodiversity conservation. Meanwhile, in Ireland, Chang et al. aimed to better understand local TB transmission between cattle and European badgers (*Meles meles*) through the development of a spatially explicit environmental transmission model that incorporated both within herd/territory and between-species transmission. The model disentangled the relationship between relative badger density and local TB transmission risk and generated the first between-herd R (reproductive ratio) map for TB that identified high-risk areas. This map provided a useful tool for identifying TB hotspots where transmission is driven primarily by badger densities, allowing to direct control strategies. In North-Eastern Lower Michigan (US), on the other hand, Dressel et al. demonstrated the feasibility of TB vaccination of free-ranging white-tailed deer (*Odocoileus virginianus*) via oral baits. The BCG vaccine delivery units included Rhodamine B

as a biomarker to subsequently quantify the achievable potential uptake coverage. This strategy demonstrated its scalability as an effective method which could spur further progress toward TB eradication in free-ranging wildlife populations and globally. Overall, these studies highlight the complexity of TB epidemiology, management, and control at the domestic-wildlife interface, as well as the importance of integrating different approaches such as genomics, risk mapping, or innovative vaccination strategies, to mitigate the risk of TB transmission and enhance public health outcomes.

In Cambodia, Porco et al. reported the first case of lumpy skin disease in an endangered banteng (*Bos javanicus*) and the subsequent initiation of a vaccination campaign in domestic cattle to mitigate the challenge of pathogen transmission at the domestic-wildlife interface. In this case, vaccination both supported local livestock-based economies and promoted biodiversity conservation. However, this is only a component of a wider and integrated solution against many other disease threats at the domestic-wildlife interface.

In addition, other studies explored the influence of wild bird communities around domestic avian farms or investigated biosecurity measures and potential risk factors related to the introduction and spread of shared pathogens at this domestic-wildlife interface. Studies on these topics are often more difficult to elaborate and their nature is not commonplace among scientists focusing on both animal and human health, particularly when addressing pathologies resulting from the interaction network that may take place among them. Sánchez-Cano et al. used a camera trapping approach to assess the effectiveness of biosecurity measures in different types of avian farms in Spain. They investigated wild bird communities that visited commercial layer and red-legged partridge farms over a one-year timeframe and assessed the occurrence of interactions. They showed that, independently of the type of farm, the house sparrow (*Passer domesticus*), a potential bridge host for several diseases, was in contact with the surveyed farms as well as with other wild bird species mostly belonging to the order Passeriformes. The most geographically extensive and costly animal health event in the history of the USA occurred in 2022-2023 as a HPAI virus H5N1 outbreak affected more than 70% of both commercial turkey and poultry farms. Knowledge of risk factors for HPAI infection became increasingly relevant because additional domestic flocks, wild birds, and other domestic and wild non-avian species have been infected (5, 6). Patyk et al. and Green et al. conducted two similarly designed case-control studies to identify potential risk factors related to the introduction of HPAI virus H5N1 into commercial meat turkey and table egg operations, respectively. In both cases, data were provided by the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS), with support from the USDA National Agricultural Statistics Service (NASS), as well as by regional/national poultry and turkey organizations. They aimed to compare farm characteristics, management practices and biosecurity methods between case and control farms. Patyk et al. included 66 case and 59 control commercial meat turkey farms from 12 different states. It should be noted that there were a few mistakes in the first published version. Thus, an Erratum (Frontiers Production Office) was also published within this Research Topic with the main goal of amending all detected errors and to update the original research article. Green et al., on their side, used data from 18 case and 22 control commercial table egg farms from eight different States, with the same goals of the previous study. Univariate and multivariable results provided a better understanding of both risk and protective factors for HPAI virus H5N1 infection that can be employed to support science-based updates to prevention and control recommendations to safeguard turkey and commercial table egg farms, respectively, in the United States. Overall, these studies emphasize the critical role of interdisciplinary approaches, robust surveillance systems, and integrated strategies in mitigating disease risks at the domestic-wildlife interface to enhance the prevention of new disease outbreaks and the preservation of both animal and public health, as well as biodiversity.

3 Conclusions

In conclusion, this Research Topic of articles provides a very interesting contribution of different studies and perspectives to improve our understanding of pathogen transmission and disease prevention and control opportunities at domestic-wildlife interfaces globally. The diversity of content highlights the multi-faceted nature and the complex dynamics of pathogen transmission between wild and domestic animals and humans. As reflected in the Research Topic, in the global north, increasing wildlife-livestock interfaces are attributed to an expansion of wildlife populations/numbers or points of contact and linked to what are described as direct threats to human productive activities including livestock-based farming or agriculture, among others. Reports from the global south associate human (with their livestock) encroachment on wildlife habitat as inducing the potential conflicts that can facilitate disease emergence among livestock, wild species, and humans. Overall, these findings highlight the importance and complexity of this topic worldwide and the growing need for improving awareness, research, and surveillance, and to develop new interdisciplinary strategies and solutions to address this growing challenge.

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References

1. Vicente J, Vercauteren KC, Gortázar C. *Diseases at the Wildlife-Livestock Interface: Research and Perspectives in a Changing World.* Cham: Springer Nature (2021).

2. Wiethoelter AK, Beltrán-Alcrudo D, Kock R, Mor SM. Global trends in infectious diseases at the wildlife-livestock interface. *Proc Nat Acad Sci.* (2015) 112:9662–7. doi: 10.1073/pnas.1422741112

3. Bacigalupo SA, Dixon LK, Gubbins S, Kucharski AJ, Drewe JA. Towards a unified generic framework to define and observe contacts between livestock and wildlife: a systematic review. *PeerJ.* (2020) 8:e10221. doi: 10.7717/peerj.10221

4. Smyser TJ, Tabak MA, Slootmaker C, Robeson MS, Miller RS, Bosse M, et al. Mixed ancestry from wild and domestic lineages contributes to the rapid expansion of invasive feral swine. *Mol Ecol.* (2020) 29:1103–19. doi: 10.1111/mec.15392

5. United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA–APHIS). *Confirmations of Highly Pathogenic Avian Influenza in Commercial and Backyard Flocks.* (2022). Available online at: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/avian/avian-influenza/hpai-2022/2022-hpai-commercial-backyard-flocks (accessed April 08, 2024).

6. United States Geological Survey (USGS). *Distribution of Highly Pathogenic Avian Influenza in North America, 2021/2022.* (2022). Available online at: https://www.usgs.gov/centers/nwhc/science/distribution-highly-pathogenic-avian-influenza-north-america-20212022 (accessed April 08, 2024).

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Phylogenomic analysis supports *Mycobacterium tuberculosis* transmission between humans and elephants

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Introduction: Tuberculosis is an infectious disease caused by a group of acid-fast bacilli known as *Mycobacterium tuberculosis* complex (MTC), which has a major impact on humans. Transmission of MTC across the human-animal interface has been demonstrated by several studies. However, the reverse zoonotic transmission from humans to animals (zooanthroponosis) has often been neglected.

Methods: In this study, we used Nanopore MinION and Illumina MiSeq approaches to sequence the whole genome of *M. tuberculosis* strains isolated from two deceased Asian elephants (*Elephas maximus*) and one human in Chitwan, Nepal. The evolutionary relationships and drug resistance capacity of these strains were assessed using the whole genome data generated by the stand-alone tool Tb-Profiler. Phylogenomic trees were also constructed using a non-synonymous SNP alignment of 2,596 bp, including 94 whole genome sequences representative of the previously described *M. tuberculosis* lineages from elephants worldwide (lineages 1 and 4) and from humans in Nepal (lineages 1, 2 and 3).

Results and Discussion: The new genomes achieved an average coverage of 99.6%, with an average depth of 55.67x. These *M. tuberculosis* strains belong to lineage 1 (elephant DG), lineage 2 (elephant PK) and lineage 4 (human), and none of them were found to have drug-resistant variants. The elephant-derived isolates were evolutionarily closely related to human-derived isolates previously described in Nepal, both in lineages 1 and 2, providing additional support for zooanthroponosis or bidirectional transmission between humans and elephants. The human-derived isolate clustered together with other published human isolates from Argentina, Russia and the United Kingdom in the lineage 4 clade. This complex multi-pathogen, multi-host system is challenging and highlights the need for a One Health approach to tuberculosis prevention and control at human-animal interface, particularly in regions where human tuberculosis is highly endemic.

KEYWORDS

***Elephas maximus*, whole-genome sequencing, one health, tuberculosis, zooanthroponosis**

1. Introduction

Tuberculosis (TB) is a significant global burden and is widely reported to be a major public health and economic problem, costing the world $617 billion between 2000 and 2015 and projected to cost $1 trillion between 2015 and 2030 (1). It is the second leading cause of death after COVID-19, with an estimated 10 million cases worldwide and 1.5 million deaths in 2020 (2). Drug-resistant TB is also a major threat to global disease control (3). It is a major contributor to global antimicrobial resistance (4). In 2021, 450,000 cases of rifampicin-resistant and multidrug-resistant (MDR/MDR-TB) TB were forecasted worldwide, of which 191,000 died (5). In Nepal, the number of cases of drug-resistant TB is estimated to be around 1,500 per year (6).

Human and animal tuberculosis is caused by a group of closely related acid-fast bacilli known as the *Mycobacterium tuberculosis* complex (MTC) (7, 8). The MTC includes several species of mycobacteria, namely *M. tuberculosis*, *Mycobacterium bovis* (Bacillus Calmette–Guerin), *Mycobacterium africanum*, *Mycobacterium caprae*, *Mycobacterium microti*, *Mycobacterium canettii* and *Mycobacterium pinnipedii* (9). *M. tuberculosis* is the human-adapted variant and is the major cause of human TB (9). However, most publications on emerging infectious agents such as MTC often focus on their zoonotic origin, but comparatively fewer reports are published on possible zooanthroponosis and the human origin of infectious diseases affecting domestic or wild animals (10). Zooanthroponosis, is an important ongoing debate regarding the transmission of pathogens from humans to animals. Evidence from a global survey showed that humans are capable of transmitting at least 21 bacterial, 12 viral and 7 fungal pathogens to animals (10). Although there is evidence that pathogens can be transmitted from humans to animals, most reported cases involve captive or domestic animals (11). Influenza is one of the best known examples of bidirectional transmission between humans and domestic pigs (12). The emergence and re-emergence of these pathogens has important implications for human and animal health (13). This risk appears to be even greater when bidirectional transmission between humans and animals is frequent, as in the case of TB in elephants (14).

Tuberculosis in elephants has been reported worldwide, although most reported cases involve captive elephants with *M. tuberculosis*, the ethological agent of human TB (15–19). In countries such as Nepal, where there is a high prevalence of active *M. tuberculosis* infection in humans (117,000 people living with TB; 20) and a large elephant population (more than 200 captive and 200–250 wild elephants; 21), this issue is particularly relevant. Elephant-human interactions are particularly high in regions where captive elephants are used for ecotourism and patrolling protected areas, such as Chitwan National Park. This poses a serious risk of zooanthroponosis and disease to both captive and wild elephant populations, as well as a risk to humans given the possibility of bidirectional transmission (15, 22). A recent study in four national parks in Nepal showed a high seroprevalence (21.56%) of TB in captive elephants, with most cases detected in Chitwan National Park (23). In addition, some evidence of zooanthroponosis has been reported from previous studies in Nepal that also isolated *M. tuberculosis* from diseased elephants using multi-locus variable number of tandem repeats (MLVA) (15, 24), large sequence polymorphism (24) or whole genome data (25). However, the latter study lacks a phylogenetic analysis of the two *M. tuberculosis* genomes (26) and their integration with previously published data (25).

In this study, a whole genome sequencing approach was used to genotype *M. tuberculosis* isolates from a human and two deceased elephants in Chitwan, Nepal. These new data, combined with genomic data from elephants and human isolates available in the literature, allowed a comprehensive evolutionary study of *M. tuberculosis* in Nepal. These results contribute to the understanding of the possible bidirectional transmission of this pathogen at human-elephant interface.

2. Methods and materials

2.1. Study area

Chitwan district is located in the southwestern part of Bagmati Province in Nepal. It covers an area of 2,238.39 km^2 and had a human population of 719,859 in 2022. Nepal's first national park, Chitwan National Park (CNP), is located in this district (Figure 1) and was established in 1973 (27). The park covers an area of 932 km^2 and is located in the subtropical Terai lowlands of south-central Nepal. It lies in a river valley basin or dun, along the floodplains of the Rapti, Reu and Narayani rivers. The Chitwan valley consists of tropical and subtropical forests. Sal forest covers 70% of the park. Sal leaves are used locally for plates in festivals and religious offerings, and are also known for their Ayurvedic use in the treatment of wounds, coughs and other ailments (28). Grasslands cover 20 per cent of the park. There are more than 50 different species of grasses, including elephant grass (*Saccharum* spp), known for its immense height. The climate is mainly dominated by the summer monsoon, and the valley experiences three distinct seasons each year: winter, summer and monsoon.

Chitwan National Park is regarded as one of the best national parks for wildlife viewing in Asia (29). It is home to 68 species of mammals, more than 576 species of birds, 49 species of reptiles and amphibians, 120 species of fish and several species of invertebrates that contribute significantly to ecosystem processes in the region. The reserve is home to many of Nepal's charismatic wildlife species, including the one-horned rhinoceros (*Rhinoceros unicornis*), Bengal tiger (*Panthera tigris*), leopard (*Panthera pardus*) and Asian elephant (*Elephas maximus*), among others (29, 30). Of the various recreational activities available in the park, elephant safaris are the most popular, especially in and around the buffer zones. Elephants are also used by the government for transport and patrolling the park. Buffer zones in the CNP were established in 1996 with the aim of involving people in the management of park resources for the conservation of biodiversity and the livelihoods of buffer zone communities (31). The buffer zone covers an area of 750 km^2 and is home to approximately 45,516 houses and 2,60,352 people (32). Buffer zones are primarily designed to create human-wildlife coexistence by providing an ecological and socio-economic buffer for communities, and in these areas, where wildlife and humans share the same landscape in close proximity, their interaction is very obvious (33).

2.2. Sample collection

Samples were collected from two deceased adult (>30 years) elephants in Sauraha (Elephant PK and Elephant DG), Chitwan,

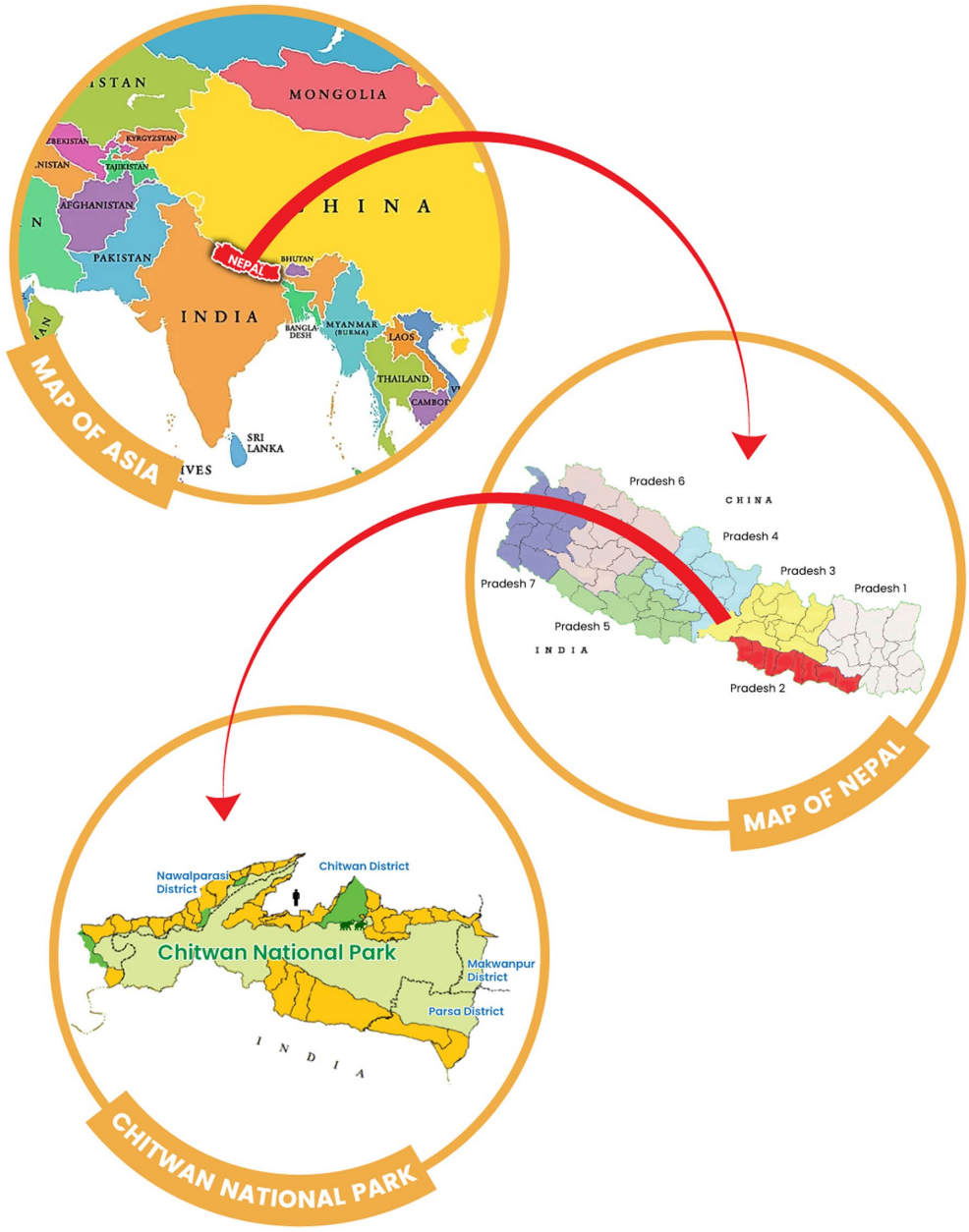

FIGURE 1
Geographical map of Nepal showing the location of Chitwan National Park (light green) and the origin of the human and elephant samples used in this study (icons). Forest communities around Chitwan National Park are shown in dark green.

owned by the government, and used for tourism, grass collection and patrolling. Sauraha is a village at the eastern entrance to Chitwan National Park, near the East Rapti River. These animals had died in captivity and at necropsy showed granulomatous lesions in the lungs compatible with tuberculosis. Tissue samples from lung biopsies (with lesions) were taken from both elephants. The human sputum sample (No. 52G111) collected in Chitwan as part of a previous study (20) was also genotyped, although it was not closely related to the diseased elephants. It was included to understand the evolutionary relationship with the *M. tuberculosis* strains isolated from elephants, as it was collected from the same region.

2.3. Molecular confirmation of *Mycobacterium tuberculosis* infection

The elephant lung tissue samples (PK and DG) and human sputum samples (52G111) were sent to GENETUP (German Nepal Tuberculosis Project microbiology lab) for culture confirmation of *M. tuberculosis* as described in (34). The heat-killed cultures were then resuspended in 100 µL of PBS for DNA extraction. The DNA extraction was performed using a DNA-SorB kit according to the manufacturer's instructions (Sacace Biotechnologies, Italy). Briefly, 300 µL of the lysis solution was mixed with 100 µL of the heat lysed *M. tuberculosis* PBS solution and incubated at 60°C for 5 min. The

samples were centrifuged at 15000 g for 5 min to separate the cell debris and DNA in the supernatant. The supernatant was then mixed with 20 µL of the sorbent to bind the DNA to the sorbent. The sorbent was washed twice, dried at 65°C for 5 min and eluted with 25 µL of DNA eluent. Elution was further enhanced by incubating the tubes with DNA eluent at 65°C for 5 min and periodically vortexing. The extracted DNA was then quantified using Qubit (Thermo Fisher Scientific, USA) and *M. tuberculosis* infection was diagnosed using the specific real-time PCR (35).

2.4. Whole-genome sequencing of elephant and human *Mycobacterium tuberculosis* isolates

Two complementary whole genome sequencing approaches were used in this study: Illumina short reads and MinION long reads. In the case of Illumina short reads, genomic DNA from the heat-killed cultures of *M. tuberculosis* was purified using AMPure beads (Beckman Coulter, United States) (1:1), quantified using Qubit (Thermo Fisher Scientific, United States) and normalised to 0.2 ng/µL. The normalised samples were tagged and indexed using the Nextera Indexing Kit (Illumina, United States). The indexed library was then purified using (0.8X) AMPure beads and quantified using HS kit (Thermo Fisher Scientific, United States). Each library was analysed using a bioanalyzer and normalised to a final concentration of 2 nM. The three normalised libraries were then pooled in equal volumes of 5 µL to give a final pooled library of 2 nM. The pooled library was spiked with 5% of 2 nM PhiX and denatured with NaOH. The denatured library was diluted to 20 pM, then 10 pM using hybridisation buffer, and the final 10 pM library was loaded onto the Illumina MiSeq platform using the MiSeq Reagent Kit V2 300-cycles (Illumina, United States), with an estimated coverage of approximately100× per sample.

For long-read sequencing in Nanopore MinION, normalised genomic DNA (0.2 ng/µL) from the heat-killed cultures of *M. tuberculosis* isolated from the two elephants was also used for long-read sequencing in Nanopore MinION, using the Nextera XT Library Preparation Kit (Illumina, USA). The 20 µL of tagged genomic DNA was end-repaired using Ultra II End Prep Buffer and Ultra II End Prep Enzyme (NEB, United Kingdom) according to the manufacturer's instructions. The end-repaired DNA was then subjected to Nanopore DNA preparation using Ligation sequencing kit (Oxford Nanopore, United Kingdom) with PCR barcoding expansion kit (Oxford Nanopore, United Kingdom). The final library was purified using 0.4X AMPure beads and Long Fragment Buffer (LFB) to obtain the eluate in 15 µL elution buffer. The adapter-ligated library was quantified using the Qubit Fluorometer and approximately 15 fm of the total library was finally loaded onto the MinION for sequencing using 15 µL of sequencing buffer and 10 µL loading beads.

2.5. Lineage and multi drug resistance status determination

Tb-Profiler version 4.1.0, a stand-alone tool (36), was used for lineage identification and drug resistance assessment of *M. tuberculosis* isolates from whole genome sequences. The tool accepts raw data from both MinION Nanopore and Illumina MiSeq sequences as well as BAM and FASTA files. It is a command-line tool that can use whole-genome data to predict lineages and identify 21 types of drug resistance (based on small variants and large deletions associated with drug resistance).

2.6. Processing and analysis of Illumina and Nanopore sequence data

Quality control and filtering of Illumina reads was performed using Fastp v0.20 (37), while NanoFilt v2.6.0 (38) was used to filter Nanopore reads. After quality filtering, the Illumina and Nanopore reads were mapped separately to the *M. tuberculosis* reference genome (GenBank accession NC_000962.3) using BWA v0.7.17 (39). SAMtools v1.15 (40) was used to convert the SAM files of the mapped reads from both Illumina and Nanopore to BAM files, and to sort and index the BAM file reads using the view, sort and index commands. SAMtools merge was then used to merge the index and sorted BAM files into a single BAM file containing both long and short reads for each sample (40).

2.7. Phylogenomic analysis

To performe a comparative phylogenomic analysis of *M. tuberculosis* isolates, we selected a sequence dataset comprising 94 full-length *M. tuberculosis* whole genomes, including data from human isolates in Nepal ($n = 8$), and a genome of *M. africanum* to use as an outgroup. These genomes were from 33 different countries and the four different geographical lineages previously described from elephants worldwide (lineages 1 and 4) and from humans in Nepal (lineages 1, 2 and 3), and they were retrieved from the NCBI Sequence Read Archive (SRA) database (Supplementary Table S1). The *prefetch* command in the SRA toolkit v2.11.0 (41) was used to download all whole-genome sequence files of our dataset in SRA format, which were later converted to FASTQ format using the *fastq-dump* command. Fastp v0.20 was used for quality control and read filtering (37). Read mapping to the reference genome (GenBank accession: NC_000962.3) was performed using BWA v0.7.17 (39). SAMtools v1.15 was used to convert the SAM files of the mapped reads to BAM files, and then to sort and index the BAM file reads (40). The SAMtools *mpileup* command generated a text pileup output, summarizing the base calls of the aligned reads to the reference sequence. The consensus call was performed using the *call* command in BCFtools v1.10 (40), which produced a VCF file that was converted to a FASTQ file using the *vcf2fq* function in the *vcfutils.pl* script (42). Seqtk v1.3 (42) was used to convert the FASTQ files to FASTA files using the *seq* command with the masking of bases with quality less than 20.

Variant calling for all the downloaded *M. tuberculosis* sequences and three *M. tuberculosis* isolates from this study was performed using PhaME v1.0.4 (43), with the H37Rv genome (GenBank accession: NC_000962.3) as the reference. The PhaME analysis tool was used to remove repeats from each genome in the dataset, and genome alignment to the reference genome was performed using the nucmer2 tool from MUMmer v3.0, as described in the pipeline (43). The output genome alignments were filtered, and SNP tables were generated for each alignment using the *delta-filter* and *show-snps* utilities,

TABLE 1 *Mycobacterium tuberculosis* lineages, sub-lineages, and drug resistance type of the two elephant and one human isolates collected and sequenced in this study and identified using TB-Profiler v4.1.0.

Sample	Host	Lineage	Sub-lineage	Drug-resistance type
52G111	Human	Lineage 4	Lineage 4.1.2.1	Sensitive
PK	Elephant	Lineage 2	Lineage 2.2	Sensitive
DG	Elephant	Lineage 1	Lineage 1.2.2.2	Sensitive

TABLE 2 Size, coverage, and mean depth of the analysed *M. tuberculosis* genomes after merging of the Illumina short reads and Nanopore long reads.

Sample	Size (bp)	Genome coverage (%)	Mean depth (*x*)
52G111	4,411,532	99.59	60.13
DG	4,411,532	99.69	39.29
PK	4,411,532	99.51	67.58

respectively, both in MUMmer v3.0. The PhaME pipeline then collated the alignment files to produce a core genome alignment and output a multiple alignment FASTA file (~200 kbp) with the variant sites. This was further filtered with SNP-sites to remove non-informative synonymous sites, resulting in a final non-synonymous SNP alignment dataset of 2,596 base pairs.

Using the non-synonymous SNP alignment data, phylogenomic analysis was performed using maximum likelihood and Bayesian inference. For maximum likelihood, the best substitution models and likelihood trees were evaluated using IQTREE v1.6.11 (44) with 500 bootstrap replicates and default parameters. Similarly, for Bayesian inference, the best nucleotide substitution model, TVM, was selected based on the Bayesian Information Criterion score in jModeltest2 v2.1.8 (45, 46), followed by tree reconstruction in MrBayes v3.2.7 (47) with 2,000,000 iterations, discarding 25% as burn-in.

3. Results

3.1. Molecular screening results

The two heat-killed culture samples from elephants yielded 1.07 ng/μL and 0.35 ng/μL DNA for DG and PK, respectively, allowing confirmation of *M. tuberculosis* by real-time PCR. The *M. tuberculosis* isolated from the human sample 52G11 was already a confirmed cultured *M. tuberculosis* with a DNA concentration of 2.34 ng/μL.

3.2. TB-profiler results

Lineage analysis using TB-Profiler showed that the three isolates belonged to three different *M. tuberculosis* lineages (Table 1). The elephant isolates DG and PK belong to lineage/sub-lineage 1.2.2.2 and 2.2, respectively, whereas the human isolate 52G111 belongs to lineage/sub-lineage 4.1.2.1. None of the three isolates had drug-resistant variants.

3.2.1. Whole genome and phylogenomic analysis

The mean coverage of the three *M. tuberculosis* genomes was 99.6%, while the mean depth was 55.67*x*, with the isolate DG having the lowest mean depth of 39*x* (Table 2). The PhaME pipeline generated a core genome alignment of ~200 kbp and a non-synonymous SNP alignment of 2,596 bp size, which included the elephant and human isolates and the genomes downloaded from the NCBI Sequence Read Archive database. The SNP alignment was used for phylogenomic analysis (Table 2).

The topology of both maximum-likelihood and Bayesian inference trees were similar, as shown in Figure 2. The phylogenomic analysis of *M. tuberculosis* sequences revealed that the two elephant-derived isolates, DG and PK, clustered into clades of two different lineages, lineage 1 and lineage 2, respectively. Similarly, the human-derived isolate, 52G111, clustered within into lineage 4 clade. This result is consistent with the lineages/sub-lineages defined by TB-Profiler. Sample DG is evolutionary closely related to two other published genomes of elephant-derived isolates sampled in Nepal (SRR14514371 and SRR14514372). These three elephant-derived sequences clustered together, in the same sub-clade, with published human-derived isolates sampled in Nepal (ERR1213884) and India (SRR5341274). Similarly, sample PK clustered closely in the same clade with published human-derived isolates sampled in Nepal (ERR553171 and ERR551520). The human sample 52G111 clustered in a clade together with other published human-derived samples from Argentina (ERR757160), Russia (ERR067670) and the United Kingdom (ERR047885). The topology of the tree also showed that previously published *M. tuberculosis* isolates derived from captive Asian elephants in the USA and Switzerland belonged to lineage 4 and clustered in a clade with human-derived isolates from nearby geographical regions. Similarly, an isolate originating from wild African elephants (SRR6487127) clustered very closely with a human-derived isolate (SRR1140950), both sampled in South Africa.

4. Discussion

In this study, we characterised elephant-derived *M. tuberculosis* isolates from two diseased animals (PK and DG), and a human-derived *M. tuberculosis* isolate (52G111) using a whole-genome sequencing approach combining Illumina short reads and Nanopore long reads, resulting in an average genome coverage of 99.6% with an average depth of 55.67*x*. Phylogenomic analyses were used to explore possible spillover of *M. tuberculosis* between humans and elephants. We also used an online-based TB profiling tool (TB-Profiler) to further identify lineages/sublineages of the sequenced isolates and to assess drug-resistant variants.

The two elephant-derived *M. tuberculosis* isolates appear to be of human origin based on both the TB-Profiler result and the phylogenomic trees. These strains are evolutionarily closely related to previous human isolates sampled in Nepal (Figure 1), suggesting a bidirectional transmission from humans to elephants. This may be due to the high prevalence of TB in humans and the continuous exposure of elephants to their handlers, the mahouts, rather than to the sporadic contact with tourists (48). Despite living in the same locality, the two elephant-derived *M. tuberculosis* isolates clustered in different clades of TB lineages, suggesting a complex and diverse *M. tuberculosis* transmission dynamics in the region. There must be multiple sources

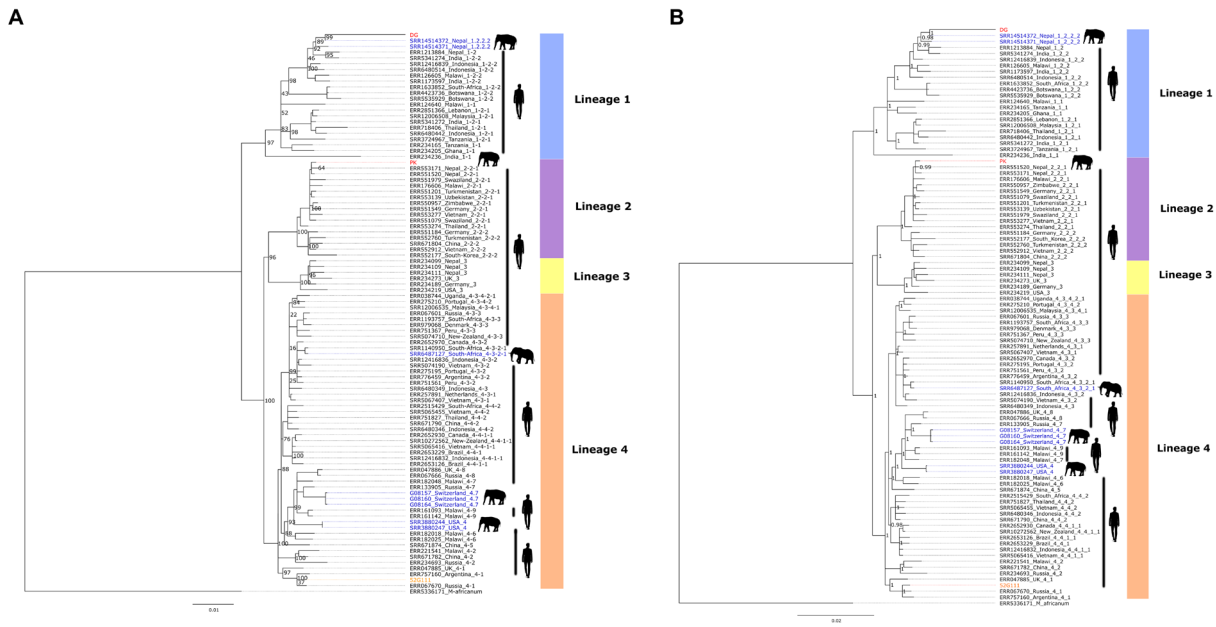

FIGURE 2
Phylogenomic trees constructed using the 2,596bp non-synonymous SNP alignment comprising the three new *M. tuberculosis* genomes described in this study and the 94 genome sequences retrieved from GenBank: **(A)** IQTREE maximum likelihood tree, the values in the nodes are 500 bootstrap scores; **(B)** MrBayes Bayesian inference tree, the values in the nodes are posterior probability scores. The elephant (PK, DG) and human (52G111) samples generated in this study are shown in red and orange font, respectively, while the published elephant and human derived samples are shown in blue and black font, respectively.

of infection and cross-species (human-mediated) transmission other than direct elephant-to-elephant transmission. This conclusion could be drawn from the fact that lineage 2 is the second most prevalent lineage in Nepal according to human samples from 2009 to 2010, while lineage 1 is the least prevalent according to a study conducted in 2018 (20). The possibility of bidirectional interspecies transmission of *M. tuberculosis* between humans and elephants has also been supported by previous reports (49–51). Our results reinforce previous evidence suggesting a close association between *M. tuberculosis* isolates from elephants and humans in Nepal using *M. tuberculosis* spoligotyping (15, 24), large sequence polymorphism (24) or whole genome data (25).

Tuberculosis is known as a re-emerging disease, but recently it has been considered a zooanthroponosis (52). The observed cases of zooanthroponosis have implications for global and national elephant conservation. Human tuberculosis already contributes to a huge annual burden of morbidity and mortality, and cases are concentrated in countries such as Nepal where poverty and high population density overlap. Multi-drug resistant *M. tuberculosis* is an additional concern, and the global burden of zoonotic TB is increasing due to the uncontrolled use of antibiotics in animals (53). Zoonotic TB is severely under-reported due to diagnostic challenges and inadequate public health surveillance (54). However, this study attempts to fill this gap in Nepal. Furthermore, the risk factors prevalent in South Asian countries, including Nepal, such as high human-animal density, close and frequent contact with infected animals, inadequate disease control measures, consumption of unpasteurised milk and milk products (55), and use of elephants in tourism (56), explain the possibility of zoonotic TB cases in humans as well as zooanthroponosis in animals. Nevertheless, a serious risk factor analysis should be undertaken to better understand the complex and dynamic transmission of TB in Nepal.

In conclusion, the prospect of an elephant potentially acting as a carrier/reservoir for the transmission of drug-resistant *M. tuberculosis* as a result of human-animal interactions is a serious concern that may pose future risks not only to humans but also to TB control in livestock and wildlife (57). A One Health approach is fundamental to understanding the spillover or transmission dynamics of infectious diseases such as TB. Therefore, regular screening and detection of these infectious diseases in elephants, other wild and domestic animals, human populations and the environment is essential. The Food and Agriculture Organization of the United Nations (FAO) has already identified in its 'Roadmap for Zoonotic Tuberculosis' (58) a wide range of actions involving a wide range of stakeholders to reduce the risk of transmission, thereby bringing economic benefits and improvements in animal welfare. The One Health Strategic Framework 2019 has already been approved in Nepal (59). In Nepal, implementation of the One Health framework appears to be possible only with a focus on zoonoses and zooanthroponosis. Therefore, it should be monitored through surveillance systems and better screening and diagnostic strategies using information on circulating *M. tuberculosis* genotypes as identified in this study. However, to limit transmission of the pathogen, oral BCG administration provides significant protection against human (60) and animal (61) TB in addition to surveillance prevention strategies. In the context of Nepal, regular *M. tuberculosis* screening of all captive elephants and people in close contact with them, especially mahouts, should be promoted to control and prevent the spread of TB. This will also help prevent the spread of TB to other endangered species in the forest (62). In addition, good practices and guidelines for tourism-related activities

involving direct human-animal interactions, such as mahout-elephant interaction, should be implemented as a preventive measure.

Data availability statement

The datasets presented in this study can be found in online repositories. The raw FASTQ genome data is available in NCBI SRA database under BioProject ID: PRJNA884899.

Ethics statement

The studies involving human participants were reviewed and approved by Ethical approval for the study was obtained from Nepal Ethical Review Board, Nepal Health Research Council (IRC number 312/2018). The patients/participants provided their written informed consent to participate in this study. Ethical review and approval was not required for the animal study because we took the samples from natural deceased animals.

Author contributions

RMR and JQ prepared the overall concept and design. RN, AG, RR, and PM performed the bioinformatics analysis, lab processing, and analysis. AP and NS contributed to the literature review. AS and RMR were involved in sample collection. RMR, NS, CG, DK, PA, JF, and JQ prepared the main writing part of the manuscript and methodology design. 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.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1133823/full#supplementary-material

References

1. Burki T. The global cost of tuberculosis. *Lancet Respir Med*. (2018) 6:13. doi: 10.1016/S2213-2600(17)30468-X

2. World Health Organization (WHO). Tuberculosis. (2021). Available at: https://www.who.int/news-room/fact-sheets/detail/tuberculosis

3. Chiang SS, Starke JR. Mycobacterium tuberculosis. *Princ Pract Pediatr Infect Dis*. (2018):790–806.e5. doi: 10.1016/B978-0-323-40181-4.00134-1

4. World Health Organization (WHO). Tackling the drug-resistant TB crisis. (2022). Available at: https://www.who.int/activities/tackling-the-drug-resistant-tb-crisis

5. Wandwalo E. Why drug-resistant tuberculosis poses a major risk to global health security. *The global fund*. (2020). Available at: https://www.theglobalfund.org/en/blog/2020-11-19-why-drug-resistant-tuberculosis-poses-a-major-risk-to-global-health-security

6. Nepal National Tuberculosis Centre. Drug resistant tuberculosis management: national guideline. (2019). Available at: https://nepalntp.gov.np/wp-content/uploads/2019/10/DR-TB-Guidelines-2019_Nepal

7. Brites D, Gagneux S. Co-evolution of mycobacterium tuberculosis and *Homo sapiens*. *Immunol Rev*. (2015) 264:6–24. doi: 10.1111/imr.12264

8. Malone KM, Gordon SV. *Mycobacterium tuberculosis* complex members adapted to wild and domestic animals. *Adv Exp Med Biol*. (2017) 1019:135–54. doi: 10.1007/978-3-319-64371-7_7

9. Talip BA, Sleator RD, Lowery CJ, Dooley JSG, Snelling WJ. An update on global tuberculosis (TB). *Infect Dis Res Treat*. (2013) 6:39–50. doi: 10.4137/IDRT.S11263

10. Messenger AM, Barnes AN, Gray GC. Reverse zoonotic disease transmission (zooanthroponosis): a systematic review of seldom-documented human biological threats to animals. *PLoS One*. (2014) 9:e89055. doi: 10.1371/journal.pone.0089055

11. Miller M, Olea-Popelka F. One health in the shrinking world: experiences with tuberculosis at the human-livestock-wildlife interface. *Comp Immunol Microbiol Infect Dis*. (2013) 36:263–8. doi: 10.1016/j.cimid.2012.07.005

12. Chastagner A, Enouf V, Peroz D, Hervé S, Lucas P, Quéguiner S, et al. Bidirectional human–swine transmission of seasonal influenza A(H1N1) pdm09 virus in pig herd, France, 2018. *Emerg Infect Dis*. (2019) 25:1940–3. doi: 10.3201/eid2510.190068

13. Epstein JH, Price JT. The significant but understudied impact of pathogen transmission from humans to animals. *Mt Sinai J Med*. (2009) 76:448–55. doi: 10.1002/msj.20140

14. Michalak K, Austin C, Diesel S, Bacon JM, Zimmerman P, Maslow JN. *Mycobacterium tuberculosis* infection as a zoonotic disease: transmission between humans and elephants. *Emerg Infect Dis*. (1998) 4:283–7. doi: 10.3201/eid0402.980217

15. Paudel S, Mikota SK, Nakajima C, Gairhe KP, Maharjan B, Thapa J, et al. Molecular characterization of *Mycobacterium tuberculosis* isolates from elephants of Nepal. *Tuberculosis*. (2014) 94:287–92. doi: 10.1016/j.tube.2013.12.008

16. Mikota SK, Peddie L, Peddie J, Lindsay W, Larsen RS, Iyer A, et al. Epidemiology and diagnosis of *Mycobacterium tuberculosis* in captive Asian elephants (*Elephas maximus*). *J Zoo Wildl Med*. (2001) 32:1–16. doi: 10.1638/1042-7260(2001)032

17. Zachariah A, Pandiyan J, Madhavilatha GK, Mundayoor S, Chandramohan B, Sajesh PK, et al. *Mycobacterium tuberculosis* in wild Asian elephants, southern India. *Emerg Infect Dis*. (2017) 23:504–6. doi: 10.3201/eid2303.161741

18. Angkawanish T, Wajjwalku W, Sirimalaisuwan A, Mahasawangkul S, Kaewsakhorn T, Boonsri K, et al. *Mycobacterium tuberculosis* infection of domesticated Asian elephants, Thailand. *Emerg Infect Dis*. (2010) 16:1949–51. doi: 10.3201/eid1612.100862

19. Payeur JB, Jarnagin JL, Marquardt JG, Whipple DL. Mycobacterial isolations in captive elephants in the United States. *Ann N Y Acad Sci*. (2002) 969:256–8. doi: 10.1111/j.1749-6632.2002.tb04388.x

20. Nepal Ministry of Health and Population. National Tuberculosis prevalence survey report 2020. (2020). Available at: https://nepalntp.gov.np/wp-content/uploads/2021/03/NTPS-Report-Bodypages

21. Shrestha S, Shrestha J. Asian elephants and their status in Nepal: a review. *J Agric Nat Resour*. (2021) 4:227–37. doi: 10.3126/janr.v4i2.33828

22. Pradhan NMB, Williams AC, Dhakal M. Current status of Asian elephants in Nepal. *Gajah*. (2011) 35:87–92.

23. Thapa J, Mikota SK, Gairhe KP, Paudel S, Singh DK, Dhakal IP, et al. Tuberculosis seroprevalence and comparison of hematology and biochemistryparameters between seropositive and seronegative captive Asian elephants of Nepal. *J Vet Med Sci*. (2021) 83:1278–83. doi: 10.1292/JVMS.21-0113

24. Paudel S, Nakajima C, Mikota SK, Gairhe KP, Maharjan B, Subedi S, et al. Mixed *Mycobacterium tuberculosis* lineage infection in 2 elephants, Nepal. *Emerg Infect Dis*. (2019) 25:1031–2. doi: 10.3201/eid2505.181898

25. Paudel S, Brenner EP, Hadi SA, Suzuki Y, Nakajima C, Tsubota T, et al. Genome sequences of two *Mycobacterium tuberculosis* isolates from Asian elephants in Nepal. *Microbiol Resour Announc*. (2021) 10:e00614–21. doi: 10.1128/MRA.00614-2

26. Martinez E, Bustamante A, Menon R, Wang Q, Jelfs P, Marais B, et al. Whole-genome sequencing of *Mycobacterium tuberculosis* for rapid diagnostics: feasibility of a decentralised model. *Lancet Respir Med*. (2016) 4:e13–4. doi: 10.1016/S2213-2600(16)00092-8

27. Chitwan District Information: Chitwan Tourism (2013). Nepal. (2013). Available at: https://www.chitwantourism.com/articles-and-information/chitwan-district-information

28. Lonely Planet. Chitwan National Park Travel—Lonely Planet. Nepal, Asia. (2013). Available at: https://www.lonelyplanet.com/nepal/the-terai-and-mahabharat-range/royal-chitwan-national-park

29. CNP. Managment plan for Chitwan National Park and it's buffer zone 2013–2017. *Chitwan Natl Park Off*. (2015) 53:1689–99.

30. CNP. Annual report Chitwan National Park. Government of Nepal. (2022). Available at: https://chitwannationalpark.gov.np

31. Bhusal NP. Buffer zone management system in protected areas of Nepal. *Third Pole J Geogr Educ*. (2014) 11:34–44. doi: 10.3126/ttp.v11i0.11558

32. Budhathoki P. Linking communities with conservation in developing countries: buffer zone management initiatives in Nepal. *Oryx*. (2004) 38:334–41. doi: 10.1017/S0030605304000584

33. Heinen JT, Mehta JN. Emerging issues in legal and procedural aspects of buffer zone management with case studies from Nepal. *J Env Dev*. (2000) 9:45–67. doi: 10.1177/107049650000900103

34. Nazarova EV, Russell DG. Growing and handling of *Mycobacterium tuberculosis* for macrophage infection assays. *Methods Mol Biol*. (2017) 1519:325–31. doi: 10.1007/978-1-4939-6581-6_22

35. Sacace Biotechnologies S.r.l. Via Scalabrini. Sacace Biotechnologies. (2022). Available at: https://www.sacace.com

36. Phelan JE, O'Sullivan DM, Machado D, Ramos J, Oppong YEA, Campino S, et al. Integrating informatics tools and portable sequencing technology for rapid detection of resistance to anti-tuberculous drugs. *Genome Med*. (2019) 11:41–7. doi: 10.1186/s13073-019-0650-x

37. Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*. (2018) 34:i884–90. doi: 10.1093/bioinformatics/bty560

38. De Coster W, D'Hert S, Schultz DT, Cruts M, Van Broeckhoven C. NanoPack: visualizing and processing long-read sequencing data. *Bioinformatics*. (2018) 34:2666–9. doi: 10.1093/bioinformatics/bty149

39. Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. (2013). *arXiv*. [Epub ahed of preprint], doi: 10.48550/arxiv.1303.3997

40. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, et al. Twelve years of SAMtools and BCFtools. *Gigascience*. (2021) 10:1–4. doi: 10.1093/gigascience/giab008

41. NCBI/NLM/NIH. Sequence read archive—download software. (2022). Available at: https://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?view=software

42. GitHub. lh3/seqtk: Toolkit for processing sequences in FASTA/Q formats. (2022). Available at: https://github.com/lh3/seqtk

43. Shakya M, Ahmed SA, Davenport KW, Flynn MC, Lo CC, Chain PSG. Standardized phylogenetic and molecular evolutionary analysis applied to species across the microbial tree of life. *Sci Rep*. (2020) 10:1723–15. doi: 10.1038/s41598-020-58356-1

44. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A, et al. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol Biol Evol*. (2020) 37:1530–4. doi: 10.1093/molbev/msaa015

45. Darriba D, Taboada GL, Doallo R, Posada D. jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods*. (2012) 9:772. doi: 10.1038/nmeth.2109

46. Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol*. (2003) 52:696–704. doi: 10.1080/10635150390235520

47. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol*. (2012) 61:539–42. doi: 10.1093/sysbio/sys029

48. Szydlowski M. Elephants in Nepal: correlating disease, tourism, and welfare. *J Appl Anim Welf Sci*. (2022) 25:126–38. doi: 10.1080/10888705.2022.2028628

49. Malla B, Stucki D, Borrell S, Feldmann J, Maharjan B, Shrestha B, et al. First insights into the phylogenetic diversity of *Mycobacterium tuberculosis* in Nepal. *PLoS One*. (2012) 7:e52297. doi: 10.1371/journal.pone.0052297

50. Abraham D, Pillai VKP. Cross-species transmission of *Mycobacterium tuberculosis* in mahouts and captive elephants: implications to health policy. *Int J Infect Dis*. (2016) 45:463. doi: 10.1016/j.ijid.2016.02.981

51. Ong BL, Ngeow YF, Razak MFAA, Yakubu Y, Zakaria Z, Mutalib AR, et al. Tuberculosis in captive Asian elephants (*Elephas maximus*) in peninsular Malaysia. *Epidemiol Infect*. (2013) 141:1481–7. doi: 10.1017/S0950268813000265

52. Shimshony A. Tuberculosis in elephants: a reverse zoonosis. *Infect Dis News*. (2008) 21:8. Available at: https://www.healio.com/news/infectious-disease/20120225/tuberculosis-in-elephants-a-reverse-zoonosis

53. Acharya KP, Wilson RT. Antimicrobial Resistance in Nepal. *Front Med*. (2019) 6:105. doi: 10.3389/fmed.2019.00105

54. Kock R, Michel AL, Yeboah-Manu D, Azhar EI, Torrelles JB, Cadmus SI, et al. Zoonotic tuberculosis—the changing landscape. *Int J Infect Dis*. (2021) 113:S68–72. doi: 10.1016/j.ijid.2021.02.091

55. Bapat PR, Dodkey RS, Shekhawat SD, Husain AA, Nayak AR, Kawle AP, et al. Prevalence of zoonotic tuberculosis and associated risk factors in central Indian populations. *J Epidemiol Glob Health*. (2017) 7:277–83. doi: 10.1016/j.jegh.2017.08.007

56. Szydlowski M. Framing conservation, colonialism and care: Captive endangered Asian elephants (*Elephas maximus*) in Nepal. (2021). *Doctoral Theses*. Available at: https://ore.exeter.ac.uk/repository/handle/10871/127765

57. Botelho A, Perdigão J, Canto A, Albuquerque T, Leal N, Macedo R, et al. Pre-multidrug-resistant *Mycobacterium tuberculosis* Beijing strain associated with disseminated tuberculosis in a pet dog. *J Clin Microbiol*. (2014) 52:354–6. doi: 10.1128/JCM.02800-13

58. Food and Agriculture Organization of the United Nations (FAO). Roadmap for zoonotic tuberculosis. (2017). Available at: https://www.fao.org/3/i7807e/i7807e

59. One Health Strategy, 2076. Ministry of Agriculture and Livestoke Development, Nepal. (2022). Available at: https://publichealthupdate.com/one-health-strategy-2076

60. Brewer TF. Preventing tuberculosis with bacillus Calmette–Guérin vaccine: a meta-analysis of the literature. *Clin Infect Dis*. (2000) 31 Suppl 3:S64–7. doi: 10.1086/314072

61. Buddle BM, Vordermeier HM, Chambers MA, de Klerk-Lorist LM. Efficacy and safety of BCG vaccine for control of tuberculosis in domestic livestock and wildlife. *Front Vet Sci*. (2018) 5:259. doi: 10.3389/fvets.2018.00259

62. Paudel S, Sreevatsan S. Tuberculosis in elephants: origins and evidence of interspecies transmission. *Tuberculosis*. (2020) 123:101962. doi: 10.1016/j.tube.2020.101962

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Investigation of risk factors for introduction of highly pathogenic avian influenza H5N1 virus onto table egg farms in the United States, 2022: a case–control study

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Introduction: The 2022–2023 highly pathogenic avian influenza (HPAI) H5N1 outbreak in the United States (U.S.) is the most geographically extensive and costly animal health event in U.S. history. In 2022 alone, over 57 million commercial and backyard poultry in 47 U.S. states were affected. Over 75% of affected poultry were part of the commercial table egg production sector.

Methods: We conducted a case–control study to identify potential risk factors for introduction of HPAI virus onto commercial table egg operations. Univariate and multivariable analyses were conducted to compare farm characteristics, management, and biosecurity factors on case and control farms.

Results: Factors associated with increased risk of infection included being in an existing control zone, sightings of wild waterfowl, mowing or bush hogging vegetation less than 4 times a month, having an off-site method of daily mortality disposal (off-site composting or burial, rendering, or landfill), and wild bird access to feed/feed ingredients at least some of the time. Protective factors included a high level of vehicle washing for trucks and trailers entering the farm (a composite variable that included having a permanent wash station), having designated personnel assigned to specific barns, having a farm entrance gate, and requiring a change of clothing for workers entering poultry barns.

Discussion: Study results improve our understanding of risk factors for HPAI infection and control measures for preventing HPAI on commercial U.S. table egg farms.

KEYWORDS

avian influenza, table egg, layers, H5N1, highly pathogenic avian influenza, case–control, biosecurity

1. Introduction

Many species of birds are susceptible to influenza A viruses. Aquatic wild birds constitute a major reservoir of these viruses, which are classified into subtypes according to their hemagglutinin (H) and neuraminidase (N) antigens. Low pathogenicity H5 and H7 influenza viruses generally cause few clinical signs or asymptomatic infections in poultry. While the clinical signs of highly pathogenic avian influenza (HPAI) viruses may vary, severe clinical signs and high mortality rates may occur (1).

Birds infected with influenza shed virus in feces and respiratory secretions. Disease may spread through direct contact with infected birds or their secretions or through contaminated feed and water. Indirect spread through fomites, such as contaminated farm equipment, can also spread the virus. As part of the U.S. response to HPAI, a 10 km control zone is established around each infected premises with birds kept primarily for the purpose of producing poultry or poultry products offered for sale or trade. Poultry, poultry products, and other materials from within a control zone require permitting before movement. Extensive surveillance of commercial and backyard flocks is conducted prior to control area release. Economic impacts of HPAI are wide-ranging, including not only loss of birds due to death or depopulation, but also the high cost of outbreak response and market losses associated with international trade restrictions (2).

The U.S. Geological Survey's National Wildlife Health Center conducts surveillance in wild birds to assist in early detection of high consequence pathogens such as HPAI (3). As described by Caliendo et al. (4), the first 2021 detection of Eurasian strain H5N1 HPAI H5N1 clade 2.3.4.4b in North America occurred in December in Newfoundland and Labrador, Canada, in a mixed species flock (4). At the time, it was noted that this was the first detection of H5 influenza virus of this lineage in the Americas since June 2015 in domestic birds and December 2016 in wild birds (5). The last introduction of viruses from this lineage to North America in 2014 ultimately resulted in what was, at the time, the largest animal health emergency in the history of the United States (6).

In February 2022, the first commercial poultry flock in the U.S. was affected with this Eurasian lineage H5N1 HPAI virus. By the end of 2022, over 57 million commercial and backyard poultry in 47 U.S. states were affected (7), resulting in over $659 million in federal expenditures for control efforts and indemnity payments. While approximately 70% of all affected commercial poultry farms in 2022 were turkey farms, the commercial table egg industry was also heavily affected. Over 75% of affected commercial poultry were table egg birds. Results of full genome sequencing indicate that independent wild bird introductions have been the primary mechanism of introduction of virus into operations in this outbreak (Youk S, Torchetti MK, Lantz K, Lenoch JB, Killian ML, Leyson C, et al., pending submission). In comparison, the severity of the 2014–2015 U.S. HPAI H5N2 outbreak, once it reached the Midwest, was heavily influenced by lateral transmission of virus between farms (8, 9).

Circulation of this highly infectious HPAI virus among North American wild birds calls for updated epidemiologic investigation of factors associated with spillover infection to domestic poultry. To explore these factors, as well as how they may differ from risk factors noted in 2015, a case-control study for H5N1 HPAI was conducted among commercial table egg layer, pullet, and breeder bird farms in Delaware, Iowa, Maryland, Minnesota, Nebraska, Ohio, Pennsylvania, and Utah. This study was conducted by the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) in collaboration with State partners, academia, and national poultry organizations. Goals of this study included identifying risk factors for HPAI on commercial table egg farms, identifying biosecurity challenges on commercial table egg farms, and providing data to assist in refining biosecurity recommendations to support prevention of HPAI. This information will improve understanding of risk factors associated with HPAI on table egg farms in the United States to support science-based guidance on farm-level preventative measures.

Unprecedented transmission of this H5N1 clade 2.3.4.4b among wild bird populations results in ongoing high risk for domestic poultry (10) and significant economic impacts for affected producers. The USDA's National Veterinary Services Laboratories perform whole genome sequencing (WGS) of the influenza virus for all confirmed positive operations and conduct analyses to help determine whether the sequence (s) are consistent with independent wild bird-origin introduction or represent the potential for lateral spread while considering all available epidemiologic data. The subset of operations likely to be infected by wild bird introduction was further examined in relation to selected farm characteristics and biosecurity-related management practices to determine possible associations with wild bird-related spillover risks for HPAI.

In addition to this commercial table egg farm study, there was a similar but independent study of the risk factors for HPAI affecting turkey farms, also conducted by USDA–APHIS. Despite similar overall objectives, the study design and target population were sufficiently different to warrant a separate report. This publication relates to the table egg layer investigation, while the turkey farm investigation is reported separately.

2. Materials and methods

2.1. Case definition and laboratory testing

Samples from poultry farms were screened for influenza A, and H5/H7 subtypes by reverse transcript real-time polymerase chain reaction (RT-PCR) by members of the National Animal Health Laboratory Network (NAHLN). Samples testing non-negative by influenza A virus (IAV) PCR were sent to the National Veterinary Services Laboratories (NVSL, Ames, Iowa, United States) for confirmation. Testing at NVSL included an H5 clade 2.3.4.4 pathotyping assay and an assay targeting N1 for neuraminidase subtyping. Whole genome sequencing was conducted directly from the samples. Influenza A viruses were sequenced directly from samples as previously described (11); RAxML was used to generate phylogenetic trees, and tables of single nucleotide polymorphisms (SNPs) were created using the vSNP pipeline.[1]

For the purposes of this study, a case farm was defined as any U.S. table egg layer, pullet, or breeder premises in Delaware, Iowa, Maryland, Minnesota, Nebraska, Ohio, Pennsylvania, or Utah from which samples were confirmed positive from February through September 2022. Control farms were defined as farms from the same states that did not have HPAI during the study period.

1 https://github.com/USDA-VS/vSNP

2.2. Questionnaire design and data collection

By September 30, 2022, more than 29 million table egg layers, pullets, and breeder birds in the participating 8 states had been lost from infection or depopulation (7). A case–control study was designed to examine risk factors associated with HPAI infection on U.S. commercial table egg farms. For the current study, the questionnaire from Garber et al. (12) was updated and condensed based on academic, field, and industry subject matter expertise. The 26-page questionnaire (included in Supplementary material) covers farm characteristics, wild birds and wildlife, biosecurity, personnel, visitors, vehicles, equipment, and management practices for the 14 days prior to detection of clinical signs or increased mortality on case farms, and for a comparable 14-day reference period on control farms. In rare situations where clinical signs or an increase in mortality were not noted, farmers were asked to provide the date that the farm was positive for HPAI based on diagnostic test results. This situation was most common when farms were located within a control or surveillance zone.

Eligible case farms included those commercial table egg layer, pullet, and breeder farms in Delaware, Iowa, Maryland, Minnesota, Nebraska, Ohio, Pennsylvania, or Utah with confirmed infection from February 22 through September 30, 2022. A total of 22 farms met the inclusion criteria. While confirmed infections also occurred in Colorado, South Dakota, and Wisconsin, these were not included due to resource constraints or lack of eligible control premises. Eligible control farms were any commercial table egg layer, pullet, or breeder farms selected from the same states as case farms, using the USDA–APHIS Veterinary Services Emergency Management Response System. Randomized lists of 10 potential controls per case were shared with interviewers in each participating state, with a goal of enrolling up to two control farms per case farm. Potential controls were contacted by interviewers via phone or email to confirm eligibility and interest in participating. To be considered eligible, control farms needed to have 50,000 or more birds, as well as birds on site for a minimum two-week window of risk within the state-specific high-risk timeframe. High-risk timeframes were determined according to reported onset of clinical signs for confirmed infections within the states. Interviewers were asked to match risk windows for cases and controls as closely as possible.

Questionnaires were administered by Federal or State veterinary medical officers via telephone interviews. Fillable pdf forms were uploaded to a secure APHIS location. Interviewers in each state only had access to their state's data. All data were treated as confidential business information; due to this requirement, results are shared in aggregate only. Producer participation was voluntary. Questionnaire administration took place between September 26 and December 28, 2022.

2.3. Data entry and management

Survey data were entered into a SAS dataset using SAS version 9.4 (SAS Institute Inc., Cary, NC). Survey responses were validated by USDA–APHIS staff prior to analysis. Validation included reviewing survey responses for consistency and logical issues such as the proper treatment of skip patterns (e.g., if a respondent reported not having a certain type of visitor on the operation but then also reported a count of more than zero visits made to the operation by that visitor type), checking for invalid responses (e.g., a response of "0" for a 1/3 response variable), reclassification of other specify responses (e.g., if the respondent chose another type of road surface but wrote in a surface type that was consistent with the listed road surface types), and other conditional logic checks. Random, single imputation was used for variables included in multiple regression modeling for which there was item nonresponse, but a valid response could not be deduced using the validation steps outlined above (13, 14).

Validation and univariable analyses were performed using SAS version 9.4. Multiple regression modeling was performed using R version 4.1.1 (15), implemented within R Studio version 1.4.1717 (16). The R packages used included AICcmodavg (17), blorr (18), BMA (19), car (20), caret (21), haven (22), Hmisc (23), leaps (24), lme4 (25), and tidyverse (26). Exact multiple logistic regression model estimates were generated using PROC LOGISTIC in SAS version 9.4.

2.4. Univariable analyses

Univariable analyses were performed to identify variables potentially associated with the presence of HPAI, and a Fisher's exact test was used for categorical variables to assess the association of each variable with HPAI infection. Numeric variables were broken into quartiles for assessment. Variables with Fisher's exact test $p \leq 0.20$ that were also biologically plausible for risk of HPAI infection and had at least 5 responses per level were considered for entry into candidate multivariable models.

The subset of farms that had WGS results consistent with wild bird introduction was further examined in relation to selected farm and biosecurity-related factors to determine which of these may be specifically associated with wild bird-related spillover risk for HPAI.

2.5. Multivariable analyses

2.5.1. Multicollinearity and confounding variables

From the pool of screened predictor variables, variance inflation factors (VIFs) (27) were computed. Variables with VIFs exceeding 3 indicated further investigation was needed. All of the predictor variables considered were binary, so the ordinary VIF was used rather than the generalized VIF (28). Groupings of variables with high similarity were identified using hierarchical clustering on a similarity matrix, calculated using proportions of observations that were positive for each pair of binary predictor variables, using complete linkage clustering (29).

Confounding and effect measure modification were assessed using three statistical methods combined with subject-matter expertise regarding likely causal relationships between predictor variables and HPAI presence (30, 31). First, the relationship between the variable of interest and the confounding variable and the relationship between the confounding variable and HPAI presence were both assessed using logistic regression modeling and assessing statistical significance at the 0.10 significance level. Secondly, the relative change in the estimated odds ratio associated with the predictor variable of interest in a multiple logistic regression model prior to and after inclusion of the potential confounding variable was assessed, with changes of more than 10 percent being indicative of potential confounding. Lastly, biological, and epidemiological

plausibility was used to determine whether the potential confounding variable was in the causal pathway between the variable of interest and HPAI presence. If a potential confounding variable passed the statistical checks and was found not to be in the causal pathway between the predictor of interest and HPAI presence, then that variable would be adjusted for in the multiple logistic regression model. Potential confounding relationships were tested to identify whether they were indicative of confounding, effect measure modification, or both (31).

2.5.2. Leave-one-out cross-validation and AICc

Leave-one-out cross-validation [LOOCV] (32) was used to rank models according to their ability to classify case and control farms. In this study, because there were approximately equal numbers of case and control farms in the dataset, the overall goodness of the multiple logistic regression model predictions was taken as the accuracy of the predictions made using the models.

The values of the accuracy of the models ranked using LOOCV were relatively coarse due to sample size. Therefore, a second model goodness criterion was used to order models within a given accuracy. The second model goodness criterion was AICc, which is a variant of Akaike's information criterion (33) with an adjustment for small sample sizes (34). Smaller values of AICc indicate models that are expected to approximate the underlying process more closely than models with higher values of AICc.

2.5.3. Multiple logistic regression modeling

During model selection, multiple logistic regression models were fit using iteratively reweighted least squares using the glm function in R, specifying the response as the indicator for whether a farm was a case (response value of 1) or a control (response value of 0) as a function of one or more predictor variables. For the final model-based estimates, an exact multiple logistic regression model wasfit using the EXACT statement in the LOGISTIC procedure within SAS, which gives conditional maximum likelihood estimates of odds ratios and their confidence intervals (35), which use permutation theory and are appropriate to use in situations in which there are small sample sizes.

Logistic regression model fit was assessed using deviance residual diagnostic plots (27) to check for influence, leverage, and overall model fit. Statistics used to describe relative model goodness included the LOOCV accuracy and AICc. McFadden's pseudo–R^2 and McFadden's adjusted pseudo-R^2 were used to assess explained variability of the model (36).

The primary inferential statistics derived included estimated odds ratios (OR), indicating the multiplicative increase in the odds of a farm being a case farm associated with a given predictor variable, given all other predictor variables remained constant. In addition, estimated 95% confidence intervals for the odds ratios were used to communicate uncertainty in the point estimates, and Type III F-test (37) value of ps were used to assess the statistical significance of model effects.

2.5.4. Bayesian model averaging

In addition to investigating potentially important predictor variables for HPAI presence using the above methods to select a single model from which to make inference, Bayesian model averaging was used to further investigate the effects of the predictor variables in the pool under consideration. Bayesian model averaging (BMA) is a statistical method that attempts to account for the uncertainty induced by the model selection problem by taking information from a broad group of models rather than from a single model (38). BMA has been shown to improve predictive ability over single-model selection methods.

Bayesian model averaged estimates of the posterior probabilities that the predictor variable effects were non-zero, odds ratios, and approximate 95% confidence intervals for the odds ratios were produced using the BMA package in R. These estimates were used to estimate the effect of each predictor variable on HPAI presence in a multiple logistic regression model setting, accounting for the spread of effect sizes those variables take across a broad range of possible models.

3. Results

Eighteen of 22 (81.8%) commercial table egg farms affected by HPAI in the 8 participating states agreed to participate in the study. An estimated 20% of potential control producers met eligibility criteria and agreed to participate. Onset of clinical signs for affected flocks ranged from March 3 to August 31, 2022. To maintain confidentiality of participating producers, cases and controls were reported by region; states in the Eastern region included Maryland, Delaware, Ohio, and Pennsylvania. States in the Midwest/Western region included Iowa, Minnesota, Nebraska, and Utah. Samples from this study were collected between March 2022 and September 2022. There were 18 case farms; 11 farms in the Eastern region (61.1% of case farms) and 7 farms in the Midwest/Western region (38.9% of case farms). There were 22 control farms, with 15 of the control farms in the Eastern region (68.2% of control farms) and 7 of the control farms (31.8% of control farms) in the Midwest/Western region. All premises were tested by PCR and confirmed with H5N1 clade 2.3.4.4b at NVSL. Based on the analysis of full genome sequences and in consideration of available epidemiologic data, each layer case farm that participated in the case–control study was categorized by likely route of introduction of virus: introductions consistent with independent wild bird-origin were identified for 61% of case farms ($n = 11$), whereas potential lateral spread or common source exposure was found for 39% of case farms ($n = 7$). The case–control analysis included 18 case farms and 22 control farms. Median flock size for case farms was 900,000 (range: 72,000–5,000,000). Median flock size for control farms was 480,000 (range: 77,000–2,900,000). Of the case farms, 83% ($n = 15$) had table egg layers, and 22% ($n = 4$) had pullets or breeders. Of the control farms, 91% ($n = 20$) had table egg layers, and 14% ($n = 3$) had pullets or breeders (Please note that there was some overlap between categories). During the study period, a total of 137 control zones were active in the 8 states that took part in the study.

3.1. Univariable analyses

Selected results of the farm-level univariable analysis are shown in Tables 1–3. These results do not include imputed values. A complete list of univariable results is available in the Supplementary material.

TABLE 1 Univariable analyses of factors considered for entry into farm-level multivariable models.

Characteristic	Number of case farms (percent)	Number of control farms (percent)	Univariable *p*-value (Fisher's exact)	Odds ratio (95% confidence interval)
In an existing control zone	8 (44.4)	2 (9.1)	0.02	8.0 (1.4, 44.9)
Flock size was large (≥500,000 birds)	11 (61.1)	11 (50.0)	0.54	1.6 (0.4, 5.6)
Closest field within 320 meters (350 yards) of farm tilled previous fall	2 (11.1)	9 (42.9)	0.04	0.2 (0.0, 0.9)
Wild waterfowl or shorebirds seen in closest field during reference period	8 (44.4)	2 (9.5)	0.03	7.6 (1.3, 42.8)
Farm entrance gated	4 (22.2)	14 (63.6)	0.01	0.2 (0.0, 0.7)
Vegetation mowed/bush hogged less than 4 times a month	11 (64.7)	9 (40.9)	0.20	2.6 (0.7, 9.8)
Lower level of vehicle washing (combination variable)	16 (88.9)	15 (68.2)	0.15	3.7 (0.7, 20.9)
Vehicle tires washed	11 (61.1)	18 (81.8)	0.17	0.3 (0.1, 1.5)
Feed trucks washed	9 (50.0)	18 (81.8)	0.05	0.2 (0.1, 0.9)
Egg trucks washed	9 (50.0)	16 (76.2)	0.11	0.3 (0.1, 1.2)
Company trucks/trailers either not shared or shared and always disinfected during reference period	12 (66.7)	20 (90.9)	0.11	0.2 (0.0, 1.2)
Permanent vehicle washing station	3 (16.7)	9 (40.9)	0.17	0.3 (0.1, 1.3)
Any vehicles either not shared or shared and always disinfected during reference period (combination variable)	7 (38.9)	6 (27.3)	0.51	1.7 (0.4, 6.4)

Number and percentage of case farms and number and percentage of control farms by farm, wild bird, and vehicle-related characteristics.

Farm characteristics (Table 1). During the 14-day reference period, more case farms were located within an existing control zone compared to control farms (44% vs. 9%, OR = 8.0, $p = 0.02$). Fewer case farms were within 320 meters (350 yards) of a field that had been tilled the previous fall (11% vs. 43%, OR = 0.2, $p = 0.04$).

Wild bird/wild animal characteristics (Table 1). Wild waterfowl or shorebirds were seen in the closest field during the 14-day reference period on 44% of case farms compared to 10% of control farms (OR = 7.6, $p = 0.03$).

Vehicle-related characteristics (Table 1). Using wash stations to wash vehicle tires was more commonly reported on control farms (82% vs. 61% of case farms, OR = 0.3, $p = 0.17$). Using wash stations for feed trucks was more commonly reported on control farms (82% vs. 50% of case farms, OR = 0.2, $p = 0.05$). Using wash stations for egg trucks was more commonly reported on control farms (76% vs. 50% of case farms, OR = 0.3, $p = 0.11$). Ninety-one percent of control operations either did not share or shared and always disinfected company trucks and trailers that might be used by another farm, as compared with 67% of case operations (OR = 0.2, $p = 0.11$). Permanent vehicle wash stations were more commonly reported by control farms (41% vs. 17% of case farms, OR = 0.3, $p = 0.17$).

Biosecurity characteristics (Tables 1, 2). Having a gated farm entrance was more commonly reported on control farms (64% vs. 22% of case farms, OR = 0.2, $p = 0.01$). Farm mowing or bush hogging less than 4 times a month was more commonly reported on case farms (65% vs. 41% of control farms, OR = 2.6, $p = 0.20$). Incineration was more commonly reported as a method of daily mortality disposal on control farms (OR = 0.2, $p = 0.10$, Table 2).

Worker and visitor-related practices (Table 2), *not all data shown.* In general, use of occasional workers was uncommon. Most farms always required the use of a clean/dirty line for workers entering barns (83% of case farms vs. 91% of control farms, $p = 0.64$). Always requiring a washable change of clothing for workers entering barns was more commonly reported by control farms (91% vs. 67% of case farms, OR = 0.20, $p = 0.11$). Nearly all farms always required a change of shoes or use of shoe covers for workers entering barns. Overall, visitors to farms were not common. High percentages of both case and control farms required visitors not to visit multiple farms in the same day (92% of case farms and 84% of control farms, $p = 1.00$). Workers being assigned to specific barns (dedicated barn personnel) was more commonly reported on control farms (OR = 0.2, $p = 0.20$).

Rodent management and wildlife feed access (Table 2). Having at least some problem with rodents was more commonly reported on case farms (72% vs. 46% of control farms, OR = 3.1, $p = 0.12$). Having wild bird access to feed or feed ingredients at least some of the time was more commonly reported on case farms (50% vs. 27% of control farms, OR = 2.7, $p = 0.19$). Having wild animal access to feed or feed ingredients at least some of the time was more commonly reported on case farms (33% vs. 9% of control farms, OR = 5.0, $p = 0.11$).

Other variables of interest, not statistically significant. Among case farms, 33% reported use of a renderer as a general practice, while this mortality disposal method was reported by 14% of control farms as a general practice ($p = 0.25$). The practice of cleaning up feed spills immediately was reported by 67% of case farms and 82% of control farms ($p = 0.30$). Half of case farms and half of control farms reported having hard top/asphalt roads as the road surface on the farms that vehicles coming onto the operation drive on.

Variables analyzed for independent wild bird introduction of HPAI (Table 3). For the univariate analysis of case farms where HPAI was independently introduced by wild birds, being located within a control zone was not significant (27% of case farms vs. 10% of control farms, OR = 3.4, $p = 0.32$). Having a structural windbreak such as a hill or other natural break present was more common for control farms (30% vs. 0% of case farms), (OR not calculated due to zero cell, $p = 0.07$). Though not statistically significant, being within 320 meters (350 yards) of a wastewater lagoon was more common among case farms (73% vs. 45% of control farms, OR = 3.3, $p = 0.26$). Having a drainage ditch visible or within 320 meters (350 yards) of the farm was more

TABLE 2 Univariable analyses ($p \leq 0.20$) of factors considered for entry into farm-level multivariable models.

Characteristic	Number of case farms (percent)	Number of control farms (percent)	Univariable *p*-value (Fisher's exact)	Odds ratio (95% confidence interval)
Change of clothing always required for workers entering poultry barns	12 (66.7)	20 (90.9)	0.11	0.2 (0.0, 1.2)
Different personnel for different barns (dedicated barn personnel)	**	5 (22.7)	0.20	0.2 (0.0, 1.9)
Severity of rodents low, moderate, or high (vs. not a problem)	13 (72.2)	10 (45.5)	0.12	3.1 (0.8, 11.8)
Wild birds able to access feed/feed ingredients at least sometimes	9 (50.0)	6 (27.3)	0.19	2.7 (0.7, 10.0)
Wild animals able to access feed/feed ingredients at least sometimes	6 (33.3)	2 (9.1)	0.11	5.0 (0.9, 28.9)
Incineration as a method of daily mortality disposal	**	6 (27.3)	0.10	0.2 (0.0, 1.5)
Off-site method of daily mortality disposal (off-site composting or burial, rendering, or landfill)	9 (50.0)	6 (27.3)	0.19	2.7 (0.7, 10.0)

Number and percentage of case farms and number and percentage of control farms by premises feed, rodent-related, worker-related, and mortality disposal management practices.
**Too few to report.

TABLE 3 Univariable analyses of selected factors analyzed for the subset of farms with independent wild bird introduction of HPAI.

Characteristic	Number of case farms (percent)	Number of control farms (percent)	Univariable *p*-value (Fisher's exact)	Odds ratio (95% confidence interval)
In an existing control zone	3 (27.3)	2 (10.0)	0.32	3.4 (0.5, 24.3)
Structural windbreak present (e.g., hill, natural break)	0 (0.0)	6 (30.0)	0.07	*
Wastewater lagoon visible or within 320 meters (350 yards) of farm	8 (72.7)	9 (45.0)	0.26	3.3 (0.7, 16.0)
Drainage ditch visible or within 320 meters (350 yards) of farm	7 (63.6)	7 (35.0)	0.15	3.3 (0.7, 15.1)
Wild waterfowl or shorebirds seen in closest field during reference period	4 (36.4)	1 (5.3)	0.05	10.3 (1.0, 108.8)
Low, moderate, or high rodent problem vs. rodents not a problem	8 (72.7)	8 (40.0)	0.14	4.0 (0.8, 19.8)
Wild birds able to access feed/feed ingredients at least sometimes	8 (72.7)	6 (30.0)	0.03	6.2 (1.2, 31.9)
Clean up feed spills immediately	5 (50.0)	16 (80.0)	0.12	0.3 (0.1, 1.4)

Number and percentage of case farms and number and percentage of control farms by premises characteristics, biosecurity, and feed-related management practices.
*OR not shown due to zero cell value for case farms.

common among case farms (64% vs. 35% of control farms, OR = 3.3, $p = 0.15$). Seeing wild waterfowl or shorebirds in the field closest to the farm during the reference period was more common among case farms (36% vs. 5% of control farms, OR = 10.3, $p = 0.05$). Having at least some problem with rodents was more common among case farms (73% vs. 40% of control farms, OR = 4.0, $p = 0.14$). Wild bird access to feed or feed ingredients at least some of the time was more common among case farms (73% vs. 30% of control farms, OR = 6.2, $p = 0.03$). Cleaning up feed spills immediately was more common among control farms (80% vs. 50% of case farms, OR = 0.3, $p = 0.12$).

3.2. Multivariable analyses

3.2.1. Variable selection

Variables that had observed cell sizes of 5 or greater and a Fisher's exact test $p \leq 0.20$ included the following 19 variables.

a) The farm was in an existing control zone on the reference date,
b) The closest crop field was tilled last fall,
c) Presence of any wild waterfowl or shorebirds in the closest crop field during the 14-day reference period,
d) Egg trucks moving eggs off the farm generally came near the barns,
e) Other business visitors (e.g., meter reader, repairman) generally came near the barns,
f) The farm had a gated entrance,
g) Frequency of mowing of vegetation on the premises,
h) Tires were washed for vehicles on the farm during the 14-day reference period,
i) Feed vehicles were washed during the 14-day reference period,
j) Egg trucks were washed during the 14-day reference period,
k) The vehicle wash station was a permanent station rather than recently put in place,
l) There was any rodent problem during the 14-day reference period,
m) Wild birds had access to feed or feed ingredients during the 14-day reference period,
n) Wild animals (such as raccoons, opossums, coyotes, or foxes) had access to feed or feed ingredients during the 14-day reference period,
o) Different personnel were always required for different barns (workers assigned to specific barns) during the 14-day reference period.
p) Workers were always required to change clothes before entering barns (washable clothes, not disposable), and
q) The farm had a high level of vehicle washing during the 14-day reference period (equal to 1 if the farm had a vehicle wash

TABLE 4 Predictor variables included in multiple regression modeling, including name, description, and calculation of the variable.

Name	Description	Calculation
Flock size	Number of birds on the farm on the reference date. Categorized as farms having 500,000 birds or more versus farms having fewer than 500,000 birds.	if sum(e204, e210) > = 500,000 then 1 else 0
Control zone	Farm was in an existing control zone on the reference date versus the farm was not in an existing control zone on the reference date.	if e205 = 1 then 1 else 0
Waterfowl presence	Any waterfowl (e.g., ducks, geese) or shorebirds seen in the closest crop field during the reference period versus no waterfowl or shorebirds seen in the closest crop field.	if e354 in (2–4) then 1 else 0
No farm entrance gate	Farm had a gated entrance versus the farm did not have a gated entrance.	if e417 = 3 then 1 else 0
Wild bird access to feed	Wild birds had any access to poultry feed or feed ingredients during the reference period versus wild birds had no access to poultry feed or feed ingredients.	if e449 in (1–3) then 1 else 0
Off-site disposal	Farm disposed of dead birds (daily mortality) using off-site methods (composting off-site, burial off-site, rendering, or landfill) versus the farm used other disposal methods.	if e1102a = 3 or e1103a = 3 or e1105 = 1 or e1106 = 1 then 1 else 0
No dedicated barn personnel	Different personnel were assigned to specific barns during the reference period versus moving between barns.	if e606 = 1 then 1 else 0
At least some rodent problems	Farm had any rodent problem (low, moderate, or high severity) during the reference period versus the farm had no rodent problem.	if e445 in (1–3) then 1 else 0
Change of clothing not always required for workers	A change of (washable) clothing was always required for workers entering barns during the reference period versus a change of clothing was not always required.	if e608 = 1 then 1 else 0
Sharing company trucks/ trailers	Farm shared and either sometimes or never cleaned and disinfected company trucks/trailers (e.g., pickup truck, trailer with supplies, supervisor truck, or similar) during the reference period versus the farm did not share company trucks/trailers or shared and always cleaned and disinfected them.	if e801 = 1 and e801a in (2, 3) then 1 else 0
Mowing less than 4 times/ month	Farm mowed or bush hogged vegetation on the premises (when vegetation is present, e.g., spring and summer) 3 or fewer times per month versus 4 or more times per month.	if e420 < = 3 then 1 else 0
Lower level of vehicle washing	Farm had a vehicle wash/spray station during the reference period and washed tires of vehicles, washed worker, feed, and egg vehicles, and the wash station was permanent (e.g., in use prior to the HPAI incident) rather than a station that was recently put into use as a response to heightened biosecurity concerns versus the farm did not practice at least one of these.	if e421 = 1 and e423 = 1 and e426 = 1 and e427 = 1 and e428 = 1 and e431 = 1 then 0 else 1

All variables are binary and, as shown, take the value 1 for the category associated with a higher risk of HPAI. The calculations refer to the item codes from the questionnaire for each variable (e.g., e205 is the item code that holds a 1 or a 3, indicating whether the farm was in a control zone on the reference date from item 2.d on the questionnaire).

station, washed tires of vehicles, washed worker, feed, and egg trucks, and had a permanent wash station rather than one that was recently put in place due to heightened biosecurity measures surrounding the HPAI outbreak, and equal to 0 otherwise),

r) Incineration was used to dispose of dead birds (daily mortality), and

s) The farm disposed of dead birds (daily mortality) off-site (equal to 1 if the farm composted off-site, buried off-site, used a renderer, or a landfill and was equal to 0 otherwise).

Two additional variables that passed the univariable screening and had sufficient cell sizes included whether a farm had egg trucks moving eggs off the farm come near the barns or whether other business visitors (e.g., a meter reader, repairman) come near the barns. These variables were not considered for multiple regression modeling because their effects appeared to be counter to an epidemiological explanation and deserve further investigation which could not be thoroughly performed due to the lack of variability in the study responses For example, all case farms ($n = 2$) and all control farms ($n = 11$) that allowed egg trucks near the barns reported washing egg trucks during the reference period. Of farms that allowed company personnel vehicles to come near the barns, 71.4% ($n = 5$) of case farms and 80.0% ($n = 8$) of control farms reported washing worker vehicles during the reference period.

One variable that did not pass the univariable screening but was included in multiple regression modeling was an indicator variable for flock size, measured as the number of birds on the farm on the reference date, where farms with fewer than 500,000 birds were considered small, and those with 500,000 birds or more were considered large. The cutoff of 500,000 birds was just below the median flock size. This variable was included as it is related to several of the poultry and farm management factors listed above and was involved in a confounding relationship with at least one of them.

3.2.2. Multicollinearity and confounding variables

Of the variables that met the cell size requirements and passed univariable screening, the four individual vehicle washing indicator variables (items h-k in the list above) were omitted in favor of the high level of washing combination variable (item q) due to the resulting high VIF values. In addition, wild animal access to feed (item n) was omitted in favor of the variable measuring wild bird access to feed (item m) due to high VIF values. The closest crop field being tilled the previous fall (item b) appeared to be multicollinear with the presence of any wild waterfowl in the closest crop field (item c), and so the former was omitted in favor of the latter. Finally, incineration as a method of dead bird disposal (item r) was omitted in favor of the combination variable assessing any off-site method of disposal (item s) due to high VIF values. This left 12 variables that were used in multiple regression modeling. Those variables are described in Table 4.

There were five confounding relationships identified using the statistical screening measures and in which the confounding effect was not believed to lie in the causal pathway between the predictor variable of interest and HPAI presence. Those included: waterfowl presence confounding flock size, control zone and the presence of a farm entrance gate were confounded, control zone confounding the sharing of company trucks/trailers, and the presence of a farm entrance gate confounded the presence of a high level of vehicle washing.

The sample size was not sufficient to adequately assess whether these relationships were confounding, effect measure modification, or both. However, for the relationships between waterfowl presence and flock size, control zone and the sharing of company trucks/trailers, and the presence of a farm entrance gate and the presence of a high level of vehicle washing, the evidence leaned more in favor of confounding relationships (both conditional ORs close to one another and both below the crude OR). The relationship between control zone and the presence of a farm entrance gate suggested there was effect measure modification present, with farms in a control zone tending to have higher ORs of having HPAI present if they did not have a farm entrance gate, while farms outside of the control zone tended to have lower (though still greater than 1.0) ORs of having HPAI present if they did not have a farm entrance gate. These effects were controlled for in multiple logistic regression modeling, but interaction effects were not included due to inadequate sample size.

3.2.3. Leave-one-out cross-validation and AICc

All possible models with no interaction terms were created using the remaining 12 variables for a total of 4,095 models (the intercept-only model was not considered). The top 15 models ranked by LOOCV accuracy and AICc are depicted in Table 5. The variables included in each of the top 15 models and the count of which models include them are also indicated. McFadden's unadjusted and adjusted pseudo-R^2 values are depicted for each model as well.

Control zone, waterfowl presence, and no farm entrance gate were included in all 15 of the top models. Flock size and not always having different personnel for different barns were included in 9 of the top 15 models, and wild bird access to feed was included in 7. The remaining variables were included in 5 or fewer models, indicating lower importance regarding their ability to predict which farms were positive, given the other variables included in the models. LOOCV accuracies ranged from 0.725–0.775 in the top 15 models. This indicates that, given an exchangeable set of new table egg layer farms in the HPAI 2022 outbreak, we would expect these models to accurately predict the HPAI infection status of between 72.5 and 77.5 percent of the farms – though there is evidence that this accuracy estimate is likely biased high (32, 39). McFadden's pseudo-R^2 values range from 0.33–0.45 for the top 15 models, with the top model having a value of 0.38. Values of McFadden's pseudo-R^2 are typically lower than those from ordinary linear regression, where values between 0.2 and 0.4 have been cited as indicating a good amount of variability explained in the response by the given logistic regression model (36). The adjusted McFadden's coefficient of determination, pseudo-R^2_{adj}, values ranged from 0.09 to 0.19, with the top model having a value of 0.16.

The model with the highest LOOCV accuracy and the lowest AICc is summarized in Table 6 using exact multiple logistic regression modeling to account for small sample size. None of the predictor variables were statistically significant at the 0.05 significance level, though whether the farm was in an existing control zone on the reference date ($p = 0.09$) was the most important variable. Control zone had an odds ratio of 10.3 (95% CI: 0.8–377.0), indicating that a farm that was in a control zone had an expected odds of being positive for HPAI 10.3 times greater than that for a farm that wasn't in a control zone. Wild waterfowl or shorebird presence in the closest crop field had an odds ratio of 5.8 (95% CI: 0.7–79.4), meaning that farms that had observed wild waterfowl or shorebirds in the closest crop field during the reference period had an expected odds of being positive for HPAI about 5.8 times greater than farms that did not, though this effect wasn't significant at the 0.05 significance level ($p = 0.12$). Not having a gate at the farm entrance had an OR of 3.8 (95% CI: 0.6–31.5), not always requiring different personnel working in different barns had an OR of 6.2 (95% CI: 0.3–427.5), and larger operations had an OR of 2.6 (95% CI: 0.3–39.5), though none of these effects were statistically significant (value of ps of 0.21, 0.34, and 0.59, respectively).

A separate, mixed effects multiple regression model was fit using the fixed effects that were included in the LOOCV top model, plus a random intercept term for state to assess state-level farm location effects. The percentage of variance explained by state in the model was 0.8%, and so it was decided that final inference would be made from the exact multiple logistic regression model, with fixed effects only, as depicted in Table 6.

3.2.4. Bayesian model averaging

The same 12 predictor variables that were used in the LOOCV model selection procedure were used in the Bayesian model averaging procedure. An image plot showing variable inclusion and whether the effect was a risk factor or protective in the given model for each top model selected by the Bayesian model averaging model search method is shown in Figure 1. Variables are ordered on the vertical axis according to the posterior probability that their effect size was non-zero. These posterior probabilities, along with posterior mean odds ratios and their approximate 95% confidence intervals, conditional on those effects being included in the model, are included in Table 7.

Comparing the LOOCV model-based selection and ranking to BMA, the most important predictor variables were similar: control zone, no farm entrance gate, waterfowl presence, wild bird access to feed, flock size, and no specific barn personnel (probabilities of 0.55, 0.53, 0.40, 0.25, 0.22, and 0.14, respectively).

There were some differences, including off-site disposal (posterior probability of 0.17) being a moderate to low effect according to BMA, but it was not included in any of the top 15 models sorted by LOOCV, and vehicle washing, which had the lowest posterior probability of 0.07 using BMA, but was a moderate to low effect using LOOCV, being included in 4 of the top 15 models. However, many of the differences in order of importance of effects were present only for the lower and moderate sized effects, while the most important effects were the same by both modeling methods.

Odds ratios were also broadly similar between the two methods. Control zone had an OR of 10.3 (95% CI: 1.1–100.5, Table 7), which was very similar to that derived using the LOOCV-based top model. Not having a farm entrance gate had a BMA-based OR of 7.0 (95% CI: 1.1–43.7), compared to 3.8 (95% CI: 0.6–31.5) in the LOOCV-based top model, which can be partly explained by the strong

TABLE 5 Predictor variables included (indicated by a black box) in the top 15 models for HPAI presence, ranked by leave-one-out cross validation (LOOCV) accuracy and AICc.

Model	LOOCV Accuracy	AICc	R^2	R^2_{adj}	Control zone	Waterfowl presence	No farm entrance gate	Flock size	No specific barn personnel	Wild bird access to feed	At least some rodent problems	Lower level of vehicle washing	Change of clothing not always required	Sharing company trucks/ trailers	Mowing less than 4 times/ mo.	Off-site disposal
1	77.5	48.8	0.38	0.16	■	■	■	■	■							
2	77.5	51.7	0.38	0.12	■	■	■	■	■			■				
3	75.0	47.5	0.35	0.17	■	■	■			■						
4	75.0	48.0	0.45	0.19	■	■	■	■		■			■			
5	75.0	48.7	0.33	0.15	■	■	■								■	
6	75.0	50.3	0.35	0.13	■	■	■			■		■				
7	75.0	50.5	0.40	0.15	■	■	■		■	■	■					
8	75.0	50.7	0.40	0.14	■	■	■	■	■		■					
9	75.0	51.0	0.45	0.16	■	■	■	■	■	■	■					
10	75.0	51.3	0.33	0.11	■	■	■						■		■	
11	75.0	51.7	0.38	0.12	■	■	■	■	■					■		
12	75.0	53.8	0.40	0.11	■	■	■	■	■		■	■				
13	75.0	54.8	0.38	0.09	■	■	■	■	■			■		■		
14	72.5	48.4	0.44	0.19	■	■	■	■		■	■					
15	72.5	48.5	0.38	0.16	■	■	■		■	■						
Count					15	15	15	9	9	7	5	4	2	2	2	0

Variables included in each model are depicted and a count of models in the top 15 in which each predictor variable is depicted in the last row. McFadden's pseudo-R^2 and McFadden's adjusted coefficient of determination, pseudo-R^2_{adj} are included for each model.

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TABLE 6 Summary of the exact multiple logistic regression model fit regressing HPAI disease status of an operation on risk factors, with the greatest leave-one-out cross validation accuracy and lowest AICc value among all models under consideration.

Variable	Level	Exact conditional test *p*-value	OR Point estimate	OR 95% Confidence Interval
Intercept			0.0	(0.0, 0.5)
Flock size (number of birds on the farm on the reference date)	Large (≥500,000)	0.59	2.6	(0.3, 39.5)
	Small (<500,000)		(referent)	
Farm in an existing control zone on the reference date	Yes	0.09	10.3	(0.8, 377.0)
	No		(referent)	
Wild waterfowl or shorebirds in closest crop field during the 14-day reference period	Yes	0.12	5.8	(0.7, 79.4)
	No		(referent)	
Was there a gate to the farm entrance	Yes	0.21	(referent)	
	No		3.8	(0.6, 31.5)
Were there always different personnel working in different barns	Yes	0.34	(referent)	
	No		6.2	(0.3, 427.5)

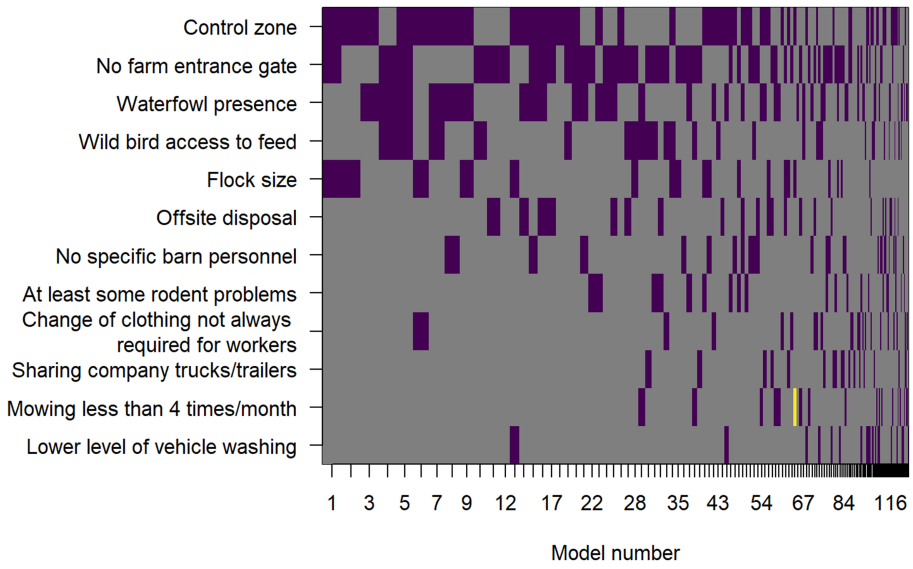

FIGURE 1
Image plot showing the variables included in the top models included in the Bayesian model averaging process. Predictor variables are on the vertical axis and models are on the horizontal axis. Each model composes a vertical stack of rectangles with width proportional to the Bayesian posterior probability of the model being selected. The rectangles are colored purple where the predictor variable was a risk factor in that given model, yellow if it was protective, and grey where that predictor variable was not included in the model.

confounding between control zone and the not having a farm entrance gate. Waterfowl presence had a similar estimate using BMA (OR = 6.2, 95% CI: 1.1–39.6) compared to LOOCV (OR = 5.8, 95% CI: 0.7–79.4) and not always having different personnel for different barns had a similar estimate using BMA (OR = 6.4, 95% CI: 0.4–97.1) compared to LOOCV (OR = 6.2, 95% CI: 0.3–27.5). Although not included in the top model by LOOCV, the BMA estimate for the OR for wild bird access to feed was 5.0 (95% CI: 0.8–30.8).

3.2.5. Model fit diagnostics

There were no substantial indicators of poor model fit for the multiple logistic regression models, after inspection of the residual diagnostic plots for the top models.

4. Discussion

The wave of H5N1 clade 2.3.4.4b HPAI that began in 2021 has been unprecedented in several regions of the world (40), including the United States. As avian influenza viruses continue to circulate in wild birds, it is critical to identify measures that may help mitigate infections in domestic poultry (41, 42). The case–control study presented here investigated the risk factors associated with infection with HPAI virus between February and September 2022 on U.S. commercial table egg farms in 8 states. Although the table egg sector is a relatively small sector of the U.S. commercial poultry industry, it has been heavily affected by this outbreak. Over 80% of affected table egg producers in participating states took part in this study—a testament to the willingness of the industry to support

TABLE 7 Summary statistics from Bayesian model averaging, including the posterior probability that the predictor variable effect is non-zero, the posterior mean odds ratio, and the approximate 95% posterior interval for the odds ratio, conditional on the predictor variable being included in the model.

Variable	Posterior probability the variable effect size is non-zero	Conditional posterior mean odds ratio	Conditional 95% posterior interval
Intercept		0.1	(0.0, 1.2)
Control zone	**0.55**	**10.3**	**(1.1, 100.5)**
No farm entrance gate	**0.53**	**7.0**	**(1.1, 43.7)**
Waterfowl presence	**0.40**	**6.2**	**(1.1, 39.6)**
Wild bird access to feed	0.25	5.0	(0.8, 30.8)
Flock size	0.22	5.9	(0.8, 44.3)
Off-site disposal	0.17	4.1	(0.7, 25.5)
No specific barn personnel	0.14	6.4	(0.4, 97.1)
At least some rodent problems	0.11	3.1	(0.6, 15.3)
Change of clothing not always required for workers	0.10	4.5	(0.4, 48.4)
Sharing company trucks/trailers	0.07	3.1	(0.4, 23.5)
Mowing less than 4 times/month	0.07	2.8	(0.4, 18.6)
Lower level of vehicle washing	0.07	2.7	(0.4, 20.0)

Predictor variables statistically significant at the 0.05 level are shown in bold.

science-based prevention efforts. It can be challenging to interpret findings from a small dataset, and so we focus on interpretation of both the outcomes of the multivariable modeling processes and the univariable analyses.

Garber et al. (12) noted that the most significant farm-level risk factor for HPAI on commercial table egg farms in 2015 was being located within an existing control zone. In the current study, this finding continues to hold true. This predictor was the closest to being significant in the exact multiple logistic regression model at the 0.05 significance level, as well as present in each of the top fifteen models produced by the LOOCV process. Farms that are located near an infected farm must be particularly diligent about biosecurity-related practices to protect flock health. Proximity to an infected farm has also been reported as a risk factor for HPAI infection in outbreaks in Europe and Japan (43, 44). Study findings confirm the need for both biosecurity and surveillance on poultry farms near an infected farm, to prevent infection and ensure rapid detection, whether the virus is likely spreading by wild birds or between farms.

Although multivariable modeling in the Garber et al. (12) study did not find an association between presence of wild birds on or around the farm and disease status, we did detect an association between sightings of wild waterfowl or shorebirds in the field closest to the farm during the reference period and farm-level disease status. Again, this predictor was present in the exact multiple logistic regression model, was in each of the top models produced by the LOOCV process, and had the third-highest posterior probability of having a non-zero effect size in BMA. Notably, this variable was a significant predictor of HPAI infection in the univariate analysis even though, as a group, all the farms that participated in the study had no other types of poultry on the farm, and none had pastured poultry or poultry with outdoor access. While this result may be due in part to recall bias by producers on case farms, producers seeking to decrease risk for HPAI may wish to work with a wildlife mitigation specialist to develop a wild bird management plan.

Included in 7 of the top models by the LOOCV process and the fourth-highest ranked predictor by BMA, any access of wild birds to feed or feed ingredients appeared to be an important predictor of HPAI farm status classification. Feed accessible to wild birds could act as a congregation point for wild birds on the farm and could increase risk of exposure to virus shed by affected wild birds. In addition, although not statistically significant, only 40% of farms that had a protocol to clean spilled feed immediately were classified as cases, while 60% of farms that had no protocol listed or a protocol to clean spilled feed less frequently were classified as cases, further supporting the need to include regular inspection of feed housing and prompt cleanup of feed spills in an overall flock management and wild bird management plan.

The presence of a farm gate was found to be protective. This predictor was present in the exact multiple logistic regression, in each of the top models produced by LOOCV, and was the predictor with the second-highest posterior probability of having a non-zero effect size by BMA. Gates were much more commonly reported on control operations than on case operations (64% vs. 22%). Having a gate may be a proxy variable for other biosecurity practices and could even be associated with a highly proactive approach to biosecurity. Gates improve control of traffic onto farms and may increase the likelihood that visitors will see posted signage and follow requested biosecurity procedures.

Flock size was non-significantly associated with increased risk in the exact multiple logistic regression ($p = 0.23$), was in 9 of the top 15 models produced by the LOOCV process, and was one of the top 5 most important predictors by BMA. This may be a finding associated with selection bias; our estimated response rate for control producers was 20%. Smaller producers may have been more likely to participate in the study.

There were two farm worker biosecurity practices that had $p \leq 0.20$ in the univariable analysis and appeared to be low to moderately important in multivariable modeling, accounting for the other modeled effects. These were always having different personnel

working in different barns (workers assigned to specific barns) and always requiring a change of clothing for workers before they enter a barn. Having workers assigned to specific barns is not a commonly reported practice, although movement of employees between barns is a known biosecurity risk (45). Having the available resources to perform biosecurity measures, such as appropriate facility design features, sufficient time, and personnel, can also affect the degree to which workers are able to carry out practices that support good biosecurity (46). Requiring a change of clothing for workers entering barns is commonly advised in biosecurity guidance, as well as in general guidance provided by United Egg Producers Animal Health and Biosecurity Committee (47).

Lack of a rodent problem was another factor more commonly reported on control farms; 28% of case farms reported having no rodent problem, whereas 54% of control farms reported no rodent problem ($p = 0.12$). The reported presence of any degree of rodent problem on-farm was a moderately important effect by both LOOCV and BMA. There is at least some evidence that rodents can transmit low-pathogenic avian influenza (48). Control of rodents is often advised to limit HPAI and other disease risks. While not meeting the criteria for confounding, mowing less frequently appeared to be related to farms having a rodent problem. That is, of farms that mowed more frequently, only 42% had a rodent problem, while of those that mowed less frequently, 71% had a rodent problem. This finding suggests that one part of an effective rodent control program could include frequent mowing of vegetation around poultry barns.

Vehicle washing was moderately important as measured by both LOOCV and BMA. This finding of moderate importance may be an artifact of the limitations of a survey-based approach; effectiveness of cleaning can be difficult to measure based on visual inspection (49). Notably, multiple vehicle wash-related variables were univariately significant ($p \leq 0.20$), including washing of feed trucks, egg trucks, washing truck tires, and having a permanent vehicle washing station.

Historic work has noted increased risks associated with use of rendering for disposal (12, 50–53). In the current study, 33.3% of case farms reported the use of rendering for dead bird disposal, while 13.6% of control farms utilized this carcass disposal practice. Rendering vehicles may transport virus via vehicle movement from farm to farm. Additionally, depending on storage of mortalities prior to renderer pickup, there is the possibility of attracting scavengers, which can include gulls, vultures, and other wild birds. Interestingly, though not a common practice among producers, use of incineration as a disposal method was significant ($p = 0.10$) in the univariate analysis. Given the recurring finding of rendering as a risk factor in multiple outbreaks, carcass disposal practices warrant further investigation. More generally, off-site disposal may increase risk due to vehicle movement between farms: off-site disposal was reported by 50% of case farms and 27% of control farms ($p = 0.19$).

The univariate analyses of the subset of cases linked to wild bird introductions suggested that topography and proximity to bodies of water can affect risk for transmission of HPAI. Presence of a structural windbreak such as a hill was protective ($p = 0.07$), and proximity to a drainage ditch was a risk factor ($p = 0.15$), though proximity to a wastewater lagoon was not statistically significant. Seeing wild waterfowl or shorebirds in the closest field during the reference period was also associated with increased risk ($p = 0.05$). While these characteristics cannot be changed, the data suggest that risk can be mitigated by limiting areas where water can pool on and around the farm and employing wildlife mitigation strategies (54). Notably, not having a rodent problem was associated with decreased risk ($p = 0.14$), as was lack of wild bird access to feed or feed ingredients ($p = 0.03$). Further study of the effectiveness of specific mitigation practices would be valuable.

5. Limitations

This study has a number of general limitations. The sample size for this investigation was relatively small, with a total size of 40 observations, 18 being case farms and 22 being control farms. However, 18 of the 22 (82%) eligible commercial table egg case farms participated in the study. The estimated rate of participation was substantially lower for control farms, so practices among this group of participants may not have been representative of unaffected commercial table egg producers overall. Relatively few table egg pullet and breeder farms were affected by HPAI during the study period, so although they were included in the study, not all findings may apply to these subgroups of the table egg sector. Recall bias is another limitation. Some respondents in the study were asked to provide responses for observations and activities that had taken place months prior to the study. Recall for some questions may have been different for case farms versus control farms. Another limitation of survey-based methods is the potential for bias associated with questions that may be considered sensitive. Respondents may be more likely to provide responses considered to be aligned with best practices, rather than reflective of actual practices. While our goal was to balance the number of completed case and control questionnaires geographically, 1:1 matching of cases and controls by state was not feasible due to variation in response rates between cases and controls, as well as a lack of eligible and interested controls.

Since predictors were pre-screened prior to performing LOOCV, estimated cross-validation accuracy of the model was likely artificially inflated (32, 39). That is, the performance of the models may be lower than they are shown to be here (72.5–77.5 percent accuracy, from Table 5) if applied to a different dataset. This was acceptable because unbiased predictive ability wasn't the end goal. Instead, the goal was to assist in selecting variables for the exact multiple logistic regression model. In addition, outbreak parameters (routes of transmission, effectiveness of control measures) can change over time or between outbreaks, therefore, the prediction error may not be directly applicable to new datasets but may serve as an adequate baseline.

Future HPAI and weather-related analysis using the 2022 outbreak data is planned across affected poultry sectors. Case–control study data will be analyzed in combination with weather conditions that occurred during the time preceding detection of HPAI infection. Weather patterns related to transmission of HPAI have been studied previously and may have had a role in the 2022 outbreak. Another direction of study underway for the turkey sector examines biosecurity investments farmers have made since 2015 and their impacts on classification of farms as cases or controls. These data will be analyzed and reported separately to identify priority areas for investment to reduce risk for HPAI. A similar study for table egg farmers may be of benefit.

Data availability statement

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

Author contributions

AG, KM, MB, VF, KP, AB, SK, AF, and AD contributed to the conception and design of the study. MB, AG, VF, KP, MV, and RM performed the statistical analysis, with MB and MV providing focused statistical expertise. AG wrote the first draft of the manuscript. MB and KP wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1229008/full#supplementary-material

References

1. Swayne DE, Suarez DL, Sims LD. Influenza In: DE Swayne, M Boulianne, C Logue, LR McDougald, V Nair and DL Suarez, editors. *Diseases of poultry. 14th* ed. Ames, IA: Wiley Publishing (2020). 210–56.

2. Seeger RM, Hagerman AD, Johnson KK, Pendell DL, Marsh TL. When poultry take a sick leave: response costs for the 2014-2015 highly pathogenic avian influenza epidemic in the USA. *Food Policy*. (2021) 102:102068. doi: 10.1016/j.foodpol.2021.102068

3. National Flyway Council. Implementation plan for avian influenza surveillance in waterfowl in the United States, summer FY 2021-winter F (2022). Available at: https://www.aphis.usda.gov/animal_health/downloads/animal_diseases/ai/2021-22-wild-bird-ai-surveillance-implementation-plan.pdf

4. Caliendo V, Lewis NS, Pohlmann A, Baillie SR, Banyard AC, Beer M, et al. Transatlantic spread of highly pathogenic avian influenza H5N1 by wild birds from Europe to north American in 2021. *Sci Rep*. (2022) 12:11729. doi: 10.1038/s41598-022-13447-z

5. OFFLU. *OFFLU statement on outbreak of H5N1 high pathogenicity avian influenza in Newfoundland*. Canada: OIE/FAO Network of Expertise on Animal Influenza (2021).

6. United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Surveillance, Preparedness, and Response Services (USDA–APHIS–VS–SPRS). (2007). Final report for the 2014-2015 outbreak of highly pathogenic avian influenza (HPAI) in the United States, revised august 11, 2016. Available at: https://www.aphis.usda.gov/animal_health/emergency_management/downloads/hpai/2015-hpai-final-report.pdf

7. United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA–APHIS). (2022) Confirmations of highly pathogenic avian influenza in commercial and backyard flocks Available at: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/avian/avian-influenza/hpai-2022/2022-hpai-commercial-backyard-flocks

8. Grear DA, Hall JS, Dusek RJ, Ip HS. Inferring epidemiologic dynamics from viral evolution: 2014-2015 Eurasian/north American highly pathogenic avian influenza viruses exceed transmission threshold, R0=1, in wild birds and poultry in North America. *Evol Appl*. (2017) 11:547–57. doi: 10.1111/eva.12576

9. Lee D, Torchetti M, Hicks J, Killian M, Bahl J, Pantin-Jackwood M, et al. Transmission dynamics of highly pathogenic avian influenza virus a(H5Nx) clade 2.3.4.4, North America, 2014–2015. *Emerg Infect Dis*. (2018) 24:1840–8. doi: 10.3201/eid2410.171891

10. Sidik SM. Why is bird flu so bad right now? *Nature*. (2022) 21. doi: 10.1038/d41586-022-03322-2

11. Bevins SN, Shriner SA, Cumbee JCJr, Dilione KE, Douglass KE, Ellis JW, et al. Intercontinental movement of highly pathogenic avian influenza a(H5N1) clade 2.3.4.4 virus to the United States, 2021. *Emerg Infect Dis*. (2022) 28:1006–11. doi: 10.3201/eid2805.220318

12. Garber L, Bjork K, Patyk K, Rawdon T, Antognoli M, Delgado A, et al. Factors associated with highly pathogenic avian influenza H5N2 infection on table egg layer farms in the Midwestern United States, 2015. *Avian Dis*. (2016) 60:460–6. doi: 10.1637/11351-121715-Reg

13. Särndal CE, Swensson B, Wretman J. *Model assisted survey sampling*. New York, NY: Springer-Verlag New York, Inc (1992).

14. Donders ART, van der Heijden GJMG, Stijnen T, Moons KGM. Review: a gentle introduction to imputation of missing values. *J Clin Epidemiol*. (2006) 59:1087–91. doi: 10.1016/j.jclinepi.2006.01.014

15. R Core Team. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing (2020) Available online at: https://www.R-project.org/.

16. R Studio Team. *RStudio: Integrated development environment for R*. Boston, MA: RStudio PBC (2020).

17. Mazerolle MJ. AICcmodavg: model selection and multimodel inference based on (Q)AIC(c). R package version 2.32 (2023). Available at: https://cran.r-project.org/package=AICcmodavg

18. Hebbali A. Blorr: tools for developing binary logistic regression models. (2020). Available at: https://CRAN.R-project.org/package=blorr

19. Raftery A, Hoeting J, Volinsky C, Painter I, Yeung KY. BMA: Bayesian Model Averaging. (2022). Available at: https://CRAN.R-project.org/package=BMA

20. Fox J, Weisberg S. *An R companion to applied regression*. Thousand Oaks, CA: Sage Publications (2019).

21. Kuhn M. Building predictive models in R using the caret package. *J Stat Softw*. (2008) 28:1–26. doi: 10.18637/jss.v028.i05

22. Wickham H, Miller E. Haven: import and export 'SPSS', 'Stata' and 'SAS' files. (2023). Available at: https://CRAN.R-project.org/package=haven

23. Harrell FE. Hmisc: Harrell Miscellaneous. (2023). Available at: https://CRAN.R-project.org/package=Hmisc

24. Lumley T, Miller A. Leaps: regression subset selection. (2020). Available at: https://CRAN.R-project.org/package=leaps

25. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *J Stat Softw*. (2015) 67:1–48. doi: 10.18637/jss.v067.i01

26. Wickham H, Averick M, Bryan J, Chang W, McGowan LDA, François R, et al. Welcome to the tidyverse. *J Open Source Software*. (2019) 4:1686. doi: 10.21105/joss.01686

27. Kutner MH, Nachtsheim CJ, Neter J, Li W. *Applied linear statistical models. 5th* ed. Blue Ridge, IL: McGraw-Hill/Irwin (2005).

28. Fox J, Monette G. Generalized collinearity diagnostics. *J Am Stat Assoc*. (1992) 87:178–83. doi: 10.1080/01621459.1992.10475190

29. Johnson RA, Wichern DW. *Applied multivariate statistical analysis. 6th* ed. Upper Saddle River, NJ: Pearson Prentice Hall (2007).

30. Hernán MA, Hernández-Diaz S, Werler MM, Mitchell AA. Causal knowledge as a prerequisite for confounding evaluation: An application to birth defects epidemiology. *Am J Epidemiol*. (2002) 155:176–84. doi: 10.1093/aje/155.2.176

31. Alexander L. K., Lopes B., Ricchetti-Masterson K., Yeatts K.B., (2015). "ERIC notebook: confounding Bias, part II and effect measure modification." Second Available at https://sph.unc.edu/wp-content/uploads/sites/112/2015/07/nciph_ERIC12.pdf

32. Hastie T, Tibshirani R, Friedman J. *Elements of statistical learning. 2nd* ed. New York, NY: Springer (2009).

33. Akaike H. A new look at the statistical model identification. *IEEE Trans Autom Control*. (1974) 19:716–23. doi: 10.1109/TAC.1974.1100705

34. Burnham KP, Anderson DR. *Model selection and multimodel inference: A practical information-theoretic approach*. New York, NY: Springer (2002).

35. Derr RE. *Performing exact logistic regression with the SAS system*. Cary, NC: SAS Institute (2009).

36. McFadden D. Conditional logit analysis of qualitative choice behavior In: P Zarembka, editor. *Frontiers in econometrics*: Academic Press (1974 (1973). 105–42.

37. Fox J. *Applied regression analysis and generalized linear models. 3rd* ed. United States: Sage Publications (2016).

38. Hoeting JA, Madican D, Raftery AE, Volinsky CT. Bayesian model averaging: a tutorial (with discussion). *Stat Sci*. (1999) 14:382–417. doi: 10.1214/ss/1009212519

39. Varma S, Simon R. Bias in error estimation when using cross-validation for model selection. *BMC Bioinformatics*. (2006) 7:91. doi: 10.1186/1471-2105-7-91

40. World Organisation for Animal Health. Statement on avian influenza and mammals (2023). Available at: https://www.woah.org/en/statement-on-avian-influenza-and-mammals/

41. Lycett SJ, Pohlmann A, Staubach C, Caliendo V, Woolhouse M, Beer M, et al. Genesis and spread of multiple reassortants during the 2016/2017 H5 avian influenza epidemic in Eurasia. *Proc Natl Acad Sci U S A*. (2020) 117:20814–25. doi: 10.1073/pnas.2001813117

42. Lewis NS, Banyard AC, Whittard E, Karibayev T, Al Kafagi T, Chvala I, et al. Emergence and spread of novel H5N8, H5N5, and H5N1 clade 2.3.4.4 highly pathogenic avian influenza in 2020. Emerg. *Microbes Infect*. (2021) 10:148–51. doi: 10.1080/22221751.2021.1872355

43. Busani L, Valsecchi MG, Rossi E, Toson M, Ferrè N, Pozza MD, et al. Risk factors for highly pathogenic H7N1 avian influenza virus infection in poultry during the 1999-2000 epidemic in Italy. *Vet J*. (2009) 181:171–7. doi: 10.1016/j.tvjl.2008.02.013

44. Nishiguchi A, Kobayashi S, Yamamoto T, Ouchi Y, Sugizaki T, Tsutsui T. Risk factors for the introduction of avian influenza virus into commercial layer chicken farms during the outbreaks caused by a low-pathogenic H5N2 virus in Japan in 2005. *Zoonoses Public Hlth*. (2007) 54:337–43. doi: 10.1111/j.1863-2378.2007.01074.x

45. Greening SS, Mulqueen K, Rawdon TG, French NP, Gates MC. Estimating the level of disease risk and biosecurity on commercial farms in New Zealand. *NZ Vet J*. (2020) 68:261–71. doi: 10.1080/00480169.2020.1746208

46. Delabbio J. J Ext (2006) 44(6). Available at: https://archives.joe.org/joe/2006december/a1.php

47. United Egg Producers. (2020). Available at: https://uepcertified.com/biosecurity/

48. Shriner SA, VanDalen KK, Mooers NL, Ellis JW, Sullivan HJ, Root JJ, et al. Low-pathogenic avian influenza viruses in wild house mice. *PLoS One*. 7:e39206. doi: 10.1371/journal.pone.0039206

49. Huneau-Salaün A, Scoizec A, Thomas R, Martenot C, Schmitz A, Pierre I, et al. Avian influenza outbreaks: evaluating the efficacy of cleaning and disinfection of vehicles and transport crates. *Poult Sci*. (2022) 101:101569. doi: 10.1016/j.psj.2021.101569

50. Selleck PW, Arzey G, Kirkland PD, Reece RL, Gould AR, Daniels PW, et al. An outbreak of highly pathogenic avian influenza in Australia in 1997 caused by an H7N4 virus. *Avian Dis*. (2003) 47:806–11. doi: 10.1637/0005-2086-47.s3.806

51. McQuiston JH, Garber LP, Porter-Spalding BA, Hahn JW, Pierson FW, Wainwright SH, et al. Evaluation of risk factors for the spread of low pathogenicity H7N2 avian influenza virus among commercial Turkey farms. *J Am Vet Med Assoc*. (2005) 226:767–72. doi: 10.2460/javma.2005.226.767

52. Kim W-H, An J-U, Kim J, Moon O-K, Bae SH, Bender JB, et al. Risk factors associated with highly pathogenic avian influenza subtype H5N8 outbreaks on broiler duck farms in South Korea. *Transbound Emerg Dis*. (2018) 65:1329–38. doi: 10.1111/tbed.12882

53. Wells SJ, Kromm MM, VanBeusekom ET, Sorley EJ, Sundaram ME, VanderWaal K, et al. Epidemiologic investigation of highly pathogenic avian influenza among upper Midwest U.S. Turkey farms, 2015. *Avian Dis*. (2017) 61:198–204. doi: 10.1637/11543-112816-Reg.1

54. Elbers ARW, Gonzales JL. Quantification of visits of wild fauna to a commercial free-range layer farm in the Netherlands located in an avian influenza hot-spot area assessed by video-camera monitoring. *Transbound Emerg Dis*. (2019) 67:661–77. doi: 10.1111/tbed.13382

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Case report: Lumpy skin disease in an endangered wild banteng (*Bos javanicus*) and initiation of a vaccination campaign in domestic livestock in Cambodia

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We describe a case of lumpy skin disease in an endangered banteng in Cambodia and the subsequent initiation of a vaccination campaign in domestic cattle to protect wild bovids from disease transmission at the wildlife-livestock interface. Lumpy skin disease virus (LSDV) was first detected in domestic cattle in Cambodia in June of 2021 and rapidly spread throughout the country. In September 2021, a banteng was seen in Phnom Tnout Phnom Pok wildlife sanctuary with signs of lumpy skin disease. Scab samples were collected and tested positive for LSDV. Monitoring using line transect surveys and camera traps in protected areas with critical banteng and gaur populations was initiated from December 2021-October 2022. A collaborative multisector vaccination campaign to vaccinate domestic livestock in and around priority protected areas with banteng and gaur was launched July 2022 and a total of 20,089 domestic cattle and water buffalo were vaccinated with Lumpyvax^{TM}. No signs of LSDV in banteng or gaur in Cambodia have been observed since this initial case. This report documents the first case of lumpy skin disease in wildlife in Cambodia and proposes a potential intervention to mitigate the challenge of pathogen transmission at the domestic-wildlife interface. While vaccination can support local livestock-based economies and promote biodiversity conservation, it is only a component of an integrated solution and One Health approach to protect endangered species from threats at the wildlife-livestock interface.

KEYWORDS

case report, lumpy skin disease, wildlife-livestock interface, vaccination, banteng, wildlife, conservation, intervention

Introduction

Lumpy skin disease virus (LSDV) is a DNA virus from the family *Poxviridae* that causes characteristic skin nodules in bovine species, such as cattle and buffalo. Because it affects the hide and can cause pyrexia, emaciation, abortions, and/or reduced milk yield in lactating cattle, it is a disease of agricultural and economic importance, and can devastate the livelihoods of farmers (1). The virus is primarily transmitted mechanically by biting arthropods, such as mosquitos, flies, and ticks, and its ability to survive in skin nodules for over a month facilitates rapid spread throughout a herd (2).

Historically, lumpy skin disease has been endemic throughout the majority of Africa. In late 2020 and early 2021, several epidemics in Asia were observed, involving China, Myanmar, and Vietnam (3). LSDV was first detected in domestic cattle in Cambodia in June of 2021. By September 2021, it had spread to all provinces throughout Cambodia and infected over 73,000 cattle and killed ~1,000 calves (personal communication GDAHP Sept 2021).

LSDV can also infect endangered and threatened wild bovids, such as banteng (*Bos javanicus*) and gaur (*Bos gaurus*). In neighboring Thailand, LSDV was detected in three gaurs in Kui Buri National Park in June 2021 and two bantengs in Huai Kha Khaeng Wildlife Sanctuary in August 2021 (4).

Globally, gaur have been listed by the IUCN as vulnerable since 1986 and banteng listed as endangered since 1996 (5, 6). There are estimated to be <1,500 banteng in Cambodia and gaur populations are too small, fragmented, and infrequently observed to estimate. The majority of these species live in isolated protected areas in the Eastern and Northern Plains of the country.

Eradication and control of LSDV in domestic livestock relies on early detection, widespread vaccination, movement restriction, and removal of infected animals. Widespread vaccination with a homologous vaccine is paramount to a LSDV control program and no country has been able to eradicate LSDV without vaccination (3).

This case of lumpy skin disease in a free-roaming wild banteng in Cambodia was detected as part of WildHealthNet, an initiative in Cambodia, Laos, and Vietnam to develop national wildlife health surveillance networks to detect, investigate, and respond to high-consequence pathogens at the wildlife-livestock-human interface (7). The rapid spread of LSDV in domestic cattle in Cambodia coupled with the susceptible populations of banteng and gaur, facilitated the need for One Health collaboration and an integrated approach to manage and control this important disease at the wildlife-livestock interface. Through the network created under WildHealthNet, government and conservation stakeholders in the region were called to action to monitor banteng and gaur populations for clinical signs of LSDV, inform targeted vaccination strategies in livestock designed to prevent transmission of LSDV to vulnerable species of wildlife, and contribute to LSDV control in domestic cattle.

Case description

Phnom Tnout Phnom Pok wildlife sanctuary in Preah Vihear province is a 42,000 hectare dry dipterocarp forest that is home to a variety of endangered wildlife species including banteng, Sunda pangolins, elongated tortoises, and several primates, such as the Indochinese silvered langur (8). There are five villages within the wildlife sanctuary and several in the surrounding area (Figure 1). Protected areas in Cambodia are not fenced and domestic cattle are allowed to roam freely within them. It is estimated that 800–1,000 domestic cattle graze within the wildlife sanctuary and ~300 share the same area as banteng (personal communication Our Future Organization April 2023). Joam Praoup, a village within the wildlife sanctuary located 5.6 km from where the banteng was found, experienced a lumpy skin disease outbreak in domestic cattle as early as June 2021.

On September 8, 2021, an adult male banteng was seen by community rangers in Phnom Tnout Phnom Pok wildlife sanctuary (Decimal degrees: *13.4665, 104.6765*). The banteng was thin, lethargic, and had multiple skin nodules on its head and flank (Figure 2). It also had a snare wound on its right front leg that appeared to be swollen and infected, causing lameness (Figure 3). Community rangers stayed with the animal to monitor its health and protect it from poachers. The banteng became progressively weaker and immobile, and eventually died on September 10 (Decimal degrees: *13.4672, 104.6771*).

Response actions were coordinated through the wildlife health surveillance network and government officials arranged for a Preah Vihear district veterinarian to travel to the site to collect samples. Whole blood and scabs from skin lesions on the head were collected. Following sample collection, the carcass was incinerated at the site.

The samples were sent to the Cambodian National Animal Health and Production Research Institute on September 13, 2021 and the scab samples were tested for LSDV via real-time-PCR following DNA extraction (Qiagen QIAamp® DNA Extraction protocol) (9, 10). Results confirmed that the banteng was positive for LSDV.

Response

Following the detection of LSDV in the banteng, conservation organizations, including Wildlife Conservation Society (WCS) and World Wide Fund for Nature (WWF), met with the Cambodian General Directorate of Animal Health and Production (GDAHP) to discuss a plan to prevent LSDV transmission to banteng and gaur. In order to protect these species from LSDV, the disease must be controlled in domestic cattle. Widespread vaccination, movement restrictions, and biosecurity in domestic cattle are essential for a LSDV control program. Unfortunately, resources and capacity for disease control in Cambodia are limited. At the time of the outbreak, GDAHP received 20,000 lumpy skin disease vaccines, however there are over 1.3 million susceptible cattle in Cambodia (personal communication GDAHP Sept 2021).

A vaccination campaign was organized in coordination with GDAHP, provincial animal health departments, WCS, and WWF to augment the national LSDV vaccination effort. All parties agreed to conduct a ring vaccination of domestic cattle in and around the Eastern and Northern Plains Landscapes. 27,000 Lumpyvax™ vaccine doses (100 doses/vial), needles and syringes were purchased

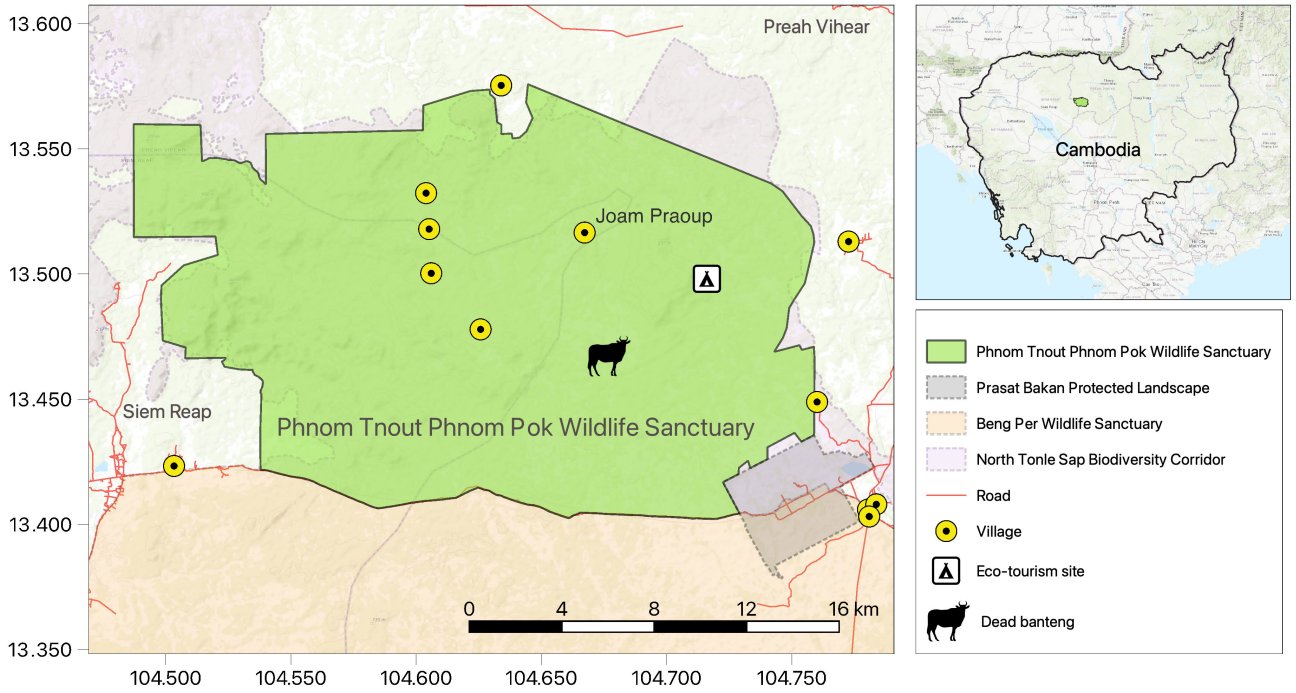

FIGURE 1
Map of Phnom Tnout Phnom Pok wildlife sanctuary with banteng case and village locations.

FIGURE 2
LSDV lesions on head and neck.

FIGURE 3
Necrotic snare wound.

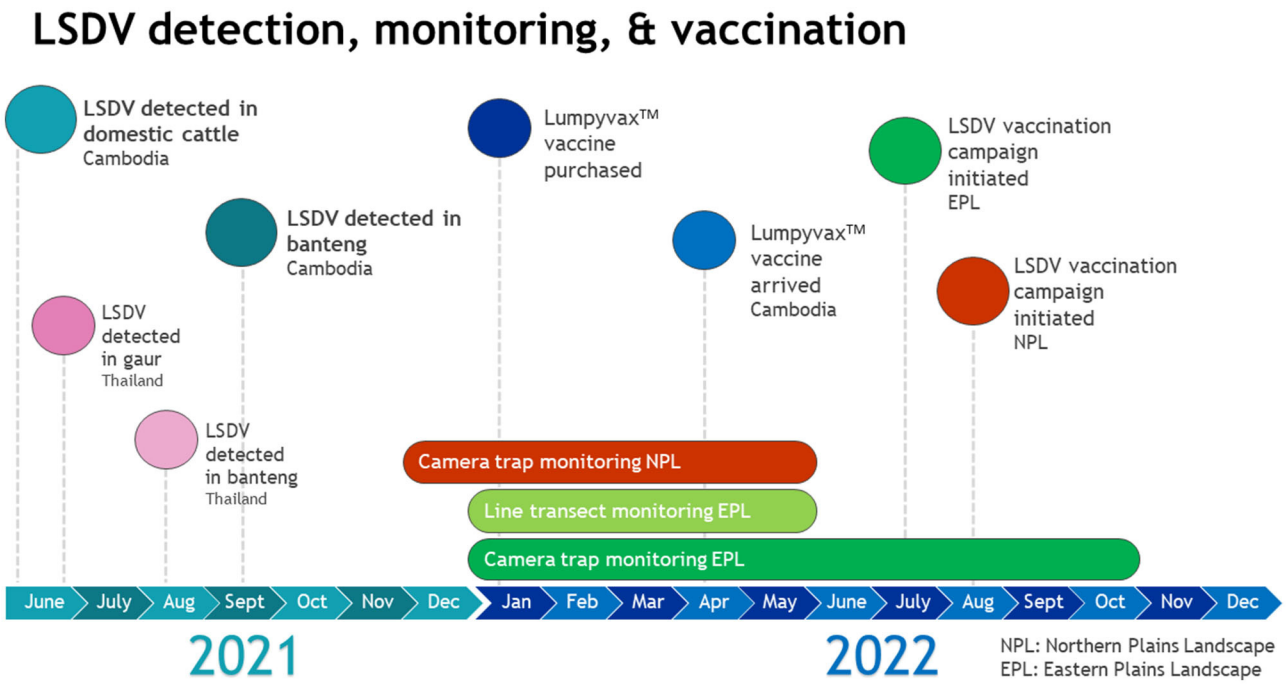

FIGURE 4
Timeline of LSDV detection, monitoring of wild ruminant populations, and vaccination campaign implementation.

in January 2022 by WCS Health program and Keo Seima REDD+ and WWF (Figure 4).

Lumpyvax™ is a homologous live attenuated Neethling strain vaccine produced by Intervet in South Africa. It was approved for use in Cambodia by GDAHP based on preliminary efficacy studies completed internally in Cambodia at the start of the outbreak in domestic cattle (personal communication GDAHP April 2023).

The Eastern Plains Landscape (Srepok, Phnom Prich, and Keo Seima wildlife sanctuaries) in Mondulkiri province were chosen as priority protected areas to implement the vaccination campaign due to their importance for banteng conservation. It was agreed that all domestic cattle in and within a 20 km radius of the protected areas would be vaccinated for LSDV. A distance of 20 km surrounding each protected area was chosen as the radius for the ring vaccination zone according to known vector range and historical vaccination recommendations (11). All remaining vaccines would be designated for distribution to the Northern Plains Landscape (Kulen Promtep, Chhaeb, Preah Roka, and Phnom Tnout Phnom Pok wildlife sanctuaries) in Preah Vihear province to vaccinate domestic cattle in select villages in close proximity to known banteng populations.

The vaccination campaign was implemented in the Eastern Plains Landscape from July 21-December 28, 2022 and in the Northern Plains Landscape from August 20-October 12, 2022. Community awareness and vaccination campaign launch events were organized by GDAHP, in coordination with WWF and WCS, in Srepok wildlife sanctuary in the Eastern Plains, and the Preah Vihear Provincial Animal Health and Production Office in Chhaeb district in the Northern Plains, at the start of each vaccination campaign, respectively. These events aimed to raise awareness among local communities about the LSDV vaccination campaign and educate village animal health workers on vaccination procedures. Twenty thousand vaccine doses were distributed to animal health authorities in the Eastern Plains Landscape and 5,000 were distributed to authorities in the Northern Plains Landscape.

Post vaccination campaign reports confirmed a total of 14,226 domestic cattle and 2,646 water buffalo were vaccinated for LSDV in the Eastern Plains and 2,676 domestic cattle and 541 water buffalo were vaccinated in the Northern Plains. The remaining 2,000 vaccine doses will be distributed to both landscapes in May 2023 to complete a second phase of vaccination. GDAHP has committed to continue vaccination for LSDV for an additional 3 years (personal communication GDAHP April 2023).

Monitoring for clinical signs of LSDV in wildlife was conducted by forest ranger patrol teams and conservation research teams in Srepok, Phnom Prich, and Keo Seima wildlife sanctuaries. A distance-sampling based line transect survey was conducted in all three Eastern Plains protected areas over the dry season (Jan-May) of 2022. During this survey, research teams directly observed banteng on seven occasions (group size ranged from 1 to 5 individuals) in Srepok. In Phnom Prich one gaur was observed and banteng were observed on seven occasions (group size ranged from 1 to 6 individuals). No signs of LSDV were seen on any of the observed wild bovids. In Keo Seima, neither banteng nor gaur were directly observed.

Camera trapping was conducted in the core zone of Srepok January-May and July-October 2022, in the core and conservation zone of Phnom Prich February-May 2022, in Keo Seima March-August 2022, and Kulen Promtep December 2021-May 2022. In Keo Seima, banteng were observed on camera trap images on two separate occasions in March (group of four) and May (group of six) 2022. No visible signs of LSDV were observed on the images. Gaur were not observed in Keo Seima. Both banteng and gaur were observed on camera trap images in Phnom Prich and Sre Pok, and banteng only were observed in Kulen Promtep, however no visible signs of LSDV were observed.

Discussion

This is the first case of LSDV reported in a banteng in Cambodia, adding to the earlier regional cases reported in Thailand. With widespread transmission of LSDV and prevalence of free-roaming domestic cattle in and around protected areas, this case epitomizes the imminent and incessant health-related threats to conservation at the wildlife-livestock interface.

In rapidly developing biodiversity-rich regions, habitat destruction, land-use change, and human encroachment into wild areas create overlapping habitats and novel interfaces with more frequent interactions between wildlife and domestic animals and increased opportunities for disease transmission. Wildlife is often blamed for spillover of diseases to humans (i.e., SARS-CoV) and domestic animals (i.e., HPAI, Nipah virus), however recognition of the risks of disease transmission to vulnerable and endangered populations already ravaged by habitat loss and wildlife trafficking is often neglected. Spillover of domestic animal diseases to wildlife represents a serious threat to conservation (12). In Mongolia, nearly 80% of the critically endangered Mongolian saiga antelope population were lost due to *Peste des Petits Ruminants* (PPR) virus, spread from sheep and goats (13). In Southeast Asia, 11 endemic wild pig species, including the Sumatran bearded pig *(Sus barbatus)*, are threatened by the widespread and catastrophic African swine fever virus epidemic in domestic swine (14, 15). In addition to primary hosts, the loss of critical keystone species due to disease transmission could have amplifying long-term consequences on the ecosystem, such as causing diminished food sources for endangered predators.

This case also highlights another formidable threat to endangered wildlife, the snaring crisis. Although the banteng developed lumpy skin disease, its snaring injury likely contributed to its inability to fight off the infection and eventual death. Wild cattle populations were severely depleted due to decades of hunting pressure historically, and currently wild bovids are severely threatened by the snaring epidemic in Southeast Asia. It is estimated that there are over 12 million snares in the protected areas of Cambodia, Laos, and Vietnam (16). Large wild bovids, including banteng and gaur, are often targeted for snaring due to the increased demand for wildlife meat, driven by wealthier classes in urban areas (17). Snaring is an enticing option for rural villagers to support their livelihoods, as snares are made from inexpensive readily available materials, such as cord and wire, and numerous snares can be deployed in a single outing (18). Often snares are not checked by the hunter who set them, leaving whatever indiscriminate species to perish due to starvation or infection from the wound (16). Despite snaring being illegal in Cambodia under the 2002 Forestry Law and 2008 Protected Areas Law, over the last decade there has been a rapid intensification of poaching, with detection rates of lethal traps by rangers increasing over 100-fold (19). Limited governance, lack of resources for law enforcement, and corruption enable the snaring crisis to persist and threaten wildlife populations.

The survival of a species is also considered at risk when confined to small, fragmented, isolated populations. Only one decade ago, the Eastern Plains in Cambodia held the largest population of banteng globally, with an estimate population size of 4,600 per IUCN with total global population estimated to be 4,000–8,000 (5, 20). The banteng population in two key protected areas within the Eastern Plains Landscape, Srepok and Phnom Prich wildlife sanctuaries, declined by a dramatic 72% in the decade between 2010 and 2020 based on a robust long-term distance-sampling based monitoring (19). By 2022, the estimated banteng population had further dropped by 89% compared to the baseline year (Groenenberg et al. unpublished data). In the adjacent Keo Seima wildlife sanctuary, line transect failed to detect any banteng in recent years despite extensive survey effort (21–23). Banteng in Phnom Tnout Phnom Pok wildlife sanctuary are estimated to be 46–119 individuals based on camera trap data, which makes it a small but globally significant population as there are only 6–8 subpopulations with over 50 animals (excluding Phnom Tnout Phnom Pok wildlife sanctuary) (8). Gaur are globally vulnerable and estimated to be 15,000–35,000 individuals worldwide (6). Cambodia harbors one of the most significant populations in the world, however surveys are currently too infrequent to estimate the population size. Evidence from other systems suggest that this case of LSDV in a banteng in Cambodia represents an additional threat to the conservation of wild ruminants in this region. We have seen that outbreaks of infectious disease can cause significant direct mortality when a pathogen enters an immunologically naive population of wildlife (13) and the presence of a pathogen circulating in a fragmented population of an endangered species can further increase extinction risk (24).

Mitigation measures to reduce disease transmission at the wildlife-livestock interface typically include biosecurity and movement control, however, these measures have limited application in wildlife populations. Vaccination of livestock in critical ecosystems is a potential intervention to prevent spillover of pathogens to susceptible wildlife, however, assessments of these types of interventions in peer-reviewed literature are limited. Recognition of the occurrence and impact of PPR in wildlife species, such as the critically endangered Saiga antelope (*Saiga tatarica mongolica*) in Mongolia, has recently been integrated into the FAO/WOAH PPR Global Eradication Programme with targeted PPR vaccination of domestic sheep and goats highlighted as critical to reducing pathogen transmission across the entire host community, including wildlife populations (25, 26). In Africa, there are disease prevention guidelines recommending immunization of protected area authorities, tourists, and other humans working in close proximity of great apes for childhood vaccines, including measles and polio, to prevent transmission of human pathogens to endangered apes (27). Direct vaccination of critically endangered wildlife is generally a last resort option. However, a canine distemper virus (CDV) vaccine was administered to foxes in Santa Catalina Island in California to successfully reestablish their population following a CDV outbreak thought to be spread by domestic dogs (28) and has been identified as a means of mitigating CDV risk to tiger populations in Asia (29).

The LSDV vaccination campaign of domestic cattle in and around protected areas in Cambodia was ultimately a successful initiative. Although we cannot conclude that the lack of detection of LSDV in banteng and gaur following the vaccination campaign was due to our efforts, it exemplified fruitful cross-sector collaboration to envision, draft, and implement a real-time animal emergency response and action plan. In addition, it increased awareness about LSDV and other infectious disease threats at the wildlife-livestock-human interface during the COVID-19 pandemic, and the downstream effects human behavior has on vulnerable wildlife populations in critical ecosystems. Perhaps most importantly, local livestock farmers and villagers were appreciative of our efforts to help protect their animals from LSDV. This opportunity for community outreach and engagement encouraged improvement of domestic animal health and fostered a relationship for future disease recognition and reporting.

Despite the campaign's successes, there were several limitations. While no additional cases of LSDV were detected in wild ruminants, cases could have gone undetected by line transect, camera traps, or in areas where monitoring does not occur. Although ring vaccination was attempted, undoubtably some cattle within the protected areas were missed, leaving opportunity for vectors to spread the virus. Of the 25,000 LSDV vaccine doses distributed, doses were inevitably lost due to vaccination handling challenges and storage logistics. Resources were limited and there were not enough vaccines for comprehensive ring vaccination to be completely effective in preventing transmission. Vaccination of domestic cattle alone is not enough to protect endangered banteng and gaur from the risk of extinction, however it is an important component of a holistic approach to address wildlife health and conservation issues.

Pathogen transmission at the wildlife-livestock interface is a complex, intricate, and frequently evolving challenge requiring intervention to prevent transboundary animal diseases from expanding their geographic range and spilling over to additional susceptible species. Spillover of livestock diseases to naïve fragmented vulnerable wildlife can have devastating consequences for endangered wildlife populations, biodiversity, and cascading effects on the ecosystem. Protecting these species from extinction requires a One Health approach and innovative integrated solutions. Vaccination is only a fraction of the solution and will not be effective without long-term commitment to conservation from government, decreased demand for wildlife meat, habitat protection, stronger law enforcement, and recognition of the role of wildlife in socioecological systems and transboundary disease response plans. The success of this multidisciplinary cross-sectional approach to detect LSDV in a banteng, activate surveillance for LSDV in critical wildlife populations, and implement a LSDV vaccination campaign in domestic cattle epitomizes the possibilities for unified One Health collaboration to protect endangered species from disease and other anthropogenic health threats.

Data availability statement

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

Ethics statement

The animal study was reviewed and approved by Wildlife Conservation Society Institutional Animal Care and Use Committee (vertebrate and invertebrate research). Written informed consent was obtained from the participant/patient(s) for the publication of this case report.

Author contributions

AP drafted manuscript. AP, MG, and CA organized vaccination campaign funding and agreements with their respective institutions. SC, SrS, BD, and SD facilitated case reporting. ST facilitated case diagnostics. VN facilitated vaccination campaign implementation. VC, CH, SaS, and SK facilitated network communication. MG, CA, BD, and SD facilitated monitoring in their respective protected areas. SO and AF reviewed and edited 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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References

1. Roche X, Rozstalnyy A, TagoPacheco D, Pittiglio C, Kamata A, Beltran Alcrudo D, et al. *Introduction and Spread of Lumpy Skin Disease in South, East and Southeast Asia*. FAO animal production and health, Paper 183 Rome, Italy: FAO (2020).

2. World Organization for Animal Health (OIE). Lumpy skin disease technical disease card (2017). Available online at: https://www.woah.org/app/uploads/2021/03/lumpy-skin-disease.pdf (accessed March 26, 2023).

3. Awada L, Lambergeon N, Morales R, Mur L, Tizzani P, Melens P, et al. Current animal health situation worldwide: analysis of events and trends. World Assembly, 88th General Session; 2021 May 24–28; Paris, France: OIE (2021). Available online at: https://web.oie.int/downld/SG/2021/A_88SG_2.pdf (accessed April 15, 2023).

4. OIE-WAHIS. Thailand-Lumpy skin disease virus (Inf. with)-Follow-up report 14 (2022). Available online at: https://wahis.woah.org/#/in-review/3644?reportId=153074&fromPage=event-dashboard-url (accessed April 30, 2023).

5. Gardner P, Hedges S, Pudyatmoko S, Gray TNE, Timmins RJ. Bos javanicus: The IUCN red list of threatened species 2016: e.T2888A46362970 (2016). Available online at: https://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS.T2888A46362970.en (accessed March 25, 2023).

6. Duckworth JW, Sankar K, Williams AC, Samba Kumar N, Timmins RJ. Bos gaurus: The IUCN red list of threatened species 2016: e.T2891A46363646 (2016). Available online at: https://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS.T2891A46363646.en (accessed March 25, 2023).

7. Pruvot M, Denstedt E, Latinne A, Porco A, Montecino-Latorre D, Khammavong K, et al. WildHealthNet: supporting the development of sustainable wildlife health surveillance networks in Southeast Asia. *Sci Total Environ.* (2023) 863:160748. doi: 10.1016/j.scitotenv.2022.160748

8. Munoz VH. *Biodiversity & environmental high conservation values, Phnom Tnout Phnom Pok wildlife sanctuary*. Our Future Organization, Cambodia (2021).

9. World Organization for Animal Health (OIE). Infection with lumpy skin disease virus. Terrestrial Animal Health Code (2022) 11.9. Available online at: https://www.woah.org/fileadmin/Home/fr/Health_standards/tahm/3.04.12_LSD.pdf (accessed June 17, 2023).

10. Bowden TR, Babiuk SL, Parkyn GR, Copps JS, Boyle DB. Capripoxvirus tissue tropism and shedding: a quantitative study in experimentally infected sheep and goats. *Virology.* (2008) 371:380–93. doi: 10.1016/j.virol.2007.10.002

11. Davies FG. Lumpy skin disease of cattle: a growing problem in Africa and the Near East. *World Anim Rev.* (1991) 68:37–42.

12. Aguilar XF, Fine AE, Pruvot M, Njeumi F, Walzer C, Kock R, et al. PPR virus threatens wildlife conservation. *Science.* (2018) 362:165–6. doi: 10.1126/science.aav4096

13. Pruvot M, Fine AE, Hollinger C, Strindberg S, Damdinjav B, Buuveibaatar B, et al. Outbreak of peste des petits ruminants among critically endangered mongolian saiga and other wild ungulates, Mongolia, 2016–2017. *Emerging Infect Dis.* (2020) 26:51–62. doi: 10.3201/eid2601.181998

14. Luskin MS, Meijaard E, Surya S, Walzer C, Linkie M. African swine fever threatens Southeast Asia's 11 endemic wild pig species. *Conserv Lett.* (2021) 14:e12784. doi: 10.1111/conl.12784

15. FAO, IUCN SSC, OIE. Conservation impacts of African swine fever in the Asia-Pacific region (2021). Available online at: https://www.fao.org/3/cb5805en/cb5805en.pdf (accessed March 25, 2023).

16. Belecky M, Gray TNF. Silence of the snares: Southeast Asia's Snaring Crisis (2020). WWF International. Available online at: https://www.worldwildlife.org/publications/silence-of-the-snares-southeast-asia-s-snaring-crisis (accessed April 15, 2023).

17. FFI. Exploring bushmeat consumption behaviors among Phnom Penh citizens (2018). Available online at: https://www.fauna-flora.org/app/uploads/2018/05/FFI_2018_Exploring-Bushmeat-Consumption-Behaviours.pdf (accessed March 25, 2023).

18. Ibbett H, Keane A, Dobson ADM, Griffin O, Travers H, Milner-Gulland EJ. Estimating hunting prevalence and reliance on wild meat in Cambodia's Eastern Plains. *Oryx.* (2021) 55:878–88. doi: 10.1017/S0030605319001455

19. Groenenberg M, Crouthers R, Yoganand K. Population Status of Ungulates in the Eastern Plains Landscape. Srepok Wildlife Sanctuary and Phnom Prich Wildlife Sanctuary, Cambodia Technical Report Phnom Penh, Cambodia: WWF-Cambodia (2020).

20. Gray TNE, Prum S, Pin C, Phan C. Distance sampling reveals Cambodia's Eastern Plains Landscape supports the largest global population of the Endangered banteng Bos javanicus. *Oryx.* (2020) 46:563–6. doi: 10.1017/S0030605312000567

21. Griffin O, Nuttall M. *Status of Key Species in Keo Seima Wildlife Sanctuary 2010–2020.* Phnom Penh, Cambodia: WCS-Cambodia (2020). doi: 10.19121/2020.Report.38511

22. Nuttall MN, Griffin O, Fewster RM, McGowan PJK, Abernethy K, O'Kelly H, et al. Long-term monitoring of wildlife populations for protected area management in Southeast Asia. *Conservation Science and Practice.* (2022) 4:e614. doi: 10.1111/csp2.614

23. Agger C. *The status of key species in Keo Seima wildlife sanctuary 2022.* Phnom Penh, Cambodia: WCS-Cambodia (2022).

24. Gilbert M, Miquelle DG, Goodrich JM, Reeve R, Cleaveland S, Matthews L, et al. Estimating the potential impact of canine distemper virus on the Amur tiger population (*Panthera tigris altaica*) in Russia. *PLoS ONE.* (2014) 9:e110811. doi: 10.1371/journal.pone.0110811

25. FAO, OIE. Overview of the plan of action, Peste des petits ruminants global eradication programme II & III (blueprint) (2022). Available online at: https://www.fao.org/3/cc2759en/cc2759en.pdf (accessed April 15, 2023).

26. Fine AE, Pruvot M, Benfield CTO, Caron A, Cattoli G, Chardonnet P, et al. Eradication of peste des petits ruminants virus and the wildlife-livestock interface. *Front Vet Sci.* (2020) 7:50. doi: 10.3389/fvets.2020.00050

27. Gilardi KV, Gillespie TR, Leendertz FH, Macfie EJ, Travis DA, Whittier CA, et al. *Best Practice Guidelines for Health Monitoring and Disease Control in Great Ape Populations.* Gland, Switzerland: IUCN SSC Primate Specialist Group (2021).

28. Kapil S, Yeary TJ. Canine distemper spillover in domestic dogs from urban wildlife. *Vet Clin North Am Small Anim Pract.* (2011) 41:1069–86. doi: 10.1016/j.cvsm.2011.08.005

29. Gilbert M, Dvornicky-Raymond Z, Bodgener J. Disease threats to tigers and their prey. *Front Ecol Evol.* (2023) 11:1135935. doi: 10.3389/fevo.2023.1135935

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Low transmission risk of African swine fever virus between wild boar infected by an attenuated isolate and susceptible domestic pigs

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African swine fever (ASF) is a lethal infectious disease that affects domestic and wild pigs. This complex virus has already affected five continents and more than 70 countries and is considered to be the main threat to the global swine industry. The disease can potentially be transmitted directly through contact with infectious animals, or indirectly by means of contaminated feed or environments. Nevertheless, the knowledge regarding the transmission patterns of different ASF virus isolates at the wildlife-livestock interface is still limited. We have, therefore, assessed the potential transmission of an attenuated ASF virus isolate between infectious wild boar and directly exposed domestic pig. We registered 3,369 interspecific interactions between animals, which were brief and mostly initiated by wild boar. The major patterns observed during the study were head-to-head contact owing to sniffing, thus suggesting a high probability of pathogen transmission. However, only one of the five domestic pigs had a short period of viremia and became serologically positive for ASF virus antibodies. It was additionally discovered that the wild boar did not transmit the virulent virus isolate to the domestic pigs, which suggests that the presence of attenuated ASF virus isolates in affected areas may control the spreading of other more virulent isolates. These outcomes may help make decisions related to large-scale targeted management actions against ASF in field conditions.

KEYWORDS

African swine fever, transmission, wild boar, domestic pig, interspecific, interactions, attenuated isolate, virulent isolate

1. Introduction

African swine fever (ASF) is a devasting hemorrhagic viral disease that affects the *Suidae* family and is harmful to domestic and wild pigs of all ages and sexes (1). The disease is caused by the African swine fever virus (ASFV), which is a large, enveloped DNA virus, and belongs to the family *Asfaviridae*. ASFV infection may appear in susceptible populations in a wide variety of clinical forms, from subclinical to severe hemorrhagic disease with a high lethality often from 90 to 100% (2). In Europe, the control of the disease is based on its rapid diagnosis and the implementation of strict sanitary measures since commercial vaccines and effective therapies are not available. The appearance of new ASF outbreaks in ASF-free countries leads to export restrictions on live animals and their products, thus triggering, huge economic losses.

ASFV, which belongs to the genotype II, reappeared in Georgia in 2007. It subsequently affected Transcaucasian countries and quickly spread to the Russian Federation, reaching European Union countries in 2014. In Europe, ASF is currently present in Lithuania, Poland, Latvia, Estonia, Moldova, Bulgaria, Hungary, Romania, Slovakia, Serbia, Greece, Germany, North Macedonia, Italy, and recently, Ukraine, Bosnia and Herzegovina and Croatia. Effective outbreak management in the Czech Republic and Belgium followed, resulting in these countries being declared ASF-free in 2019 and 2020, respectively (3). Nevertheless, after an absence of more than 3 years, ASF has re-emerged in the Czech Republic, where the carcasses of infected wild boar have been found (4). In August 2018, the ASF crisis expanded throughout Asia, where the first outbreak of ASF was reported in the world's largest pig producing country, China (5). The virus has since spread rapidly and has, to date, affected neighboring countries such as Mongolia, Vietnam, Cambodia, Hong Kong, the Democratic People's Republic of Korea, Laos, Myanmar, Philippines, the Republic of Korea, Timor-Leste, Indonesia, India, Malaysia, Thailand and Papua New Guinea in Oceania (4). The most recent update from the World Organization for Animal Health (WOAH) confirmed new cases of ASF in the Western hemisphere for the first time in ∼40 years. The ASF-positive pig samples were confirmed in the Dominican Republic and Haiti as a part of a cooperative surveillance program between the United States and the Dominican Republic (4).

The epidemiology of ASF is complex and varies according to the environment, types of production system, the presence or absence of wild pigs, competent tick vectors, and human behavior (1). The circulation of the virus in the natural ecosystem has, in the last decade, developed into a self-sustained epidemiological cycle with the implication of the wild suids (6). The involvement of wild boar (*Sus scrofa*) population in ASFV maintenance, spread, and transmission is of particular concern on the European and Asian continents owing to its extensive presence in these territories (5, 7). All efforts directed toward ASF control should, therefore, consider the important role played by wild boar in pathogen transmission.

The clinical course of the ASFV infection depends on multiple variables, such as, the virulence of the virus and the individual immunological characteristics of the host (8–12). The principal routes of ASFV transmission are related to blood, excretions, secretions, or the carcasses of infected wild boar (13). It has been demonstrated that direct contact is an effective ASFV transmission route between infected and susceptible suids (9, 14, 15). With regard to the virulence of the virus, wild boar infected with virulent ASFV isolates developed serious clinical signs similar to those observed in domestic pigs, and died within seven to nine days (9, 16, 17). Furthermore, other less virulent ASFV isolates, such as Lv17/WB/Rie1 (genotype II) or NH/P68 (genotype I), have been shown to produce mild to absent clinical signs with transitory fever, and obtained good results as regards protective immunity against virulent isolates (18–21). These attenuated isolates are currently being evaluated in the UE-funded research project H2020 VACDIVA (Grant Agreement n° 862874) which focuses on the development and assessment of vaccine candidates as a safe and effective tool for wild boar and domestic pig populations. However, the use of live attenuated vaccines (LAVs) based on naturally attenuated virus isolates may, overall, entail a series of concerns related to the shedding of the vaccine virus, reversion to virulence, or the generation of new variants with wild-type viruses (22). These safety concerns of LAVs should be reduced to a minimum, and it is for this reason, that deletion mutants based on naturally attenuated virus isolates are currently the most promising options with which to control the spread of ASFV and reduce the risk of the devasting consequences of this disease for swine producers worldwide (23–25).

The issue with LAVs is their ability to occasionally transmit the attenuated virus to susceptible animals. The potential shedding of attenuated virus isolates such as Lv17/WB/Rie1 has already been investigated in domestic pigs (26) and wild boar (27). The results described in these studies suggest that the risk of oral shedding, which is the natural route of infection, is much lower with attenuated viruses than with highly virulent or moderate virulence isolates (15, 26). However, animals infected with attenuated isolates have demonstrated the capacity to transmit the virus to sentinel animals (19, 21). For instance, the attenuated virus Lv17/WB/Rie1 was transmitted to sentinel wild boar within 2 weeks (21), while NH/P68 was transmitted to sentinel pigs within 3 or 4 weeks after the initial exposure (19). In both cases, animals previously infected with these LAVs did not transmit the virulent challenge virus to sentinel animals. This demonstrated that susceptible animals were successfully protected by direct contact, which could have beneficial effects for control strategies (13, 28).

In the present study, we investigated the transmission rate of the attenuated ASFV isolate Lv17/WB/Rie1 between wild boar and domestic pigs. A group of sentinel domestic pigs was, therefore, exposed through direct contact with wild boar infected with the attenuated ASFV isolate. All details of the interspecific interactions between the two subspecies and their clinical consequences are described herein.

2. Material and methods

2.1. Animals

The experiment was carried out using four 4–5-month-old female and one male wild boar weighing 20–25 kg, and five castrated 2-month-old Large White breed male pigs, weighing 15–20 kg. The wild boar were obtained from a commercial farm in Sevilla, Spain, while the domestic pigs were from an authorized breeding farm in Segovia, Spain. The experiment was performed in biosafety level 3 (BSL-3) facilities at the VISAVET Health Surveillance Centre at the University Complutense of Madrid, Spain. Animal care, management and sampling procedures were conducted according to national and European regulations and the experimental protocol was previously approved by the Ethics Committee of the Complutense University of Madrid and the Community of Madrid (reference PROEX 159/19). The protocol included a detailed description of efforts to prevent and avoid the animals' unnecessary suffering, including humane endpoints and guidelines regarding euthanasia, following the EC Directive 2010/63/UE. All procedures were designed and performed by specially trained personnel and veterinarians (animal experimentation categories B, C, and D) following the Directive

TABLE 1 The allocation period of each wild boar with susceptible domestic pigs during the study (days post-infection).

Wild boar ID	Days post-infection (dpi)
WB 1	12–40
WB 2	40–74
WB 3	0–40
WB 4	40–74
WB 5	0–12

2003/65/EC and Spanish laws RD53/2013. Guidelines for ARRIVE 2.0 for the care and use of laboratory animals were also followed.

Upon arrival, the animals were individually ear-tagged and acclimated for one week before the experiment began. Access to food and water was provided *ad libitum* throughout the study. These animals were not vaccinated against any pathogen and tested negative for ASFV and the main porcine pathogens in the region: *Mycoplasma hyopneunoniae, Mycobacterium bovis,* porcine reproductive and respiratory syndrome (PRRS) virus, porcine circovirus type 2.

2.2. ASFV isolates

The wild boar were infected using the attenuated non-hemoadsorbing p72 genotype II ASFV Lv17/WB/Rie1 isolate. This isolate has previously been tested on domestic pigs and wild boar, showing promising results in terms of effectiveness against the challenge with a highly virulent ASFV isolate, Armenia 2007 (20, 21, 29). The virus was grown for 7 days in porcine blood monocytes (PBM) and was collected as described previously by Barasona et al. (21). Viral titer was defined as the amount of virus causing cytopathic effects in 50% of infected cultures ($TCID_{50}$/mL), estimated by employing immunoperoxidase staining (19).

The challenge virus employed was the virulent and hemadsorbing p72 genotype II ASFV Armenia 2007 (Arm07) isolate. The virus was propagated in PBM as previously described by Gallardo et al. (19), and the viral titer was defined as the amount of virus causing hemadsorption in 50% of infected cultures (HAD_{50}/mL).

Both isolates were provided by the European Union Reference Laboratory (EURL) for ASF (CISA-INIA, Valdeolmos, Spain).

2.3. Wild boar infection

After the acclimatization period, the five wild boar were orally infected with a 10^4 $TCID_{50}$ dose of the attenuated ASFV Lv17/WB/Rie1 isolate. This infection dose demonstrated to be effective in previous studies where wild boar were orally inoculated (21, 29). Eighteen days after prime inoculation, these wild boar received a second dose of the ASFV Lv17/WB/Rie1 isolate with the same dose and route of administration. The five susceptible domestic pigs were housed jointly and exposed to direct contact with the infected wild boar in order to evaluate the transmission of the attenuated isolate. It was not possible to allocate more than two wild boar with all the susceptible pigs at the same time owing to space limitations in the pen. The order of the contact of the wild boar was determined randomly (Table 1).

After the infection period of 42 days, all the wild boar were intramuscularly inoculated with 10 HAD_{50} of the virulent ASFV isolate, Arm07. The infection period, which could be understood as the period between the prime inoculation and the challenge was expressed in days post-infection (dpi) for the wild boar and days post-exposure (dpe) for the domestic pigs. The infected wild boar and susceptible domestic pigs were maintained together for 32 days post-challenge (dpc), a total of 74 days.

2.4. Interspecific interactions between wild boar and domestic pigs

The interactions between the infected wild boar and the susceptible pigs sharing the same pen were monitored by means of a video surveillance system (Hikvision iVMS-4200, Hikvision®, Hangzhou, China) throughout the study. Direct contact occurred through a metal livestock fence that separated the two compartments of the pen in order to prevent fights between these subspecies (Figure 1). During the experimental period, all details of interspecific interactions was specified using video surveillance. We registered the animals that participated in each interaction (subspecies and identification number), the type of interaction, and its duration. The type of interaction was considered at five levels: 1-simple approaches (<30 cm), 2-sniffing, 3-skin contact, 4-mucocutaneous contact, and 5-grooming or bites. The degrees of interaction levels 3, 4, and 5 (skin and mucocutaneous contact, grooming or bites) were considered high-risk contacts. The time of interaction was expressed in four time ranges: <30 s, 30 s - 1 min, 1 min - 5 min, more than 5 min. We also registered the number of animals involved in contact and which animal initiated the contact.

2.5. Clinical monitoring

The animals were observed daily throughout the trial in order to monitor their health status, by means of a video surveillance system and direct inspections carried out by veterinarians. Clinical signs, including rectal temperature, were expressed individually in terms of a quantitative clinical score (CS) specific to ASFV infection in domestic pigs (19, 30) and in wild boar (31). Fever was defined as a rectal temperature ≥40°C.

This CS considers nine parameters, which are rectal temperature, behavior, body condition, skin alterations, ocular/nasal discharge, swelling of joints, respiratory symptoms, digestive symptoms, and neurological symptoms. All clinical observations were recorded on a daily basis, with the exception of temperature, which was taken during the sampling (once a week during the infection period and twice a week during the challenge period), in order to minimize animal handling and stress.

Clinical evaluations were also monitored so as to ensure the animals' welfare. The humane endpoint was pre-defined as animals

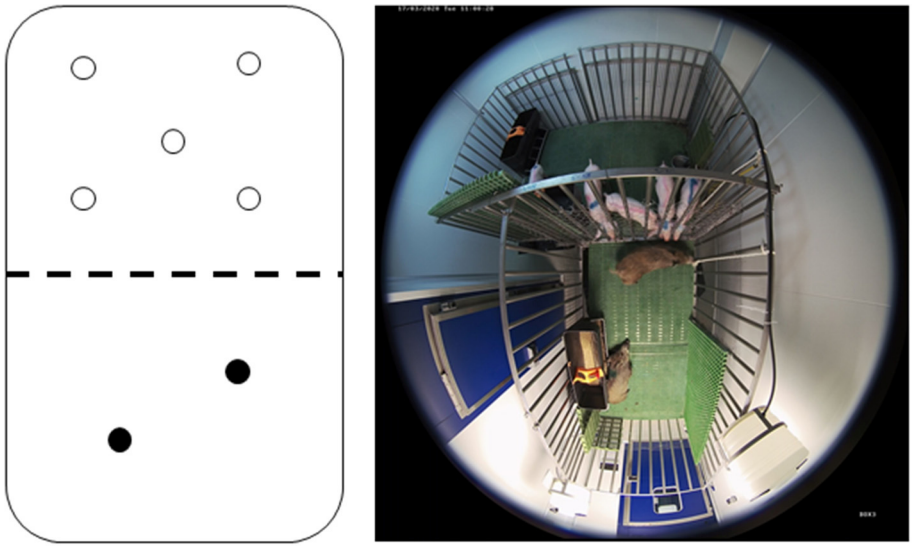

FIGURE 1
Schematic illustration and photo of the pen taken by a camera from the video surveillance system. Susceptible domestic pigs (transparent circle; $n = 5$) coming into contact with the infected wild boar (black circle; $n = 2$) through a metal livestock fence that separates the compartments of the pen.

with a CS > 18, and animals with severe clinical signs (level 4) of fever, behavior, body condition, respiratory and digestive signs for more than two consecutive days were also included, following the standards described by Cadenas-Fernández et al. (31). Any animals undergoing unacceptable suffering without reaching the pre-defined humane endpoint were also euthanized on the basis of veterinarian criteria.

2.6. Sample collection, ASFV DNA, and antibody detection

Paired EDTA blood and sera were obtained from all the animals once a week during the infection period and twice a week during the challenge period.

Additionally, oral fluid and feces were collected from the wild boar in order to detect ASFV DNA. This was done by employing quantitative PCR (qPCR). Feces were collected using cotton swabs (Deltalab, Barcelona, Spain) and oral fluid was obtained using sponge swabs (ZIZNBA, Guangdong, China).

Viral DNA was extracted from each sample using the High Pure Template Preparation Mix Kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. The detection of ASFV DNA from different types of samples (blood, oral fluid, feces) was performed using the Universal Probe Library (UPL) real-time quantitative PCR (qPCR) previously described by Fernández-Pinero et al. (32). A positive result for the qPCR was determined by identifying the threshold cycle value (cycle of quantification: Cq) at which a reporter dye emission appeared above the background within 40 cycles. Negative control samples were collected on day 0, which was the day of prime inoculation.

The sera samples were tested in order to detect antibodies. This was done using a commercial ELISA test (Ingenasa-Ingezim PPA Compac K3; Ingenasa, Madrid, Spain) and an indirect immunoperoxidase test (IPT).

At the end of the observation period (74 dpi/dpe), any surviving animals were anesthetized by means of an intramuscular injection of a combination of tiletamine-zolazepam (Zoletil® 100 mg/ml, Virbac, France) and medetomidine (Medetor®, Virbac, France) (33), and were then euthanized by employing intravenous injection of T61® (Intervet, Spain).

2.7. Statistical analysis

The records obtained from the video-surveillance monitoring of the trial, the sanitary results of the clinical inspections and laboratory analyses were unified in a dataset for a preliminary exploration. Overall, descriptive statistics were used for the evaluation of the interspecific interaction parameters and comparison to pathogen transmission findings. The relationships between the different categorical variables were assessed by using the Chi-square test (χ^2) with a significance level of 95%. Continuous variables quantifying the number of contacts per species and individuals were assessed using the Kolmogorov-Smirnov test (KSt) to confirm the absence or presence of statistical normality. The Student's *t*-test and the Mann–Whitney *U*-test were used to detect differences between continuous variables according to KSt screening. The statistical evaluation of continuous variables concerning the occurrence of contacts and the degree of interaction was carried out using the Spearman rank correlation test. The statistical

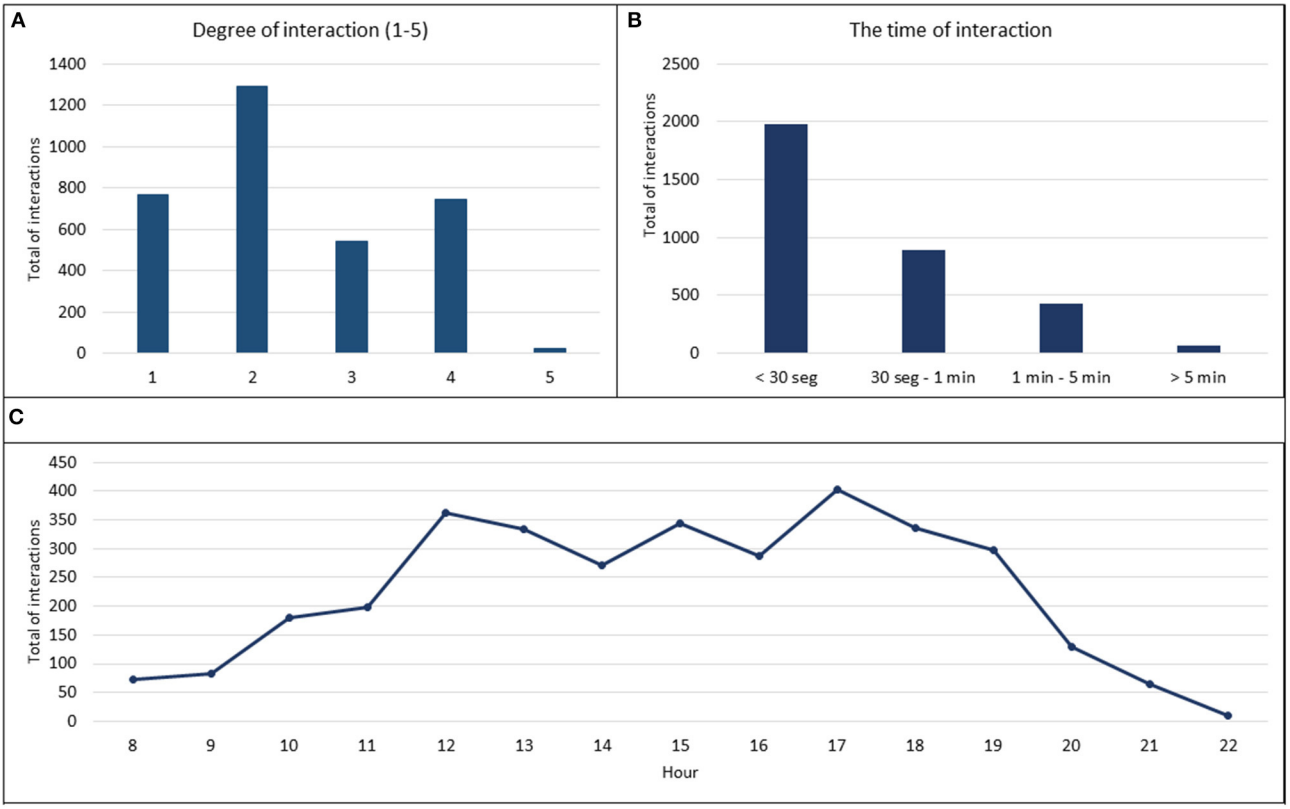

FIGURE 2
Results of interspecific interactions between infected wild boar and susceptible pigs. **(A)** The type of interaction was evaluated on a five degree scale: 1-simple approaches (<30 cm), 2-sniffing, 3-skin contact, 4-mucocutaneous contact, and 5-grooming or bites, and the results were expressed in a total number of interactions. **(B)** The time of interaction was expressed in four time ranges and the results were expressed as the total number of interactions. **(C)** Daily activity profile of susceptible pigs and infected wild boar, expressed as the total number of interactions by an hour of the day.

analysis was caried out using SPSS Statistics Version 25 (IBM Corporation, USA).

3. Results

3.1. Interspecific interactions between wild boar and domestic pigs

Overall, we registered 3,369 interspecific interactions between the infected wild boar and the susceptible pigs. The major pattern observed during the study period was head-to-head contact and sniffing (38% of total interactions). We registered 39% of high-risk transmission contacts (degrees 3, 4, and 5) (Figure 2A). Interestingly, 60% of these high-risk contacts were initiated by the wild boar. With regard to the duration of interaction, the percentage of the appearance of contact was inversely proportional to the duration of contact (Spearman's correlation test R = −95%; $p < 0.001$). In this respect, short periods of contact of <30 s predominated in most of the observations (59%). Only 2% of the interactions lasted 5 min or more, and were registered frequently when the animals fell asleep on either side of the metal fence (Figure 2B).

The daily activity register showed increased activity between 11:30 and 18:30 h with two peaks of interactions at 12 and 17 h (Figure 2C). This pattern was maintained as regards the interactions initiated by the wild boar and those initiated by the domestic pigs. The days with the highest daily activity were registered after the introduction of new wild boar into the pen, with an average number of registered interactions of 158 ± 45 in comparison to 64 ± 24 registered on a usual day.

With regard to which species favored the initiation of the interaction, 52% of the observations were initiated by the wild boar and 31% by the pigs. For the remaining percentage, it was not possible to identify which species initiated the interaction. In this respect, it was possible to observe a statistical difference with a major tendency for the wild boar to initiate interactions when compared to the domestic pigs (Student's t-test; $t = 8.88$; $p < 0{,}001$). Furthermore, almost half of the observations (49%) showed that the domestic pigs interacted in-group, while this was the case of the wild boar in only 16% of the total observations (Chi-square test; $\beta = 860.6$; $p = 0.001$). There are significant differences in the contact rate observed at the individual level. Wild boar WB 1 participated significantly more in interspecific contacts when compared with the other individuals ($\beta = 19.9$; $p < 0.001$). Pig P1 had significantly more interactions (both individual and in-group) then pigs P2 ($\beta = 5.41$; $p = 0.02$) and P4 ($\beta = 6.32$; $p = 0.01$). In addition, marginally significant differences were observed in the comparison between pig P1 and the other individuals ($\beta = 3.68$; $p = 0.05$).

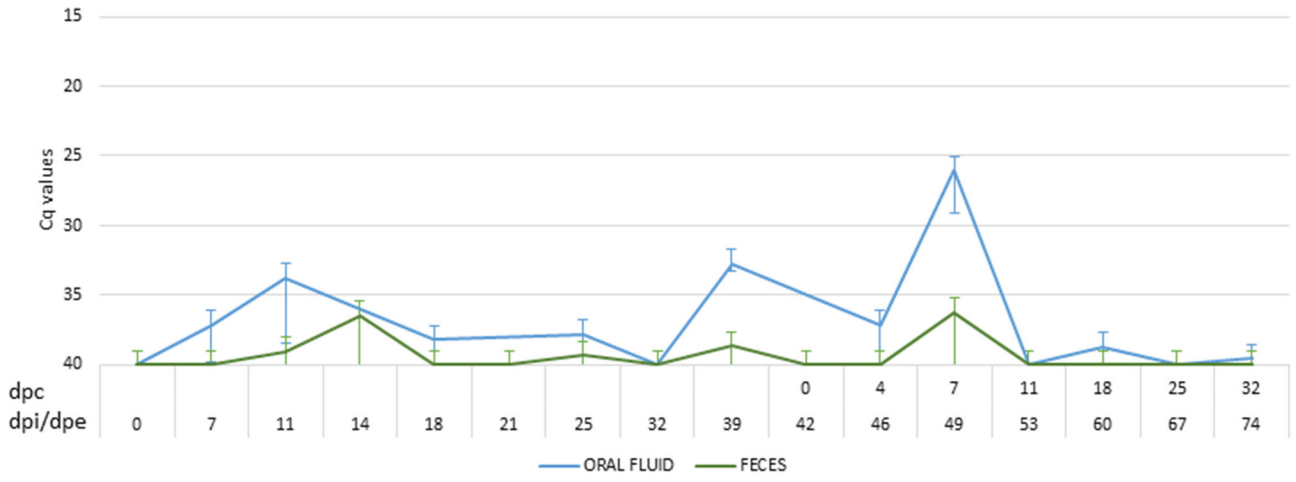

FIGURE 3
Mean of the DNA African swine fever virus (ASFV) load excreted by oral fluid (blue) and feces (green) of wild boar infected with Lv17/WB/Rie1 ASFV isolate. The viral load was expressed in cycles of quantification values (Cq) obtained by employing real-time PCR. The period between the prime inoculation and the challenge was expressed in days post-infection (dpi) for wild boar and days post-exposure (dpe) for domestic pigs, while the period after the challenge was expressed in days post-challenge (dpc).

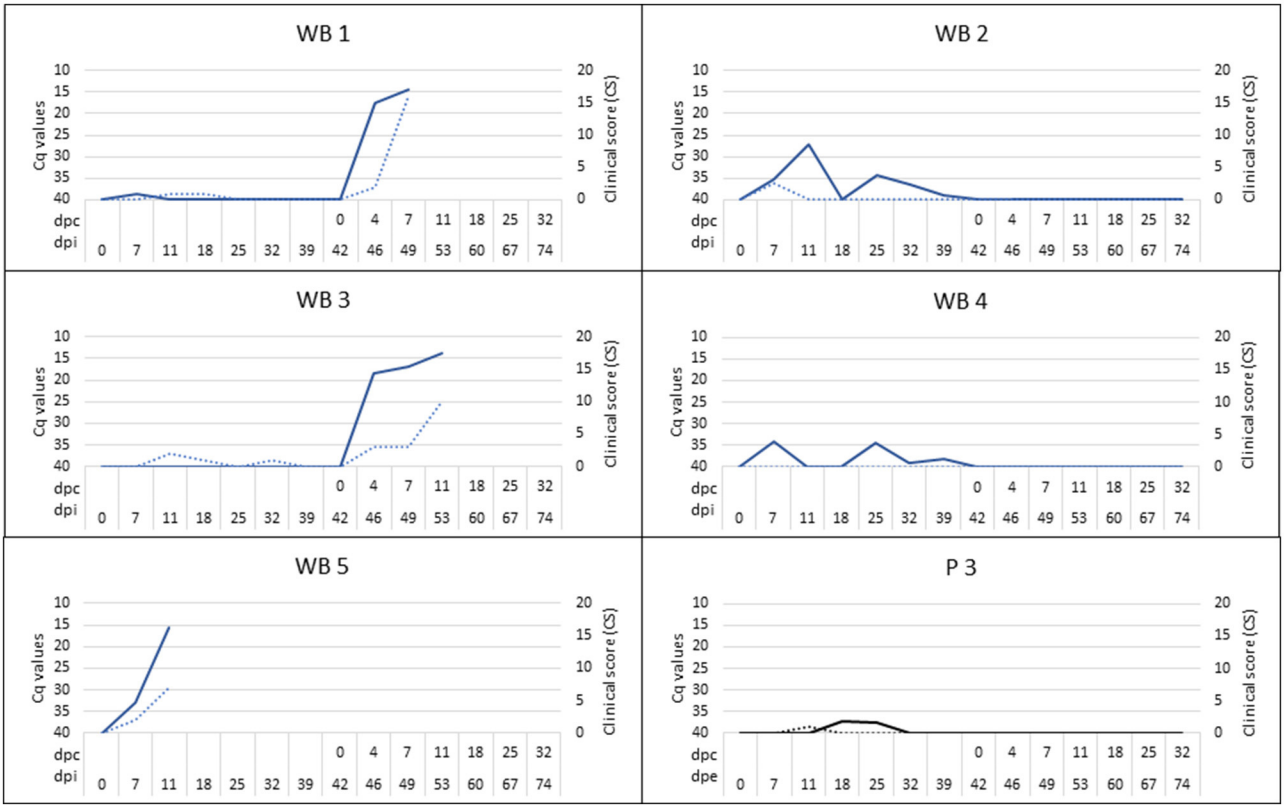

FIGURE 4
Clinical score (CS; dashed line) and viremia (continuous line) were determined by means of real-time PCR and expressed in cycles of quantification (Cq) for infected wild boar (WB 1-WB 5) and susceptible pig (P 3) exposed by direct contact. Four of the five susceptible pigs (P1, P2, P4, P5) did not have clinical signs or viremia. The period between the prime inoculation and the challenge was expressed in days post-infection (dpi) for wild boar and days post-exposure (dpe) for domestic pigs, while the period after the challenge was expressed in days post-challenge (dpc).

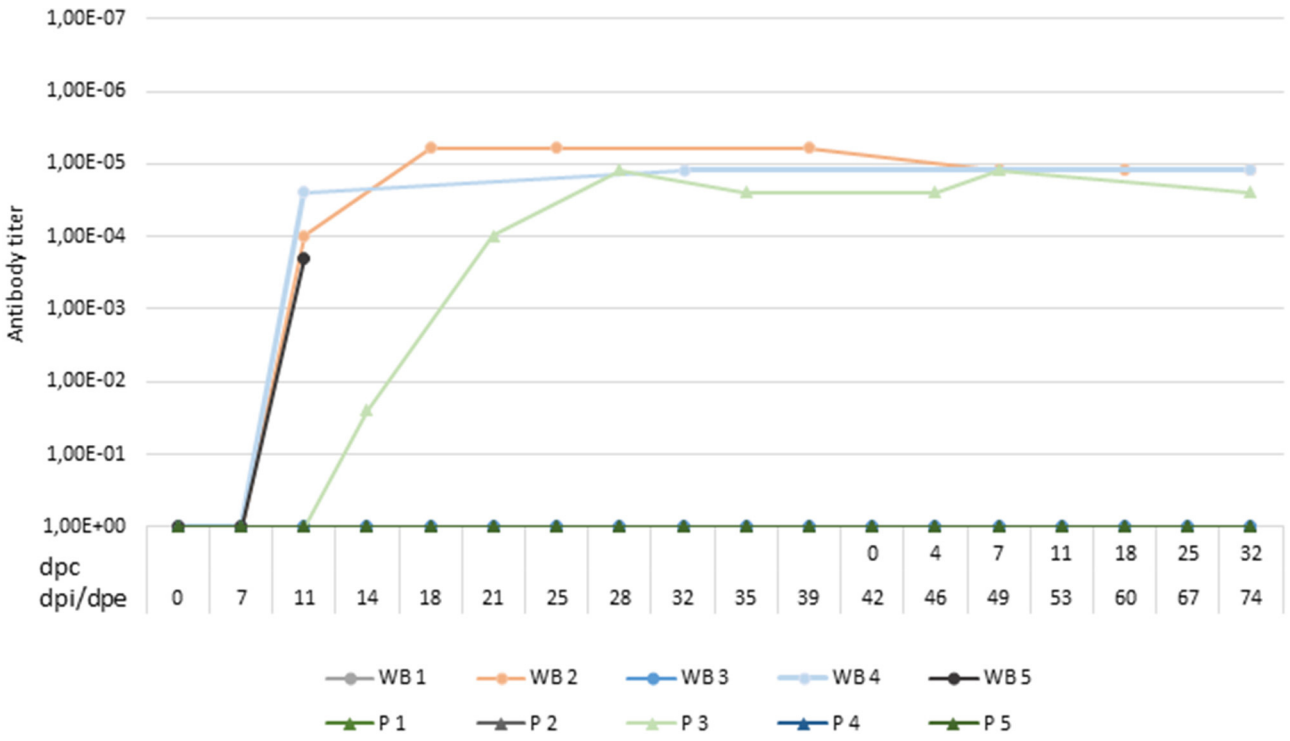

FIGURE 5
Titers of antibodies against African swine fever virus in wild boar infected with Lv17/WB/Rie1isolate (WB1, WB2, WB3, WB4, WB5) and domestic pigs exposed through contact with the infected animals (P1, P2, P3, P4, P5). A positive antibody response was not observed in two of the five wild boar infected. Titers were determined using the indirect immunoperoxidase test. The period between the prime inoculation and the challenge was expressed in days post-infection (dpi) for wild boar and days post-exposure (dpe) for domestic pigs, while the period after the challenge was expressed in days post-challenge (dpc).

3.2. Clinical and laboratory analysis

After coming into contact with the attenuated isolate, three of the five wild boar (60%) were successfully infected and had a positive antibody response, confirmed by ELISA and IPT, starting at 12 dpi. During the infection period, the wild boar had two clear peaks of viremia at 12 dpi (Cq = 33.66 ± 7.97) and 25 dpi (Cq = 37.13 ± 2.86), following prime and boost infection (Figure 4). In this period, three of the five wild boar had slight fever (40.00 ± 0.28°C) and occasionally lethargy, accompanied by viremia (Cq = 37.75 ± 4.23). Viral DNA was detected intermittently in wild boar excretions with a low viral load in oral fluid (Cq = 36.98 ± 3.89) and feces (Cq = 39.34 ± 1.22) (Figure 3).

One of the infected wild boar (WB 5), which appeared to be clinically healthy, but proved to be viremic (Cq = 27.11), did not recover after a handling procedure and anesthesia and was euthanized at 12 dpi. At the moment of death, this wild boar had significant viremia (Cq = 15.45; Figure 4), fever (40.9°C), and a low titer of ASFV-specific antibodies, which was detected using IPT (Figure 5). This animal was replaced with another in order to always maintain two wild boar in contact with the susceptible pigs.

At 14 dpe, two of the five susceptible pigs underwent a slight increase in body temperature (40.2°C) and suffered from slight depression. Viral DNA was detected in the blood (Cq = 37.38) of one of them (P3), which was maintained only in the next sampling. This pig also had a positive antibody response based on ELISA and IPT, reaching a high titer of antibodies, similar to those obtained for the infected wild boar (Figure 5).

Two of the infected and successfully protected wild boar, which were allocated with susceptible domestic pigs, survived the challenge carried out with the virulent ASFV isolate, Arm07. We did not detect fever or any other clinical signs that could be compatible with ASFV infection, and no ASFV DNA was detected in their blood. All of the susceptible pigs survived the challenge period and did not have any clinical signs of infection or fever, and no ASFV DNA was detected in their blood. One of the five susceptible pigs (P3) remained positive for the detection of specific antibodies against ASFV after the challenge period.

4. Discussion

Understanding the transmission mechanisms and sources of infection of ASFV has become a research priority in the current Eurasian context, in which wild boar play a key role in the epidemiology of the infection (7). In addition to this, with regard to the urgent need to develop and evaluate of multiple attenuated vaccine candidates in an attempt to prevent the advance of this infection (24), exploring the transmission capacity of these isolates is an important advance as regards deciphering gaps in research. This is, to the best of our knowledge, the first study to assess

the potential transmission of an attenuated ASFV between wild boar and domestic pigs under experimental conditions. We have specifically, evaluated the spread of the ASFV Lv17/WB/Rie1 isolate in susceptible domestic pigs exposed by contact through a simple partition, simulating a livestock fence, with infected wild boar. Overall, we have determined high contact rates among the groups studied, suggesting a high probability of pathogen transmission. However, two of the susceptible pigs showed signs of infection, and only one had a short period of viremia and became serologically positive for ASFV antibodies. The results confirmed that the transmission capacity of this attenuated ASFV isolate is relatively lower than that of other genotype II isolates previously studied (14).

In this work, we have focused on carrying out a detailed assessment of direct interactions between wild boar and domestic pigs in order to understand potential cross-species pathogen transmission. Extensive monitoring, with more than 890 h of video-surveillance, has been performed to obtain detailed interactions between animals. This monitoring effort made it possible not only to discover interactions at a quantitative level but also to provide additional qualitative information related to the intensity, type and duration of these interactions. In general, most of the contacts were brief, with periods of <30 s, during which there was no aggressive behavior but rather curiosity. This result supports other studies carried out in the field, in which mostly indirect interactions between these subspecies were observed, and when direct interactions existed, they were for short periods (34, 35). Despite the brevity of these interactions, the relative risk of transmission could be high owing to a high proportion of oral and nasal mucosal contacts. In the case of highly or moderately virulent isolates of ASFV, this would be sufficient to achieve effective transmission (9, 16). However, in the case of the attenuated isolate studied, this has been observed to a lesser extent. We have also registered a low number of longer interactions (> 5 min), but these contacts corresponded to the occasions on which the animals were resting on either side of the metal fence, which was likely owing to space constraints inherent to an experiment in controlled laboratory conditions. Interestingly, the wild boar initiated contact through the fence more frequently than the pigs, despite the lower proportion of wild boar kept simultaneously in the pen throughout the experiment for animal welfare reasons. In this respect, the wild boar were more predisposed to a single contact as an individual, while the pigs tended to make contact in a group. This could be explained by the natural social behavior of pigs, based on the imitation of certain patterns observed in other individuals (36). The daily pattern of interaction by hours determined a higher risk between 11:30h and 18:30h, which is consistent with field studies in temperate environments in seasons with low thermal fluctuation, such as spring and autumn in Mediterranean scenarios (37, 38).

These results should be considered with caution, because they may be highly influenced by the experimental conditions of our study, which could differ from real scenarios. In the natural environment, interspecific interactions can be monitored through the use of tracking technologies such as GPS-collars, proximity loggers, or photo-trapping. As mentioned previously, these field studies indicate that direct interactions between livestock and wildlife occur rarely, and animals most often interact indirectly during the common use of water sources and supplementary feeding points (37–40). Although the data regarding contacts obtained in this study may have been altered by the experimental design, and there may be many factors affecting these interaction patterns, our results suggest that this may be a first approach with which to predict pathogen transmission between wild boar and domestic swine, and its applicability to reduce the consequences of these interspecific interactions. These results should be verified and extended in further field studies under controlled conditions and with a larger number of groups and animals.

With regard to the individual differences observed, the male wild boar (WB 1) had a higher number of interactions when compared to the females, which is mainly explained by a higher activity described in behavioral studies for this gender, along with sexual interest as a sign of early puberty. Furthermore, one of the susceptible domestic pigs (P1) showed a significantly higher number of interactions than the other pigs. However, this increase in interactions was not sufficient to cause infection with the attenuated ASFV isolate, despite increased exposure to infected wild boar. The results obtained in this study suggest a very sporadic transmission of the ASFV Lv17/WB/Rie1 isolate, which means that a higher transmission risk pathway would be needed to initiate transmission with this isolate from the wild boar to the domestic pig than would be observed with simple oro-nasal contact. This observation confirms that this attenuated isolate has the potential to be disseminated. However, the shedding pattern is limited, as also confirmed by previous studies (21, 26, 27). In this respect, the transfer/consumption of blood from an infected animal to a susceptible animal may be the cause of transmission, as previously suggested in the case of isolates of moderate to low virulence (26, 41). We can not rule out that blood consumption (e.g., through a bite from a viremic wild boar) occurred only in the case of the one pig (P3) that became infected with this attenuated isolate. This single infected pig, together with the other boars, was not able to spread the disease to the rest of the sentinel pigs beyond day 14 dpe, thus suggesting a null capacity to maintain carriers with transmission capacity. This outcome coincides with those of two long-term studies, which determined that animals infected with moderately virulent ASFV did not transmit the virus to commingled sentinel pigs after clinical recovery from ASF (42, 43).

Another important result of this study is the fact that the wild boar were successfully protected with the administration of the Lv17/WB/Rie1 isolate, did not transmit the virulent virus isolate to the susceptible pigs for 32 days, and survived the challenge with the virulent ASFV isolate, Arm07, without ASF-compatible pathognomonic signs or associated viremia. This observation could indicate that the infection of wild boar or the presence of attenuated ASFV isolates in ASF-affected areas reduces the spreading of the virulent isolates and ASFV introduction into the domestic pig value chain. This identifies a need for future research into the evolution/stability of these low virulence ASFV isolates, molecular epidemiology, and immunology in sympatric populations of endemic areas. Knowledge concerning the role of gene deletion mutants

in ASFV transmission among both wild and domestic compartments is also lacking and further investigation is, therefore, required.

Data availability statement

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

Ethics statement

The animal study was reviewed and approved by the Ethics Committee of the Complutense University of Madrid and the Community of Madrid (reference PROEX 159/19). The study was conducted in accordance with the local legislation and institutional requirements. No potentially identifiable images or data are presented in this study.

Author contributions

Conceptualization, validation, and supervision: JS-V and JB. Methodology, writing—review and editing, and research: AK, LB, EC-F, SB-A, JB, and JS-V. Formal analysis, data cleansing, software, and visualization: AK, LB, and JB. Resources: JS-V. Writing—original draft preparation: AK and JB. All authors have read and agreed to the published version of the manuscript and 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.

Publisher's note

References

1. Costard S, Wieland B, Glanville D, Jori W, Rowlands F, Vosloo R, et al. African swine fever: how can global spread be prevented? *Philos Trans Roy Soc Biol Sci.* (2009) 364:2683–2696. doi: 10.1098/rstb.2009.0098

2. Pikalo J, Zani L, Hühr J, Beer M, Blome S. Pathogenesis of African Swine fever in domestic pigs and european wild boar – lessons learned from recent animal trials. *Virus Res.* (2019) 271:197614. doi: 10.1016/j.virusres.2019.04.001

3. Sauter-Louis C, Schulz K, Richter M, Staubach C, Mettenleiter TC, Conraths FJ, et al. African swine fever: why the situation in germany is not comparable to that in the Czech Republic or Belgium. *Transbound Emerg Dis.* (2021) 5:1–8. doi: 10.1111/tbed.14231

4. WOAH. *WAHIS World Organisation for Animal Health*. (2023). Available online at: https://wahis.woah.org/#/dashboards/country-or-disease-dashboard (accessed July 13, 2023).

5. Ito S, Bosch J, Martínez-Avilés M, Sánchez-Vizcaíno JM. The evolution of African swine fever in China: a global threat? *Front Vet Sci.* (2022) 9:1–10. doi: 10.3389/fvets.2022.828498

6. FAO. *African Swine Fever in Wild Boar Ecology and Biosecurity. FAO Animal Production and Health Manual.* Rome :FAO (2019).

7. Lewis JS, Farnsworth ML, Burdett CL, Theobald DM, Gray MRS. Biotic and abiotic factors predicting the global distribution and population density of an invasive large mammal. *Sci Rep.* (2017) 7:44152. doi: 10.1038/srep44152

8. Sehl J, Pikalo J, Schäfer A, Franzke K, Pannhorst K, Elnagar A, et al. Comparative pathology of domestic pigs and wild boar infected with the moderately virulent african swine fever virus strain "estonia 2014." *Pathogens.* (2020) 9:662. doi: 10.3390/pathogens9080662

9. Pietschmann J, Guinat C, Beer M, Pronin V, Tauscher K, Petrov A, et al. Course and transmission characteristics of oral low-dose infection of domestic pigs and European wild boar with a caucasian african swine fever virus isolate. *Arch Virol.* (2015) 160:1657–67. doi: 10.1007/s00705-015-2430-2

10. Pikalo J, Schoder M, Sehl J, Breithaupt A, Tignon M, Cay AB, et al. The African swine fever virus isolate Belgium 2018/1 shows high virulence in european wild boar. *Transbound Emerg Dis.* (2020) 2019: tbed.13503. doi: 10.1111/tbed.13503

11. Howey EB, O'Donnell V, Carvalho F, Borca HC, Arzt MVJ. Pathogenesis of highly virulent african swine fever virus in domestic pigs exposed via intraoropharyngeal, intranasopharyngeal, and intramuscular inoculation, and by direct contact with infected pigs. *Virus Res.* (2013) 178:328–39. doi: 10.1016/j.virusres.2013.09.024

12. Barroso-Arévalo S, Barasona JA, Cadenas-Fernández E, Sánchez-Vizcaíno JM. The Role of interleukine-10 and interferon-γ as potential markers of the evolution of African swine fever virus infection in wild boar. *Pathog.* (2021) 10:757. doi: 10.3390/pathogens10060757

13. Arias M, de la Torre A, Dixon L, Gallardo C, Jori F, Laddomada A, Martins C. Approaches and perspectives for development of African swine fever virus vaccines. *Vaccines.* (2017) 5:35. doi: 10.3390/vaccines5040035

14. Guinat C, Gogin A, Blome S, Keil G, Pollin R, Pfeiffer DU, et al. Transmission routes of African swine fever virus to domestic pigs: current knowledge and future research directions. *Vet Rec.* (2016) 178:262–7. doi: 10.1136/vr.103593

15. Carvalho F, Backer HC, Weesendorp JA, Klinkenberg E, Stegeman D, Loeffen JA, et al. Transmission rate of african swine fever virus under experimental conditions. *Vet Microbiol.* (2013) 165:296–304. doi: 10.1016/j.vetmic.2013.03.026

16. Gabriel C, Blome S, Malogolovkin A, Parilov S, Kolbasov D, Teifke JP, et al. Characterization of african swine in european wild boars. *Emerg Infect Dis.* (2011) 17:2342–5. doi: 10.3201/eid1712.110430

17. Blome S, Gabriel C, Beer M. *Pathogenesis of African Swine Fever in Domestic Pigs and European Wild Boar. Virus Research*. Amsterdam: Elsevier, 122–130.

18. Leitão A, Cartaxeiro C, Coelho R, Cruz B, Parkhouse RME, Portugal FC, et al. The non-haemadsorbing african swine fever virus isolate ASFV/NH/P68 provides a model for defining the protective anti-virus immune response. *J Gen Virol.* (2001) 82:513–23. doi: 10.1099/0022-1317-82-3-513

19. Gallardo C, Sánchez EG, Pérez-Núñez D, Nogal M, León AL, Carrascosa P, et al. African swine fever virus (ASFV) protection mediated by nh/p68 and nh/p68 recombinant live-attenuated viruses. *Vaccine.* (2015) 36:2694–704. doi: 10.1016/j.vaccine.2018.03.040

20. Gallardo C, Soler A, Rodze I, Nieto R, Jovita CC, Arias FM, et al. attenuated and non - haemadsorbing (non - HAD) genotype II african swine fever virus (ASFV) isolated in Europe. *Latvia*. (2019) 2018:1399–404. doi: 10.1111/tbed.13132

21. Barasona JA, Gallardo C, Cadenas-Fernández E, Jurado C, Rivera B, Rodríguez-Bertos A, et al. First oral vaccination of eurasian wild boar against african swine fever virus genotype II. *Front Vet Sci.* (2019) 6:1–10. doi: 10.3389/fvets.2019.00137

22. Rock DL. Challenges for african swine fever vaccine development —"perhaps the end of the beginning." *Vet Microbiol.* (2017) 206:52–8. doi: 10.1016/j.vetmic.2016.10.003

23. Sang H, Miller G, Lokhandwala S, Sangewar N. Progress toward development of effective and safe african swine fever virus *Vaccines*. (2020) 7:1–9. doi: 10.3389/fvets.2020.00084

24. Bosch-Camós L, López E, Rodriguez F. African swine fever vaccines: a promising work still in progress. *Porc Heal Manag.* (2020) 6:1–14. doi: 10.1186/s40813-020-00154-2

25. Gladue DP, Borca MV. Recombinant ASF live attenuated virus strains as experimental vaccine candidates. *Viruses*. (2022) 14:878. doi: 10.3390/v14050878

26. Gallardo C, Soler A, Nurmoja I, Cano-Gómez C, Cvetkova S, Frant M. Dynamics of african swine fever virus (ASFV) infection in domestic pigs infected with virulent, moderate virulent and attenuated genotype II ASFV european isolates. *Transbound. Emerg Dis.* (2021) 68:2826–41. doi: 10.1111/tbed.14222

27. Kosowska A, Cadenas-Fernández E, Barroso S, Sánchez-Vizcaíno JM, Barasona JA. Distinct african swine fever virus shedding in wild boar infected with virulent and attenuated isolates. *Vaccines.* (2020) 8:1–12. doi: 10.3390/vaccines8040767

28. Rossi S, Staubach C, Blome S, Guberti V, Thulke HH, Vos A, et al. Controlling of CSFV in european wild boar using oral vaccination: a review. *Front Microbiol 6.* (2015) 1–11. doi: 10.3389/fmicb.2015.01141

29. Barasona JA, Cadenas-Fernández E, Kosowska A, Barroso-Arévalo S, Rivera B, Sánchez R, et al. Safety of african swine fever vaccine candidate lv17/wb/rie1 in wild boar: overdose and repeated doses. *Front Immunol.* (2021) 12:1–11. doi: 10.3389/fimmu.2021.761753

30. Gallardo C, Nieto R, Soler A, Pelayo V, Fernández-Pinero J, Markowska-Daniel I, et al. Assessment of African swine fever diagnostic techniques as a response to the epidemic outbreaks in eastern european union countries: how to improve surveillance and control programs. *J Clin Microbiol.* (2018) 53:2555–65. doi: 10.1128/JCM.00857-15

31. Cadenas-Fernández E, Sánchez-Vizcaíno JM, Kosowska A, Rivera B, Mayoral-Alegre F, Rodríguez-Bertos A, et al. Adenovirus-vectored african swine fever virus antigens cocktail is not protective against virulent arm07 isolate in eurasian wild boar. *Pathogens.* (2020) 9:171. doi: 10.3390/pathogens9030171

32. Fernández-Pinero J, Gallardo C, Elizalde M, Robles A, Gómez C, Bishop R, et al. Molecular diagnosis of african swine fever by a new real-time PCR using universal probe library. *Transbound Emerg Dis.* (2013) 60:48–58. doi: 10.1111/j.1865-1682.2012.01317.x

33. Barasona J, López-Olvera J, Beltrán-Beck B, Gortázar C, Vicente J. Trap-effectiveness and response to tiletamine-zolazepam and medetomidine anaesthesia in Eurasian wild boar captured with cage and corral traps. *BMC Vet Res.* (2013) 9:107. doi: 10.1186/1746-6148-9-107

34. Cowie CE, Hutchings MR, Barasona JA, Gortázar C, Vicente J, White PCL, et al. Interactions between four species in a complex wildlife: livestock disease community: implications for mycobacterium Bovis maintenance and transmission. *Eur J Wildlife Res.* (2016) 62:51–4. doi: 10.1007/s10344-015-0973-x

35. Triguero-Ocaña R, Laguna E, Jiménez-Ruiz S, Fernández-López J, García-Bocanegra I, Barasona JÁ, et al. The wildlife-livestock interface on extensive free-ranging pig farms in central Spain during the "montanera" period. *Transbound Emerg Dis.* (2021) 68:2066–78. doi: 10.1111/tbed.13854

36. Graves HB. Behavior and ecology of wild and feral swine (*Sus scrofa*). *J Anim Sci.* (1984) 58:482–92. doi: 10.2527/jas1984.582482x

37. Kukielka E, Barasona JA, Cowie CE, Drewe JA, Gortazar C, Cotarelo I, et al. Spatial and temporal interactions between livestock and wildlife in south central spain assessed by camera traps. *Prev Vet Med.* (2013) 112:213–21. doi: 10.1016/j.prevetmed.2013.08.008

38. Cadenas-Fernández E, Sánchez-Vizcaíno JM, Pintore A, Denurra D, Cherchi M, Jurado C, et al. Free-ranging pig and wild boar interactions in an endemic area of african swine fever. *Front Vet Sci.* (2021) 2019:6. doi: 10.3389/fvets.2019.00376

39. Kukielka EA, Jori F, Martínez-López B, Chenais E, Masembe C, Chavernac D, et al. Wild and domestic pig interactions at the wildlife-livestock interface of murchison falls national park, uganda, and the potential association with african swine fever outbreaks. *Front Vet Sci.* (2016) 3:31. doi: 10.3389/fvets.2016.00031

40. Triguero-Ocaña RI, Barasona JA, Carro F, Soriguer RC, Vicente J, Acevedo P, et al. Spatio-temporal trends in the frequency of interspecific interactions between domestic and wild ungulates from Mediterranean Spain. *J Pone.* (2019) 14: e211216. doi: 10.1371/journal.pone.0211216

41. Sereda AD, Balyshev VM, Kazakova AS, Imatdinov AR, Kolbasov DV. Protective properties of attenuated strains of african swine fever virus belonging to seroimmunotypes I-Viii. *Pathogens.* (2020) 9:4. doi: 10.3390/pathogens9040274

42. Nurmoja I, Petrov A, Breidenstein C, Zani L, Forth JH, Beer M, et al. Biological characterization of african swine fever virus genotype II strains from north-eastern estonia in european wild boar. *Transbound Emerg Dis.* (2017) 64:2034–41. doi: 10.1111/tbed.12614

43. Petrov A, Forth JH, Zani L, Beer M, Blome S. No evidence for long-term carrier status of pigs after african swine fever virus infection. *Transbound Emerg Dis.* (2018) 65:1318–28. doi: 10.1111/tbed.12881

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Investigation of risk factors for introduction of highly pathogenic avian influenza H5N1 infection among commercial turkey operations in the United States, 2022: a case-control study

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Introduction: The 2022–2023 highly pathogenic avian influenza (HPAI) H5N1 outbreak in the United States (U.S.) is the largest and most costly animal health event in U.S. history. Approximately 70% of commercial farms affected during this outbreak have been turkey farms.

Methods: We conducted a case-control study to identify potential risk factors for introduction of HPAI virus onto commercial meat turkey operations. Data were collected from 66 case farms and 59 control farms in 12 states. Univariate and multivariable analyses were conducted to compare management and biosecurity factors on case and control farms.

Results: Factors associated with increased risk of infection included being in an existing control zone, having both brooders and growers, having toms, seeing wild waterfowl or shorebirds in the closest field, and using rendering for dead bird disposal. Protective factors included having a restroom facility, including portable, available to crews that visit the farm and workers having access and using a shower at least some of the time when entering a specified barn.

Discussion: Study results provide a better understanding of risk factors for HPAI infection and can be used to inform prevention and control measures for HPAI on U.S. turkey farms.

KEYWORDS

avian influenza, biosecurity, case control, H5N1, highly pathogenic avian influenza, risk factors, turkey

1 Introduction

Avian influenza viruses (AIV) are distributed worldwide (1, 2). Wild waterfowl are primary natural reservoirs and have an important role in the maintenance and dispersal of AIVs, including H5 and H7 subtypes, that have the potential to result in outbreaks in domestic poultry (3–5). Spillover of AIVs from wild birds to poultry may occur through direct (i.e., direct exposure to birds infected with AIV) or indirect (e.g., exposure to contaminated soil, water,

fomites, aerosols, or droplets) routes of transmission (6, 7). Outbreaks of AIVs in domestic poultry can result in high morbidity and mortality among poultry and serious economic impacts due to the loss of birds from death or depopulation, outbreak response costs, and trade restrictions (8).

On 20 December 2021, HPAI H5N1 was detected in a mixed species flock on an exhibition farm in Newfoundland, Canada, following a period of rapid, increased mortality in the flock, and retrospective testing identified virus in a wild black-backed gull from a nearby pond that had died in November 2021 (9). Phylogenetic analysis indicated that these A/Goose/Guangdong/1/1996 lineage (GsGD) viruses belonged to HPAI clade 2.3.4.4b and were likely spread to Newfoundland from Europe by migratory birds (9). In late December 2020 and January 2021, GsGD lineage clade 2.3.4.4b H5N1 HPAI was detected in several wild bird species sampled in the Atlantic Flyway in North Carolina and South Carolina as part of the routine AIV surveillance program (10). The first U.S. commercial poultry flock was detected in Indiana in February of 2022, and detections of Eurasian H5 2.3.4.4b GsGD viruses have subsequently occurred in commercial and backyard poultry flocks, wild birds, and wild mammals across the United States (11, 12).

In 2022 alone, over 57 million commercial and backyard poultry on over 700 farms across 47 U.S. states were affected, resulting in over $659 million in federal expenditures for control efforts and indemnity payments. Commercial turkey farms comprised the highest percentage of affected commercial poultry farms, with approximately 70% of all affected commercial farms being turkey farms. Results of full genome sequencing indicated that independent wild bird introductions were the primary mechanism of introduction of virus into operations in this outbreak (Youk et al., in preparation). In comparison, the severity of the 2014–2015 U.S. HPAI H5N2 and H5N8 outbreak was heavily influenced by lateral transmission of virus between farms (13, 14). Several studies conducted during the 2014–2015 outbreak explored potential risk factors for transmission of virus within and between farms (15–17). The differences in spread mechanism, as well as the larger geographic scope of the 2022 outbreak, as compared to the 2014–2015 outbreak, necessitated further examination into risk factors for introduction and biosecurity practices.

The goal of this study was to investigate potential risk factors for introduction of HPAI virus onto commercial turkey farms. To address this goal, the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS), with support from the USDA National Agricultural Statistics Service (NASS), as well as from State and national poultry organizations, conducted a case-control study among commercial meat turkey operations. The study objectives were to (1) identify risk factors for infection with HPAI, (2) identify biosecurity challenges on turkey farms, and (3) refine biosecurity recommendations to support prevention of infection on farms. This information will improve understanding of the risk factors associated with introduction of HPAI on turkey farms and will be valuable for informing enhancements of on-farm preventive measures.

Abbreviations: AIV, Avian influenza virus; CI, Confidence interval; HPAI, Highly pathogenic avian influenza; OR, Odds ratio; USDA, United States Department of Agriculture.

2 Materials and methods

2.1 Study design

A case-control study was designed to examine risk factors associated with HPAI infection on U.S. commercial turkey farms. Commercial turkey farms that raised meat turkeys between 1 January and 17 October 2022, and that raised more than 30,000 meat turkeys annually, were eligible to participate in the study. Commercial turkey breeder farms and backyard farms with turkeys were excluded from the study.

Case farms were defined as commercial meat turkey farms that met the USDA's HPAI case definition during the study time frame (18). Farms were tested for HPAI during the outbreak in accordance with USDA HPAI response plans. For farms being tested, samples were screened for influenza A and H5/H7 subtypes by real-time reverse transcription polymerase chain reaction (RT-PCR) by members of the National Animal Health Laboratory Network (NAHLN). Samples testing non-negative by influenza A virus (IAV) PCR were forwarded to the National Veterinary Services Laboratories (NVSL, Ames, Iowa, United States) for confirmation. Testing at NVSL included an H5 clade 2.3.4.4 pathotyping assay and an assay targeting N1 for neuraminidase subtyping and whole genome sequencing was conducted directly from the samples. Influenza A viruses were sequenced directly from samples as previously described (10), RAxML was used to generate phylogenetic trees, and tables of single nucleotide polymorphisms (SNPs) were created using the vSNP pipeline.[1] For purposes of the case-control study, the reference date was the date of onset of clinical signs, or if not available/applicable, the date of a presumptive diagnosis based on the USDA's case definition (18) on the farm.

Control farms were defined as commercial meat turkey farms that did not meet the USDA's case definition for HPAI during the study period. For each case farm, 2 to 5 control farms located in the same state were randomly selected. Enumerators were instructed to move to the next case after they had gotten 1 to 2 completed controls for a single case. Contact information for case and control farms was obtained from the USDA Veterinary Services Emergency Management Response System (EMRS), from Thomson Reuters® CLEAR software, from State databases where available, and from poultry company representatives. At the start of the study, the potential sampling pool consisted of 161 HPAI-affected commercial meat turkey farms in 13 states (CA, IA, IN, KY, MI, MN, MO, NC, ND, PA, SD, UT, WI). A total of 153 case farms from all 13 states were contacted for participation. Eight case farms were excluded due to a lack of availability of contact information within the study timeline.

2.2 Data collection and sources

A 24-page questionnaire (Supplementary material) was administered to farm managers or supervisors on each participating farm by telephone interview by trained NASS enumerators or USDA–APHIS epidemiologists, or by mail. The questions focused on farm

1 https://github.com/USDA-VS/vSNP

characteristics, wild birds and wildlife, biosecurity, personnel, visitors, vehicles and equipment, and management practices for the 14 days prior to the reference date on a case farm and a comparable 14-day reference period on control farms. The length of the reference period was chosen based on the flock-level 14-day incubation period recognized by the World Organization for Animal Health for HPAI (19). Control farms were provided with a tentative 14-day reference period that was the same as the 14-day reference period for a case farm located in the same state. If a control farm did not have turkeys on the farm for the tentative 14-day period, they were asked to identify the closest 14-day period to the tentative reference period in 2022 during which they had turkeys on the farm. The 14 days identified were then used as the 14-day reference period when answering questions. Some questions asked about practices for the entire farm, and some asked about practices for a "selected barn." The selected barn on case farms was the first barn on the farm to be confirmed HPAI positive, and for control farms, respondents were asked to identify a single barn at random to be designated as the selected barn.

Data collection took place between 7 November 2022 and 27 February 2023. Following data entry, survey responses were validated to identify logical inconsistencies in the data. Validation identified numeric extremes, improper categorical responses, and erroneous skip patterns, and relational checks were performed. Errors were evaluated by two analysts. Where deductions from other survey responses could not be made, appropriate solutions were implemented as agreed upon by the two analysts, and, for some errors, enumerators followed up with producers or cross-checked reported results using EMRS.

The zone status for each case and control farm during their 14-day reference period was determined using information from EMRS. Following detection of infection of HPAI on a farm, a 10 km radius control zone is established around the infected farm for purposes of outbreak response. Zones remain in place for a duration consistent with the HPAI outbreak response plan, typically 4 to 5 weeks for this outbreak. Zone status (i.e., whether a farm was located inside or outside of an existing control zone) was included as a covariate in the multivariable analysis.

2.3 Statistical analyses

Data were analyzed to identify statistical associations between infected status (case vs. control) and farm or selected barn characteristics, such as management practices. The percentages of case and control farms having each characteristic were calculated. Univariate analyses were performed to identify variables potentially associated with the presence of HPAI at the farm/selected barn level.

For the univariate analyses, Fisher's exact test was used for categorical variables and the Score test for continuous variables to assess the association of each variable with HPAI infection. Variables with p-values ≤ 0.20 and where the relationship was biologically plausible for risk of HPAI infection were considered for entry into candidate multivariable models.

The subsets of farms that had either lateral transmission/common source or wild bird introduction were evaluated via univariate analyses, while a multivariable model was only created for wild bird introduction due to the low number of cases associated with lateral transmission/common source exposure between farms.

To address item non-response, random, single imputation was performed on the variables that entered the multiple logistic regression model as candidate variables. Multivariable results are reported using the imputed data, whereas univariate results are reported using the non-imputed data. Hierarchical cluster analysis of predictor variables using PROC VARCLUS was used to help guide final model selection, and variance inflation factors (VIFs) were computed to help identify issues with multicollinearity, with VIFs exceeding 3 indicating further investigation was needed (20).

Multivariable logistic regression models were fit using PROC LOGISTIC in SAS version 9.4. Forward-, backward-, and step-wise selection procedures were carried out via PROC HPGENSELECT to select a final model from which to make inference, using the AICc criterion, which is a variant of Akaike's information criterion with an adjustment for small sample sizes (21, 22). The final model results using imputation matched the results using the non-imputed data. Primary model outputs included estimated odds ratios (ORs) and their 95% confidence intervals, along with Type III F-test p-values to assess statistical significance of effects (23). Factors were considered statistically significant in final multivariable models if $p < 0.05$.

All statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, United States).

3 Results

Questionnaires were completed for 67 case farms and 61 control farms across 12 states (CA, IA, IN, MI, MN, MO, NC, ND, PA, SD, UT, WI). One case and one control questionnaire were excluded because the farms had only breeder turkeys on-site during the 14-day reference period. One case farm completed both a case and a control questionnaire; the control questionnaire was excluded from this analysis. After excluding the farms without meat turkeys and adjusting for case-control status, there were completed questionnaires for 66 case farms and 59 control farms across 12 states. The sample included 30 company farms, 50 contract farms including lessees, and 44 independent farms; 1 farm had a missing response for this question.

Case farms had a median of 37,356 birds (range: 8,000–300,000), and control farms had a median of 39,350 birds (range: 6,000–200,000). The phylogenetic analyses provided evidence for independent introductions of virus from wild birds on 77% of case farms ($n = 51$) and suggested lateral spread or common source exposure on 23% of case farms ($n = 15$).

3.1 Univariable analysis

Selected results from the univariate analyses are shown in Tables 1–5. A complete list of univariable results is available in Supplementary material.

3.1.1 Premises characteristics

During the 14-day reference period, more case farms were located within an existing control zone compared to control farms (32% vs. 12%, $p = 0.01$; Table 1). Having both brooder and grower production on the farm was associated with case status (52% vs. 27%; $p < 0.01$). Also, having toms as the market-type bird on the farm was associated with case status (86% vs. 67%; $p = 0.02$).

TABLE 1 Univariate analyses of premises characteristics ($p \leq 0.20$) considered for entry into the multivariable model.

Characteristic	Level	Number of case farms (%)	Number of control farms (%)	Univariate *p*-value
In an existing control zone[A]		21 (31.8)	7 (11.9)	0.01
Stage of production	Only brooder or only grower	32 (48.5)	43 (72.9)	<0.01
	Brooder and grower	34 (51.5)	16 (27.1)	
Hens on the farm		14 (21.2)	28 (48.3)	<0.01
Toms on the farm		57 (86.4)	39 (67.2)	0.02
Other poultry on farm		13 (19.7)	3 (5.2)	0.02
Farm type	Independent	29 (43.9)	15 (25.9)	0.04
	Other	37 (56.1)	43 (74.1)	
Wetland or swamp within 320 m (350 yards) of farm		17 (25.8)	8 (13.8)	0.12

Percentage of case farms and percentage of control farms by premises characteristics. [A]During the 14-day reference period.
Percentage of case farms and percentage of control farms by premises characteristics.

TABLE 2 Univariate analyses of wild animal characteristics ($P \leq 0.20$) considered for entry into the multivariable model.

Characteristic[A]	Number of case farms (%)	Number of control farms(%)	Univariate *p*-value
Waterfowl/shorebirds seen on bodies of water within 320 m (350 yards) of farm[B]	23 (34.8)	9 (15.3)	0.01
Waterfowl/shorebirds seen on closest body of water[B]	30 (45.5)	14 (23.7)	0.01
Waterfowl/shorebirds seen in closest field[B]	20 (30.3)	7 (11.9)	0.02
Waterfowl seen within 91.4 m (100 yards) of the barns[C]	33 (51.6)	16 (27.6)	<0.01
Wild mammals near barns[D]	13 (21.3)	19 (35.2)	0.14

Percentage of case farms and percentage of control farms by wild animal characteristics. [A]During the 14-day reference period.
[B]Any birds present (tens, hundreds, thousands) vs. none/do not know.
[C]Birds often or sometimes seen within 91.4 m (100 yards) of the barn vs. never.
[D]Yes vs. no.

3.1.2 Wild animal characteristics

In the univariate analysis, several variables were related to wild birds and nearby water bodies (Table 2). Cluster analysis showed several of these variables clustered together and were candidates for the multivariable analysis.

Wild waterfowl or shorebirds were seen in the closest field during the 14-day reference period on 30% of case farms, compared to 12% of control farms ($p = 0.02$). During the 14-day reference period, case farms also reported more commonly seeing wild waterfowl or shorebirds on water bodies found within 320 meters (350 yards) of the farm (35% vs. 15%; $p = 0.01$), seeing wild waterfowl or shorebirds on the closest body of water (45% vs. 24%; $p = 0.01$), and seeing waterfowl within 91.4 meters (100 yards) of the outside of the barns (52% vs. 28%; $p < 0.01$). Case farms also more commonly reported a wetland or swamp visible or within 320 meters (350 yards) of the farm (Table 1; 26% vs. 14%; $p = 0.12$). Control farms were more likely to see wild mammals (such as raccoons, opossums, skunks, coyotes, or foxes) or evidence of them in or around the barns (35% vs. 21%, $p = 0.14$).

3.1.3 Biosecurity characteristics

Having a restroom facility (including portable) always or sometimes available to crews that visit the farm was more common on control farms compared to case farms (70% vs. 46%; $p = 0.02$; 13% of respondents did not answer this question; Table 3). Control farms were more likely to always use a visitor log (78% vs. 60%; $p = 0.05$).

Neither case nor control farms had many visitors overall, and there were no notable significant differences in the types of visitors coming to the farm. If visitors came onto the farm, they also had limited access to the turkey barns. There were not many differences reported in worker biosecurity practices. Control farms were more likely to have workers who used showers when entering the selected barn (26% vs. 11%; $p = 0.04$) and washed hands or used hand sanitizer before entering the selected barn (91% vs. 83%; $p = 0.19$).

During the 14-day reference period, the use of and sharing of vehicles, including company trucks or trailers, feed trucks, and bird delivery vehicles, or equipment, such as gates/panels and skid-steer loaders, was also not associated with case status. For case and control farms, vehicles and equipment were not shared frequently.

3.1.4 Dead bird disposal

Use of rendering for dead bird disposal was more common on case farms than on control farms (28% vs. 14%; $p = 0.08$; Table 4). On the other hand, incineration (17% vs. 6%; $p = 0.09$) and use of a landfill (7% vs. too few to report; $p = 0.19$) as dead bird disposal methods were

TABLE 3 Univariate analyses of biosecurity practices ($p \leq 0.20$) considered for entry into the multivariable model.

Biosecurity Practice	Level	Case farms (*N*/%)	Control farms (*N*/%)	Univariate *p*-value
Workers showered[A]		7 (10.8)	15 (25.9)	0.04
Workers washed hands or used hand sanitizer[A]		54 (83.1)	53 (91.4)	0.19
Visitor log used	Always	39 (60.0)	45 (77.6)	0.05
	Sometimes/never	26 (40.0)	13 (22.4)	
Restroom facility available to crews visiting farm	Always/sometimes	30 (45.5)	41 (69.5)	0.02
	Never	26 (39.4)	12 (20.3)	
	Unknown (skipped question)	10 (15.2)	6 (10.2)	

Percentage of case farms and percentage of control farms by biosecurity practices.
[A]Workers always, most of the time, or sometimes used the practice before entering the barn vs. never or not available.
This question was asked specifically for the selected barn and for the 14-day reference period.

more common on control farms than case farms. Case farms were more likely to have visits from vehicles used for rendering than control farms (32% vs. 19%; $p = 0.14$).

3.1.5 Variables of interest found to be non-significant

Several factors found to be risk factors in previous outbreaks were explored but not found to be significant in this study (Table 5). There was not a significant difference between case and control farms in the use of a wash station or spray area for vehicles, and no significant differences in wash station practices were reported. Barn ventilation type and percentage of time the curtains were open were also not related to case status. Approximately 97% of case and control farms had gravel or dirt roads compared to hard top/asphalt roads as the road surface on the farms that vehicles coming onto the operation drive on. Use of landscape fabric (weed barrier) on curtains or air inlets, a relatively new practice, was also not found to be associated with case status.

3.2 Multivariable analysis

The 20 variables that passed the univariate screening, having Fisher's exact test $p \leq 0.20$, and were biologically plausible included the following. All variables a-s were categorical; the last variable t, was discrete numeric.

a. Farm being in a control zone.
b. Having both brooder and grower production on the farm.
c. Having market toms on the farm.
d. Having any other poultry on the farm.
e. Farm was independent (vs. being a company, contract, or other type of farm).
f. Water treatments (such as chlorination) given in poultry drinking water continuously (vs. intermittently or not at all).
g. Wetland or swamp was within 320 meters (350 yards) of the farm.
h. Any wild waterfowl or shorebirds seen on water bodies within 320 meters (350 yards) of the farm.
i. Any wild waterfowl or shorebirds seen on the closest body of water to the farm.
j. Any wild waterfowl or shorebirds seen on the closest crop field to the farm.
k. Any wild waterfowl seen on the farm within approximately 91.4 meters (100 yards) of the outside of the barns.
l. Any wild mammals (e.g., raccoons, opossums, skunks, coyotes, or foxes) or evidence of their presence seen in or around poultry barns.
m. Workers shower before entering the selected barn.
n. Workers wash their hands or use hand sanitizer before entering the selected barn.
o. Use of a visitor log to record visitor traffic onto the farm.
p. Availability of a restroom facility (including portable) for crews that visit the farm.
q. Use of rendering as a dead bird (daily mortality) disposal method.
r. Use of incineration as a dead bird (daily mortality) disposal method.
s. Use of landfill as a dead bird (daily mortality) disposal method.
t. The general weekly number of vehicles (including employee vehicles) that entered the farm (coming near the barns or not).

To avoid collinearity, a single variable (j) from the cluster of g–k was selected and offered into the multivariable models to represent wild bird exposure, and rendering (q) was selected from the list of daily mortality disposal methods because it was a risk factor [incineration (r) and landfill (s) were protective, see Supplementary material].

In the final model, imputation was only used for 2 variables: worker biosecurity includes shower before entering the barn and render dead birds. These variables were missing 2 and 3 responses out of 125 (2%), respectively, and were divided between cases and controls.

Seven variables remained in the final multivariable model (Table 6). Farms within an existing control zone had increased odds of being a case [odds ratio (OR) = 3.68, 95% confidence interval (CI) = 1.06–12.74]. Other factors associated with increased odds of H5N1 HPAI infection included having both brooder and grower turkey production on the farm (OR = 7.35, CI = 2.51–21.54) and having toms as the sex market type on the farm (OR = 6.86, CI = 1.83–25.79). Seeing wild waterfowl or shorebirds in the closest field was also associated with increased odds of infection (OR = 6.02, CI = 1.83–19.78). The use of rendering for dead bird disposal during the 14-day reference period was associated with increased odds of infection

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TABLE 4 Univariate analyses of management practices and vehicle characteristics ($p \leq 0.20$) considered for entry into the multivariable model.

Characteristic	Level	Number of case farms (%) or median*	Number of control farms (%) or median*	Univariate *p*-value
Bird drinking water treated (e.g., chlorination)	Continuously treated	60 (90.9)	46 (80.7)	0.10
	Intermittently/not treated	6 (9.1)	11 (19.3)	
Number vehicles entering the farm per week		5.0*	4.0*	0.10
Render dead birds[A]		18 (28.1)	8 (13.8)	0.08
Incinerate dead birds[A]		4 (6.3)	10 (17.2)	0.09
Landfill for dead bird disposal[A]		**	4 (6.9)	0.19
Vehicles for rendering	Come to the farm[B]	20 (32.3)	11 (19.0)	0.14

Percentage of case farms and percentage of control farms by management practices and vehicle characteristics.
[A]During the 14-day reference period.
[B]Includes to the perimeter of the farm, enter the farm but not near the barns, and come near the barns.
*Median.
**Too few to report.

TABLE 5 Univariate analyses of selected factors of interest not found to be associated with HPAI H5N1 infection ($p > 0.20$).

Characteristic	Level	Number of case farms (%)	Number of control farms (%)	Univariate *p*-value
Non-asphalt roads		62 (96.9)	56 (96.6)	1.00
Use of vehicle wash/spray station[A]		40 (60.6)	40 (69.0)	0.35
Closest field actively worked (e.g., tilled)[A]		6 (9.8)	6 (13.3)	0.76
Wild birds observed around dead bird collection area[A]		20 (32.8)	18 (31.6)	1.00
Ventilation type[B]	Curtain ventilated	29 (46.0)	22 (40.7)	0.90
	Environmental control/tunnel ventilation	16 (25.4)	14 (25.9)	
	Side doors	3 (4.8)	4 (7.4)	
	Other	15 (23.8)	14 (25.9)	
Percentage of time curtains open (for curtain ventilated)[A,B]	Less than 20%	7 (25.0)	3 (13.6)	0.48
	20% or more	21 (75.0)	19 (86.4)	
Use of landscape fabric on air inlets or along curtains[A,B]	Not used	44 (71.0)	36 (66.7)	0.72
	Used without disinfectant spray	8 (12.9)	10 (18.5)	
	Used with disinfectant	10 (16.1)	8 (14.8)	

[A]During the 14-day reference period.
[B]This question was asked specifically for the selected barn.

TABLE 6 Results of multivariable logistic regression analysis of factors associated with HPAI H5N1 infection on U.S. commercial meat turkey farms.

Characteristic	% Case farms	% Control farms	Odds ratio (95% CI)	*p*-value
In an existing control zone	31.8	11.9	3.68 (1.06–12.74)	0.04
Both brooder and grower stages on farm	51.5	27.1	7.35 (2.51–21.54)	<0.01
Sex: toms	86.4	67.8	6.86 (1.83–25.79)	<0.01
Waterfowl/shorebirds seen in closest field	30.3	11.9	6.02 (1.83–19.78)	<0.01
Worker biosecurity includes shower before entering barn[A]	10.6	27.1	0.29 (0.09–0.98)	0.05
Restroom facility available to crews visiting farm	45.5	69.5	0.32 (0.10–1.05)[B]	0.05
Render dead birds	30.3	13.6	8.26 (2.25–30.34)	<0.01

[A]Workers always, most of the time, or sometimes showered vs. never or shower not available. This question was asked specifically for the selected barn.
[B]Odds ratio is for comparison between always/sometimes available vs. never available.

(OR = 8.26, CI = 2.25–30.34). Factors found to have a protective effect included workers entering the selected barn using a shower during the 14-day reference period at least some of the time (OR = 0.29, CI = 0.09–0.98) and having a restroom facility available to crews who visit the farm (OR = 0.32, CI = 0.10–1.05).

Biologically plausible, first-order interactions were assessed but were not significant. A region variable (east, central, west) was offered for inclusion in the final model as a fixed effect to test for confounding by region, but no confounding was seen, so it was excluded from the final model.

A multivariate model based on data from the subset of farms linked to wild bird introductions was similar to the risk factors identified from the farm-level model described above, other than the control zone becoming non-significant (data not shown).

4 Discussion

The United States has experienced an unprecedented outbreak of HPAI H5N1 beginning in late 2021, with the first detections of Eurasian H5 2.3.4.4b GsGD in wild birds and followed by the first confirmed infected commercial poultry premises in early 2022. Subsequently, this outbreak has resulted in the loss of millions of commercial and backyard poultry and detections in many species of wild birds and wild mammals across the country, in addition to having severe financial consequences. With the ongoing global circulation of AIVs that have repeatedly caused large outbreaks, there remains a need to identify actions that may be helpful to prevent infection on farms (4, 24, 25). The case-control study presented here investigated the risk factors associated with infection with HPAI virus between February and October 2022 on U.S. meat turkey farms.

Our results indicated that being inside a control zone increased the odds of a farm being infected with HPAI. Proximity of a farm to the nearest infected farm was a risk factor for HPAI infection in outbreaks in Europe and Japan (26–29); and the most significant risk factor for infection on table egg layer farms during the 2014–2015 HPAI H5N2 Midwestern U.S. outbreak was the farm being located within an existing control zone (16). When analyzing the subset of data from farms likely infected by independent wild bird introductions, however, control zone did not remain in the final model. This finding may highlight the importance and effectiveness of control measures implemented inside of control zones, including rapid depopulation following detection. These measures may have minimized transmission by lateral spread, a spread mechanism implicated in a much smaller percentage of cases during 2022. Overall, our findings corroborate the importance of biosecurity and surveillance for farms located in close proximity to an infected farm to prevent infection and ensure rapid detection.

Other factors associated with HPAI infection were related to the stages of production on the farm and sex of birds. Case farms were more likely to raise toms and were more likely to have both brooder and grower stages on farm. Age of birds has been shown to impact susceptibility to virus (30, 31). Toms are typically grown several weeks longer than hens prior to movement to slaughter, and production practices, such as changes in ventilation, may differ during those extra weeks that toms are on farm. The increased age and additional time on farm, which includes further exposures to fomites such as personnel and vehicles, may account for the increased risk of case status for farms with toms. Similarly, farms with birds of differing ages, such as brooders and growers, may be uniquely susceptible. Although production stage and sex of birds raised on a particular farm may not be easily changed due to the structure of the poultry industry, this information regarding risk could be used to inform surveillance activities and guide the implementation of increased biosecurity on farms raising toms and multiple stages of production.

Rendering as a method of dead bird disposal for normal daily mortality during the 14-day reference period was also a risk factor for infection. Various methods of dead bird disposal are used by poultry farms, including on-farm approaches, such as burial and composting, and off-farm approaches, such as rendering and landfill. Rendering requires the regular removal of dead bird carcasses from the farm and movement to a renderer, where carcasses are converted to useable by-products. Rendering has been reported as an important risk factor in previous AIV outbreaks (16, 17, 32–34). Movement of virus in carcasses and feathers from a farm to the renderer prior to detection and vehicle movements are possible modes of transmission. We found that rendering vehicles coming onto the farm vs. not coming to the farm at all was significant ($p = 0.14$) in the univariate analysis. We also asked several follow-up questions to respondents using rendering on farm to better understand the risk of this practice, including covering the carcass bin, means of transport, and how frequently carcasses are moved to the renderer; however, none of these variables were significant in the analysis. Interestingly, when data were analyzed by introduction route, rendering remained a risk factor for infection even when analyzing only farms infected as a result of independent wild bird introductions. Given this finding and the continued finding of rendering as a risk factor in multiple outbreaks, dead bird disposal practices should be investigated further. Future studies should consider adding more detailed questions to identify specific risk factors and protective factors for all methods of dead bird disposal, not just rendering. For example, if disposal is on-farm vs. off-farm, if methods are shared with other farms, how carcasses are moved from the barn to either a holding area or to disposal, whether carcass handling attracts wildlife or wild birds, frequency of movement to disposal, and any equipment or vehicles used in association with rendering and their disinfection.

Two biosecurity measures remained in the final model and were found to be protective: workers having access to and using a shower at least some of the time when entering the selected barn and having a restroom facility (including portable) available to crews that visit the farm. No follow-up questions were asked about this management practice, but the availability of a restroom may improve hand hygiene and reduce human movements on the farm, particularly movements in and around barns and surrounding areas. Although not retained in the final model, workers on control farms were also more likely to wash hands or use hand sanitizer before entering the barn. Contaminated fomites, such as hands and clothing, can contribute to viral spread (6, 29, 35). Worker shower use, hand washing, and the availability of a restroom are biosecurity practices that can help reduce the indirect transmission of virus into poultry barns. The importance of on-farm biosecurity has been previously highlighted, especially for personnel moving between poultry

farms and for those not only coming onto poultry farms but also entering poultry barns (16, 17, 29, 34, 35). Providing restroom facilities could improve hygiene measures and restrict human movements and barn entry to only those barns or areas where work is being performed. Implementing on-farm biosecurity measures for workers and visitors is an essential component of disease prevention, and these findings may be considered in the development of farm biosecurity plans.

Phylogenetic analyses indicated that independent wild bird introductions were the predominant route of introduction of virus onto turkey farms in the U.S. in 2022. This is in contrast to the 2014–2015 outbreak, which was predominated by lateral (farm-to-farm) transmission (5, 13). Most introductions are likely due to indirect contact with wild birds or undefined mechanisms, although direct contact cannot be ruled out, particularly in instances where birds have access to the outdoors and there is a possibility of mingling with wild birds (6, 36–39). Case farms were more likely to report observations of wild birds in proximity to farms and nearby fields and waterbodies, and to report wild bird habitat such as wetlands or swamps within 320 meters (350 yards) of the farm. Farm proximity to water and wild bird habitat, as well as presence of high densities of migratory wild waterfowl, have been identified as risk factors in previous outbreaks (37, 38, 40, 41). Concentrations of domestic poultry in combination with high densities of wild birds provides a potential interface for viral transmission and spill-over events; and necessitates the identification and implementation of protective biosecurity measures to limit introduction. When data from this case-control study were analyzed by sub-setting those farms likely infected by wild bird introductions, few changes were observed in the risk or protective factors for being a case farm. The similarities between the full and sub-set models are likely explained by the predominance of wild bird introductions in the full dataset. Although some factors, for example, farm location near bodies of water, cannot be changed, measures can be undertaken to mitigate the possibility of associated direct or indirect exposures. We asked some questions about measures taken to minimize wildlife and wild bird activity on-farm and entry into barns, but given the important role of wild birds in the dynamics of the U.S. HPAI H5N1 outbreak, additional work to explore on-farm protective measures is needed. Practices such as reducing water pooling, minimizing wildlife attractants and food sources, using trained dogs, implementing laser technology, and using decoys have been used to prevent direct and indirect contacts at the interface of wild birds and poultry. Additional studies to elucidate the utilization and effectiveness of these practices would be useful (42–44).

It is important to acknowledge the limitations of this study. Recall bias is a consideration for data collection via surveys. Respondents in the current study were asked to provide responses for activities and observations that had taken place an average of 7 months prior, with the difference between the date of the interview and the beginning of the reference period as little as 2 months and as long as 12 months prior. It is also possible that recall and observations may have been different for case farms vs. control farms, as case farms may have been more likely to reflect on the time period prior to detection of infection. Another limitation of survey methodology is bias associated with questions that may be considered sensitive, providing responses that may be considered more favorable or that follow biosecurity plans vs. being reflective of actual practices. While attempts were made to balance the numbers of completed case and control questionnaires geographically, it was not possible to perfectly match cases and controls by state due to non-response and, in some situations, a lack of sufficient control farms. Finally, the results of this study are representative of U.S. production practices and of the viral dynamics of the 2022 HPAI H5N1 outbreak and, therefore, may not be directly applicable to production systems in other countries or future outbreaks with different viruses.

Future work may help further improve our understanding of the complex epidemiology of avian influenza transmission at the interface of wild birds and domestic poultry. Two additional topics were included in the case-control questionnaire but were not reported here. One section of the questionnaire was related to biosecurity investments, including questions regarding ongoing biosecurity expenses and permanent and temporary improvements made since 2015 that impact farm biosecurity. These data will be analyzed and reported separately to identify priority areas for investment in biosecurity measures to reduce risk for HPAI. The questionnaire also included challenge-level questions asking for producers' opinions on level of challenge of certain topics, including biosecurity-, personnel-, and equipment-related challenges. Finally, weather conditions and patterns related to AIV transmission have been examined previously and could have played a role in the outbreak in 2022 (45–47). Future work could expand upon the case-control study presented here to incorporate historical weather data such as temperature, relative humidity, precipitation, and wind speed in the time preceding detection to investigate the role of weather on risk of HPAI infection.

5 Conclusion

This study compared management and biosecurity factors on case and control meat turkey farms in the U.S. during the HPAI H5N1 outbreak in 2022. Knowledge of risk factors for infection has become increasingly important as this outbreak continues into 2023 and as additional domestic poultry flocks, wild birds, and wildlife species are detected. Study results identified the following key risk factors: location of farms within an existing control zone, multiple stages of production on farm, toms as the sex market type on farm, waterfowl/shorebirds seen in the closest field, gaps in worker biosecurity measures such as lack of availability of a shower before entering the barn or a restroom facility for visiting crews, and the use of rendering for dead bird disposal. These risk factors were found to be associated with HPAI infection on farms and provide information that can be directly applied to support science-based updates to prevention and control recommendations to safeguard turkey farms in the United States.

Data availability statement

The datasets presented in this article are not readily available because the data are protected from release under the Confidential

Information Protection and Statistical Efficiency Act (CIPSEA, P.L. 115-435, Title III). Anonymized data may be made available upon request, for statistical purposes only, and completion of non-disclosure training, forms, and review of any output products. Requests to access the datasets should be directed to KP.

Author contributions

VF, KP, AB, AG, MB, AF, AD, and KM conceptualized the research and study design. KP, VF, AB, and AG designed the questionnaire. RM, MB, AB, VF, and KP supported data collection. MT and KL led diagnostic sampling and interpretation of results. VF, KP, AB, RM, MB, and AG participated in the data analysis and visualization. KP and VF wrote the primary draft of the manuscript. KP, VF, AB, RM, AG, MB, AD, KM, and MT contributed to reviewing and editing the final 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.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1229071/full#supplementary-material

References

1. Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. Evolution and ecology of influenza a viruses. *Microbiol Rev*. (1992) 56:152–79. doi: 10.1128/mr.56.1.152-179.1992

2. Swayne DE. The global nature of avian influenza In: DE Swayne, editor. *Animal Influenza*. Hoboken, NJ: Wiley-Blackwell (2016). 117–201.

3. Easterday BC, Trainer DO, Tůmová B, Pereira HG. Evidence of infection with influenza viruses in migratory waterfowl. *Nature*. (1968) 219:523–4. doi: 10.1038/219523a0

4. Olsen B, Munster VJ, Wallensten A, Waldenström J, Osterhaus ADME, Fouchier RAM. Global patterns of influenza a virus in wild birds. *Science*. (2006) 312:384–8. doi: 10.1126/science.1122438

5. Li L, Bowman AS, DeLiberto TJ, Killian ML, Krauss S, Nolting JM, et al. Genetic evidence supports sporadic and independent introductions of subtype H5 low-pathogenic avian influenza a viruses from wild birds to domestic poultry in North America. *J Virol*. (2018) 92:e000913–8. doi: 10.1128/JVI.00913-18

6. Sims LD, Weaver J, Swayne DE. Epidemiology of avian influenza in agricultural and other man-made systems In: DE Swayne, editor. *Animal Influenza*. Hoboken, NJ: Wiley-Blackwell (2016). 302–36.

7. Swayne DE, Suarez DL, Sims LD. Influenza. in Diseases of Poultry. Eds. DE Swayne, M Boulianne, CM Logue, MD LR, V Nair and DL Suarez et al. (Hoboken, NJ: John Wiley & Sons, Inc.) (2020)

8. Seeger RM, Hagerman AD, Johnson KK, Pendell DL, Marsh TL. When poultry take a sick leave: response costs for the 2014-2015 highly pathogenic avian influenza epidemic in the USA. *Food Policy*. (2021) 102:102068. doi: 10.1016/j.foodpol.2021.102068

9. Caliendo V, Lewis NS, Pohlmann A, Baillie SR, Banyard AC, Beer M, et al. Transatlantic spread of highly pathogenic avian influenza H5N1 by wild birds from Europe to North America in 2021. *Sci Rep*. (2022) 12:11729. doi: 10.1038/s41598-022-13447-z

10. Bevins SN, Shriner SA, Cumbee JC Jr, Dilione KE, Douglass KE, Ellis JW, et al. Intercontinental movement of highly pathogenic avian influenza a (H5N1) clade 2.3.4.4 virus to the United States, 2021. *Emerg Infect Dis*. (2022) 28:1006–11. doi: 10.3201/eid2805.220318

11. United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA–APHIS). Confirmations of highly pathogenic avian influenza in commercial and backyard flocks. (2022). Available at: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/avian/avian-influenza/hpai-2022/2022-hpai-commercial-backyard-flocks (Accessed 5 April 2023).

12. United States Geological Survey (USGS). Distribution of highly pathogenic avian influenza in North America, 2021/2022. (2022). Available at: https://www.usgs.gov/centers/nwhc/science/distribution-highly-pathogenic-avian-influenza-north-america-20212022 (Accessed 5 April 2023).

13. Grear DA, Hall JS, Dusek RJ, Ip HS. Inferring epidemiologic dynamics from viral evolution: 2014-2015 Eurasian/north American highly pathogenic avian influenza viruses exceed transmission threshold, R_0=1, in wild birds and poultry in North America. *Evol Appl*. (2017) 11:547–57. doi: 10.1111/eva.12576

14. Lee D, Torchetti M, Hicks J, Killian M, Bahl J, Pantin-Jackwood M, et al. Transmission dynamics of highly pathogenic avian influenza virus a(H5Nx) clade 2.3.4.4, North America, 2014–2015. *Emerg Infect Dis*. (2018) 24:1840–8. doi: 10.3201/eid2410.171891

15. Dargatz D, Beam A, Wainwright S, McCluskey B. Case series of turkey farms from the H5N2 highly pathogenic avian influenza outbreak in the United States during 2015. *Avian Dis*. (2016) 60:467–72. doi: 10.1637/11350-121715-Reg

16. Garber L, Bjork K, Patyk K, Rawdon T, Antognoli M, Delgado A, et al. Factors associated with highly pathogenic avian influenza H5N2 infection on table-egg layer farms in the midwestern United States, 2015. *Avian Dis*. (2016) 60:460–6. doi: 10.1637/11351-121715-Reg

17. Wells SJ, Kromm MM, VanBeusekom ET, Sorley EJ, Sundaram ME, VanderWaal K, et al. Epidemiologic investigation of highly pathogenic H5N2 avian influenza among upper Midwest U.S. turkey farms, 2015. *Avian Dis*. (2017) 61:198–204. doi: 10.1637/11543-112816-Reg.1

18. United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA–APHIS). Case definition: Avian influenza (AI). (2022). Available at: https://www.aphis.usda.gov/animal_health/monitoring_surveillance/avian-influenza-case-definition.pdf

19. World Organization for Animal Health (WOAH). Terrestrial animal health code: Chapter 10.4. Infection with high pathogenicity avian influenza viruses. Terrestrial code online access (2023). Available at: https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmfile=chapitre_avian_influenza_viruses.htm

20. Kutner MH, Nachtsheim CJ, Neter J, Li W. *Applied linear statistical models* 5th ed. New York, NY: McGraw-Hill/Irwin (2005).

21. Akaike H. A new look at the statistical model identification. *IEEE Trans Autom Control*. (1974) 19:716–23. doi: 10.1109/TAC.1974.1100705

22. Burnham KP, Anderson DR. *Model selection and multimodel inference: A practical information-theoretic approach*. New York, NY: Springer (2002).

23. Fox J. *Applied regression analysis and generalized linear models. 3rd* ed Thousand Oaks, California: Sage Publications (2016).

24. Lycett SJ, Pohlmann A, Staubach C, Caliendo V, Woolhouse M, Beer M, et al. Genesis and spread of multiple reassortants during the 2016/2017 H5 avian influenza epidemic in Eurasia. *Proc Natl Acad Sci U S A*. (2020) 117:20814–25. doi: 10.1073/pnas.2001813117

25. Lewis NS, Banyard AC, Whittard E, Karibayev T, al Kafagi T, Chvala I, et al. Emergence and spread of novel H5N8, H5N5 and H5N1 clade 2.3.4.4 highly pathogenic avian influenza in 2020. *Microbes Infect*. (2021) 10:148–51. doi: 10.1080/22221751.2021.1872355

26. Stegeman A, Bouma A, Elbers ARW, de Jong MCM, Nodelijk G, de Klerk F, et al. Avian influenza a virus (H7N7) epidemic in the Netherlands in 2003: course of the epidemic and effectiveness of control measures. *J Infect Dis*. (2004) 190:2088–95. doi: 10.1086/425583

27. Mannelli A, Ferrè N, Marangon S. Analysis of the 1999-2000 highly pathogenic avian influenza (H7N1) epidemic in the main poultry-production area in northern Italy. *Prev Vet Med*. (2006) 73:273–85. doi: 10.1016/j.prevetmed.2005.09.005

28. Busani L, Valsecchi MG, Rossi E, Toson M, Ferrè N, Pozza MD, et al. Risk factors for highly pathogenic H7N1 avian influenza virus infection in poultry during the 1999-2000 epidemic in Italy. *Vet J*. (2009) 181:171–7. doi: 10.1016/j.tvjl.2008.02.013

29. Nishiguchi A, Kobayashi S, Yamamoto T, Ouchi Y, Sugizaki T, Tsutsui T. Risk factors for the introduction of avian influenza virus into commercial layer chicken farms during the outbreaks caused by a low-pathogenic H5N2 virus in Japan in 2005. *Zoonoses Public Hlth*. (2007) 54:337–43. doi: 10.1111/j.1863-2378.2007.01074.x

30. Elbers ARW, Holtslag JB, Bouma A, Koch G. Within-flock mortality during the high-pathogenicity avian influenza (H7N7) epidemic in the Netherlands in 2003: implications for an early detection system. *Avian Dis*. (2007) 51:304–8. doi: 10.1637/7579-040106R.1

31. Jerry C, Stallknecht DE, Leyson C, Berghaus R, Jordan B, Pantin-Jackwood M, et al. Age-associated changes in recombinant H5 highly pathogenic and low pathogenic avian influenza hemagglutinin tissue binding in domestic poultry species. *Animals*. (2021) 11:2223. doi: 10.3390/ani11082223

32. Selleck PW, Arzey G, Kirkland PD, Reece RL, Gould AR, Daniels PW, et al. An outbreak of highly pathogenic avian influenza in Australia in 1997 caused by an H7N4 virus. *Avian Dis*. (2003) 47:806–11. doi: 10.1637/0005-2086-47.s3.806

33. McQuiston JH, Garber LP, Porter-Spalding BA, Hahn JW, Pierson FW, Wainwright SH, et al. Evaluation of risk factors for the spread of low pathogenicity H7N2 avian influenza virus among commercial poultry farms. *J Am Vet Med Assoc*. (2005) 226:767–72. doi: 10.2460/javma.2005.226.767

34. Kim W-H, An J-U, Kim J, Moon O-K, Bae SH, Bender JB, et al. Risk factors associated with highly pathogenic avian influenza subtype H5N8 outbreaks on broiler duck farms in South Korea. *Transbound Emerg Dis*. (2018) 65:1329–38. doi: 10.1111/tbed.12882

35. Yoo D, Lee K, Chun B, Lee H, Park H, Kim J. Preventive effect of on-farm biosecurity practices against highly pathogenic avian influenza (HPAI) H5N6 infection on commercial layer farms in the Republic of Korea during the 2016-17 epidemic: a case-control study. *Prev Vet Med*. (2022) 199:105556. doi: 10.1016/j.prevetmed.2021.105556

36. Gonzales JL, Stegeman JA, Koch G, de Wit S, Elbers ARW. Rate of introduction of a low pathogenic avian influenza virus infection in different poultry production sectors in the Netherlands. *Influenza Other Respir Viruses*. (2013) 7:6–10. doi: 10.1111/j.1750-2659.2012.00348.x

37. Galletti G, Santi A, Guberti V, Paternoster G, Licata E, Loli Piccolomini L, et al. A method to identify the areas at risk for the introduction of avian influenza virus into poultry flocks through direct contact with wild ducks. *Transbound Emerg Dis*. (2018) 65:1033–8. doi: 10.1111/tbed.12838

38. Ramey AM, DeLiberto TJ, Berhane Y, Swayne DE, Stallknecht DE. Lesson learned from research and surveillance directed at highly pathogenic avian influenza a viruses in wild birds inhabiting North America. *Virology*. (2018) 518:55–63. doi: 10.1016/j.virol.2018.02.002

39. Grant M, Bröjer C, Zohari S, Nöremark M, Uhlhorn JDS. Highly pathogenic avian influenza (HPAI H5Nx, clade 2.3.4.4.B) in poultry and wild birds in Sweden: synopsis of the 2020-2021 season. *Vet Sci*. (2020) 9:344. doi: 10.3390/vetsci9070344

40. Duvauchelle A, Huneau-Salaün A, Balaine L, Rose N, Michel V. Risk factors for the introduction of avian influenza virus in breeder duck flocks during the first 24 weeks of laying. *Avian Pathol*. (2013) 42:447–56. doi: 10.1080/03079457.2013.823145

41. Bowstra R, Gonzales JL, de Wit S, Stahl J, Fouchier RAM, Elbers ARW. Risk for low pathogenicity avian influenza virus on poultry farms, the Netherlands, 2007-2013. *Emerg Infect Dis*. (2017) 23:1510–6. doi: 10.3201/eid2309

42. Werner SJ, Clark L. Effectiveness of a motion-activated laser hazing system for repelling captive Canada geese. *Wildl Soc Bull*. (2006) 34:2–7. doi: 10.2193/0091-7648(2006)34[2:EOAMLH]2.0.CO;2

43. Castelli PM, Sleggs SE. Efficacy of border collies to control nuisance Canada geese. *Wildl Soc Bull*. (2000) 28:385–92.

44. Elbers ARW, Gonzales JL. Quantification of visits of wild fauna to a commercial free-range layer farm in the Netherlands located in an avian influenza hot-spot area assessed by video-camera monitoring. *Transbound Emerg Dis*. (2019) 67:661–77. doi: 10.1111/tbed.13382

45. Liu C-M, Lin S-H, Chen Y-C, Lin KC-M, Wu T-SJ, King C-C. Temperature drops and the onset of severe avian influenza a H5N1 virus outbreaks. *PLoS One*. (2007) 2:e191. doi: 10.1371/journal.pone.0000191

46. Si Y, de Boer WF, Gong P. Different environmental drivers of highly pathogenic avian influenza H5N1 outbreaks in poultry and wild birds. *PLoS One*. (2013) 8:e53362. doi: 10.1371/journal.pone.0053362

47. Chen W, Zhang X, Zhao W, Yang L, Wang Z, Bi H. Environmental factors and spatiotemporal distribution characteristics of the global outbreaks of the highly pathogenic avian influenza H5N1. *Environ Sci Pollut Res*. (2022) 29:44175–85. doi: 10.1007/s11356-022-19016-1

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Erratum: Investigation of risk factors for introduction of highly pathogenic avian influenza H5N1 infection among commercial turkey operations in the United States, 2022: a case-control study

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KEYWORDS

avian influenza, biosecurity, case control, H5N1, highly pathogenic avian influenza, risk factors, turkey

An Erratum on

Investigation of risk factors for introduction of highly pathogenic avian influenza H5N1 infection among commercial turkey operations in the United States, 2022: a case-control study

by Patyk, K. A., Fields, V. L., Beam, A. L., Branan, M. A., McGuigan, R. E., Green, A., Torchetti, M. K., Lantz, K., Freifeld, A., Marshall, K., and Delgado, A. H. (2023). *Front. Vet. Sci.* 10:1229071. doi: 10.3389/fvets.2023.1229071

Due to a production error, there was a mistake in Table 3 as published. "Visitor log used" and "Restroom facility available to crews visiting farm" should have been in different rows.

"Visitor log" should have had levels of "Always" and "Sometimes/never".

"Restroom facility available to crews visiting farm" should have had levels "Always/sometimes", "Never", "Unknown (skipped question)."

The corrected Table 3 appears below.

Due to a production error, the word "production" was misspelled as "productteion".

A correction has been made to the section **Conclusion,** Paragraph Number: 1.

"This study compared management and biosecurity factors on case and control meat turkey farms in the U.S. during the HPAI H5N1 outbreak in 2022. Knowledge of risk factors for infection has become increasingly important as this outbreak continues into 2023 and as additional domestic poultry flocks, wild birds, and wildlife species are detected. Study results identified the following key risk factors: location of farms within an existing control zone, multiple stages of production on farm, toms as the sex market type on farm, waterfowl/shorebirds seen in the closest field, gaps in worker biosecurity measures such as lack of availability of a shower before entering the barn or a restroom facility for visiting crews, and the use of rendering for dead bird disposal. These risk factors were found to be associated with HPAI infection on farms and provide information that can be directly applied to support science-based updates to prevention and control recommendations to safeguard turkey farms in the United States."

The publisher apologizes for these mistakes. The original article has been updated.

TABLE 3 Univariate analyses of factors ($p \leq 0.20$) considered for entry into the multivariable model.

Biosecurity practice	Level	Case farms (*N*/%)	Control farms (*N*/%)	Univariate *p*-value
Workers showered[A]		7 (10.8)	15 (25.9)	0.04
Workers washed hands or used hand sanitizer[A]		54 (83.1)	53 (91.4)	0.19
Visitor log used	Always	39 (60.0)	45 (77.6)	0.05
	Sometimes/never	26 (40.0)	13 (22.4)	
Restroom facility available to crews visiting farm	Always/sometimes	30 (45.5)	41 (69.5)	0.02
	Never	26 (39.4)	12 (20.3)	
	Unknown (skipped question)	10 (15.2)	6 (10.2)	

Percentage of case farms and percentage of control farms by biosecurity practices.
[A]Workers always, most of the time, or sometimes used the practice before entering the barn vs. never or not available.
This question was asked specifically for the selected barn and for the 14-day reference period.

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Mathematical modeling at the livestock-wildlife interface: scoping review of drivers of disease transmission between species

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Modeling of infectious diseases at the livestock-wildlife interface is a unique subset of mathematical modeling with many innate challenges. To ascertain the characteristics of the models used in these scenarios, a scoping review of the scientific literature was conducted. Fifty-six studies qualified for inclusion. Only 14 diseases at this interface have benefited from the utility of mathematical modeling, despite a far greater number of shared diseases. The most represented species combinations were cattle and badgers (for bovine tuberculosis, 14), and pigs and wild boar [for African (8) and classical (3) swine fever, and foot-and-mouth and disease (1)]. Assessing control strategies was the overwhelming primary research objective (27), with most studies examining control strategies applied to wildlife hosts and the effect on domestic hosts (10) or both wild and domestic hosts (5). In spatially-explicit models, while livestock species can often be represented through explicit and identifiable location data (such as farm, herd, or pasture locations), wildlife locations are often inferred using habitat suitability as a proxy. Though there are innate assumptions that may not be fully accurate when using habitat suitability to represent wildlife presence, especially for wildlife the parsimony principle plays a large role in modeling diseases at this interface, where parameters are difficult to document or require a high level of data for inference. Explaining observed transmission dynamics was another common model objective, though the relative contribution of involved species to epizootic propagation was only ascertained in a few models. More direct evidence of disease spill-over, as can be obtained through genomic approaches based on pathogen sequences, could be a useful complement to further inform such modeling. As computational and programmatic capabilities advance, the resolution of the models and data used in these models will likely be able to increase as well, with a potential goal being the linking of modern complex ecological models with the depth of dynamics responsible for pathogen transmission. Controlling diseases at this interface is a critical step toward improving both livestock and wildlife health, and mechanistic models are becoming increasingly used to explore the strategies needed to confront these diseases.

KEYWORDS

mechanistic, epizootic, transmission, review, interface, domestic, livestock, wildlife

Introduction

Modeling of infectious diseases at the domestic-wildlife interface is a unique niche within mathematical modeling. Requiring cross-disciplinary competence in infectious disease epidemiology, domestic animal health and livestock production, and wildlife ecology, these models seek to unravel the complex mechanisms behind both disease transmission between ecosystems and disease emergence in novel ecosystems. Developing models at this interface carries its own unique set of challenges. Indeed, entire articles have been written on the subject (1, 2). Simply estimating transmission between species is a burdensome task. There exists difficulty even in defining what constitutes an epidemiologically-relevant contact, as laboratory-based forced contact is different than that experienced under natural circumstances, and observing natural contacts to infer model parameters is a challenging ecological task (1). Further, spillover events are rarely observed but their frequency must be indirectly inferred, so as to inform the means of disease transmission in the non-reservoir population (2).

Transmission drivers for a wide range of pathogens have been well studied among human and domestic animal populations, for which specific epidemiological studies were set-up. In contrast, the transmission dynamics of infectious agents among wildlife species is more difficult to assess (3, 4). Wildlife characteristics ranging from descriptions of movement patterns and contact networks to simply quantifications of host population size are less certain (3, 5–8). The difficulty of observing wildlife species further affects the ability to obtain accurate measurements of disease frequency—and even simply of host population distribution—due to biases among sampled and non-sampled subsets of wildlife populations (3, 9). These uncertainties inherently affect the ability to quantify the transmission potential of a disease among its host population, and these uncertainties must be recognized and accounted for when developing mechanistic models for infectious agents in the context of wildlife populations.

Modeling disease transmission at the interface between domestic and wildlife species, therefore, is a complex equation system involving multiple distinct host and pathogen factors. Within these mathematical models, the modeling frameworks used to represent the transmission dynamics in such context need to account for the specificities of both host populations. However, models also need to remain parsimonious in terms of parameterization, not to add unnecessary uncertainty into the system. Therefore, a balance between model complexity and host population representation needs to be found to capture the transmission dynamics in regard to the available data. This review aims to examine the means of representation of livestock and wildlife species, drivers and mechanisms of transmission in the models, and the main challenges that are yet to be overcome in this field.

Materials and methods

The literature search was conducted *via* the PubMed and Web of Science databases on 28 February 2023 and performed in accordance with PRISMA guidelines (10). Constructed to capture all articles of mechanistic modeling that accounted for transmission between major livestock species and wildlife, the search—within keywords, title, and abstract—was comprised of the following query: (*livestock* OR *cattle* OR *cow* OR *ruminant* OR *bovine* OR *swine* OR *pig* OR *porcine* OR *sheep* OR ovine OR *goat* OR *caprine*) AND (*wild** OR *"wild boar"* OR *buffalo* OR *bison* OR *deer* OR *elk* OR *ibex* OR *badger*) AND *transmission* AND (*simulation* OR *math** OR *stochastic* OR *estimation* OR *inference*) AND *model*. The search was restricted to mammalian species, as the ecological processes behind the drivers of transmission of non-mammalian epizootic diseases of major concern, notably highly-pathogenic avian influenza, were considered too distinct and deserving of their own independent review. No date limitation was specified, and the English language was indirectly specified through search terminology.

A total of 709 articles were retrieved (PubMed 398, Web of Sciences 311) (Figure 1). Following removal of duplicates (149), 560 articles were considered for preliminary title and abstract screening. All original research describing mechanistic models between mammalian wildlife and livestock were included.

Preliminary review resulted in the exclusion of 501 articles. These articles only considered a single species, did not include interaction between livestock and mammalian (i.e., non-avian) wildlife, were an exclusively within-host study (i.e., molecular, microbiological, immunological, or genomic model), were of phylogenetic or phylodynamic models, used purely statistical, economic or decision-analysis models, were a review or editorial, or were experiments or field studies that did not include mechanistic modeling.

Of the 59 articles that qualified for full-text review, four articles were excluded following full-text assessment for not modeling transmission at the livestock-wildlife interface (11–14). All 10 calibration articles were captured in the search query (15–24). One article not identified in the initial search but previously known to the authors was subsequently included (25), yielding 56 articles for data extraction. Author, date, domestic and wildlife species, disease, location, domestic and wildlife model frameworks, means of domestic and wildlife representation, source of model calibration, main driver of transmission between species, interaction process between species, direction of transmission, primary research objective and main hurdles challenges or limitations were extracted.

Model frameworks were classified either by the author classification or, if not specified, the classification that best approximated the described model. Individual-based models (IBMs)—synonymous with agent-based models but chosen for its nomenclature preference in ecology—were those where populations are simulated through the complex interactions of individuals with distinct properties (26, 27). Whether individual animals or herds, in these spatially-explicit models each individual unit interacts with its environment. Conversely, population-based models—commonly referred to as compartmental models—reflected the dynamics at a population scale without accounting for individual heterogeneity. Geographic automata were a generalization of the cellular automata structure, relying on the same principles of local grid-based neighbor interactions, but no longer constraining animal populations to a uniformly-spaced lattice (28, 29). Metapopulation models were defined as those models that connect multiple subpopulations, where in the simplest form infectives in one patch can simply transmit disease to susceptibles in either their or another patch (30). Lastly, models were classified as network models when the framework relied on individual or herd connectivity through explicit networks. Of note, no standard methodology for describing

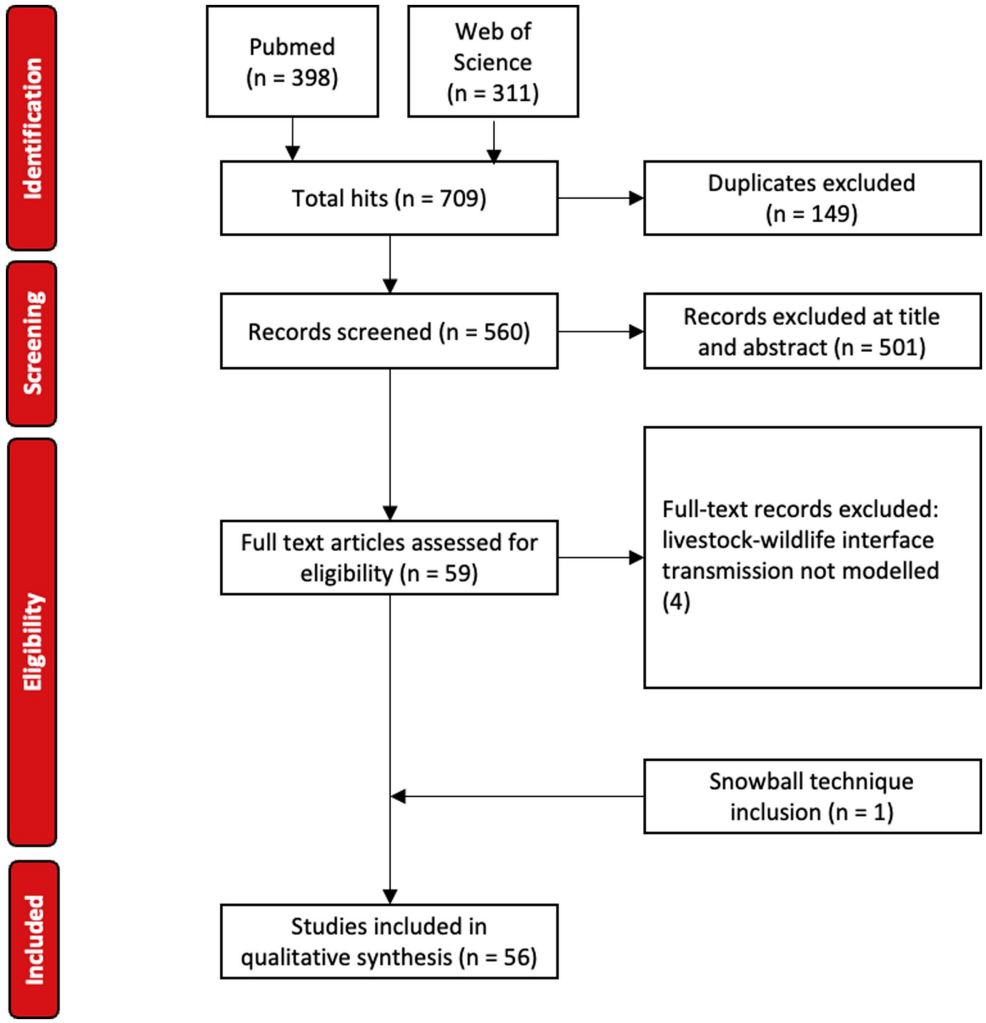

FIGURE 1
PRISMA flow diagram for article selection.

individual-based epidemic models exists, which has led to irregularities and inconsistencies among model descriptions (31). Though protocols have been proposed for describing model structures in a standardized way, they are not specific to disease modeling nor are they consistently followed (31, 32).

Results

Epidemiological characteristics

Publication dates ranged from 2001 to 2023 (Supplementary Table S1). Cattle were the predominant domestic species represented—being included in 32 models—followed by pigs (14), sheep (9), nonspecific livestock (5), and goats (4) (Figure 2). Combinations of livestock species (cattle, goats, pigs and sheep or cattle, goats, and sheep, or cattle and sheep, or goats and sheep) were present in four models. Among explicitly modeled wildlife, wild boar (16), badgers (13), nonspecific wildlife (9), deer (6), and buffalo (3) were most commonly represented with one model including both wild boar and deer (Figure 2). Additional wildlife was represented only once each: bharal, bighorn sheep, bison, feral cats, feral pigs, impala, possums, Saiga antelopes, stray dogs, wildebeest, and zebra.

Viral, bacterial, and parasitic diseases were represented among the models (Figure 3). Bovine tuberculosis (bTB) was the most frequently modeled disease (19) followed by African swine fever (ASF) (8), foot-and-mouth disease (FMD) (7), brucellosis (3), classical swine fever (CSF) (3), trypanosomiasis (3), and nematodiasis (2) (Supplementary Table S1). Babesiosis, echinococcosis, louping ill, neosporosis, toxoplasmosis, trichostrongylosis, and paratuberculosis were each represented a single time (Supplementary Table S1). Of the locations explicitly modeled, the United Kingdom (UK) and United States of America (USA) were represented the most frequently (13 and 10, respectively), and a total of 19 unique countries or regions across Africa, Europe, North America, and Oceania were represented among the studies (Supplementary Table S1). One set of studies occurred on a fictitious island for the purposes of the ASF modeling Challenge (3) (33), and nine models were not of a specific location.

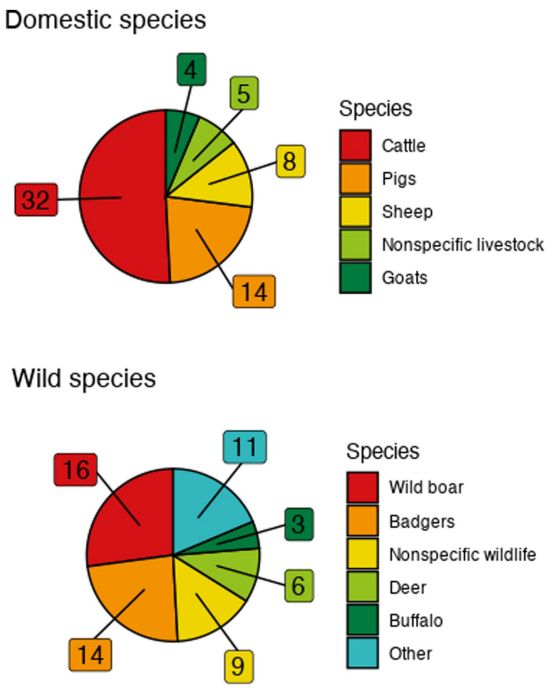

FIGURE 2
Frequency of represented species in the included models. "Other" category includes singularly-represented species consisting of bharal, bighorn sheep, bison, feral cats, feral pigs, impala, possums, Saiga antelopes, stray dogs, wildebeest, and zebra.

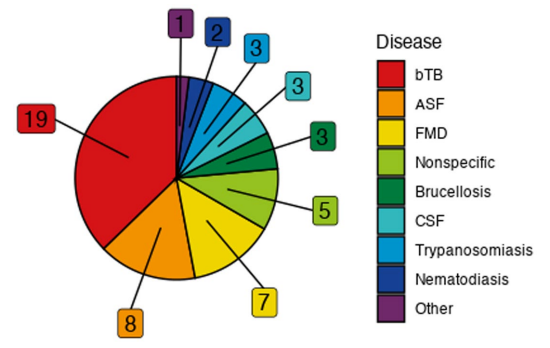

FIGURE 3
Frequency of represented diseases in the included models. "Other" category includes singularly-represented diseases of babesiosis, echinococcosis, louping ill, neosporosis, toxoplasmosis, trichostrongylosis, and paratuberculosis. Abbreviations: African swine fever (ASF), bovine tuberculosis (bTB), classical swine fever (CSF), foot and mouth disease (FMD).

Model objectives, frameworks, and representation of hosts

The majority of primary objectives were to assess control strategies in a multihost population (26), estimate transmission risk to livestock from wildlife (9), or explain observed transmission dynamics while considering the effects of multiple hosts (8), though estimating transmission parameters (4), determining consequences of hypothetical outbreak scenarios (4), nowcasting of multihost epidemics (3), and comparing the impact of model assumptions on transmission in a multihost environment (1) were also represented (Supplementary Table S2). Models that assessed control strategies were mostly concerned with the outcomes of control strategies on livestock, whether the strategy was applied to wild hosts (10) (16, 20, 22, 34–40) or wild and domestic hosts (5) (23, 41–44). These studies were heavily focused on bTB (11) in the UK (7), ascertaining the outcomes of control strategies applied to badgers on either cattle (4) or cattle and badgers (5). Other studies examined the outcomes on domestic hosts of interventions applied to domestic hosts while accounting for transmission from wildlife, as for babesiosis, louping-ill, nematodiasis, and CSF (45–48).

Five model frameworks were used among domestic or wildlife species in the included articles: individual-based models (IBMs), population-based models (PBMs), cellular or geographic automata (CA), metapopulation, and network models. Individual-based models were the most popular framework for domestic hosts (25), whereas population-based models were the most prominent framework for wildlife species (25). The majority of models used the same frameworks for both the domestic and wildlife populations, though five articles used different model frameworks for each species. Here, network models for domestic species were used in combination with a wildlife metapopulation model (49) or PBM (50), or domestic IBMs were used with a wildlife metapopulation model (51) or wildlife PBMs (39, 52).

Among individual-based frameworks, a variety of approaches were taken to represent hosts, with point locations of farms, herds or production sites being the most common epidemiological unit for both domestic species (9) and raster cells being the most used method for wildlife (17). Indeed, representing domestic species by point locations and wildlife through a raster was the most common model combination seen in the included articles (7). Among wildlife, raster cells were predominantly based on habitat (10), though home ranges (43), host density (21, 53), and contiguous social groups (23, 41, 42, 44) were also used to define them. The models in ten articles represented species through mobile agents across a simulated landscape, with five of them using individual mobile agents for both domestic and wildlife models. In these models, mobile agents were programmed to roam over home-range polygons (54), a habitat raster (17, 45), or a lattice of cells without habitat characteristics (20, 55). Alternatively, five studies provided movement attributes to only one host, with four studies representing domestic hosts through raster cells or polygons while wildlife were represented *via* mobile agents (22, 34, 35, 40).

Population-based frameworks were used for a variety of diseases, including ASF, brucellosis, bTB, CSF, FMD, louping ill, and multiple parasitic diseases (echinococcosis, nematodiasis, neosporosis, toxoplasmosis, trichostrongylosis, and trypanosomiasis). These models represented domestic and wildlife species through parameters quantifying host abundance, though population density (16, 47, 56, 57), host presence (58), and recruitment rate (59) were also used.

Three studies used metapopulation approaches to represent wildlife, two of which were designed explicitly for the ASF Modeling Challenge (49, 60). Here, wildlife was represented through a habitat raster (49), home range polygon (51), or host-presence patches (60),

while domestic species were represented through network models, metapopulation models, or IBMs using farms as location-specific network nodes (49, 60) or polygons of herd locations (51), respectively.

Cellular automata models—or their complexification to geographic automata models—were used to model FMD in Australia and the USA (19, 29, 61). A density distribution over a cellular lattice (19, 29) or a raster of herds (61) was used to represent domestic species, while wildlife species were represented *via* seasonal habitat or land cover density over a cellular lattice (19, 29) or habitat raster (61).

Network models were used to simulate ASF (49), bTB (50, 62) and brucellosis (63) transmission. Network nodes were used to represent farms, pastures, or herd types of domestic hosts and home ranges or statistic reservoirs of wildlife hosts.

Drivers of disease transmission, representations of host interaction processes, and model calibration

Disease transmission was predominately driven through the overlap of livestock and wildlife habitat, home range, or shared pasture (18) (Supplementary Table S3). In some cases, modeled transmission was driven through wildlife escaping their home range and contacting livestock (64) or from wildlife explicitly seeking food and water sources (17). When explicit overlap was not considered, the proximity of livestock to wildlife areas or cases was used, as seen in models of ASF and CSF (24, 48, 60, 65, 66). Livestock proximity to forests (15) or livestock adjacency to hunting areas (35) was also used to drive transmission. Population-based models, frequently of parasitic disease, relied on host abundance or density to drive transmission between species (Supplementary Table S2). Wildlife dispersal in response to applied control strategies was also seen to drive transmission between species, as modeled in Byrom et al. (34) and Lintott et al. (67).

The models in this review examined transmission in all directions, with unidirectional transmission from wildlife to livestock (26) or bidirectional transmission between wildlife and livestock (25) being most frequent (Supplementary Table S3). Two models examined unidirectional disease transmission from livestock to wildlife (22, 51), and three models looked at transmission of disease between both wildlife and livestock to humans (36, 37, 63).

Different functional representations of the interaction processes between the different host populations were seen throughout the models in the included studies. Transmission rates, corresponding to the average number of new infections produced by one infectious unit per unit of time, are widely used in the literature for all modeling paradigms. This key parameter in epidemiology governs the force of infection, which might reflect either direct transmission between host or indirect transmission through vectors or environment. The transmission rate can also be defined as the product of the contact rate and the transmission probability whenever a contact occurs with an infectious unit. A few studies disentangled these two parameters to evaluate the relative impact of external factors on the different mechanisms of transmission (15, 29, 53, 61). When transmission rates were not used to represent host interaction, if the data was available, transmission or contact probability, or contact rate were also used to represent the host interaction process.

Of the 56 included studies, 37 models were calibrated *via* published literature. Only 10 of the models were calibrated to a real epidemic, and seven of those were specific to bTB (16, 18, 50, 55, 58, 62, 68). The other two real epidemics modeled were ASF in the Republic of Korea (24) and CSF in Japan (48, 65). Three more articles did model ASF, but as part of the ASF challenge and with synthetic data (49, 60, 66). Four studies included a field component that was used in model calibration (25, 34, 57, 69).

Main hurdles

While each model had its own limitations unique to the specific scenario for which it was designed, four main classes of hurdles were identified: Lack of empirical parameter estimates, limited wildlife location data, defining what constitutes livestock-wildlife contact, and balancing model complexity with utility (Supplementary Table S3). By far, a lack of empirical parameter estimates was the primary limitation in 31 studies. The lack of empirical parameter estimates needed for model calibration could be further divided between parameters for disease transmission (17), wildlife behavior (8), livestock-wildlife contact (2), wildlife prevalence (2), interspecies control strategy effects (1), and host management (1). Even when an explicit interhost transmission study was conducted, its occurrence under controlled laboratory conditions limits extrapolation of these parameters to natural conditions (25). Limited data on wildlife locations was the primary limitation in 14 models. Here, a lack of wildlife density and/or distribution data (11), lack of environmental reservoir locations (2), or uncertainties regarding wildlife habitat use as a function of preference versus availability (1) were identified. Indeed, even with fine-grain wildlife habitat data, understanding if such habitat is preferred or simply available limits the generalizability of a model (45).

Beyond a lack of parameters for quantifying livestock-wildlife contact, even defining what constitutes an epidemiological relevant contact was the primary hurdle of 3 models (29, 64, 69). Balancing model complexity with utility was the main hurdle in 4 models. Among multihost models of vector-borne disease, incorporating explicit vector population dynamics was the primary limitation even when parameterization data was available, due to its effects on model complexity and generalizability (36, 37, 68). Conversely, one of the ASF challenge models—where all data was synthetic—was more limited by the trade-off between model complexity and computational time required for real-time modeling than any explicit wildlife parameter gaps (60).

Discussion

Modeling disease transmission between wild and domestic species is a complex task that has been achieved through a multitude of methods but for only a few disease scenarios. Indeed, of the 118 diseases at the wildlife-livestock interface represented in the literature body, only 14 have been explored through mathematical modeling (70). From selecting model frameworks and host representations to determining the drivers of transmission that are to be included in the models, distinct populations—often with drastically differing

population dynamics—must be accurately represented. These choices of methodology are a reflection of the skill set of the researcher team, the research question being addressed by the model and the availability of data. Domestic species were defined through explicit herd locations, and further delineated by additional parameters of herd density, defined pasture area, habitat and abundance. In contrast, wildlife species were often modeled through variables of habitat potential, density distribution, or population abundance. Only in a few models of badgers were the exact burrow locations known, but even then only the underground dens were identified and surrounding home ranges still had to be inferred (23, 41, 42). In choosing a paradigm to represent a system one must consider the trade-offs between complexity, comprehensibility, and underlying assumptions. Though a model should be a realistic representation, deciding on the degree of realism required—and keeping in mind that models are only synthetic representations of a phenomenon—is part of the art of model selection. The parsimony principle should always be kept in mind, especially in situations involving wildlife where parameters are difficult to document, require a high level of data for inference, or are highly variable, due to the influence of the interaction of multiple factors.

Habitability is often used as a proxy to represent wild host populations, as was the case in 11 models (15, 19, 22, 35, 40, 45, 49, 61, 65, 66, 71). Defining such suitability can involve the incorporation of landcover maps, abundance data from hunting records, expert opinion, and previously-published species distribution models. In the context of models examined in this review, species distribution is a means to the end for representing disease transmission through multiple populations, and simplifications of a species' true distribution—especially wildlife—are evident in all livestock-wildlife disease transmission models. Combined with data limitations among wildlife species, this invariably results in wildlife disease transmission models that contain more uncertainties than those of domestic animal species (4). Indeed, monitoring infectious diseases in wild populations is far more demanding in terms of resources and time required than for livestock. Though there are innate assumptions that may not be fully accurate when using habitat suitability to represent wildlife presence, for the given modeling objectives these assumptions are acceptable. While sensitivity analyses within the selected articles focused on model parameters (e.g., transmission detection and contact rates, mortality, and initial infection location) and not the representation of the distribution of wildlife, Birch et al. (50) did assess the sensitivity of their model to the number of environmental reservoirs—identifying that that parameter was more constrained than that of the environment-to-livestock transmission rate.

By far the most-represented transmission driver was that of overlapping habitat. Whether livestock were modeled as discrete farms or mobile herds, most disease transmission was driven by locations that intersected with wildlife habitats or home ranges. Agricultural intensification, wildlife habitat fragmentation and encroachment on wild animal habitats are known global drivers of disease emergence, and these drivers are reflected in these models (72, 73). Local drivers, such as water and food-seeking behaviors of wildlife, pasture sharing between livestock and native wildlife, and outdoor husbandry, were also reflected among the models (73). Control strategies themselves can also be implicit in driving transmission, as culling can have an opposite-as-intended effect increasing both disease prevalence and number of infectious individuals (74, 75). This was reflected in models of bTB transmission as when Smith et al. (43) used a perturbation parameter to account for the increase in transmission from culling, or studied by Lintott et al. (67) to quantify the impact of dispersal following disease control.

Included studies that focused on control strategy assessments invariably quantified the number of infected herds, as explicitly stated in Pineda-Krch et al. (53), Ramsey et al. (40), and Smith et al. (41, 43), but certain methodologies precluded the ability to determine the relative contribution of species to overall spread. For instance, when foot-and-mouth disease was investigated among feral pigs and livestock, a single-layer cellular automata model was used (19). Therefore, multiple species had to be mutated into a composite herd that varied based on a species-specific infectivity parameter (depending on the type and number of each specie). Though effective at discerning the overall epizootic spatio-temporal pattern, such a method did not allow for the disentangling of individual species' contribution. Of the models that tried to explain observed transmission dynamics, the relative contribution of involved species to epizootic propagation was only ascertained in a few models (18, 24, 50, 58). Indeed, mechanistic models that are based on specific spatio-temporal case data and uncertain population distributions (particularly for wildlife), and for which inter-species transmission events are not directly observable, may be very challenging to estimate relative contributions. More direct evidence of disease spill-over, as can be obtained through genomic approaches based on pathogen sequences, could be a useful complement to further inform such models (76).

The challenges of multispecies modeling have been extensively reviewed in the literature. Whether focused on the human-wildlife (2) or the domestic-wildlife (3, 6) interface, or more broadly examining modeling of multihost systems (1, 77), all reviews espouse that though hurdles have been overcome, many more challenges remain in need of address. Huyvaert et al. (3) identified that these challenges fall into three broad categories relating to host and pathogen distribution and movement, transmission pathways and rates, and the effects of disease and mitigation on host populations. Five years later, these hurdles continue to be represented in the 14 included studies published since 2018. Investments in ecological research with project planning input from ecological modelers, infectious disease specialists (including epidemiologists, veterinarians, and virologists) and wildlife managers—among many additional critical fields at this interface—may help to overcome these challenges, through enabling the studies needed to elucidate the parameters needed for modeling this interface.

The need for additional modeling at the livestock-wildlife interface is supported by the ever-increasing interactions between wildlife and livestock. Livestock production systems constitute the largest use of land in the world, and increasing global food demand invariably results in the expansion of these systems (73). The consequent deforestation that makes room for these enterprises results in the juxtaposition of livestock with wildlife, increasing the areas of interaction between the two (72, 73). Climate change has had profound effects at both global and local scales. Large-scale shifts in vector distributions have resulted in outbreaks of diseases that were formerly confined to tropical regions, as seen with bluetongue virus (73, 78). Locally, water scarcity in arid and semi-arid regions has resulted in mixed congregations around available water sources for pastoral livestock and wildlife (73).

In the majority of rural communities, backyard farming and small-scale animal production systems constitute the primary

livelihoods and food sources (79). These low-biosecurity operations permit regular contact between livestock and wildlife, and have often been central to outbreaks of diseases shared at this interface—including ASF, CSF, FMD, brucellosis, and rabies (73, 80). Improved animal welfare in high-income countries has also resulted in increases in the number of outdoor and open-air production systems, which also puts livestock at higher risk of wildlife contacts (73). The livestock-wildlife interface acts as an important area of infectious disease propagation, and mathematical models are able to investigate and quantify the involved dynamics, helping to improve our understanding of these drivers of transmission and contribute to the conception of holistic control strategies.

Data availability statement

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

Author contributions

BH, TV, MA, and NR contributed to conception and design of the study. BH performed the data extraction, analysis, and composed the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

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

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1225446/full#supplementary-material

References

1. Buhnerkempe MG, Roberts MG, Dobson AP, Heesterbeek H, Hudson PJ, Lloyd-Smith JO. Eight challenges in modelling disease ecology in multi-host, multi-agent systems. *Epidem Chall Model Infect Dis Dyn*. (2015) 10:26–30. doi: 10.1016/j.epidem.2014.10.001

2. Roberts M, Dobson A, Restif O, Wells K. Challenges in modelling the dynamics of infectious diseases at the wildlife–human interface. *Epidemics*. (2021) 37:100523. doi: 10.1016/j.epidem.2021.100523

3. Huyvaert KP, Russell RE, Patyk KA, Craft ME, Cross PC, Garner MG, et al. Challenges and opportunities developing mathematical models of shared pathogens of domestic and wild animals. *Vet Sci*. (2018) 5:E92. doi: 10.3390/vetsci5040092

4. McCallum H. Models for managing wildlife disease. *Parasitology*. (2016) 143:805–20. doi: 10.1017/S0031182015000980

5. Cowled B, Garner G. A review of geospatial and ecological factors affecting disease spread in wild pigs: considerations for models of foot-and-mouth disease spread. *Prev Vet Med*. (2008) 87:197–212. doi: 10.1016/j.prevetmed.2008.03.012

6. Craft ME. Infectious disease transmission and contact networks in wildlife and livestock. *Philos Trans R Soc Lond Ser B Biol Sci*. (2015) 370:20140107. doi: 10.1098/rstb.2014.0107

7. Cross PC, Creech TG, Ebinger MR, Heisey DM, Irvine KM, Creel S. Wildlife contact analysis: emerging methods, questions, and challenges. *Behav Ecol Sociobiol*. (2012) 66:1437–47. doi: 10.1007/s00265-012-1376-6

8. Russell RE, Katz RA, Richgels KLD, Walsh DP, Grant EHC. A framework for Modeling emerging diseases to inform management. *Emerg Infect Dis*. (2017) 23:1–6. doi: 10.3201/eid2301.161452

9. Gilbert AT, Fooks AR, Hayman DTS, Horton DL, Müller T, Plowright R, et al. Deciphering serology to understand the ecology of infectious diseases in wildlife. *EcoHealth*. (2013) 10:298–313. doi: 10.1007/s10393-013-0856-0

10. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med*. (2009) 6:e1000100. doi: 10.1371/journal.pmed.1000100

11. Barron MC, Tompkins DM, Ramsey DSL, Bosson MAJ. The role of multiple wildlife hosts in the persistence and spread of bovine tuberculosis in New Zealand. *N Z Vet J*. (2015) 63:68–76. doi: 10.1080/00480169.2014.968229

12. Mateus-Anzola J, Wiratsudakul A, Rico-Chávez O, Ojeda-Flores R. Simulation modeling of influenza transmission through backyard pig trade networks in a wildlife/livestock interface area. *Trop Anim Health Prod*. (2019) 51:2019–24. doi: 10.1007/s11250-019-01892-4

13. Santos N, Richomme C, Nunes T, Vicente J, Alves PC, de la Fuente J, et al. Quantification of the animal tuberculosis multi-host community offers insights for control. *Pathog Basel Switz*. (2020) 9:421. doi: 10.3390/pathogens9060421

14. Smith GC. Persistence of disease in territorial animals: insights from spatial models of tb. *N Z J Ecol*. (2006) 30:35–41.

15. Boklund A, Goldbach SG, Uttenthal A, Alban L. Simulating the spread of classical swine fever virus between a hypothetical wild-boar population and domestic pig herds in Denmark. *Prev Vet Med*. (2008) 85:187–206. doi: 10.1016/j.prevetmed.2008.01.012

16. Cox DR, Donnelly CA, Bourne FJ, Gettinby G, McInerney JP, Morrison WI, et al. Simple model for tuberculosis in cattle and badgers. *Proc Natl Acad Sci U S A*. (2005) 102:17588–93. doi: 10.1073/pnas.0509003102

17. Dion E, Van Schalkwyk L, Lambin EF. The landscape epidemiology of foot-and-mouth disease in South Africa: a spatially explicit multi-agent simulation. *Ecol Model*. (2011) 222:2059–72. doi: 10.1016/j.ecolmodel.2011.03.026

18. Donnelly CA, Nouvellet P. The contribution of badgers to confirmed tuberculosis in cattle in high-incidence areas in England. *PLoS Curr*. (2013) 5:60998. doi: 10.1371/currents.outbreaks.097a904d3f3619db2fe78d24bc776098

19. Doran RJ, Laffan SW. Simulating the spatial dynamics of foot and mouth disease outbreaks in feral pigs and livestock in Queensland, Australia, using a susceptible-infected-recovered cellular automata model. *Prev Vet Med*. (2005) 70:133–52. doi: 10.1016/j.prevetmed.2005.03.002

20. Manlove KR, Sampson LM, Borremans B, Cassirer EF, Miller RS, Pepin KM, et al. Epidemic growth rates and host movement patterns shape management performance for pathogen spillover at the wildlife-livestock interface. *Philos Trans R Soc B-Biol Sci*. (2019) 374:343. doi: 10.1098/rstb.2018.0343

21. Mur L, Sánchez-Vizcaíno JM, Fernández-Carrión E, Jurado C, Rolesu S, Feliziani F, et al. Understanding African swine fever infection dynamics in Sardinia using a spatially explicit transmission model in domestic pig farms. *Transbound Emerg Dis*. (2018) 65:123–34. doi: 10.1111/tbed.12636

22. Taylor RA, Podgórski T, Simons RRL, Ip S, Gale P, Kelly LA, et al. Predicting spread and effective control measures for African swine fever-should we blame the boars? *Transbound Emerg Dis*. (2021) 68:397–416. doi: 10.1111/tbed.13690

23. Wilkinson D, Smith G, Delahay R, Cheeseman C. A model of bovine tuberculosis in the badger *Meles meles*: an evaluation of different vaccination strategies. *J Appl Ecol*. (2004) 41:492–501. doi: 10.1111/j.0021-8901.2004.00898.x

24. Yoo DS, Kim Y, Lee ES, Lim JS, Hong SK, Lee IS, et al. Transmission dynamics of African swine fever virus, South Korea, 2019. *Emerg Infect Dis*. (2021) 27:1909–18. doi: 10.3201/eid2707.204230

25. Pietschmann J, Guinat C, Beer M, Pronin V, Tauscher K, Petrov A, et al. Course and transmission characteristics of oral low-dose infection of domestic pigs and European wild boar with a Caucasian African swine fever virus isolate. *Arch Virol*. (2015) 160:1657–67. doi: 10.1007/s00705-015-2430-2

26. DeAngelis DL, Grimm V. Individual-based models in ecology after four decades. *F1000Prime Rep*. (2014) 6:39. doi: 10.12703/P6-39

27. Vincenot CE. How new concepts become universal scientific approaches: insights from citation network analysis of agent-based complex systems science. *Proc R Soc B Biol Sci*. (2018) 285:20172360. doi: 10.1098/rspb.2017.2360

28. Wolfram S. Universality and complexity in cellular automata. *Phys Nonlinear Phenom*. (1984) 10:1–35. doi: 10.1016/0167-2789(84)90245-8

29. Laffan SW, Wang Z, Ward MP. The effect of neighbourhood definitions on spatio-temporal models of disease outbreaks: separation distance versus range overlap. *Prev Vet Med*. (2011) 102:218–29. doi: 10.1016/j.prevetmed.2011.07.009

30. van den Driessche P. Spatial structure: patch models In: F Brauer, P van den Driessche and J Wu, editors. *Mathematical epidemiology, Lecture Notes in Mathematics*. Berlin, Heidelberg: Springer (2008). 179–89.

31. Orbann, C, Sattenspiel, L, Miller, E, and Dimka, J. Defining epidemics in computer simulation models: How do definitions influence conclusions? *Epidemics*. (2017) 19:24–32.

32. Grimm, V, Berger, U, Bastiansen, F, Eliassen, S, Ginot, V, Giske, J, et al. A standard protocol for describing individual-based and agent-based models. *Ecol Model*. (2006) 198:115–26.

33. Picault S, Vergne T, Mancini M, Bareille S, Ezanno P. The African swine fever modelling challenge: objectives, model description and synthetic data generation. *Epidemics*. (2022) 40:100616. doi: 10.1016/j.epidem.2022.100616

34. Byrom AE, Anderson DP, Coleman M, Thomson C, Cross ML, Pech RP. Assessing movements of brushtail possums (*Trichosurus vulpecula*) in relation to depopulated buffer zones for the Management of Wildlife Tuberculosis in New Zealand. *PLoS One*. (2015) 10:e0145636. doi: 10.1371/journal.pone.0145636

35. Cosgrove MK, O'Brien DJ, Ramsey DSL. Baiting and feeding revisited: Modeling factors influencing transmission of tuberculosis among deer and to cattle. *Front Vet Sci*. (2018) 5:306. doi: 10.3389/fvets.2018.00306

36. Hargrove JW, Ouifki R, Kajunguri D, Vale GA, Torr SJ. Modeling the control of trypanosomiasis using trypanocides or insecticide-treated livestock. *PLoS Negl Trop Dis*. (2012) 6:1615. doi: 10.1371/journal.pntd.0001615

37. Kajunguri D, Hargrove JW, Ouifki R, Mugisha JYT, Coleman PG, Welburn SC. Modelling the use of insecticide-treated cattle to control tsetse and Trypanosoma brucei rhodesiense in a multi-host population. *Bull Math Biol*. (2014) 76:673–96. doi: 10.1007/s11538-014-9938-6

38. Mateus-Pinilla N, Hannon B, Weigel R. A computer simulation of the prevention of the transmission of toxoplasma gondii on swine farms using a feline T-gondii vaccine. *Prev Vet Med*. (2002) 55:17–36. doi: 10.1016/S0167-5877(02)00057-0

39. Odeniran PO, Onifade AA, Mac Leod ET, Ademola IO, Alderton S, Welburn SC. Mathematical modelling and control of African animal trypanosomosis with interacting populations in West Africa-could biting flies be important in maintaining the disease endemicity? *PLoS One*. (2020) 15:e0242435. doi: 10.1371/journal.pone.0242435

40. Ramsey DSL, O'Brien DJ, Smith RW, Cosgrove MK, Schmitt SM, Rudolph BA. Management of on-farm risk to livestock from bovine tuberculosis in Michigan, USA, white-tailed deer: predictions from a spatially-explicit stochastic model. *Prev Vet Med*. (2016) 134:26–38. doi: 10.1016/j.prevetmed.2016.09.022

41. Smith G, Cheeseman C, Clifton-Hadley R, Wilkinson D. A model of bovine tuberculosis in the badger *Meles meles*: an evaluation of control strategies. *J Appl Ecol*. (2001) 38:509–19. doi: 10.1046/j.1365-2664.2001.00609.x

42. Smith G, Cheeseman C, Wilkinson D, Clifton-Hadley R. A model of bovine tuberculosis in the badger *Meles meles*: the inclusion of cattle and the use of a live test. *J Appl Ecol*. (2001) 38:520–35. doi: 10.1046/j.1365-2664.2001.00610.x

43. Smith GC, Delahay RJ, McDonald RA, Budgey R. Model of selective and non-selective Management of Badgers (*Meles meles*) to control bovine tuberculosis in badgers and cattle. *PLoS One*. (2016) 11:e0167206. doi: 10.1371/journal.pone.0167206

44. Smith GC, McDonald RA, Wilkinson D. Comparing badger (*Meles meles*) management strategies for reducing tuberculosis incidence in cattle. *PLoS One*. (2012) 7:e39250. doi: 10.1371/journal.pone.0039250

45. Agudelo MS, Grant WE, Wang H-H. Effects of white-tailed deer habitat use preferences on southern cattle fever tick eradication: simulating impact on "pasture vacation" strategies. *Parasit Vectors*. (2021) 14:102. doi: 10.1186/s13071-021-04590-z

46. Porter R, Norman R, Gilbert L. Controlling tick-borne diseases through domestic animal management: a theoretical approach. *Theor Ecol*. (2011) 4:321–39. doi: 10.1007/s12080-010-0080-2

47. Walker JG, Evans KE, Rose Vineer H, van Wyk JA, Morgan ER. Prediction and attenuation of seasonal spillover of parasites between wild and domestic ungulates in an arid mixed-use system. *J Appl Ecol*. (2018) 55:1976–86. doi: 10.1111/1365-2664.13083

48. Yang Y, Nishiura H. Assessing the geographic range of classical swine fever vaccinations by spatiotemporal modelling in Japan. *Transbound Emerg Dis*. (2022) 69:1880–9. doi: 10.1111/tbed.14171

49. Beaunée G, Deslandes F, Vergu E. Inferring ASF transmission in domestic pigs and wild boars using a paired model iterative approach. *Epidemics*. (2023) 42:100665. doi: 10.1016/j.epidem.2023.100665

50. Birch CPD, Goddard A, Tearne O. A new bovine tuberculosis model for England and Wales (BoTMEW) to simulate epidemiology, surveillance and control. *BMC Vet Res*. (2018) 14:273. doi: 10.1186/s12917-018-1595-9

51. Carpenter TE, Coggins VL, McCarthy C, O'Brien CS, O'Brien JM, Schommer TJ. A spatial risk assessment of bighorn sheep extirpation by grazing domestic sheep on public lands. *Prev Vet Med*. (2014) 114:3–10. doi: 10.1016/j.prevetmed.2014.01.008

52. Smith LA, Marion G, Swain DL, White PCL, Hutchings MR. Inter-and intra-specific exposure to parasites and pathogens via the faecal-oral route: a consequence of behaviour in a patchy environment. *Epidemiol Infect*. (2009) 137:630–43. doi: 10.1017/S0950268808001313

53. Pineda-Krch M, O'Brien JM, Thunes C, Carpenter TE. Potential impact of introduction of foot-and-mouth disease from wild pigs into commercial livestock premises in California. *Am J Vet Res*. (2010) 71:82–8. doi: 10.2460/ajvr.71.1.82

54. Ward MP, Garner MG, Cowled BD. Modelling foot-and-mouth disease transmission in a wild pig-domestic cattle ecosystem. *Aust Vet J*. (2015) 93:4–12. doi: 10.1111/avj.12278

55. Moustakas A, Evans MR. Coupling models of cattle and farms with models of badgers for predicting the dynamics of bovine tuberculosis (TB). *Stoch Environ Res RISK Assess*. (2015) 29:623–35. doi: 10.1007/s00477-014-1016-y

56. Greenman JV, Hoyle AS. Exclusion of generalist pathogens in multihost communities. *Am Nat*. (2008) 172:576–84. doi: 10.1086/590967

57. Khanyari M, Suryawanshi KR, Milner-Gulland EJ, Dickinson E, Khara A, Rana RS, et al. Predicting parasite dynamics in mixed-use trans-Himalayan pastures to underpin Management of Cross-Transmission between Livestock and Bharal. *Front Vet Sci*. (2021) 8:714241. doi: 10.3389/fvets.2021.714241

58. Brooks-Pollock E, Wood JLN. Eliminating bovine tuberculosis in cattle and badgers: insight from a dynamic model. *Proc Biol Sci*. (2015) 282:20150374. doi: 10.1098/rspb.2015.0374

59. Mugabi F, Duffy KJ. Exploring the dynamics of African swine fever transmission cycles at a wildlife-livestock interface. *Nonlinear Anal-Real World Appl*. (2023) 70:103781. doi: 10.1016/j.nonrwa.2022.103781

60. Dankwa EA, Lambert S, Hayes S, Thompson RN, Donnelly CA. Stochastic modelling of African swine fever in wild boar and domestic pigs: epidemic forecasting and comparison of disease management strategies. *Epidemics*. (2022) 40:100622. doi: 10.1016/j.epidem.2022.100622

61. Ward MP, Laffan SW, Highfield LD. Disease spread models in wild and feral animal populations: application of artificial life models. *Rev Sci Tech Int Off Epizoot*. (2011) 30:437–46. doi: 10.20506/rst.30.2.2042

62. Bouchez-Zacria M, Courcoul A, Durand B. The distribution of bovine tuberculosis in cattle farms is linked to cattle trade and badger-mediated contact networks in South-Western France, 2007-2015. *Front Vet Sci*. (2018) 5:173. doi: 10.3389/fvets.2018.00173

63. Roy S, McElwain TF, Wan Y. A network control theory approach to modeling and optimal control of zoonoses: case study of brucellosis transmission in sub-Saharan Africa. *PLoS Negl Trop Dis*. (2011) 5:e1259. doi: 10.1371/journal.pntd.0001259

64. Jori F, Etter E. Transmission of foot and mouth disease at the wildlife/livestock interface of the Kruger National Park, South Africa: can the risk be mitigated? *Prev Vet Med*. (2016) 126:19–29. doi: 10.1016/j.prevetmed.2016.01.016

65. Hayama Y, Shimizu Y, Murato Y, Sawai K, Yamamoto T. Estimation of infection risk on pig farms in infected wild boar areas-epidemiological analysis for the reemergence of classical swine fever in Japan in 2018. *Prev Vet Med*. (2020) 175:104873. doi: 10.1016/j.prevetmed.2019.104873

66. Muñoz F, Pleydell DRJ, Jori F. A combination of probabilistic and mechanistic approaches for predicting the spread of African swine fever on Merry Island. *Epidemics*. (2022) 40:100596. doi: 10.1016/j.epidem.2022.100596

67. Lintott RA, Norman RA, Hoyle AS. The impact of increased dispersal in response to disease control in patchy environments. *J Theor Biol*. (2013) 323:57–68. doi: 10.1016/j.jtbi.2013.01.027

68. O'Hare A, Orton RJ, Bessell PR, Kao RR. Estimating epidemiological parameters for bovine tuberculosis in British cattle using a Bayesian partial-likelihood approach. *Proc Biol Sci*. (2014) 281:20140248. doi: 10.1098/rspb.2014.0248

69. Moreno-Torres KI, Pomeroy LW, Moritz M, Saville W, Wolfe B, Garabed R. Host species heterogeneity in the epidemiology of Nesopora caninum. *PLoS One*. (2017) 12:e0183900. doi: 10.1371/journal.pone.0183900

70. Wiethoelter AK, Beltrán-Alcrudo D, Kock R, Mor SM. Global trends in infectious diseases at the wildlife-livestock interface. *Proc Natl Acad Sci U S A*. (2015) 112:9662–7. doi: 10.1073/pnas.1422741112

71. Patterson L, Belkhiria J, Martínez-López B, Pires AFA. Identification of high-risk contact areas between feral pigs and outdoor-raised pig operations in California: implications for disease transmission in the wildlife-livestock interface. *PLoS One*. (2022) 17:e0270500. doi: 10.1371/journal.pone.0270500

72. Jones BA, Grace D, Kock R, Alonso S, Rushton J, Said MY, et al. Zoonosis emergence linked to agricultural intensification and environmental change. *Proc Natl Acad Sci U S A*. (2013) 110:8399–404. doi: 10.1073/pnas.1208059110

73. Jori F, Hernandez-Jover M, Magouras I, Dürr S, Brookes VJ. Wildlife–livestock interactions in animal production systems: what are the biosecurity and health implications? *Anim Front Rev Mag Anim Agric*. (2021) 11:8–19. doi: 10.1093/af/vfab045

74. Choisy M, Rohani P. Harvesting can increase severity of wildlife disease epidemics. *Proc Biol Sci*. (2006) 273:2025–34. doi: 10.1098/rspb.2006.3554

75. Prentice JC, Fox NJ, Hutchings MR, White PCL, Davidson RS, Marion G. When to kill a cull: factors affecting the success of culling wildlife for disease control. *J R Soc Interface*. (2019) 16:20180901. doi: 10.1098/rsif.2018.0901

76. Guinat C, Vergne T, Kocher A, Chakraborty D, Paul MC, Ducatez M, et al. What can phylodynamics bring to animal health research? Trends Ecol. *Evolution*. (2021) 36:837–47. doi: 10.1016/j.tree.2021.04.013

77. Cross, PC, Prosser, DJ, Ramey, AM, Hanks, EM, and Pepin, KM. Confronting models with data: the challenges of estimating disease spillover. *Philos Trans R Soc Lond B Biol Sci.* (2019) 374:20180435.

78. Jacquot M, Nomikou K, Palmarini M, Mertens P, Biek R. Bluetongue virus spread in Europe is a consequence of climatic, landscape and vertebrate host factors as revealed by phylogeographic inference. *Proc Biol Sci*. (2017) 284:20170919. doi: 10.1098/rspb.2017.0919

79. Committee on Considerations for the Future of Animal Science Research. *Global considerations for animal agriculture research, critical role of animal science research in food security and Sustainability*. Washington, DC: National Academies Press (2015).

80. WOAH. (2022). Implementing stronger biosecurity to avoid disease spread to new areas. WOAH-world organ. Anim. Health URL. Available at: https://www.woah.org/en/implementing-stronger-biosecurity-to-avoid-disease-spread-to-new-areas/ (Accessed July 27, 2022).

81. Kilpatrick AM, Gillin CM, Daszak P. Wildlife-livestock conflict: the risk of pathogen transmission from bison to cattle outside Yellowstone National Park. *J Appl Ecol*. (2009) 46:476–85. doi: 10.1111/j.1365-2664.2008.01602.x

82. Marion G, Smith LA, Swain DL, Davidson RS, Hutchings MR. Agent-based modelling of foraging behaviour: the impact of spatial heterogeneity on disease risks from faeces in grazing systems. *J Agric Sci*. (2008) 146:507–20. doi: 10.1017/S0021859608008022

83. Morgan ER, Medley GF, Torgerson PR, Shaikenov BS, Milner-Gulland EJ. Parasite transmission in a migratory multiple host system. *Ecol Model*. (2007) 200:511–20. doi: 10.1016/j.ecolmodel.2006.09.002

84. Nyerere N, Luboobi LS, Mpeshe SC, Shirima GM. Modeling the impact of seasonal weather variations on the infectiology of brucellosis. *Comput Math Methods Med*. (2020) 2020:8972063. doi: 10.1155/2020/8972063

85. Phepa PB, Chirove F, Govinder KS. Modelling the role of multi-transmission routes in the epidemiology of bovine tuberculosis in cattle and buffalo populations. *Math Biosci*. (2016) 277:47–58. doi: 10.1016/j.mbs.2016.04.003

86. Rong X, Fan M, Zhu H, Zheng Y. Dynamic modeling and optimal control of cystic echinococcosis. *Infect Dis Poverty*. (2021) 10:38. doi: 10.1186/s40249-021-00807-6

87. Wilkinson D, Bennett R, McFarlane I, Rushton S, Shirley M, Smith GC. Cost-benefit analysis model of badger (*Meles meles*) culling to reduce cattle herd tuberculosis breakdowns in Britain, with particular reference to badger perturbation. *J Wildl Dis*. (2009) 45:1062–88. doi: 10.7589/0090-3558-45.4.1062

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Inferring bovine tuberculosis transmission between cattle and badgers via the environment and risk mapping

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Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is one of the most challenging and persistent health issues in many countries worldwide. In several countries, bTB control is complicated due to the presence of wildlife reservoirs of infection, i.e. European badger (*Meles meles*) in Ireland and the UK, which can transmit infection to cattle. However, a quantitative understanding of the role of cattle and badgers in bTB transmission is elusive, especially where there is spatial variation in relative density between badgers and cattle. Moreover, as these two species have infrequent direct contact, environmental transmission is likely to play a role, but the quantitative importance of the environment has not been assessed. Therefore, the objective of this study is to better understand bTB transmission between cattle and badgers via the environment in a spatially explicit context and to identify high-risk areas. We developed an environmental transmission model that incorporates both within-herd/territory transmission and between-species transmission, with the latter facilitated by badger territories overlapping with herd areas. Model parameters such as transmission rate parameters and the decay rate parameter of *M. bovis* were estimated by maximum likelihood estimation using infection data from badgers and cattle collected during a 4-year badger vaccination trial. Our estimation showed that the environment can play an important role in the transmission of bTB, with a half-life of *M. bovis* in the environment of around 177 days. Based on the estimated transmission rate parameters, we calculate the basic reproduction ratio (R) within a herd, which reveals how relative badger density dictates transmission. In addition, we simulated transmission in each small local area to generate a first between-herd R map that identifies high-risk areas.

KEYWORDS

bovine tuberculosis, environmental transmission, domestic wildlife interface, R map, next-generation matrix method

1. Introduction

Bovine tuberculosis (bTB) is one of the most complicated, persistent, and expensive health issues globally. While its primary impact is on bovines, it can infect many other mammals, including humans and wildlife animals (1). bTB is very persistent in livestock globally due to the involvement of several wildlife species in bTB transmission. Notable examples include badgers in the UK and Ireland, brushtail possums in New Zealand, wild boars in Spain (2), red deer in Austria (3), and African buffalo in South Africa (4). Although pasteurization of milk can reduce human infection, *Mycobacterium bovis* is estimated to cause ∼10% of total human TB cases in developing countries (5, 6). The impact of bTB extends beyond public health with substantial economic consequences, costing approximately US$3 billion globally (7). In the Republic of Ireland (bTB) alone, more than 15,000 cattle have been removed annually over the last decade. In 2020, the total programme expenditure cost was €97 million and is rising year-on-year (8).

The Irish national bTB eradication programme is underpinned by a test-and-removal strategy, leading to the slaughter of all cattle that are positive to the single intradermal comparative tuberculin test (SICTT), performed at least annually in each Irish herd (9). This strategy has been successful in eradicating bTB in some countries, such as Australia and some northern European countries (10). In Ireland, however, progress has stalled in the national eradication programme (11, 12), at least in part due to the presence of other reservoirs of infection, including badgers (*Meles meles*; 13). Badger vaccination has proven effective at reducing badger susceptibility, both in pen and field studies (13–15), and a badger vaccination programme is now being progressively incorporated into a national programme (16, 17).

A number of different approaches have been used in recent studies to investigate the role of badgers in bTB transmission and persistence. In Republic of Ireland (ROI), badger culling trials resulted in a significant decrease in cattle incidence in areas of badger culling compared to reference areas (13, 18, 19). In Britain, the Randomized Badger Cull Trial (RBCT) found evidence for decreased risk of bTB breakdown in proactive cull areas; however, *post-hoc* analysis suggested that a transitory increased risk to neighboring areas could occur (20). Using a case–control design, badger relative abundance in the vicinity of cattle herds was identified as an important risk factor for bTB herd breakdown risk in Britain (21) and Ireland (22). In addition, studies of road-killed badgers found strong evidence that badgers and cattle are colonized by the same *M. bovis* strain in the same area (23, 24). Most recently, genomic epidemiology has been used to understand transmission direction between species, generally suggesting that within-species transmission is more common than between-species transmission in study areas (25–28). The relative importance of cattle and badgers appears to be context specific (26, 28–30). Although these studies provide important insight that badger bTB is associated with cattle bTB, a quantitative understanding of how relative badger density impacts bTB transmission in this cattle and badger episystem is still lacking.

The main transmission routes of bTB are believed to be droplets, aerosols, and fecal to oral transmission (31). These three transmission mechanisms are intrinsically similar, involving an environmental vehicle such as droplets, aerosols, feces, urine, etc. *M. bovis*-laden droplets and aerosols may also settle onto pastures and contribute to the subsequent environment for oral transmission. The distinction between these transmission routes lies in the duration between the shedding moment and the time point of inhaling or ingesting *M. bovis*. Buddle et al. (32) have proposed a role for environmental transmission as an explanation for the variable efficacy observed in an overview of vaccine trials for the control of tuberculosis in cattle, wildlife, and humans. *Mycobacterium tuberculosis* complex (MTBC) has been demonstrated to be present at the wildlife–environment–livestock interface in Spain (33) and Italy (34), and more specifically, *M. bovis* has been detected in badger feces in the UK (35) and experimentally infected cattle (36). In recent global positioning system (GPS) studies, badgers barely have direct contact with cattle, suggesting that environmental transmission may indeed play an important role in bTB transmission (37, 38). However, to this point, the quantitative importance of bTB transmission via the environment has barely been considered (39).

Therefore, this study aims to gain a better understanding of the quantitative role of badgers and cattle in TB transmission via environmental transmission and quantify the impact of relative badger density on bTB transmission in a spatial context. With this information, we can identify high-risk areas for transmission where bTB might sustain locally and assess whether badger vaccination along with the test-and-removal strategy is sufficient to control transmission in different areas.

2. Materials and methods

In this study, we aim to understand the local transmission of bTB in a cattle and badger system. To this end, we develop an environmental transmission model that incorporates both within-herd/territory transmission and between-species transmission.

In Section 2.1, we present the structure of an environmental transmission model for the cattle and badger system. The model parameterisation, which is partially drawn from existing literature, is described in Section 2.2, and the estimation of transmission and decay rate parameters from time-series infection data is presented in Section 2.3. The infection data used in the estimation are explained in Section 2.4. With the estimated parameters, we use the next-generation matrix (NGM) method to calculate the basic reproduction ratio for the within-herd transmission and investigate the impact of the relative badger density on the within-herd R (Section 2.5.1). Furthermore, we use simulation to generate between-herd R maps (Section 2.5.2).

2.1. Model description

We developed a stochastic compartmental model with environmental transmission for a cattle and badger system. In this system, a herd of cattle and a social group of badgers refer to the animals of interest, whereas a farm and a badger territory each refer to a spatial unit. A farm is a spatial location for a herd, with all cattle in the herd registered to the same herd identifier. In Ireland, a farm can consist of several fragments of land that can be spatially dispersed, and we assume that cattle spend time on each fragment

proportionally to its area. A badger territory is an area where a social group of badgers primarily resides, which usually contains a main sett and several outlier setts. The model incorporates a geographic overlay of these two spatial units, where the between-species transmission and the spatial spread are assumed to occur.

2.1.1. A completely shared area with one farm and one badger sett territory

To explain this environmental transmission model, we first look at a conceptual spatial structure in a small local area where one farm and one badger territory are completely overlapping (Figure 1). In this local area, individual badgers from one social group and individual cattle from one herd share the same environment (light blue circle in Figure 1). Cattle, unvaccinated badgers, and vaccinated badgers are the three types of animals in the model, abbreviated as c, ub, and vb in subscripts. Vaccinated and unvaccinated badgers can exist in the same area because of the ongoing vaccination programme, and they are assumed to differ in terms of susceptibility but not infectivity (15). All individual animals are classified into three compartments: susceptible (S), latent (O), and infectious (I). Susceptible individuals can get infected by the same species or another species at a certain transmission rate after being exposed to *M. bovis*. When infection becomes established, animals can become infectious, although the length of the latent period is controversial. Infectious animals can shed *M. bovis* into the environment of their spatial units. We assume that *M. bovis* in the environment (denoted as E_c, E_b) are distributed evenly in the farm and the badger territory, which is the same area in this example (light blue circle in Figure 1). Since the vaccination is assumed not to reduce badgers' infectivity (15), the amount of *M. bovis* shed by infectious badgers is represented by compartment E_b, regardless of whether the infectious badgers are vaccinated or unvaccinated.

The transmission rate from cattle to cattle is $\beta_{c,c}S_c\frac{E_c}{N_c}$. The $\beta_{c,c}$ represents the cattle transmission rate parameter per contact with one unit of E_c per day. Here, we use cattle number N_c to represent the area size, hence for each susceptible bovine, the probability that the contact with E_c is made is equal to $\frac{E_c}{N_c}$. The same rules apply to all the other transmission rates. For example, the transmission rate from badger to cattle is $\beta_{b,c}S_c\frac{E_b}{N_c}$ in which the probability that the contact with E_b is made for each susceptible badger is $\frac{E_b}{N_c}$. We use one denominator in both cattle and badgers to have a unified representation of the area in this two-host system. In transmission rate parameter $\beta_{b,ub}$ and $\beta_{b,vb}$, we do not distinguish whether the infection source badger is vaccinated or unvaccinated (the first b of the subscript) because the vaccination is assumed not to reduce the infectivity, and environmental contamination from the vaccinated or unvaccinated badgers is not distinguished in E_b.

Infected animals (O compartment) can develop further into infectious state (I compartment) at a rate of $\lambda_c I_c$ and $\lambda_b I_b$. Infectious animals are removed at a rate of $\alpha_c I_c$ and $\alpha_b I_b$, caused by cattle test-and-removal and by bTB-induced badger death, respectively. We assume the background death rate parameters are equal to the birth rate of animals (α_c, α_b) and that all newborn animals are susceptible.

Infectious animals can shed *M. bovis* into the environment, where it subsequently decays. The shedding and decay of *M. bovis* are modeled deterministically as follows:

$$\frac{dE_{c(i)}}{dt} = \varphi I_{c(i)} - \mu E_{c(i)} \tag{1}$$

$$\frac{dE_{b(j)}}{dt} = \varphi I_{ub(j)} + \varphi I_{vb(j)} - \mu E_{b(j)} \tag{2}$$

where i, j denote the index for farm and badger territories, respectively. We assume that the decay of *M. bovis* has the same decay rate parameter μ despite the different infection source and strains (μE_c for cattle and μE_b for badgers). The shedding rate parameter φ is scaled as a function of the decay rate parameter ($\frac{\mu^2}{-1+e^{-\mu}+\mu}$) (40). The reason for this scaling is that the shedding rate parameter φ and the transmission rate parameter β are structurally not jointly identifiable from infection data (41). Therefore, we choose to fix the shedding rate parameters and estimate the different transmission rate parameters from infection data (more details in Eqs. 5, 6). With the standardization ($\varphi = \frac{\mu^2}{-1+e^{-\mu}+\mu}$), the transmission rate parameters represent the transmission rate from one typical infectious individual to a susceptible individual during one interval starting in a clean environment (40).

2.1.2. Many farms and many badger territories that partially overlap

We then consider the spatial structure of badger territories and farms in the full model. Badger territories can overlap with several farms; hence, badgers act as vectors that facilitate between-herd transmission. Similarly, herds can overlap with several badger territories and facilitate transmission between different badger social groups (Figure 2). To account for the spatial structure in the model, the exposure from the other species is weighted by the ratio of (the total area of overlap between farms and badger territories) and (the total area of farms or badger territories). The denominator in the transmission rate for badgers is also adjusted with the weighted cattle number as a representation of the badger territory area. The ordinary differential equation version of the transmission is presented in Eqs. 3, 4.

$$\frac{dO_{c(i)}}{dt} = \beta_{c,c}S_{c(i)}\frac{E_{c(i)}}{N_{c(i)}} + \beta_{b,c}S_{c(i)}\frac{\sum_{j=1,..k}E_{b(j)}\frac{A_{(ij)}}{AT_{(j)}}}{N_{c(i)}} - \lambda_c O_{c(i)} \tag{3}$$

$$\begin{aligned}\frac{dO_{b(j)}}{dt} = {} & VC\left(\beta_{b,vb}S_{vb(j)}\frac{E_{b(j)}}{\sum_{i=1..m}N_{c(i)}\frac{A_{(ij)}}{AF_{(i)}}}\right.\\ & \left.+\beta_{c,vb}S_{vb(j)}\frac{\sum_{i=1,..m}E_{c(i)}\frac{A_{(ij)}}{AF_{(i)}}}{\sum_{i=1..m}N_{c(i)}\frac{A_{(ij)}}{AF_{(i)}}}\right)\\ & +(1-VC)\left(\beta_{v,ub}S_{ub(j)}\frac{E_{b(j)}}{\sum_{i=1..m}N_{c(i)}\frac{A_{(ij)}}{AF_{(i)}}}\right.\\ & \left.+\beta_{c,ub}S_{ub(j)}\frac{\sum_{i=1,..m}E_{c(i)}\frac{A_{(ij)}}{AF_{(i)}}}{\sum_{i=1..m}N_{c(i)}\frac{A_{(ij)}}{AF_{(i)}}}\right) - \lambda_b O_{b(j)}\end{aligned} \tag{4}$$

Farms and badger territories are the two spatial units in the model where i, j denote the index for farm and badger territories, respectively. $A_{(ij)}$ denotes the total area of overlap between farm

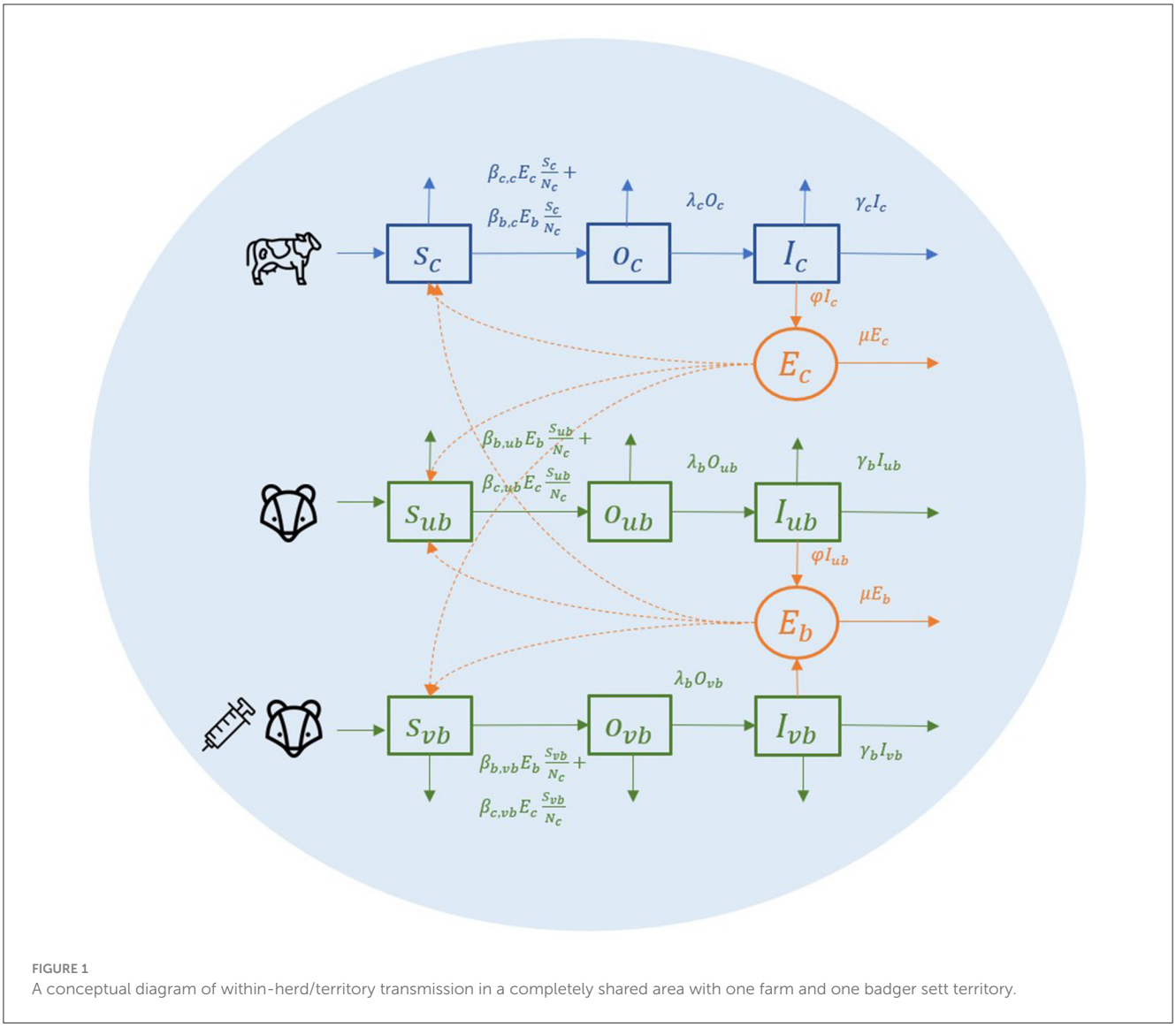

FIGURE 1
A conceptual diagram of within-herd/territory transmission in a completely shared area with one farm and one badger sett territory.

i and territory j. $\frac{A_{(ij)}}{AF_{(i)}}$ represents the proportion of farm i that overlaps with territory j. Similarly, $\frac{A_{(ij)}}{AT_{(j)}}$ is the proportion of territory j that overlaps with farm i.

Cattle on farm i can get infected by *M. bovis* on the farm excreted by cattle ($E_{c(i)}$) at rate $\beta_{c,c}S_{c(i)}\frac{E_{c(i)}}{N_{c(i)}}$ or excreted by badgers whose territories overlap with the farm i at rate $\beta_{b,c}S_{c(i)}\frac{\sum_{j=1..k}E_{b(j)}\frac{A_{(ij)}}{AT_i}}{N_{c(i)}}$. Multiple badger territories ($j = 1..k$) can overlap with farm i, so the contribution from these territories ($j = 1..k$) are summed. For each territory j, only the part of the territory that is located inside farm i can pose a threat on infecting cattle; hence, each $E_{b(j)}$ is adjusted to $E_{b(j)}\frac{A_{(ij)}}{AT_i}$.

Similarly, badgers can get infected by badgers in their own territory j or by cattle in farms that overlap with j. As mentioned in Section 2.1.1, we use a unified representation of the area, namely the number of cattle in that area. Therefore, the area of badger territory is represented by the weighted number of cattle as $\sum_{i=1..m}N_{c(i)}\frac{A_{(ij)}}{AF_{(i)}}$, as territory j overlaps with different farms ($i = 1..m$). A proportion of the badgers are vaccinated, denoted as VC (vaccination coverage). Vaccinated badgers are assumed to have reduced susceptibility but the same infectivity as the unvaccinated badgers. Therefore, transmission from infectious badgers to vaccinated badgers is modeled as $(VC)\beta_{b,vb}S_{vb(j)}\frac{E_{b(j)}}{\sum_{i=1..m}N_{c(i)}\frac{A_{(ij)}}{AF_{(i)}}}$ and transmission from infectious badgers to unvaccinated badgers as $(1-VC)\beta_{b,ub}S_{ub(j)}\frac{E_{b(j)}}{\sum_{i=1..m}N_{c(i)}\frac{A_{(ij)}}{AF_{(i)}}}$. For cattle-to-badger transmission, only part of farm i is located inside the badger territory j, so $E_{c(j)}$ is adjusted with $E_{c(j)}\frac{A_{(ij)}}{AF_i}$. Therefore, the cattle-to-badger transmission rate is denoted as $(VC)\beta_{c,vb}S_{vb(j)}\frac{\sum_{i=1,..m}E_{c(i)}\frac{A_{(ij)}}{AF_{(i)}}}{\sum_{i=1..m}N_{c(i)}\frac{A_{(ij)}}{AF_{(i)}}}$ for vaccinated badgers and $(1-VC)\beta_{c,ub}S_{ub(j)}\frac{\sum_{i=1,..m}E_{c(i)}\frac{A_{(ij)}}{AF_{(i)}}}{\sum_{i=1..m}N_{c(i)}\frac{A_{(ij)}}{AF_{(i)}}}$ for unvaccinated badgers.

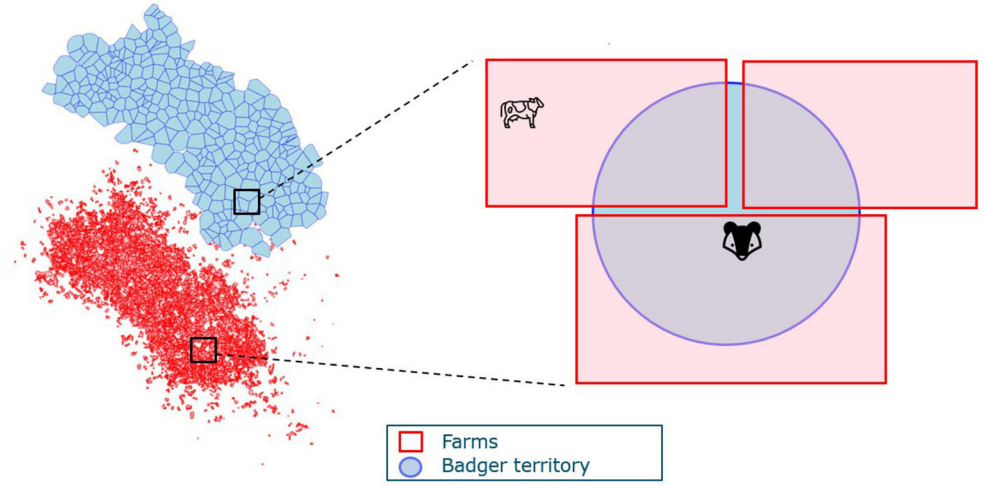

FIGURE 2
An example of the spatial structure of farms and badger territories. The blue map represents badger territories, and the red irregular shapes delineate farm boundaries.

2.2. Model parameterisation

There are 14 parameters in this model. Six model parameters were estimated from the literature (Table 1). The details on the explanation and references for those parameters can be found in Supplementary Table 1. In addition, the transmission rate and decay rate parameters of *M. bovis* in the environment are estimated by fitting time-series infection data into a dose–response function (Section 2.3).

TABLE 1 Model parameters.

Parameter	Description	Value
$\beta_{c,c}$	Transmission rate parameter from cattle to cattle	Estimated
$\beta_{b,c}$	Transmission rate parameter from badges to cattle	Estimated
$\beta_{b,ub}\frac{N_b}{N_c}$	Transmission rate parameter from badger to unvaccinated badger	Estimated
$\beta_{c,ub}\frac{N_b}{N_c}$	Transmission rate parameter from cattle to unvaccinated badger	Estimated
$\beta_{b,vb}\frac{N_b}{N_c}$	Transmission rate parameter from badger to vaccinated badger	Estimated
$\beta_{c,vb}\frac{N_b}{N_c}$	Transmission rate parameter from cattle to vaccinated badger	Estimated
φ	The shedding rate parameter of *M. bovis*	Standardized
μ	*M. bovis* decay rate parameter	Estimated
$\frac{1}{\gamma_c}$	Infectious period for cattle	101 days
$\frac{1}{\gamma_b}$	Infectious period for badgers	365 days
$\frac{1}{\lambda_c}$	Latent period for cattle	1.8 days
$\frac{1}{\lambda_b}$	Latent period for badgers	90 days
α_c	The cattle background death rate	9.13e-4 day^{-1}
α_b	The badger natural death rate	7.52e-4 day^{-1}

2.3. Statistical analysis

We estimate transmission rate and decay rate parameters by fitting time-series infection data into the model. The core of this method is to relate exposure to hazards and the hazards to the infection probability (40). We first reconstruct the exposure in this two-host environmental transmission model (Section 2.3.1) and then fit the cattle and badger infection data and exposure to the statistical model to estimate transmission rate and decay rate parameters (Section 2.3.2).

2.3.1. Reconstruction of exposure

From Eq. 1, we derive the environmental contamination ($E(t)$) as a function of time and the number of infectious individuals (Eq. 5). The exposure to the environmental contamination during one time interval is the integral of $E(t)$ as $\int_0^1 E\left(t|I_t, E_0\right)$ (Eq. 4).

$$E\left(t|I_t, E_0\right) = \frac{(1 - {}^{-t\mu})\mu}{-1 + {}^{-\mu} + \mu} I_t + {}^{-t\mu} E_0 \tag{5}$$

$$\int_0^1 E\left(t|I_t, E_0\right) = I_t + \frac{1 - {}^{-\mu}}{\mu} E_0 \tag{6}$$

Here, E_0 denotes the environmental contamination of at the start of an interval and I_t denotes the number of infectious individuals (cattle or badgers) during this interval. These equations were used to construct E_c and E_b and exposure by integrating each farm and territory.

2.3.2. Likelihood function

The number of new cases over each observation time interval $(\tau, \tau + \Delta)$ follows a binomial distribution with a binomial total susceptible individual number at each time interval. The

probability used in the binomial distribution is the probability of getting infected. From Eqs. 3, 4, the probability of getting infected can be derived for cattle and badgers, respectively, as follows:

$$P_c = 1 - e^{-}(\beta_{c,c}\frac{\int_{\tau}^{\tau+\Delta} E_{c(i)}(t|I_{c(i)\tau}, E_{c(i)}(\tau))dt}{N_{c(i)}} + \beta_{b,c}\frac{\sum_{j=1..k}(\int_{\tau}^{\tau+\Delta}(E_{b(j)}(t|I_{b(j)\tau}, E_{b(j)}(\tau))^{*}\frac{A_{(ij)}}{AT_{(j)}})dt)}{N_{c(i)}} \quad (7)$$

$$P_{ub} = 1 - e^{-}(\beta_{b,ub}\frac{\int_{\tau}^{\tau+\Delta} E_{b(j)}(t|I_{b(j)\tau}, E_{b(j)}(\tau))dt}{\sum_{i=1..m} N_{c(i)}\frac{A_{(ij)}}{AF_{(i)}}} + \beta_{c,ub}\frac{\sum_{i=1..m}(\int_{\tau}^{\tau+\Delta}(E_{c(i)}(t|I_{c(i)\tau}, E_{c(i)}(\tau))^{*}\frac{A_{(ij)}}{AF_{(i)}})dt)}{\sum_{i=1..m} N_{c(i)}\frac{A_{(ij)}}{AF_{(i)}}}) \quad (8)$$

$$P_{vb} = 1 - e^{-}(\beta_{b,vb}\frac{\int_{\tau}^{\tau+\Delta} E_{b(j)}(t|I_{b(j)\tau}, E_{b(j)}(\tau))dt}{\sum_{i=1..m} N_{c(i)}\frac{A_{(ij)}}{AF_{(i)}}} + \beta_{c,vb}\frac{\sum_{i=1..m}(\int_{\tau}^{\tau+\Delta}(E_{c(i)}(t|I_{c(i)\tau}, E_{c(i)}(\tau))^{*}\frac{A_{(ij)}}{AF_{(i)}})dt)}{\sum_{i=1..m} N_{c(i)}\frac{A_{(ij)}}{AF_{(i)}}}) \quad (9)$$

where $I_{c(i)\tau}$, $I_{ub(j)\tau}$, and $I_{vb(j)\tau}$ represent the I_c at farm I, I_{ub}, and I_{vb} at territory j at the beginning of $(\tau, \tau+)$. $I_{c(i)\tau}$, $I_{ub(j)\tau}$, and $I_{vb(j)\tau}$ are integers and change discretely in jumps of 1. $E_{c(i)}(\tau)$ and $E_{b(j)}(\tau)$ represent $E_{c(i)}$ and $E_{b(j)}$ at time τ. $E_{c(i)}(\tau)$ and $E_{b(j)}(\tau)$ change continuously.

The likelihood as a function of transmission rate parameters and decay rate parameters is given by:

$$\mathcal{L}(\theta) = \prod_{x}(P)^{cases_x}(1-P)^{(S_x - cases_x)} \quad (10)$$

where P represents either P_c, P_{ub}, or P_{vb} from Eqs. 7–9.

2.4. Data

The infection data and geographic data for cattle and badgers are extracted to quantify parameters as described in Section 2.3. The new cases in each observation interval are used to calculate the probability of infection in each interval in Eqs. 7–9 and the prevalence at the beginning of each observation time interval in each spatial unit is used to reconstruct the exposure as described in Section 2.3.

2.4.1. Badger data

The badger vaccination trial ran from 2009 to 2013 in the Kilkenny area (42). A 750 km^2 study area was divided into three zones (A, B, and C) from north to south. Badger setts were identified and their locations recorded. Badgers were captured using cages or restraints. Blood samples were collected at each capture and tested using enzyme-linked immunosorbent assay (ELISA; (43)). Captured badgers were assigned to the sett closest to where they were trapped, with most captures taking place directly outside sett entrances. All the captured badgers in Zone A and 50% of the captured badgers in Zone B received a placebo. Half of the captured badgers in Zone B and all the captured badgers in Zone C received oral BCG vaccine (Danish strain 1331, at dose 10^8 cfu).

Details of the badger infection dataset from the vaccination trial and the location of badger territories were described elsewhere (15, 44). In total, there were 1759 trapping records. Each record contains the information from the trapping of a single badger: badger ID, sett ID, infection status, date of examination, vaccine status, date of vaccination, vaccine code, etc. From all the trapping records, we extracted 440 pairs of trapping records from badgers that were captured more than once. Each pair of capture records consists of two examination results, namely the serology status at the beginning and the end of the interval, with the infectious status being negative at the beginning. Each pair of capture records has an outcome of 0 or 1 infection, which can be used to calculate the probability of infection during an interval, namely P_{ub} and P_{vb} in Eqs. 7–9.

In addition, the number of infectious badgers in each territory j at the time x ($I_{b(j)x}$) is needed on the right side of Eqs. 7–9. We calculated $I_{b(j)x}$ by multiplying the badger bTB prevalence by the number of badgers per territory. The number of badgers per territory was calculated using the minimum number alive. Badger prevalence was calculated from 1759 trapping results. The spatial and temporal resolution in the model is at territory and day levels, while the data are limited compared to the resolution in this model. Therefore, we fitted badger bTB prevalence at the territory level at different time points with several generalized additive models (GAMs) and then used the best-fitting GAM to predict the badger bTB prevalence for each day in each territory (see details about the GAMs in Supplementary Table 3). In addition, a sensitivity analysis was conducted to assess the impact of uncertainty in badger prevalence on the parameter estimation (see Supplementary Table 4).

2.4.2. Cattle data

Cattle data were extracted from the Animal Health Computer System (AHCS) dataset and the Land Parcel Identification System (LPIS) of the Irish Government's Department of Agriculture, Food and the Marine (DAFM). The AHCS dataset comprises bTB test records on more than 98% of herds, including single intradermal comparative tuberculin test (SICTT), interferon-gamma array, ELISA test, and slaughterhouse inspection results. Herds are tested by the SICTT at least once a year. In this study, the sensitivity of tests was assumed to be 100%. Positive cattle are removed within 2–4 weeks of testing by staff from DAFM. In the AHCS dataset, each record consists of the number of cattle tested, the date of the test, the type of the test, the number of positive cattle, the number of inconclusive cattle, etc. When there are inconclusive tests in the herd, field veterinarians re-test the cattle or the herd within 3 months. From 2009 to 2013, there were 6787 test records from 1335 herds in this badger vaccination trial area. In all these events, 696 records from 390 herds were positive. In each data line, the number of new cases in a herd during an interval is the P_c in Eq. (7). The number of infected animals at the start of the interval time x ($I_{c(i)\tau}$) is used to construct the exposure (right side of Eq. 7).

The LPIS dataset delineates the land parcels making up each farm. Many Irish farms consist of several land fragments (45–47).

For historic and topological reasons, the extent of fragmentation varies within Ireland. In the region of this study, approximately 20% of farms are single-fragment farms. The remaining 80% of farms have an average of five fragments, with a mean distance between same-farm fragments of 3.3 km. The movement within a herd but amongst different fragments was not recorded. Therefore, we assume that the time cattle spend on each fragment is proportional to the area of the fragment.

2.5. Basic reproduction ratio

2.5.1. Within-herd R

The next-generation matrix (NGM) is a commonly used method to derive the basic reproduction ratio for a compartmental model (48). With the estimated transmission and decay rate parameters, we can calculate the basic reproduction ratio for this cattle badger system in a theoretical local area as: $\begin{bmatrix} R_{c,c}, R_{b,c} \\ R_{c,b}\frac{N_b}{N_c}, R_{b,b}\frac{N_b}{N_c} \end{bmatrix}$, where

$$
\begin{aligned}
R_{c,c} &= \frac{\beta_{c,c}\mu}{(-1+e^{-\mu}+\mu)}\frac{\lambda_c}{(\alpha_c+\lambda_c)}\frac{1}{\alpha_c+\gamma_c} \\
R_{b,c} &= \frac{\beta_{c,c}\mu}{(-1+e^{-\mu}+\mu)}\frac{\lambda_c}{(\alpha_c+\lambda_c)}\frac{1}{\alpha_b+\gamma_b} \\
R_{c,b} &= VC^*\left(\frac{\beta_{c,vb}\mu}{(-1+e^{-\mu}+\mu)}\frac{\lambda_b}{(\alpha_b+\lambda_b)}\frac{1}{\alpha_c+\gamma_c}\right) \\
&\quad + (1-VC)^*\frac{\beta_{c,ub}\mu}{(-1+e^{-\mu}+\mu)}\frac{\lambda_b}{(\alpha_b+\lambda_b)}\frac{1}{\alpha_c+\gamma_c} \\
R_{b,b} &= VC^*\frac{\beta_{b,vb}\mu}{(-1+e^{-\mu}+\mu)}\frac{\lambda_b}{(\alpha_b+\lambda_b)}\frac{1}{\alpha_b+\gamma_b} \\
&\quad + (1-VC)^*\frac{\beta_{b,ub}\mu}{(-1+e^{-\mu}+\mu)}\frac{\lambda_b}{(\alpha_b+\lambda_b)}\frac{1}{\alpha_b+\gamma_b}.
\end{aligned}
$$

$\frac{N_b}{N_c}$ represents the relative badger density compared to cattle in a local area. We used this term rather than the term relative abundance because in our model, N_c is a proxy of the area under consideration, with the implicit assumption that cattle density is spatially uniform. Thus, the relative badger density cannot be reduced by simply increasing the number of cattle, as such an increase would mean an enlargement of the land area. *VC* represents the vaccination coverage, and (1 – *VC*) represents the proportion of unvaccinated badgers. We use $VC = 0\%$ and 100% to calculate the partial reproduction ratio in unvaccinated and fully vaccinated areas. The largest eigenvalue of this matrix is the basic reproduction ratio within this local area, which is derived as follows:

$$
\begin{aligned}
R = \frac{1}{2}\left(R_{c,c} + R_{b,b}\frac{N_b}{N_c}\right) \\
+ \frac{1}{2}\sqrt{\left(R_{c,c} + R_{b,b}\frac{N_b}{N_c}\right)^2 - 4\left(R_{c,c}R_{b,b}\frac{N_b}{N_c} - R_{c,b}R_{b,c}\frac{N_b}{N_c}\right)} \quad (11)
\end{aligned}
$$

R represents the average number of new infections per case within this isolated local area, such as a farm with a badger territory lying completely inside the farm.

However, in reality, badgers' territories connect multiple local areas. Badgers act as vectors in the sense that they get infected by one herd and transmit infection to cattle in other herds. When an infectious bovine is introduced to a herd or an infectious badger comes into contact with a herd, there is a risk that infection will be spread to neighboring herds by badgers. To control bTB spread, we need to evaluate both within- and between-herd transmission.

2.5.2. Between-herd R

The average number of neighboring herds infected by a single newly infected farm is denoted by the between-herd R. To calculate the between-herd R, a stochastic metapopulation model for each herd and its neighboring herds was developed with the same model structure as described in Figure 1 using the SimInf package in R (49). All the infection and vital dynamic processes are modeled stochastically using the Gillespie Algorithm, while *M. bovis* dynamic shedding and decay in the environment are modeled deterministically in Eqs. 1, 2. The spatial structure was accounted for according to Eqs. 3–4. In the Kilkenny area, there are a total of 1335 herds. For each herd, we simulated the transmission between the herd itself, the connected badger territories, and the herds that are directly connected (i.e. those that share a connected badger territory with the initial herd). In total, 1335 different spatial configurations were simulated, each with 200 repetitions.

Parameter estimations obtained in the analyses in Sections 2.2 and 2.3 were used in this simulation. In the initial state, one infectious bovine is introduced to a herd. Badgers are considered fully susceptible, and there is no contamination in the environment. The resulting distribution for the number of infected neighboring herds represents the between-herd R distribution. The average number of infected herds is the between-herd R.

3. Results

3.1. Parameter estimations

The decay rate parameter is estimated as 0.0039 day^{-1} with CI (0.0036, 0.0041), which means the half-life of *M. bovis* is 178 days, ranging from 169 to 192 days. Transmission rate parameters are estimated with a unit of per day for one infectious individual (Table 2). In addition, our parameter estimation is robust across the varying assumptions used to calculate badger prevalence (Supplementary Table 4).

We transform β_{cc} to a yearly rate per infected individual $(\frac{\beta_{cc}\mu}{(-1+e^{-\mu}+\mu)})^*365)$ for comparison with other transmission models that use direct contact assumptions. One infectious bovine can infect on average 1.97 cattle per year in a fully susceptible herd with CI (1.82, 1.97). This estimation is slightly lower than estimations in New Zealand, the Netherlands, and Argentina, ranging from 2.2 to 5.2 per year (50–53).

The transmission rate parameter for badgers ($\beta_{b,vb}$, $\beta_{c,vb}$, $\beta_{b,ub}$, and $\beta_{c,vb}$) need to be interpreted with a multiplication of the local relative badger density (see NGM in Section 2.5), hence they cannot be directly compared with transmission rate parameters for cattle ($\beta_{c,c}$, $\beta_{b,c}$). For example, in an area with $\frac{N_b}{N_c} = 0.01$, an infectious

TABLE 2 Parameter estimation.

Parameter	Estimation (per day per E unit)	CI	Transformed value (per individual per year)	CI
$\beta_{c,c}$	1.01e-5	(9.7e-6, 1.07e-5)	1.89	(1.82, 1.97)
$\beta_{b,c}$	3.977e-6	(3.78e-6, 4.19e-6)	0.756	(0.71, 0.78)
$\beta_{b,vb}\frac{N_b}{N_c}$	5.14e-5 $\frac{N_b}{N_c}$	(3.34e-5, 7.28e-5) $\frac{N_b}{N_c}$	9.63 $\frac{N_b}{N_c}$	(6.26, 13.64) $\frac{N_b}{N_c}$
$\beta_{c,vb}\frac{N_b}{N_c}$	4.43e-4 $\frac{N_b}{N_c}$	(2.64e-4, 6.62e-4) $\frac{N_b}{N_c}$	82.95 $\frac{N_b}{N_c}$	(49.62, 124.07) $\frac{N_b}{N_c}$
$\beta_{b,ub}\frac{N_b}{N_c}$	9.19e-5 $\frac{N_b}{N_c}$	(6.44e-5, 1.23e-4) $\frac{N_b}{N_c}$	17.22 $\frac{N_b}{N_c}$	(12.09, 23.21) $\frac{N_b}{N_c}$
$\beta_{c,ub}\frac{N_b}{N_c}$	5.07e-4 $\frac{N_b}{N_c}$	(2.98e-4, 7.62e-4) $\frac{N_b}{N_c}$	95.13 $\frac{N_b}{N_c}$	(55.83, 142.87) $\frac{N_b}{N_c}$

bovine can infect on average 0.95 unvaccinated badgers per year with CI (0.56, 1.42).

3.2. Within-herd R

In an isolated farm that does not connect to other farms, the within-herd R can be derived based on the methods presented in Section 2.5.1. When badgers are unvaccinated, the NGM for this farm is $\begin{bmatrix} 0.49, 0.59 \\ 22.11\frac{N_b}{N_c}, 14.04\frac{N_b}{N_c} \end{bmatrix}$, where $\frac{N_b}{N_c}$ represents the relative badger density in the farm. When badgers are vaccinated, the NGM is $\begin{bmatrix} 0.49, 0.59 \\ 20.02\frac{N_b}{N_c}, 8.22\frac{N_b}{N_c} \end{bmatrix}$. When an infectious bovine is introduced on this isolated farm, it will infect 0.49 cattle on average during its infectious period. In comparison, when an infectious badger is introduced, it will infect, on average, 0.59 cattle. The shorter infectious period of cattle than badgers leads to a smaller R_{cc} than R_{bc}. However, a relaxation of the test-and-removal strategy will lead to a longer cattle infectious period and thus increase R_{cc}.

The number of infected badgers in this system depends on the relative badger density ($\frac{N_b}{N_c}$). In addition, the impact of badger vaccination on within-herd R depends on the $\frac{N_b}{N_c}$. For example, in a herd with 100 cattle and three unvaccinated badgers, the within-herd R for this local area is 1.08. If all badgers are vaccinated in this local area, the within-herd R is 0.97 (Figure 3). For example, to control R <1 within an isolated area that accommodates 100 cattle, the relative badger density should be less than 2.5 unvaccinated badgers or 3.2 vaccinated badgers. As the relative badger density and the system R are highly correlated (with a correlation coefficient of 0.999), we fit them into a linear regression. In estimated linear relationships, R increases by 0.134 when the $\frac{N_b}{N_c}$ increases by 0.01 in an unvaccinated area. With all the badgers being vaccinated, this increase in R per 0.01 $\frac{N_b}{N_c}$ is reduced to 0.084.

3.3. Between-herd R

In real life, herds are not isolated but connected with each other by badger territories. Even if each isolated area has an R below 1, bTB might still spread from one local area to another. Therefore, we used simulations to calculate the average number of herds that get infected if an infectious bovine is introduced or tested positive in an index herd.

In between-herd R maps (Figure 4), herds in yellow are expected to spread bTB to fewer than 1 neighboring herd, while herds in orange and red are expected to spread to more than 1 neighboring herd. Red areas are mostly clustered on the north and east sides of the study area due to the higher density of badgers. Some sporadic red dots lie in the yellow area because of the farm fragmentation, where high R herds have some land parcels in the low R herd clusters. By comparing the two maps, vaccination reduces the average between-herd R from 1.21 to 0.85. It is worth noting that the average between-herd R is being used to allow a quantitative comparison between maps but does not infer the bTB persistence in a whole area. Despite a 10% decrease in herds with R >1, there are still 30% of herds that can transmit bTB to more than 1 herd with the badger vaccination (Figure 4).

4. Discussion

The quantification of bTB transmission between wildlife and cattle is critical for efforts to eradicate bTB. In Ireland and the UK, recent studies have provided evidence that badgers are involved in maintaining bTB transmission; however, a quantitative understanding of how relative badger density influences transmission in this cattle and badger episystem has so far been lacking. To address this gap, this study quantifies the role of badgers, cattle, and the environment in bTB transmission and disentangles how relative badger density may contribute to the spatial heterogeneity in bTB transmission. To achieve this objective, we developed a novel environmental transmission model that incorporates both within-herd/badger territory transmission and between-species transmission. This approach is guided by the overlap of badger territories with cattle herds.

In this two-host transmission system, the partial reproduction ratio R_{bc} is higher than R_{cc}. This is because badgers likely remain infectious for a longer period than cattle, given the test and removal policy in cattle in place. Therefore, any relaxation of the test-and-removal policy can lead to higher R_{cc}. The partial reproduction ratios $R_{c,(u/v)b}\frac{N_b}{N_c}$ and $R_{b,(u/v)b}\frac{N_b}{N_c}$ depend on the local relative badger density ($\frac{N_b}{N_c}$). As a result, we quantified the relationship between local relative badger density and the R for the system. In unvaccinated areas, within-herd R increases by 0.134 for every 0.01 increase in the $\frac{N_b}{N_c}$. This increase is reduced to 0.084 per 0.01 increase in $\frac{N_b}{N_c}$ when badgers are vaccinated.

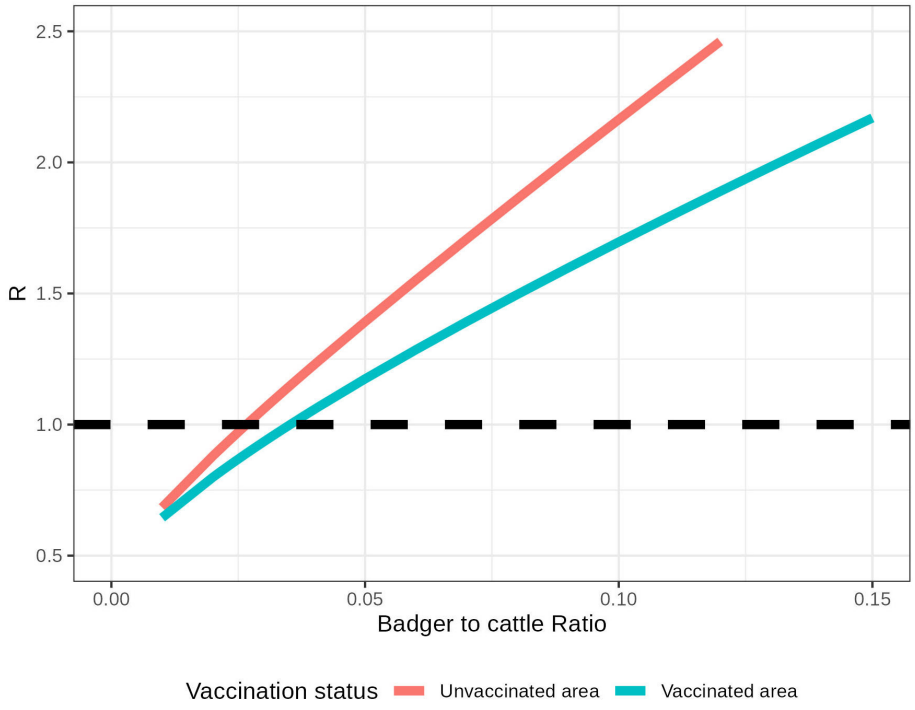

FIGURE 3
Within-herd R in an isolated herd with different relative badger densities (Nb/Nc). The pink line represents the within-herd R without badger vaccination, and the blue line represents the within-herd R with badger vaccination. The black dashed line represents the threshold R = 1.

Our transmission model adopts a single transmission route, incorporating an environmental compartment. We simplified droplet, aerosol, fecal to oral transmissions to one environmental transmission route as they are intrinsically similar. The primary distinction lies in the duration between the shedding moment and the time point of inhaling or ingesting *M. bovis*. Shortly after being shed into the environment, *M. bovis* cells may pose an infection risk to other animals. This infection risk decreases over time because viable *M. bovis* decays over time in the environment. We unified these three transmission routes into one and assumed an exponential decay of *M. bovis* with a specified decay rate. This unification simplifies the model structure while still capturing the significance of historic infections. In addition, badger-to-badger transmission via biting may represent a secondary route of infection, which has not been considered in this study. Previous studies have shown that transmission via biting can cause a more rapid and progressive infection with generalized pathology (54). The simplification of transmission routes might lead to an underestimation of badger-to-badger transmission and an overestimation of cattle-to-badger transmission. However, it is not our goal to distinguish badger infection via biting or the other three mechanisms, as the data to distinguish the contribution of different mechanisms are lacking.

Previous studies on the within-herd transmission of bTB have exploited either frequency- or density-dependent models (50, 52, 55). A study in US dairy herds found that a frequency-dependent model can predict risk significantly better than a density-dependent model (55). Additionally, Conlan et al. (56) measured the strength of the density dependence of transmission and found a non-linear dependence with herd size. Therefore, our model adopts a frequency-dependent model and uses the number of cattle as a proxy for the area in transmission rates (Eqs. 3, 4). This approximation is valid in areas where badger territories and farms dominate a significant portion of the region frequented by badgers, as in this study area. However, when a significant portion of the region consists of woodlands, rivers, and urban areas, it becomes crucial to modify this proxy. This adjustment is necessary to avoid underestimating the denominator in the badger-to-badger transmission rate, which could otherwise result in an overestimation of the badger-to-badger transmission rate parameter. In addition to using cattle numbers as a proxy for area, one can consider alternative denominators such as the number of badgers or the sum of cattle and badgers. Our assessment showed that models with Nc or Nc + Nb as the denominator in the transmission rates (in Eqs. 3, 4) provided similar results in fitting the data (see Supplementary Table 5).

The significance of the environment in the transmission of *M. bovis* is emphasized in our model, which estimates a half-life of 6 months. Our estimation of the half-life of *M. bovis* in the environment is five times higher in comparison to other modeling studies (39), although still within the range of experimental studies (31, 57). We also conducted a sensitivity analysis of the decay rate using the estimates from (39). A shorter survival time of *M. bovis* can lead to an increase in transmission rate parameters, but the outcome of this study with respect to NGM, R, and the threshold for relative badger density remain largely unaffected (see Supplementary Table 6).

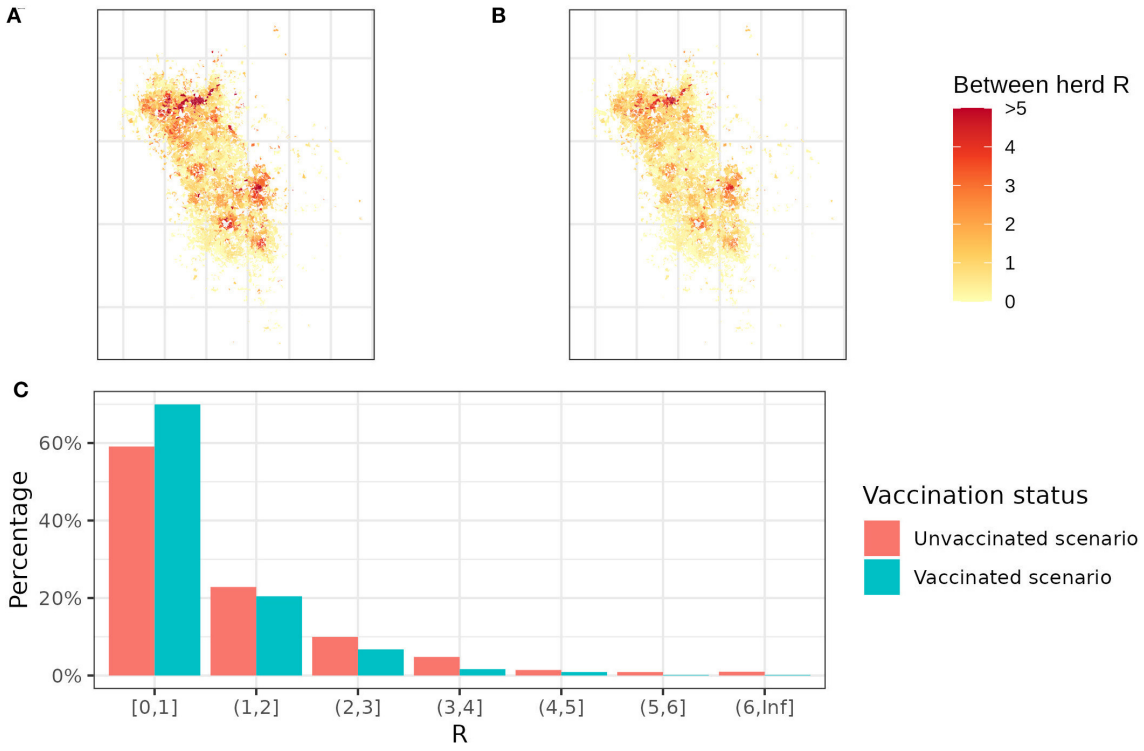

FIGURE 4
Between-herd R maps and R distribution. **(A)** The between-herd R map without any badger vaccination and **(B)** the between-herd R map with 100% vaccination coverage. Yellow herds represent between-herd R below 1, while orange and red herds represent between-herd R >1. **(C)** The distribution of between-herd R with and without badger vaccination. Each bar represents the percentage of herds falling within a specific between-herd R range. For example, the first bar indicates that 70% of herds have between-herd R < 1 in the vaccination scenario and 60% in the un-vaccination scenario.

The parameters defining the duration of intermediate stages of the disease (latent periods) were derived from the literature (see Supplementary Table 1). We did not estimate them from infection data because previous modeling studies have not been able to distinguish models with differing assumptions regarding these intermediate stages (SORI or SOR model) based on model fit (56). The most debated parameter is the latent period for cattle. Conventionally, it is believed that *M. bovis* can cause a long latent period similar to human TB. However, an animal challenge study showed that acute infection may occur (58). In addition, a recent review also suggests that *M. bovis* can frequently cause acute infection in cattle (59). Therefore, we also assume a short latent period for cattle. In this model, assuming a different latent period for cattle or badgers would impact the transmission rate parameter estimates. However, such a variation would not influence the values for R and NGM since the modifications to these β and λ would counterbalance each other within the R formula as described in Section 2.5.1. In addition, the sensitivity of tests for cattle and badgers is assumed to be perfect in this model. Infected but undetected animals shed *M. bovis*, which causes an underestimation of environmental contamination. On the other hand, these hidden infections cause an underestimation of the new cases. Both the left and right sides of Eqs. 7–9 were underestimated, whose effects are likely to be canceled out and therefore have a limited impact on the transmission rate parameter.

In this model, cattle and badgers are assumed to spend their time homogenously distributed within their spatial units. This is a simplification of reality, as some parcels of farms might not be used for grazing, or not all of the time, and badgers may spend more time near setts than elsewhere in their territories. However, as cattle and badger numbers and infection data are available at the farm and territory level, we used this as the spatial resolution for our model. Within-farm and within-territory heterogeneity might lead to an underestimation of the actual densities at the location of an infected animal, which in turn leads to an underestimation of the within-herd R by the model. However, heterogeneity in densities may also lead to less overlap in areas used by cattle and badgers, which would have the opposite effect. In addition, the assumption that animals are restricted to their spatial units, might attribute movement-mediated transmission to between-species transmission in the model. This can result in an overestimation of the between-species transmission. Future studies could relax this assumption and capture the effect of cattle movements using detailed cattle movement data.

In conclusion, this model disentangles the quantitative relationship between relative badger density and local transmission risks. Estimating transmission rate parameters improves our

understanding of badgers as a vector in this two-host system. In addition, the model produces the first between-herd R map for bTB considering badger, cattle, and environment. These R maps identify high-risk areas as clusters of farms with between-herd R >1 and demonstrate how relative badger density determines the local transmission risk. Our results suggest that badger vaccination can maximally reduce the average between-herd R in Kilkenny to 0.85; however, despite this, 30% of herds will still have an R value >1 and so, if infected, have a high potential risk of transmitting bTB to their neighbors. Whether these 30% of herds with a high between-herd R can sustain the bTB spread in a large area, such as the whole Kilkenny area, is unknown and requires further research.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: GitHub; https://git.wur.nl/chang025/btb_transmission_and_r_map.

Author contributions

YC, NH, and MdJ contributed to the conception and design of the study. YC performed the statistical analysis, interpreted the results, and drafted the manuscript. NH and MdJ participated in the data analysis and manuscript preparation. AB, EG, GM, and JT organized and provided datasets for this study. PB, SM, AB, EG, GM, and JT critically revised the manuscript. All authors contributed to the manuscript revision and approved the submitted version.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1233173/full#supplementary-material

References

1. O'Reilly LM, Daborn CJ. The epidemiology of Mycobacterium bovis infections in animals and man: a review. *Tubercle Lung Dis.* (1995) 76:1–46. doi: 10.1016/0962-8479(95)90591-X

2. Naranjo V, Gortazar C, Vicente J, de La Fuente J. Evidence of the role of European wild boar as a reservoir of Mycobacterium tuberculosis complex. *Vet Microbiol.* (2008) 127:1–9. doi: 10.1016/j.vetmic.2007.10.002

3. Nigsch A, Glawischnig W, Bago Z, Greber N. Mycobacterium caprae infection of red deer in western austria-optimized use of pathology data to infer infection dynamics. *Front Vet Sci.* (2018) 5:350. doi: 10.3389/fvets.2018.00350

4. Palmer MV. Mycobacterium bovis: characteristics of wildlife reservoir hosts. *Transbound Emerg Dis.* (2013) 60:1–13. doi: 10.1111/tbed.12115

5. Etchechoury I, Valencia GE, Morcillo N, Sequeira MD, Imperiale B, López M, et al. Molecular typing of mycobacterium bovis isolates in argentina: first description of a person-to-person transmission case. *Zoonoses Public Health.* (2010) 57:375–81. doi: 10.1111/j.1863-2378.2009.01233.x

6. Srinivasan S, Easterling L, Rimal B, Niu XM, Conlan AJK, Dudas P. Prevalence of bovine tuberculosis in india: a systematic review and meta-analysis. *Transbound Emerg Dis.* (2018) 65:1627–40. doi: 10.1111/tbed.12915

7. Waters WR, Palmer MV, Buddle BM, Vordermeier HM. Bovine tuberculosis vaccine research: historical perspectives and recent advances. *Vaccine.* (2012) 30:2611–22. doi: 10.1016/j.vaccine.2012.02.018

8. Committee of Public Accounts. *Examination of the 2019 Appropriation Account for Vote 30 – Agriculture, Food and the Marine, and related financial matters. 33/CPA/015.* (2022). Available online at: https://data.oireachtas.ie/ie/oireachtas/committee/dail/33/committee_of_public_accounts/reports/2022/2022-11-08_examination-of-the-2019-appropriation-account-for-vote-30-agriculture-food-and-the-marine-and-related-financial-matters_en.pdf (accessed November, 2022).

9. DAFM. *Ireland's Bovine TB Eradication Program.* (2018). Available online at: https://www.agriculture.gov.ie/media/migration/animalhealthwelfare/diseasecontrols/tuberculosistbandbrucellosis/tbforum/IrelandsBovineTBEradicationProgramme2018Overview210818.pdf

10. Schiller I, Oesch B, Vordermeier HM, Palmer MV, Harris BN, Orloski KA. Bovine tuberculosis: a review of current and emerging diagnostic techniques in view of their relevance for disease control and eradication. *Transboundary Emerg Dis.* (2010) 57:205–20. doi: 10.1111/j.1865-1682.2010.01148.x

11. Watchorn RC. Bovine Tuberculosis Eradication Scheme 1954-1965 (1965).

12. More SJ, Good M. The tuberculosis eradication programme in Ireland: a review of scientific and policy advances since 1988. *Vet Microbiol.* (2006) 112:239–51. doi: 10.1016/j.vetmic.2005.11.022

13. Griffin JM, Williams DH, Kelly GE, Clegg TA, O'Boyle I, Collins JD. The impact of badger removal on the control of tuberculosis in cattle herds in Ireland. *Prev Vet Med.* (2005) 67:237–66. doi: 10.1016/j.prevetmed.2004.10.009

14. Gormley E, Bhuachalla D, O'Keeffe J, Murphy D, Aldwell FE, Fitzsimons T. Oral vaccination of free-living badgers (Meles meles) with bacille calmette guérin (BCG) vaccine confers protection against tuberculosis. *PLoS ONE.* (2017) 12:e0168851. doi: 10.1371/journal.pone.0168851

15. Aznar I, Frankena K, More SJ, O'Keeffe J, McGrath G, de Jong MCM. Quantification of Mycobacterium bovis transmission in a badger vaccine field trial. *Prev Vet Med.* (2018) 149:29–37. doi: 10.1016/j.prevetmed.2017.10.010

16. Byrne AW, Kenny K, Fogarty U, O'Keeffe JJ, More SJ, McGrath G. Spatial and temporal analyses of metrics of tuberculosis infection in badgers (Meles meles) from the Republic of Ireland: Trends in apparent prevalence. *Prev Vet Med.* (2015) 122:345–54. doi: 10.1016/j.prevetmed.2015.10.013

17. Martin SW, O'Keeffe J, Byrne AW, Rosen LE, White PW, McGrath G. Is moving from targeted culling to BCG-vaccination of badgers (Meles meles) associated with an unacceptable increased incidence of cattle herd tuberculosis in the Republic of Ireland? A practical non-inferiority wildlife intervention study in the Republic of Ireland (2011-2017). *Prev Vet Med.* (2020) 179:105004. doi: 10.1016/j.prevetmed.2020.105004

18. Eves JA. Impact of badger removal on bovine tuberculosis in east County Offaly. *Ir Vet J.* (1999) 52:199–203.

19. Kelly GE, Condon J, More SJ, Dolan LA, Higgins IM, Eves J. A long-term observational study of the impact of badger removal on herd restrictions due to bovine TB in the Irish midlands during 1989–2004. *Epidemiol Infect.* (2008) 136:1362–73. doi: 10.1017/S0950268807000027

20. Bourne FJ, Morrison WI. Bovine TB: *The Scientific Evidence. Final Report of the Independent Scientific Group on Cattle TB*. London, UK (2007)

21. Bessell PR, Orton R, White PCL, Hutchings MR, Kao RR. Risk factors for bovine tuberculosis at the national level in Great Britain. *BMC Vet Res.* (2012) 8:1–9. doi: 10.1186/1746-6148-8-51

22. Byrne AW, White PW, McGrath G, O'Keeffe J, Martin SW. Risk of tuberculosis cattle herd breakdowns in Ireland: effects of badger culling effort, density and historic large-scale interventions. *Vet Res.* (2014) 45:1–12. doi: 10.1186/s13567-014-0109-4

23. Sandoval Barron E, Swift B, Chantrey J, Christley R, Gardner R, Jewell C. A study of tuberculosis in road traffic-killed badgers on the edge of the British bovine TB epidemic area. *Sci Rep.* (2018) 8:17206. doi: 10.1038/s41598-018-35652-5

24. Milne G, Allen A, Graham J, Kirke R, McCormick C, Presho E. Mycobacterium bovis population structure in cattle and local badgers: co-localisation and variation by farm type. *Pathogens.* (2020) 9:592. doi: 10.3390/pathogens9070592

25. Crispell J, Zadoks RN, Harris SR, Paterson B, Collins DM, De-Lisle GW. Using whole genome sequencing to investigate transmission in a multi-host system: bovine tuberculosis in New Zealand. *BMC Genomics.* (2017) 18:1. doi: 10.1186/s12864-017-3569-x

26. Crispell J, Benton CH, Balaz D, De Maio N, Ahkmetova A, Allen A. Combining genomics and epidemiology to analyse bi-directional transmission of Mycobacterium bovis in a multi-host system. *Elife.* (2019) 8:45833. doi: 10.7554/eLife.45833

27. Crispell J, Cassidy S, Kenny K, McGrath G, Warde S, Cameron H. Mycobacterium bovis genomics reveals transmission of infection between cattle and deer in Ireland. *Microb Genomics.* (2020) 6:388. doi: 10.1099/mgen.0.000388

28. Van Tonder AJ, Thornton MJ, Conlan AJK, Jolley KA, Goolding L, Mitchell AP. Inferring Mycobacterium bovis transmission between cattle and badgers using isolates from the Randomised Badger Culling Trial. *PLoS Pathog.* (2021) 17:e1010075. doi: 10.1371/journal.ppat.1010075

29. Rossi G, Crispell J, Brough T, Lycett SJ, White PCL, Allen A. Phylodynamic analysis of an emergent Mycobacterium bovis outbreak in an area with no previously known wildlife infections. *J Appl Ecol.* (2021) 59:210–22. doi: 10.1111/1365-2664.14046

30. Akhmetova A, Guerrero J, McAdam P, Salvador LCM, Crispell J, Lavery J, et al. Genomic epidemiology of Mycobacterium bovis infection in sympatric badger and cattle populations in Northern Ireland. *Microb Genom.* (2023) 9:1023. doi: 10.1099/mgen.0.001023

31. Allen AR, Ford T, Skuce RA. (2021). Does mycobacterium tuberculosis var bovis survival in the environment confound bovine tuberculosis control and eradication? A literature review. *Vet Med Int.* (2021). doi: 10.1155/2021/8812898

32. Buddle BM, Parlane NA, Wedlock DN, Heiser A. Overview of vaccination trials for control of tuberculosis in cattle, wildlife and humans. *Transbound Emerg Dis.* (2013) 60:136–46. doi: 10.1111/tbed.12092

33. Barasona JA, Acevedo P, Diez-Delgado I, Queiros J, Carrasco-Garcia R, Gortazar C. Tuberculosis-associated death among adult wild boars, Spain, 2009-2014. *Emerg Infect Dis.* (2016) 22:2178–80. doi: 10.3201/eid2212.160677

34. Tagliapietra V, Boniotti MB, Mangeli A, Karaman I, Alborali G, Chiari M. Mycobacterium microti at the environment and wildlife interface. *Microorganisms.* (2021) 9:10. doi: 10.3390/microorganisms9102084

35. Courtenay O, Reilly LA, Sweeney FP, Hibberd V, Bryan S, Ul-Hassan A. Is Mycobacterium bovis in the environment important for the persistence of bovine tuberculosis? *Biol Lett.* (2006) 2:460–2. doi: 10.1098/rsbl.2006.0468

36. Palmer S, Williams GA, Brady C, Ryan E, Malczewska K, Bull TJ. Assessment of the frequency of Mycobacterium bovis shedding in the faeces of naturally and experimentally TB infected cattle. *J Appl Microbiol.* (2022) 133:1832–42. doi: 10.1111/jam.15677

37. Woodroffe R, Donnelly CA, Ham C, Jackson SY, Moyes K, Chapman K. Badgers prefer cattle pasture but avoid cattle: implications for bovine tuberculosis control. *Ecol Lett.* (2016) 19:1201–8. doi: 10.1111/ele.12654

38. Woodroffe R, Donnelly CA, Chapman K, Ham C, Moyes K, Stratton NG. Successive use of shared space by badgers and cattle: implications for Mycobacterium bovis transmission. *J Zool.* (2021) 314:132–42. doi: 10.1111/jzo.12863

39. Brooks-Pollock E, Roberts GO, Keeling MJ. A dynamic model of bovine tuberculosis spread and control in Great Britain. *Nature.* (2014) 511:228–31. doi: 10.1038/nature13529

40. Chang Y, de Jong MCM. A novel method to jointly estimate transmission rate and decay rate parameters in environmental transmission models. *Epidemics.* (2023) 42:100672. doi: 10.1016/j.epidem.2023.100672

41. Brouwer AF, Eisenberg MC, Love NG, Eisenberg JN. Persistence-infectivity trade-offs in environmentally transmitted pathogens change population-level disease dynamics. *arXiv preprint* arXiv:1802.05653 (2018).

42. Aznar I, McGrath G, Murphy D, Corner LA, Gormley E, Frankena K. Trial design to estimate the effect of vaccination on tuberculosis incidence in badgers. *Vet Microbiol.* (2011) 151:104–11. doi: 10.1016/j.vetmic.2011.02.032

43. Aznar I, Frankena K, More SJ, Whelan C, Martin W, Gormley E. Optimising and evaluating the characteristics of a multiple antigen ELISA for detection of mycobacterium bovis infection in a badger vaccine field trial. *PLoS ONE.* (2014) 9:e100139. doi: 10.1371/journal.pone.0100139

44. Byrne AW, O'Keeffe J, Martin SW. Factors affecting european badger movement lengths and propensity: evidence of density-dependent effects? *Small Carniv Evol Ecol Behav Conserv.* (2022) 5:173–93. doi: 10.1002/9781118943274.ch8

45. Zimmermann J, Fealy RM, Lydon K, Mockler EM, O'Brien P. The irish land-parcels identification system (LPIS)-experiences in ongoing and recent environmental research and land cover mapping. *Biology and Environment: Proceedings of the Royal Irish Academy* (Royal Irish Academy) (2016) 116. doi: 10.1353/bae.2016.0025

46. Milne G, Byrne AW, Campbell E, Graham J, McGrath J, Kirke R. Quantifying land fragmentation in northern irish cattle enterprises. *Land.* (2022) 11:402. doi: 10.3390/land11030402

47. Milne G, Graham J, McGrath J, Kirke R, McMaster W, Byrne AW. Investigating farm fragmentation as a risk factor for bovine tuberculosis in cattle herds: a matched case-control study from Northern Ireland. *Pathogens.* (2022) 11:299. doi: 10.3390/pathogens11030299

48. Diekmann O, Heesterbeek JA, Roberts MG. The construction of next-generation matrices for compartmental epidemic models. *J R Soc Interface.* (2010) 7:873–85. doi: 10.1098/rsif.2009.0386

49. Widgren S, Bauer P, Eriksson R, Engblom S. SimInf: an R package for data-driven stochastic disease spread simulations. *arXiv preprint* arXiv:1605.01421 (2016).

50. Barlow ND, Kean JM, Hickling G, Livingstone PG, Robson AB. A simulation model for the spread of bovine tuberculosis within New Zealand cattle herds. *Prev Vet Med.* (1997) 32:57–75. doi: 10.1016/S0167-5877(97)00002-0

51. Perez AM, Ward MP, Charmandarián A, Ritacco V. Simulation model of within-herd transmission of bovine tuberculosis in Argentine dairy herds. *Prev Vet Med.* (2002) 54:361–72. doi: 10.1016/S0167-5877(02)00043-0

52. Fischer EA, van Roermund HJ, Hemerik L, van Asseldonk MA, de Jong MC. Evaluation of surveillance strategies for bovine tuberculosis (Mycobacterium bovis) using an individual based epidemiological model. *Prev Vet Med.* (2005) 67:283–301. doi: 10.1016/j.prevetmed.2004.12.002

53. Alvarez J, Bezos J, de la Cruz ML, Casal C, Romero B, Dominguez L. Bovine tuberculosis: within-herd transmission models to support and direct the decision-making process. *Res Vet Sci.* (2014) 97:S61–68. doi: 10.1016/j.rvsc.2014.04.009

54. Gormley E, Corner LAL. pathogenesis of mycobacterium bovis infection: the badger model as a paradigm for understanding tuberculosis in animals. *Front Vet Sci.* (2017) 4:247. doi: 10.3389/fvets.2017.00247

55. Smith RL, Schukken YH, Lu Z, Mitchell RM, Grohn YT. Development of a model to simulate infection dynamics of Mycobacterium bovis in cattle herds in the United States. *J Am Vet Med Assoc.* (2013) 243:411–23. doi: 10.2460/javma.243.3.411

56. Conlan AJK, McKinley TJ, Karolemeas K, Pollock EB, Goodchild AV, Mitchell AP. Estimating the hidden burden of bovine tuberculosis in Great Britain. *PCBI J.* (2012) 24:e1002730. doi: 10.1371/journal.pcbi.1002730

57. Rodríguez-Hernández E, Pizano-Martínez OE, Canto-Alarcón G, Flores-Villalva S, Quintas-Granados LI, Milián-Suazo F. Persistence of Mycobacterium bovis under environmental conditions: is it a real biological risk for cattle? *Rev Res Med Microbiol.* (2016) 27:20–4. doi: 10.1097/MRM.0000000000000059

58. Kao RR, Gravenor MB, Charleston B, Hope JC, Martin M, Howard CJ. Mycobacterium bovis shedding patterns from experimentally infected calves and the effect of concurrent infection with bovine viral diarrhoea virus. *J R Soc Interface.* (2007) 4:545–51. doi: 10.1098/rsif.2006.0190

59. García JS, Bigi MM, Klepp LI, García EA, Blanco FC, Bigi F. Does Mycobacterium bovis persist in cattle in a non-replicative latent state as Mycobacterium tuberculosis in human beings? *Vet Microbiol.* (2020) 247:108758. doi: 10.1016/j.vetmic.2020.108758

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Risk of African swine fever virus transmission among wild boar and domestic pigs in Poland

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Introduction: African swine fever (ASF) is a notifiable disease of swine that impacts global pork trade and food security. In several countries across the globe, the disease persists in wild boar (WB) populations sympatric to domestic pig (DP) operations, with continued detections in both sectors. While there is evidence of spillover and spillback between the sectors, the frequency of occurrence and relative importance of different risk factors for transmission at the wildlife-livestock interface remain unclear.

Methods: To address this gap, we leveraged ASF surveillance data from WB and DP across Eastern Poland from 2014–2019 in an analysis that quantified the relative importance of different risk factors for explaining variation in each of the ASF surveillance data from WB and DP.

Results: ASF prevalence exhibited different seasonal trends across the sectors: apparent prevalence was much higher in summer (84% of detections) in DP, but more consistent throughout the year in WB (highest in winter with 45%, lowest in summer at 15%). Only 21.8% of DP-positive surveillance data included surveillance in WB nearby (within 5 km of the grid cell within the last 4 weeks), while 41.9% of WB-positive surveillance samples included any DP surveillance samples nearby. Thus, the surveillance design afforded twice as much opportunity to find DP-positive samples in the recent vicinity of WB-positive samples compared to the opposite, yet the rate of positive WB samples in the recent vicinity of a positive DP sample was 48 times as likely than the rate of positive DP samples in the recent vicinity of a positive WB sample. Our machine learning analyses found that positive samples in WB were predicted by WB-related risk factors, but not to DP-related risk factors. In contrast, WB risk factors were important for predicting detections in DP on a few spatial and temporal scales of data aggregation.

Discussion: Our results highlight that spillover from WB to DP might be more frequent than the reverse, but that the structure of current surveillance systems challenge quantification of spillover frequency and risk factors. Our results emphasize the importance of, and provide guidance for, improving cross-sector surveillance designs.

KEYWORDS

African swine fever, wild boar, domestic pigs, surveillance, wildlife-livestock

1. Introduction

Understanding the risks of pathogen transmission across the wildlife-livestock interface is key to mitigating threats to human health (1), food security (2), and endangered wildlife (3). Pathogen transmission from wildlife to domestic hosts or the reverse results from a combination of epidemiological, ecological and

behavioral drivers of pathogen pressure in the reservoir host, pathogen exposure in the receiving host, and structural barriers to contact at the interface between them (4, 5). Force of infection at the interface will depend on the pathogen prevalence in the donor host population, contact rate between the reservoir and recipient host, and probability of infection given contact (6). On the donor host side, pathogen pressure is generated by the interaction of host ecology (population distribution, connectivity, and density, host movements and contact structure) and pathogen ecology (routes of transmission, survival in the environment) which determine prevalence, persistence and spread (4, 7). If wildlife and domestic host populations have similar susceptibility and transmission ability to a particular pathogen, transmission between the two can be bidirectional (8–10), yet with distinct disease dynamics in each population due to differences in ecological context. Surveillance systems that include data from each host population jointly are important for understanding transmission risk at the wildlife-livestock interface (7, 11).

African swine fever (ASF) is a highly transmissible viral disease of swine that impacts global trade of swine and pork products. In Eurasia, ASF occurs in wildlife (wild boar; WB) and domestic pigs (DP) (8). In domestic pig populations, circulation is maintained through direct transmission between pigs within farms (12) as well as indirect transmission through fomites (e.g., contaminated feed, material, equipment) (13) or soft tick vectors in areas where the vectors exist (14). Proximity to infected farms and local density of DP are risk factors of farm ASF incidence in the Italian island of Sardinia (15, 16), Nigeria (17), Romania (18), Russia (19, 20), and globally (21). Between-farm transmission, involving transport of infected animals (direct transmission), equipment, feed and other fomites (indirect transmission), is closely related to trade and contact networks (22). For example, density of regional road networks is the most important risk factor for ASF occurrence in DP in Russia (19). Introduction of stringent regulations regarding domestic pig movements in the infected areas of the European Union (European Commission Implementing Regulation 2023/594) has reduced the risk associated with transport of live animals in relation to transmission through fomites.

In WB, ASF circulation is thought to occur through host-to-host contacts (direct transmission) and contaminated environments and infectious carcasses (indirect transmission). Patterns in ASF surveillance data in WB and modeling of ASF in WB suggests that the disease can persist endemically in the WB in some conditions (23–27). WB population density and habitat quality appear to drive patterns of ASF occurrence (26–30). High WB abundance enhances direct transmission (26), while carcass-based transmission is thought to be a key mechanism allowing low-level and long-term ASF persistence in WB populations, particularly at low densities (26, 29, 31). Studies in several different countries estimated an effective or basic reproduction number of ~1.5 between groups of WB from ASF surveillance data (11, 32–34) supporting the notion of endemic transmission levels in WB. However, these studies did not consider the potential role of DP in the estimates of effective reproductive numbers.

While DP and WB populations could each maintain ASF independently, bidirectional cross-cycle transmission is thought to occur (35). A primary mechanism of emergence of ASF in WB in new areas likely occurs through inappropriate disposal of infectious domestic pig carcasses or pork products in WB habitat followed by transmission among WB (12, 36). Once ASF occurs in WB populations, it is thought that the most likely routes of transmission from WB to DP is through contaminated feed or environments, and through direct contact depending on husbandry practices (12). Several studies have pointed to WB as an important risk factor for outbreaks in DP (13, 37), both in low-biosecurity backyard farms (18, 38) and high-biosecurity commercial farms (39). But, two important gaps remain: (1) determining if repeated transmission from DP to WB is important for explaining the patterns of detection in WB populations and, if so, how much transmission is important for persistence in WB (26, 40), and (2) whether transmission from WB to DP occurs and, if so, at what frequency. For example, Lange et al. (40) found little evidence of spatio-temporal clustering of WB detections suggesting lack of autonomous persistence in WB populations while Podgórski et al. (27) found substantial evidence for spatio-temporal clustering of detections in WB suggesting endemic transmission within WB populations. Pepin et al. (26) found autonomous persistence was likely in WB but depended on WB density and the frequency of carcass-based transmission, but this study did not include the potential for transmission from DP throughout the study area.

Few studies have examined surveillance data from WB alongside DP (e.g., 20, 32, 39) making it difficult to understand the occurrence and drivers of transmission dynamics among these host populations. While transmission from WB has been often implicated in ASF outbreaks in DP (18, 39), transmission from DP to WB has been rarely studied (20). Here, we integrate ASF surveillance data from WB and DP populations to better understand potential transmission between these host contexts. Our main objectives were to characterize risk factors of ASF occurrence in WB and DP populations and determine the relative frequency of transmission in each direction: WB-to-DP vs. DP-to-WB. We addressed these objectives by considering risk for WB and DP separately using covariates from the other population. We expected to observe greater transmission risk from WB to DP than the reverse based on the numerous reports of transmission risk from WB to DP, widespread occurrence of detections in WB, and free-roaming lifestyle of WB. Our analysis highlights important considerations for surveillance design at the wildlife-livestock interface.

2. Materials and methods

2.1. Surveillance data

ASF surveillance in Poland is regulated by international and national legislation [e.g., European Commission Implementing Regulation (EU) 2021/605 of April 7, 2021]. Intensity of surveillance and control measures follows a zoning system of restricted areas: zone III (ASF in DP and WB), zone II (ASF in WB), zone I (ASF high risk area, bordering zone II or III). Obligatory testing of all hunted (active surveillance), found dead, and road-killed (passive surveillance) WB is implemented in zones I (PCR test), II and III (PCR and ELISA/IPT tests), where our study area is contained (41). DP are tested in zones I, II and III

TABLE 1 Number of data points within 2 × 2 km grid cells for different temporal aggregations.

Aggregation	Any sample	≥ 1 WB sample	≥ 1 DP sample	≥ 1 WB & 1 DP sample	≥ 1 WB positive	≥ 1 DP positive	≥ 1 WB & 1 DP positive
2 × 2 km grid cell by serial week	516,105	127,835	403,813	16,121	5,457	279	12
2 × 2 km grid cell by year and season	152,405	91,873	94,975	34,642	3,979	191	23
2 × 2 km grid cell across all time	21,136	20,552	15,727	15,153	2,881	182	55

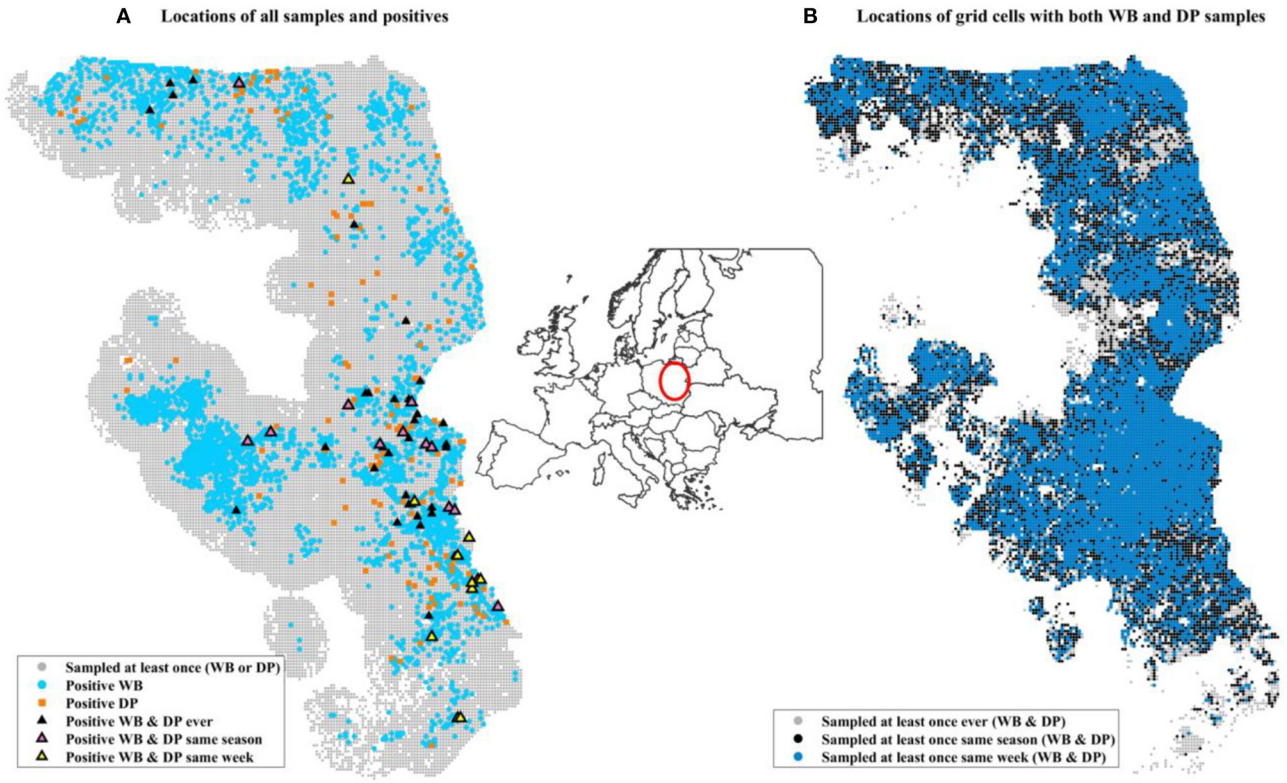

FIGURE 1
Maps of data included in the analysis. The inset map of Europe illustrates the location in Poland of the surveillance data using a red circle. **(A)** Includes all grid cells within 20 km (Euclidian distance) of a grid cell that was positive at some point during 2014–2019 (the entire time frame of our surveillance data). Small gray circles indicate a grid cell that was sampled at least once but never found to be positive. Light blue circles are locations of at least one positive WB sample during the 6 years. Orange squares are locations of at least one positive domestic pig. Black triangles are grid cells where at least one positive WB and one positive domestic pig was found at some point during the 6 years, not necessarily during the same time. **(B)** Shows the sampling design for grid cells that had sampling of both WB and DP at different time scales. The three colors distinguish the time frames within which at least one sample from each of WB and DP was collected: ever (gray, 106,247 unique grid cells), same season (black, 86,336), same week (blue, 7590 unique grid cells). The distribution of WB and DP samples collected in the same week (blue) is similar to that over broader temporal scales (gray and black).

(PCR) when moved from the holding to abattoir (all animals) and randomly within the holding (number of animals sampled scaled by holding size according to "Procedure for collecting and sending samples for laboratory diagnostics for African swine fever," Chief Veterinary Officer, Poland, April 2020).Samples collected by veterinary services and hunters were analyzed by the National Reference Laboratory for ASF diagnostics at the National Veterinary Research Institute in Puławy, Poland. All positive results were confirmed by the European Reference Laboratory for ASF in Valdeolmos, Spain. We used surveillance data collected in 2014–2019 which totaled 1,244,117 test results of DP (including 2,184 ASF-positive results from 261 focal outbreaks, i.e., pig holdings) and 196,800 test results of WB (including 9,213 ASF-positive results, i.e., individual cases). Geographic coordinates were available for all domestic pig samples and positive WB samples. Negative WB samples were available aggregated at the level of commune, the smallest administrative unit in Poland. To make this subset of data compatible with the rest, we created a number of locations equal to negative WB tests in a given commune and assigned them randomly-generated geographic coordinates.

TABLE 2 Covariates used in the analyses.

Variable	Description and mechanism	Processing	References for source data
Area developed	Description: surface of the developed area in each grid cell Mechanism: drives contact rates between WB and DP	Total surface area of all built-up and developed areas within the cell (km^2)	Topographic Objects Database (BDOT10k), Head Office of Geodesy and Cartography (https://bit.ly/3Ji1Mdh)
Human population density	Description: density of human population in each grid cell Mechanism: contamination between WB and DP through humans as a vector	Each grid cell was assigned with a density of human population (ind/ km^2) from the commune (mean size of 126.2 km^2) the cell was in	Statistics Poland (https://stat.gov.pl/en/)
WB habitat suitability	Description: quality of available habitats (QAH) based on global land cover vegetation (GLOBCOVER) Mechanism: higher quality habitats will sustain higher WB numbers and drive transmission	Average value of QAH was assigned to each grid cell. Input database categorized QAH into 7 levels at 300 × 300 m resolution.	(28)
Extensive farming of DP (more land to increase yield – e.g., holdings at lower density and biosecurity over more land)	Description: density of pigs bred in an extensive system. Mechanism: extensive pig farming facilitates transmission between WB and DP	Pig density (ind./km^2) assigned to each grid cell from the FAO database available at the 5 minutes of arc (49.3 km^2)	Food and Agriculture Organization (FAO) Gridded Livestock of the World; Global pigs distribution in 2015 (43, 44)
Hunter harvest	Description: number of WB harvested in each year and each grid cell. Mechanism: high WB numbers and hunting activity drive transmission	Each grid cell was assigned a hunting bag from the hunting ground the cell was in (mean size of a hunting ground was 6128 km^2).	Forest Data Bank (https://bit.ly/3WDVlnJ)
Season	Description: Season that the sample was collected. Mechanism: transmission is higher at particular times of the year.	A level 1–4 was assigned to each data point. 1) December–February (weeks 49–53, 1–9) 2) March–May (weeks 10–22) 3) June–August (weeks 23–35) 4) September–November (weeks 36–48)	See Processing step
Sample size	Description: Number of surveillance samples submitted. Mechanism: Affects detection probability.	The count of surveillance samples submitted by grid and week for the response variable	Derived from surveillance data
Prevalence in neighborhood DP	Description: Recent prevalence in DP in neighboring grid cells. Mechanism: Proximity to infectious individuals drives infection.	The number of PCR+ samples from DP within the last 4 weeks within 5 km of the grid cell divided by the total number of samples in the same time/space scale.	Derived from surveillance data
Prevalence in neighborhood WB	Description: Recent prevalence in WB in neighboring grid cells. Mechanism: Proximity to infectious individuals drives infection.	The number of PCR+ samples from WB within the last 4 weeks within 5 km of the grid cell divided by the total number of samples in the same time/space scale.	Derived from surveillance data
Closest positive in DP	Description: Closest distance of recent positive detections in DP. Mechanism: Proximity to infectious individuals drives infection.	Minimum distance between a focal grid cell and the location of a PCR+ domestic pig sample within the last 4 weeks.	Derived from surveillance data
Closest positive in WB	Description: Closest distance of recent positive detections in WB. Mechanism: Proximity to infectious individuals drives infection.	Minimum distance between a focal grid cell and the location of a PCR+ WB sample within the last 4 weeks.	Derived from surveillance data

All variables were continuous except for season which was categorical variable with 4 levels.

2.2. Data processing

Surveillance data (number of positives by PCR and number of samples collected) were aggregated at a weekly scale on a 2 km by 2 km grid cell resolution across all of Poland. Only grid cells that were ever positive themselves during the time frame of surveillance (2014–2019) or within 20 km of a positive were included in the analysis to control for biased weighting of landscape covariates that were never in the vicinity of disease. We also excluded an isolated western cluster in Lubuskie Voivodeship because it was small and contained and not thought to be involved in driving the dynamics in the eastern side of Poland (42). This resulted in 516,105 grid-cell-by-week combinations of data. DP samples were collected in 403,813 grid-cell-by-week data points, while WB samples were collected in 127,835 grid grid-cell-by-week data points (Table 1). There were 21,136 unique grid cells with at least 1 surveillance sample of any kind, and 15,153 of these unique grid cells had at least 1 surveillance sample

collected from each of DP and WB during the 6 years of data (Table 1, Figure 1).

The independent variables used in the analyses, rationale for including them, and data sources are presented in Table 2. The response variable (binary) was the presence of positive samples within grid cell k in week t – a 0 if none of the samples in grid cell k in week t were positive and a 1 if at least one sample in grid cell k at week t was positive. We analyzed the data at different temporal scales of aggregation (week, season, or over all time) because timescales of disease persistence in carcasses remain poorly understood and we wanted to examine how the effects of covariates depended on the temporal aggregation of the response data. For the weekly aggregation, we created 4 response variables that we analyzed separately: all samples from WB (WB full model), all samples from DP (DP full model), samples from WB that occurred within 5 km of a PCR+ sample in DP in the last 4 weeks (WB submodel), and samples from DP that occurred within 5 km of a PCR+ sample in WB in the last 4 weeks (DP submodel). The last two responses allowed us to test for potential factors driving transmission between host populations (WB submodel, DP submodel) without noise from data points that were too far away to be linked to transmission. We chose 5 km distance because between-sounder contact and transmission is most likely within this distance (45, 46). For the season aggregation, we summed surveillance data for 4 separate seasons as specified in Table 2. Finally, we analyzed the data by summing over all time, thus only effects of spatial covariates were tested.

2.3. Analyses

All analyses were conducted in Matlab R2021b (The Mathworks Inc, Natick, Massachusetts) using the Statistics and Machine Learning Toolbox. We modeled the data using boosted regression trees with a least-squares boosting loss function. We chose this approach because we expected complex interrelationships among independent variables in their effects on the response data and non-linear relationships. We optimized hyperparameters for each regression ensemble model using Bayesian optimization and 10-fold cross-validation aimed at minimizing the mean squared error implemented in fitrensemble using the Optimize Hyperparameters option. We co-optimized the following hyperparameters using the following specified prior ranges: number of learning cycles [50, 2000] (except DP week full model was [50, 1000] for computational feasibility), learning rate [0.0001, 0.1] (except DP week full model was [0.001, 0.1] for computational feasibility), minimum leaf size [50, 100], maximum number of splits [1, 60] (Table 3). The small range on maximum number of splits and high minimum value on minimum leaf size was chosen to reduce overfitting. The optimization was run for 5000 iterations on each data set except for the DP full model that was run for only 1000 iterations (because the dataset was very large and preliminary runs showed early convergence).

We then fit the final models with the optimal hyperparameters to estimate variable importance and make inferences about the effects of independent variables on the responses for each model.

TABLE 3 Optimal hyperparameters for each model.

Model	N	PCR+	Total iterations	Iteration where lowest MSE was reached	Optimal hyperparameter estimates			
					Learning rate	Learning cycles	Minimum leaf size	Maximum number of splits
WB week submodel	792	194	5000	151	0.0084	1968	50	2
DP week submodel	45,128	141	3000	1,459	0.1000	1934	94	15
WB week full model	127,835	5,457	3000	133	0.0998	106	69	59
DP week full model	403,813	279	1000	562	0.0983	980	62	27
WB season	91,873	3,979	3000	318	0.0990	50	100	43
DP season	94,975	191	3000	1,565	0.0515	361	50	31
WB all time	20,552	2881	3000	535	0.0058	651	93	55
DP all time	15,727	182	3000	931	0.0962	931	97	2

N is the number of data points in the model; PCR+ are the number of samples that tested positive for ASFv with the PCR assay.

3. Results

3.1. Spatial and temporal trends in cases

Of the 15,153 unique grid cells that had at least one surveillance sample from each of WB and DP during the 6 years of surveillance, 2,881 cells had at least one positive WB sample, while only 182 cells had at least one positive DP sample (Table 1). Only 55 grid cells found at least one positive WB and DP sample in the same grid cell during the 6 years of surveillance. Of the 16,121 surveillance data points that involved collection of at least 1 WB and 1 DP sample in the same grid cell on a given week, 5,457 grid cell-by-week data points had at least one WB positive sample, while only 279 had at least one positive sample for DP. There were only 12 grid-cell-by-week data points and 23 grid-cell-by-season data points that had at least 1 positive sample for each of WB and DP samples (Table 1, Figure 1). Thus, given the wide spatial extent of WB positive samples (2,881 unique grid cells), and numerous data points where at least one WB and one DP sample were collected in the same grid cell in the same week (16,121), most WB positive samples did not temporally overlap with the unique grid cells where positive DP samples were found. Only 6.6% (12/182) occurred in the same grid cell in the same week, 12.6% (23/182) occurred in the same grid cell in the same season, and 30% (55/182) occurred in the same grid cell ever (across 6 years).

Of the 279 grid-cell-by-week data points that had at least one DP positive sample, there were only 61 (21.8%) with at least one surveillance sample from WB within 5 km of the grid cell within the last 4 weeks, and only 15 of those instances (25% of WB samples, 5.4% of the DP-positive grid-cell-by-weeks) had at least one positive WB sample. There were 217 DP-positive grid cells that had at least 1 DP surveillance sample in the vicinity (77.8%), but only 10 of those had a DP-positive sample (10/217 = 4.6% of DP-positive samples with DP surveillance samples or 3.6% of the total DP-positive grid-cell-by-weeks). Thus, the large majority of DP positive surveillance data did not include surveillance in WB nearby, despite including DP surveillance nearby, but when surveillance did occur nearby it was more likely that the WB samples were positive relative to the DP samples.

In contrast, of the 5,457 grid-cell-by-week data points that had at least one WB positive sample, 2,287 (41.9%) had at least one surveillance sample from DP within 5 km of the grid cell within the last 4 weeks, but only 12 (0.52% of DP samples, 0.22% of the WB-positive grid-cell-by-weeks) had at least one positive surveillance sample from DP. Thus, the surveillance design afforded twice as much opportunity to find DP-positive samples in the recent vicinity of WB-positive samples compared to the opposite (compare 41.9 to 21.8%), yet the reverse occurred – the rate of positive WB samples in the recent vicinity of a positive DP sample was 48 times as likely than the rate of positive DP samples in the recent vicinity of a positive WB sample (divide 25% for WB-positive samples collected in the recent vicinity of a DP positive by 0.52% of the DP-positive samples collected in the recent vicinity of a WB-positive sample).

Cases in WB were detected throughout the year at similarly high levels with the highest detection rates occurring in winter (45%; 4150/9198) and spring (25%; 2351/9198) relative to summer (14.9%; 1366/9198) and fall (14.5%; 1331/9198) (Figures 2A, C). In contrast, cases in DP showed a distinct seasonality with most cases (84.2%; 1832/2174) occurring in summer despite a similar number of samples being collected during each season with the most being collected in the fall (Figures 2B, D).

3.2. Correlation with potential risk factors

The distribution of cases in WB tracked the distribution of independent variable values closely (Figures 3A–D, H, J) except there were: (1) a small number of very high values for DP extensive and hunter harvest where WB cases were not found (Figures 3E, F), (2) visible trends of more cases at closer distances of recent cases in WB and higher neighborhood prevalence in WB (Figures 3G, I), and (3) visible trends of higher prevalence at larger sample size (Figure 3K). The distribution of cases in DP showed similar trends relative to independent variables except that cases clustered toward the closer distances of cases in both WB and DP (Figure 4).

3.3. Risk factors involving DP were not important for predicting ASF detection in WB

In the WB week submodel, which limited the data only to WB samples that occurred within 5 km of a PCR+ sample in DP in the last 4 weeks, the number of WB harvested and closest distance to the nearest WB-positive detection were the most important risk factors, followed by sample size (Figure 5A). However, when all the WB samples were considered in the model, the only important risk factor of WB positivity was the neighborhood prevalence in WB (Figure 5C). When aggregating the data at seasonal scale both the neighborhood prevalence in WB and distance to the nearest WB sample were important risk factors (Figure 5E), whereas when all data was pooled, only distance to the nearest WB sample was an important risk factor (Figure 5G).

3.4. Risk factors involving WB were important for predicting ASF detection in DP

In the DP week submodel, which limited the data only to DP samples that occurred within 5 km of a PCR+ sample in WB in the last 4 weeks, the number of WB harvested (a proxy for WB density) was the most important risk factor, followed by distance to the closest DP-positive sample, recent neighborhood prevalence in WB, sample size, and season (Figure 5B). However, in the full model for DP, the most important variable was neighborhood prevalence of DP, followed by neighborhood prevalence of WB (Figure 5D), although the best full model did not fit the data very well (AUC = 0.61). When aggregating the data to the season scale neighborhood prevalence in WB was the strongest risk factor, followed by neighborhood prevalence in DP and sample size (Figure 5F), whereas when the data were aggregated across all time, only proximity to DP-positive samples was an important risk factor (Figure 5H).

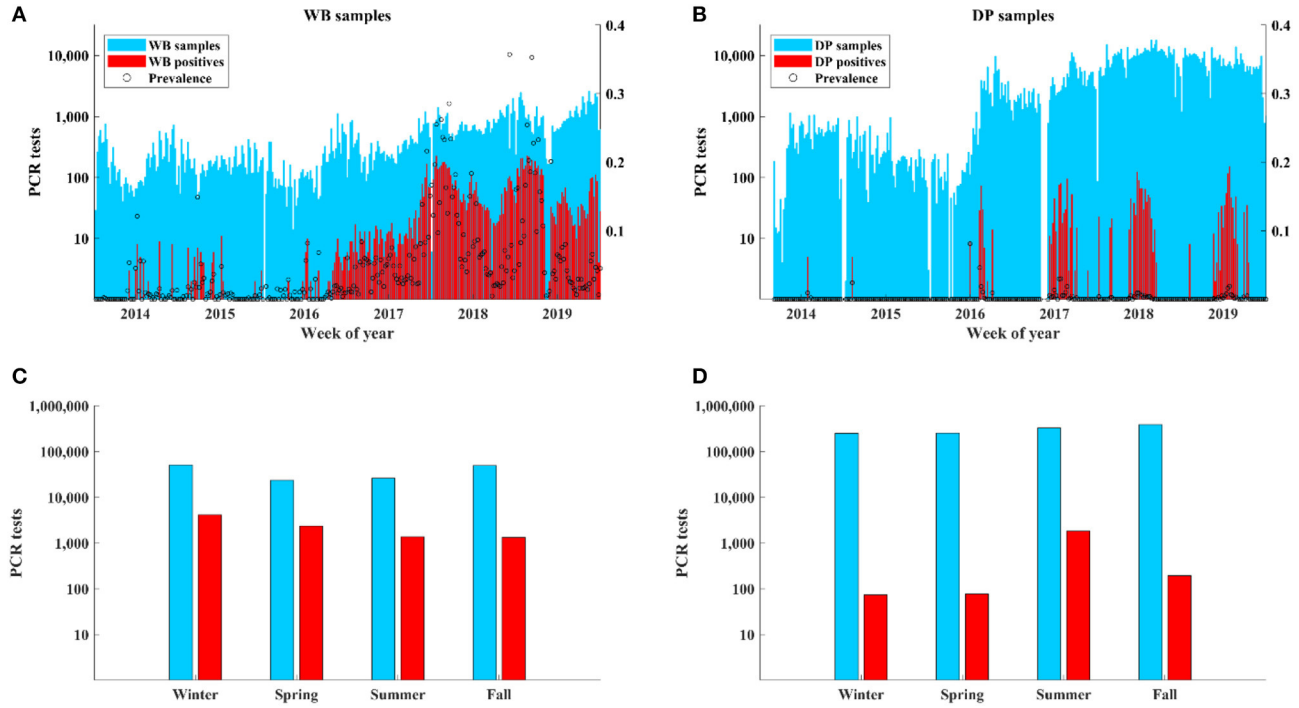

FIGURE 2
ASF surveillance data over time.

4. Discussion

Our analysis revealed surprisingly few surveillance data containing samples for both WB and DP (12.6% for WB and 4.0% DP data) on spatial and temporal scales that are most relevant to transmission (e.g., within 4 km^2 and the same week). This emphasizes that active surveillance of WB around DP premises might be needed in addition to passive surveillance to understand transmission routes and frequency between WB and DP host populations from ASF surveillance data. We addressed the surveillance design issues by examining effects of risk factors across a variety of spatial and temporal scales. At all scales, positive samples in WB were predicted by WB-related risk factors (e.g., recent proximity to WB-positive samples, hunter-harvest samples - a proxy for WB density, WB sample size), but not to DP-related risk factors. In contrast, WB risk factors were important for predicting detections in DP on a few spatial and temporal scales. These trends occurred even though the sampling design afforded more opportunity to detect DP as a risk factor of ASF detection in WB relative to the ability to detect WB as a risk factor of ASF detection in DP.

In several studies, the presence of ASF-infected WB in close vicinity to DP holdings has been cited as a main risk factor for ASF outbreaks in DP (47). Our results are consistent with these findings. However, we also addressed the gap (48, 49) of whether DP pose a risk of transmission to WB. Our results in eastern Poland suggested that the WB were almost 50 times more likely to pose a transmission risk to DP than the other way around. Experimental infection data show that WB and DP are equally susceptible to ASF virus when inoculated through similar routes (50) thus these differences are likely due to ecological or behavioral factors at the wildlife-livestock interface (51). For example, WB are social and disruptions of/in social structure affects movement behavior (52). It is possible that in areas with wide circulation of virulent strains of ASF or high hunting pressure WB change their movement behavior and seek interaction with other swine, even DP (51), or escape disturbance (53, 54). This could lead to higher rate of transmission among WB and from WB to DP. Also, studies that investigated interaction frequency between WB and DP have documented a wide range of interaction rates depending on the husbandry practices, biosecurity levels, and ecological context (49, 51, 55). Some of these studies documented high rates of direct contact while others mainly indirect contacts. It is also likely that indirect contact routes pose different transmission risks between WB to DP relative to the reverse. Accounting for variation within these contextual factors in analyses of ASF surveillance data would be valuable for quantifying the frequency of WB-DP transmission by different routes.

Similar to previous work we found that seasonal peaks of ASF detections in WB and DP were not synchronized (winter-spring for WB vs. summer for DP) (15, 56), but quantitative information on how much transmission varies seasonally is currently missing. Our results in eastern Poland suggest that 85% of transmission among DP occurred in summer, while only 15% of transmission in WB occurred in each of summer and fall. The low rates of detection in DP in fall and winter but

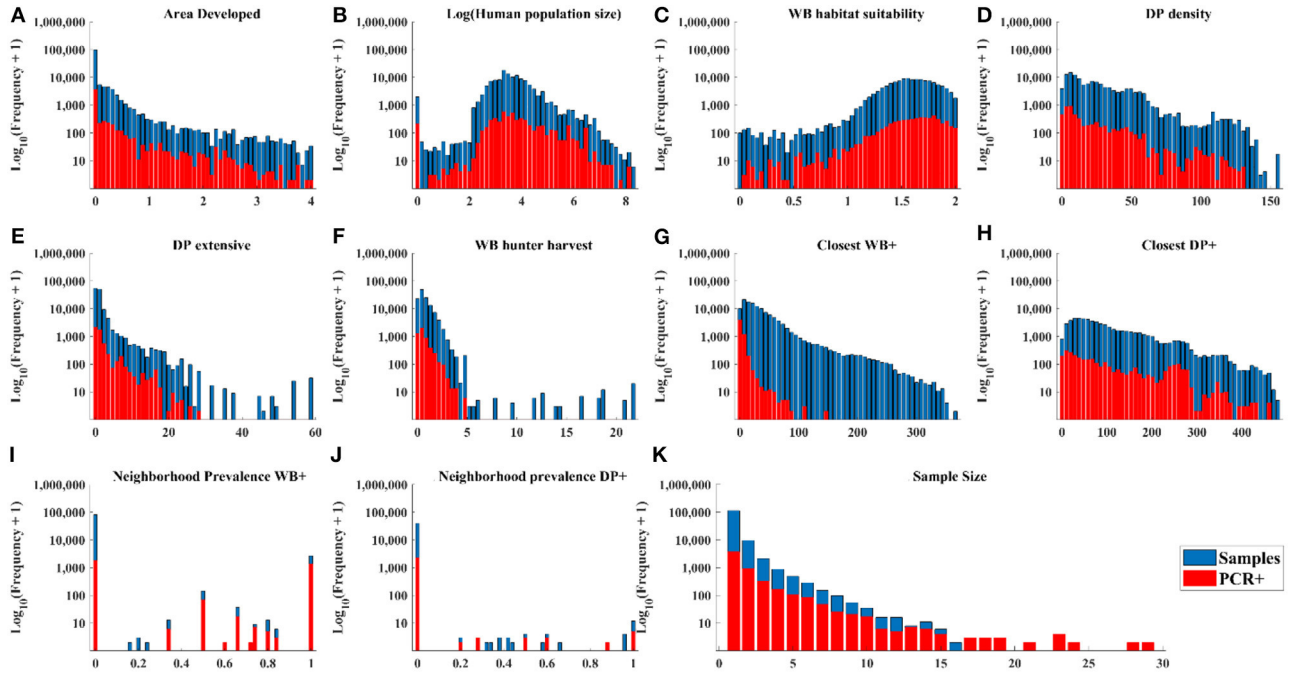

FIGURE 3
Distribution of WB samples and positive tests by PCR within the range of each independent variable.

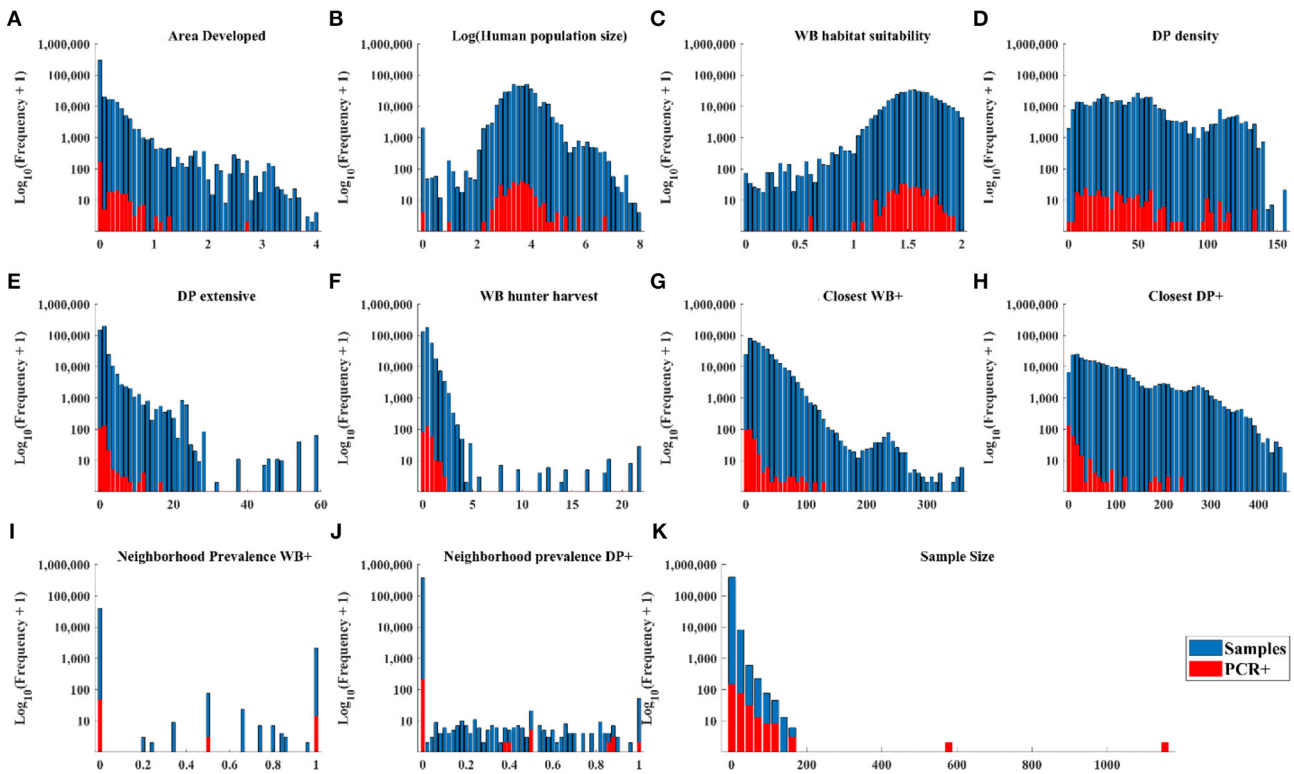

FIGURE 4
Distribution of DP samples and positive tests by PCR within the range of each independent variable.

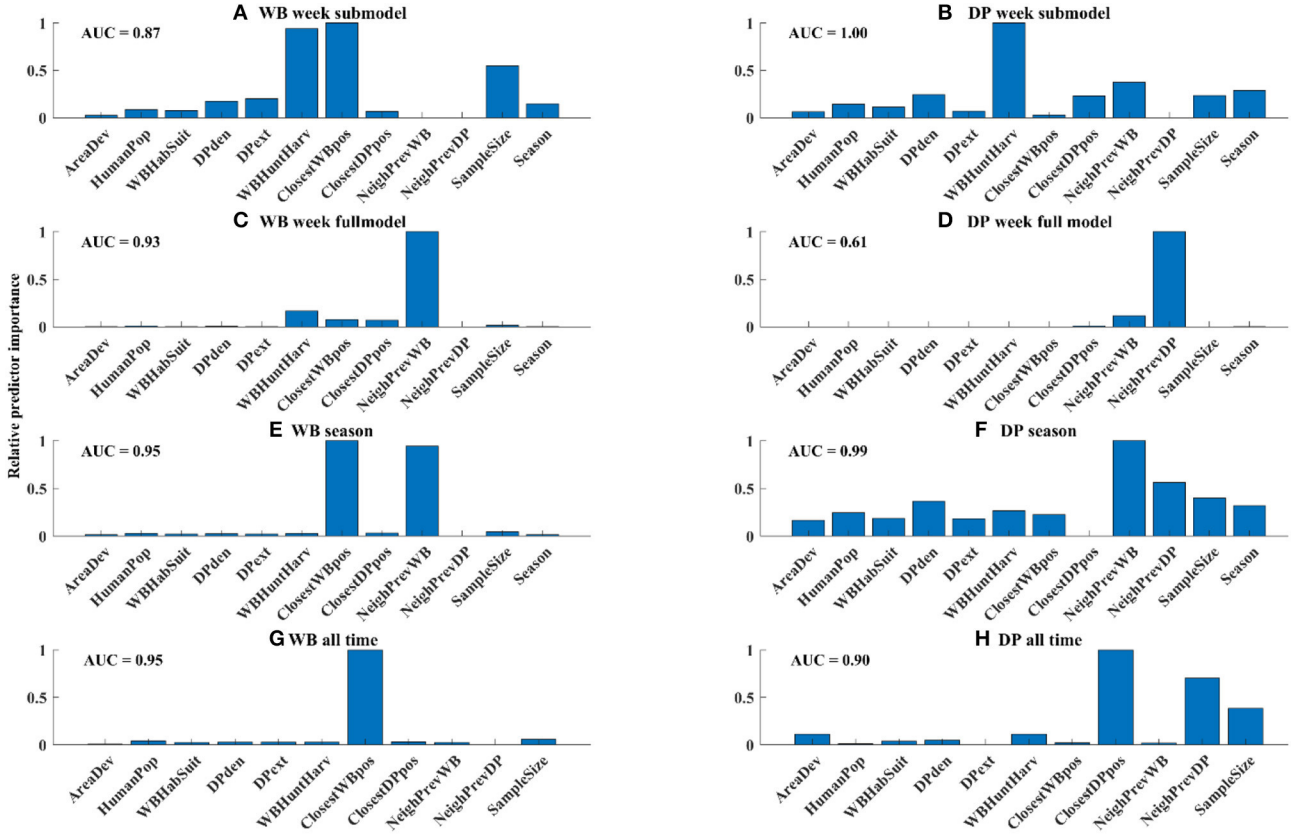

FIGURE 5
Relative variable importance for each model. Absolute goodness-of-fit for each model was measured by AUC and is shown in the upper left of each panel.

high rates of detection in WB in winter further supports our finding of a low rate of transmission from DP to WB. In contrast, detections in DP were highest in summer, which follows the highest season of detections in WB and supports the higher frequency of transmission from WB to DP. It has been hypothesized that summer peaks in DP are driven by indirect transmission from the surrounding environment (through movements of contaminated feed, bedding, equipment during intensive field work) (39) while winter-spring peaks in WB are driven by seasonal factors, such as longer carcass persistence and birth pulses that introduce susceptible individuals.

The sampling design made it difficult to estimate the relative frequency of transmission from WB to DP and the reverse. One gap is that locations of negative WB samples are imprecise - georeferenced to the commune instead of GPS coordinates of collection. GPS coordinates for negative WB samples would allow for more precise estimates of the distance between DP and WB samples. It is important to understand the spatial sampling design for all samples for quantifying risk. Secondly, for DP premises with ASF outbreaks, it was uncommon for WB samples to be collected within 5 km, making it challenging to assess the potential role of WB in seeding DP outbreaks. Epidemiological investigations that coordinate veterinary and wildlife agencies in surveillance sampling around DP premises will help to better understand potential transmission routes between WB and DP. Relatedly, a mechanism for capturing husbandry practices or biosecurity actions around premises would provide additional information for inferring transmission routes. These gaps in surveillance design are not due to a lack of WB presence near DP. For example, there were > 16,121 grid-cell-by-week data points and 34,642 grid-cell-by-season data points that had at least one DP and one WB sample. Thus, it appears there is opportunity to conduct targeted surveillance in WB and DP populations around positive cases. Another valuable approach could be to conduct targeted risk-based surveillance where higher numbers of DP and WB samples would be collected on and around premises where outbreaks have a higher likelihood of occurring based on historical data or other risk assessments conducted at a fine spatial resolution (e.g., premises-level and within 5 km of premises). These surveillance approaches could be paired with other important metadata (i.e., husbandry and biosecurity practices).

A challenging gap to address is the poor understanding of long-range movements in both DP and WB populations. Our analysis does not address long-range connectivity in either WB, DP, or their interface. Most WB movement and contact is thought to be close (45) but there may be some longer distance WB movements (57) or other mechanisms (fomites, contaminated meat products) that spread ASF over longer distances in WB (42). However, DP may be moved over longer distances more regularly. Data describing

these movements are important for quantifying the role of WB in outbreaks in DP and potential for the reverse.

Data availability statement

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

Author contributions

KP: Conceptualization, Formal analysis, Investigation, Visualization, Writing—original draft, Writing—review & editing. TB: Data curation, Investigation, Writing—review & editing. MF: Data curation, Writing—review & editing. KP: Data curation, Writing—review & editing. TP: Conceptualization, Data curation, Investigation, Visualization, Writing—original draft, Writing—review & editing.

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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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References

1. Taylor LH, Latham SM, Woolhouse MEJ. Risk factors for human disease emergence. *Philos T R Soc B.* (2001) 356:983–9. doi: 10.1098/rstb.2001.0888

2. Mason-D'Croz D, Bogard JR, Herrero M, Robinson S, Sulser TB, Wiebe K, et al. Modelling the global economic consequences of a major African swine fever outbreak in China. *Nat Food.* (2020) 1:221–8. doi: 10.1038/s43016-020-0057-2

3. Luskin MS, Meijaard E, Surya S, Sheherazade, Walzer C, Linkie M. African Swine Fever threatens Southeast Asia's 11 endemic wild pig species. *Conserv Lett.* (2021) 14:e12784. doi: 10.1111/conl.12784

4. Plowright RK, Parrish CR, McCallum H, Hudson PJ, Ko AI, Graham AL, et al. Pathways to zoonotic spillover. *Nat Rev Microbiol.* (2017) 15:502–10. doi: 10.1038/nrmicro.2017.45

5. Sokolow SH, Nova N, Pepin KM, Peel AJ, Pulliam JRC, Manlove K, et al. Ecological interventions to prevent and manage zoonotic pathogen spillover. *Philos Trans R Soc Lond B Biol Sci.* (2019) 374:20180342. doi: 10.1098/rstb.2018.0342

6. Lloyd-Smith JO, George D, Pepin KM, Pitzer VE, Pulliam JRC, Dobson AP, et al. Epidemic dynamics at the human-animal interface. *Science.* (2009) 326:1362–7. doi: 10.1126/science.1177345

7. Cross PC, Prosser DJ, Ramey AM, Hanks EM, Pepin KM. Confronting models with data: the challenges of estimating disease spillover. *Philos Trans R Soc Lond B Biol Sci.* (2019) 374:20180435. doi: 10.1098/rstb.2018.0435

8. Chenais E, Stahl K, Guberti V, Depner K. Identification of wild boar-habitat epidemiologic cycle in african swine fever epizootic. *Emerg Infect Dis.* (2018) 24:810–2. doi: 10.3201/eid2404.172127

9. Pepin KM, Miller RS, Wilber MQ. A framework for surveillance of emerging pathogens at the human-animal interface: pigs and coronaviruses as a case study. *Prev Vet Med.* (2021) 188:105281. doi: 10.1016/j.prevetmed.2021.105281

10. Rhyan JC, Spraker TR. Emergence of diseases from wildlife reservoirs. *Vet Pathol.* (2010) 47:34–9. doi: 10.1177/0300985809354466

11. Pepin KM, Golnar A, Podgorski T. Social structure defines spatial transmission of African swine fever in wild boar. *J R Soc Interface.* (2021) 18:20200761. doi: 10.1098/rsif.2020.0761

12. Guinat C, Gogin A, Blome S, Keil G, Pollin R, Pfeiffer DU, et al. Transmission routes of African swine fever virus to domestic pigs: current knowledge and future research directions. *Vet Rec.* (2016) 178:262–7. doi: 10.1136/vr.103593

13. Bergmann H, Dups-Bergmann J, Schulz K, Probst C, Zani L, Fischer M, et al. Identification of risk factors for african swine fever: a systematic review. *Viruses-Basel.* (2022) 14:2107. doi: 10.3390/v14102107

14. Boinas FS, Wilson AJ, Hutchings GH, Martins C, Dixon LJ. The persistence of African swine fever virus in field-infected *Ornithodoros erraticus* during the ASF endemic period in Portugal. *PLoS ONE.* (2011) 6:e20383. doi: 10.1371/journal.pone.0020383

15. Iglesias I, Rodriguez A, Feliziani F, Rolesu S, De la Torre A. Spatio-temporal analysis of African swine fever in sardinia (2012-2014): trends in domestic pigs and wild boar. *Transbound Emerg Dis.* (2017) 64:656–62. doi: 10.1111/tbed.12408

16. Rolesu S, Mandas D, Loi F, Oggiano A, Dei Giudici S, Franzoni G, et al. African swine fever in smallholder sardinian farms: last 10 years of network transmission reconstruction and analysis. *Front Vet Sci.* (2021) 8:692448. doi: 10.3389/fvets.2021.692448

17. Fasina FO, Agbaje M, Ajani FL, Talabi OA, Lazarus DD, Gallardo C, et al. Risk factors for farm-level African swine fever infection in major pig-producing areas in Nigeria, 1997-2011. *Prev Vet Med.* (2012) 107:65–75. doi: 10.1016/j.prevetmed.2012.05.011

18. Boklund A, Dhollander S, Chesnoiu Vasile T, Abrahantes JC, Botner A, Gogin A, et al. Risk factors for African swine fever incursion in Romanian domestic farms during 2019. *Sci Rep.* (2020) 10:10215. doi: 10.1038/s41598-020-66381-3

19. Gulenkin VM, Korennoy FI, Karaulov AK, Dudnikov SA. Cartographical analysis of African swine fever outbreaks in the territory of the Russian Federation and computer modeling of the basic reproduction ratio. *Prev Vet Med.* (2011) 102:167–74. doi: 10.1016/j.prevetmed.2011.07.004

20. Vergne T, Gogin A, Pfeiffer DU. Statistical exploration of local transmission routes for african swine fever in pigs in the russian federation, 2007-2014. *Transbound Emerg Dis.* (2017) 64:504–12. doi: 10.1111/tbed.12391

21. Jiang D, Ma T, Hao MM, Ding FY, Sun K, Wang Q, et al. Quantifying risk factors and potential geographic extent of African swine fever across the world. *PLoS ONE.* (2022) 17:e0267128. doi: 10.1371/journal.pone.0267128

22. Sánchez-Vizcaino JM, Mur L, Gomez-Villamandos JC, Carrasco L. An update on the epidemiology and pathology of African swine fever. *J Comp Pathol.* (2015) 152:9–21. doi: 10.1016/j.jcpa.2014.09.003

23. Chenais E, Depner K, Guberti V, Dietze K, Viltrop A, Stahl K. Epidemiological considerations on African swine fever in Europe 2014-2018. *Porcine Health Manag.* (2019) 5:6. doi: 10.1186/s40813-018-0109-2

24. EFSA, Banos JV, Boklund A, Gogin A, Gortazar C, Guberti V, et al. Epidemiological analyses of African swine fever in the European Union: (September 2020 to August 2021). *EFSA J.* (2022) 20:e07290. doi: 10.2903/j.efsa.2022.7290

25. EFSA, Boklund A, Cay B, Depner K, Foldi Z, Guberti V, et al. Epidemiological analyses of African swine fever in the European Union (November 2017 until November 2018). *EFSA J.* (2018) 16:e05494. doi: 10.2903/j.efsa.2018.5494

26. Pepin KM, Golnar AJ, Abdo Z, Podgorski T. Ecological drivers of African swine fever virus persistence in wild boar populations: insight for control. *Ecol Evol.* (2020) 10:2846–59. doi: 10.1002/ece3.6100

27. Podgórski T, Borowik T, Lyjak M, Wozniakowski G. Spatial epidemiology of African swine fever: Host, landscape and anthropogenic drivers of disease occurrence in wild boar. *Prev Vet Med.* (2020) 177:104691. doi: 10.1016/j.prevetmed.2019.104691

28. Bosch J, Iglesias I, Munoz MJ, de la Torre A. A cartographic tool for managing african swine fever in eurasia: mapping wild boar distribution based on the quality of available habitats. *Transbound Emerg Dis.* (2017) 64:1720–33. doi: 10.1111/tbed.12559

29. Gervasi V, Guberti V. African swine fever endemic persistence in wild boar populations: Key mechanisms explored through modelling. *Transbound Emerg Dis.* (2021) 68:2812–25. doi: 10.1111/tbed.14194

30. Nurmoja I, Schulz K, Staubach C, Sauter-Louis C, Depner K, Conraths FJ, et al. Development of African swine fever epidemic among wild boar in Estonia - two different areas in the epidemiological focus. *Sci Rep UK.* (2017) 7:12562. doi: 10.1038/s41598-017-12952-w

31. O'Neill X, White A, Ruiz-Fons F, Gortazar C. Modelling the transmission and persistence of African swine fever in wild boar in contrasting European scenarios. *Sci Rep UK.* (2020) 10:5895. doi: 10.1038/s41598-020-62736-y

32. Iglesias I, Munoz MJ, Montes F, Perez A, Gogin A, Kolbasov D, et al. Reproductive ratio for the local spread of African swine fever in wild boars in the Russian federation. *Transbound Emerg Dis.* (2016) 63:e237–e45. doi: 10.1111/tbed.12337

33. Loi F, Di Sabatino D, Baldi I, Rolesu S, Gervasi V, Guberti V, et al. Estimation of R(0) for the spread of the first ASF epidemic in italy from fresh carcasses. *Viruses.* (2022) 14:2240. doi: 10.3390/v14102240

34. Marcon A, Linden A, Satran P, Gervasi V, Licoppe A, Guberti V. R(0) Estimation for the African swine fever epidemics in wild boar of Czech Republic and Belgium. *Vet Sci.* (2019) 7:E2. doi: 10.3390/vetsci7010002

35. Mazur-Panasiuk N, Wozniakowski G, Niemczuk K. The first complete genomic sequences of African swine fever virus isolated in Poland. *Sci Rep.* (2019) 9:4556. doi: 10.1038/s41598-018-36823-0

36. Denstedt E, Porco A, Hwang J, Nga NTT, Ngoc PTB, Chea S, et al. Detection of African swine fever virus in free-ranging wild boar in Southeast Asia. *Transbound Emerg Dis.* (2021) 68:2669–75. doi: 10.1111/tbed.13964

37. Bellini S, Casadei G, De Lorenzi G, Tamba M. A review of risk factors of African swine fever incursion in pig farming within the European union scenario. *Pathogens.* (2021) 10:84. doi: 10.3390/pathogens10010084

38. Mur L, Sanchez-Vizcaino JM, Fernandez-Carrion E, Jurado C, Rolesu S, Feliziani F, et al. Understanding African swine fever infection dynamics in sardinia using a spatially explicit transmission model in domestic pig farms. *Transbound Emerg Dis.* (2018) 65:123–34. doi: 10.1111/tbed.12636

39. Nurmoja I, Motus K, Kristian M, Niine T, Schulz K, Depner K, et al. Epidemiological analysis of the 2015-2017 African swine fever outbreaks in Estonia. *Prev Vet Med.* (2020) 181:104556. doi: 10.1016/j.prevetmed.2018.10.001

40. Lange M, Siemen H, Blome S, Thulke H-H. Analysis of spatiotemporal patterns of African swine fever cases in Russian wild boar does not reveal an endemic situation. *Prev Vet Med.* (2014) 117:317–25. doi: 10.1016/j.prevetmed.2014.08.012

41. Frant MP, Gal-Cison A, Bocian L, Zietek-Barszcz A, Niemczuk K, Wozniakowski G, et al. African swine fever in wild boar (Poland 2020): passive and active surveillance analysis and further perspectives. *Pathogens.* (2021) 10:1219. doi: 10.3390/pathogens10091219

42. Wozniakowski G, Pejsak Z, Jablonski A. Emergence of African Swine fever in Poland (2014-2021). Successes and failures in disease eradication. *Agriculture.* (2021) 11:738. doi: 10.3390/agriculture11080738

43. Gilbert M, Cinardi G, Da Re D, Wint WGR, Wisser D, Robinson TP. *Global Pigs Distribution in 2015 (5 Minutes of Arc).* Cambridge, MA: Harvard Dataverse, V1 (2022).

44. Gilbert M, Nicolas G, Cinardi G, Van Boeckel TP, Vanwambeke SO, Wint GRW, et al. Global distribution data for cattle, buffaloes, horses, sheep, goats, pigs, chickens and ducks in 2010. *Sci Data.* (2018) 5:180227. doi: 10.1038/sdata.2018.227

45. Podgórski T, Apollonio M, Keuling O. Contact rates in wild boar populations: Implications for disease transmission. *J Wildlife Manage.* (2018) 82:1210–8. doi: 10.1002/jwmg.21480

46. Podgórski T, Pepin K, Radko A, Podbielska A, Lyjak M, Wozniakowski G, et al. How do genetic relatedness and spatial proximity shape African swine fever infections in wild boar? *Transbound Emerg Dis.* (2022) 69:2656–66. doi: 10.1111/tbed.14418

47. Sauter-Louis C, Conraths FJ, Probst C, Blohm U, Schulz K, Sehl J, et al. African Swine fever in wild boar in Europe-a review. *Viruses.* (2021) 13:1717. doi: 10.3390/v13091717

48. Hayes BH, Andraud M, Salazar LG, Rose N, Vergne T. Mechanistic modelling of African swine fever: a systematic review. *Prev Vet Med.* (2021) 191:105358. doi: 10.1016/j.prevetmed.2021.105358

49. Kosowska A, Barasona JA, Barroso-Arévalo S, Leon LB, Cadenas-Fernández E, Sánchez-Vizcaíno JM. Low transmission risk of African swine fever virus between wild boar infected by an attenuated isolate and susceptible domestic pigs. *Front Vet Sci.* (2022) 9:844209. doi: 10.3389/fvets.2023.1177246

50. Sanchez-Cordon PJ, Nunez A, Neimanis A, Wikstrom-Lassa E, Montoya M, Crooke H, et al. African swine fever: disease dynamics in wild boar experimentally infected with ASFV isolates belonging to genotype I and II. *Viruses.* (2019) 11:852. doi: 10.3390/v11090852

51. Jori F, Petit G, Civil N, Decors A, Charrier F, Casabianca F, et al. A questionnaire survey for the assessment of wild-domestic pig interactions in a context oedema disease outbreaks among wild boars (Sus scrofa) in South-Eastern France. *Transbound Emerg Dis.* (2022) 69:4009–15. doi: 10.1111/tbed.14704

52. Clontz LM, Yang AN, Chinn SM, Pepin KM, VerCauteren KC, Wittemyer G, et al. Role of social structure in establishment of an invasive large mammal after translocation. *Pest Manag Sci.* (2023) 12:3819–29. doi: 10.1002/ps.7567

53. Scillitani L, Monaco A, Toso S. Do intensive drive hunts affect wild boar (*Sus scrofa*) spatial behaviour in Italy? Some evidences and management implications. *Eur J Wildlife Res.* (2010) 56:307–18. doi: 10.1007/s10344-009-0314-z

54. Thurfjell H, Spong G, Ericsson G. Effects of hunting on wild boar *Sus scrofa* behaviour. *Wildlife Biol.* (2013) 19:87–93. doi: 10.2981/12-027

55. Cadenas-Fernandez E, Sanchez-Vizcaino JM, Pintore A, Denurra D, Cherchi M, Jurado C, et al. Free-ranging pig and wild boar interactions in an endemic area of african swine fever. *Front Vet Sci.* (2019) 6:376. doi: 10.3389/fvets.2019.00376

56. EFSA, Stahl K, Boklund A, Podgorski T, Vergne T, Abrahantes JC, et al. Epidemiological analysis of African swine fever in the European Union during 2022. *EFSA J.* (2023) 21:8016. doi: 10.2903/j.efsa.2023.8016

57. Morelle K, Podgorski T, Prevot C, Keuling O, Lehaire F, Lejeune P. Towards understanding wild boar Sus scrofa movement: a synthetic movement ecology approach. *Mammal Rev.* (2015) 45:15–29. doi: 10.1111/mam.12028

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African swine fever at the wildlife-livestock interface: challenges for management and outbreak response within invasive wild pigs in the United States

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African swine fever (ASF) causes significant morbidity and mortality in both domestic and wild suids (*Sus scrofa*), and disease outbreaks convey profound economic costs to impacted industries due to death loss, the cost of culling exposed/infected animals as the primary disease control measure, and trade restrictions. The co-occurrence of domestic and wild suids significantly complicates ASF management given the potential for wild populations to serve as persistent sources for spillover. We describe the unique threat of African swine fever virus (ASFV) introduction to the United States from epidemiological and ecological perspectives with a specific focus on disease management at the wild-domestic swine interface. The introduction of ASF into domestic herds would require a response focused on containment, culling, and contact tracing. However, detection of ASF among invasive wild pigs would require a far more complex and intensive response given the challenges of detection, containment, and ultimately elimination among wild populations. We describe the state of the science available to inform preparations for an ASF response among invasive wild pigs, describe knowledge gaps and the associated studies needed to fill those gaps, and call for an integrated approach for preparedness that incorporates the best available science and acknowledges sociological attributes and the policy context needed for an integrated disease response.

KEYWORDS

African swine fever, domestic-wildlife interface, emergency response, management implications, policy

Introduction

African swine fever (ASF) causes significant morbidity and mortality in swine (*Sus scrofa domesticus*) and can cause profound economic costs for the pork industry due to death loss, the cost of disease control, and trade restrictions imposed on ASF positive regions (1). Additionally, this disease impacts animal welfare, rural development, and food security across local, national, and international markets (2). Managing this hemorrhagic virus in domestic swine exclusively is challenging and complex; however, wild boar (*S. scrofa*) and invasive or feral suids are also susceptible to African swine fever virus (ASFV) and are now recognized to play an important role in the spread and maintenance of ASFV throughout affected regions (3, 4). The potential for ASFV transmission across the wild-domestic interface necessitates a holistic approach for disease management to prevent wild or feral populations from posing a persistent threat of disease spillover, particularly for countries with the risk of large economic consequences if the disease is not controlled (5, 6).

African swine fever virus is a large double stranded DNA virus in the family *Asfarviridae* (7, 8) that exclusively impacts members of Suidae (9). ASFV is transmitted through direct and indirect contact and can be vectored through competent soft-bodied ticks of the *Ornithodoros* genus (10). Numerous strains of ASFV can be found across the globe with clinical presentation ranging from mild to severe, although most of the strains currently circulating in epizootic regions cause moderate to severe disease. Infected swine typically develop a high fever, inappetence, and lethargy with most (~95%) animals succumbing within a week of infection (10). ASFV is endemic across most of the African continent, Eastern Europe, China, and much of southeast Asia (11); however, in recent years other parts of Europe have experienced ASFV outbreaks including Belgium in 2018 (12) (although Belgium has since eradicated the virus) (13); Germany in 2020 (14); Italy in 2022 (15); and Sweden in 2023 (16). Additionally, ASFV was identified in the Western Hemisphere for the first time in nearly 40 years with an ongoing outbreak on the island of Hispaniola (representing the counties Dominican Republic and Haiti) since 2021 (17). Aside from the acute lethality and the numerous source populations for ASFV distributed across the globe, there are several other attributes of ASFV that make it a particularly challenging pathogen to contain and control.

ASFV poses a significant threat to global food security and nutrition as 113 million tons of pork were consumed in 2022 (18). In addition to the production losses and morbidity/mortality caused by the virus, outbreaks of ASFV have significantly altered global export markets for pork products and have negatively impacted the swine industry in affected countries (19). The economic impacts of an ASFV introduction to the U.S. would be significant considering 27.5% of U.S. pork production was exported in 2022, representing a US$7.7 billion economy (20). An ASFV detection in either domestic or wild swine populations could trigger a halt to export activities, and the time needed to recover some or all exports is unknown and would be largely dependent upon the scale of the outbreak. Preliminary estimates suggest losses to the U.S. pork industry could be US$15 billion and US$50 billion for 2- and 10-year scenarios, respectively (21). Given the unique risk wild suids pose as a source for ASFV spillover to domestic herds, we describe the challenges for the control and management of this pathogen among invasive wild pigs from epidemiological and ecological perspectives and identify knowledge gaps that could complicate an effective outbreak response.

Challenges of disease control among domestic populations

Pathogens at the livestock-wildlife interface are unique in that the spillover-spillback dynamics create their own epidemiological scenario that are often not well understood (22). The response plan to contain and control ASFV among domestic pigs in the U.S. establishes biosecurity procedures that swine producers are expected to follow during an ASFV event to prevent transmission. Additionally, individual states may also impose additional biosecurity requirements. As a primary means for control, the response plan establishes a 5-km control area and a minimum of a 5-km surveillance zone around ASFV affected domestic swine premises (i.e., domestic pig production operations) as well as around infected wild pigs or wild pig carcasses (23, 24). Within this response zone, pathogen control and surveillance activities would be targeted and prioritized. Regardless of whether only domestic swine or only wild pigs are affected, all domestic swine premises within the control area would be subject to quarantine, movement restrictions, permitted movement requirements, and surveillance due to the potential risk of exposure and transmission. Domestic swine premises located in the surveillance zone—the movement-free area (hereafter, free area) surrounding the control area—would not be under quarantine/movement restrictions but would be subject to enhanced surveillance and biosecurity requirements.

Depending on the geographic region of the outbreak, the movement restrictions to domestic swine for premises located in a control area, even if the outbreak is restricted to wild pigs, could have significant implications for animal welfare. Specifically, the commercial swine industry is highly vertically integrated, requiring regular movements among different production stages (25), with most animals moving from farrowing to finishing to slaughter in the same cohort (26). Production facilities are designed for specific stocking densities for animals of a certain body size and interruptions in the supply cannot be readily absorbed (27). Thus, should movement restrictions for domestic swine be imposed due to an ASFV outbreak in wild pigs, producers may be unable to move animals to slaughter, which may necessitate euthanasia and carcass disposal at the production facility or risk animals experiencing welfare concerns due to their growing too large to live comfortably in the available production space. Thus, an outbreak of ASFV that soley occurs in wild pigs can still result in significant economic impacts to the domestic swine industry.

The primary means to control an ASFV outbreak involving domestic or wild swine will be culling of infected, exposed, or at-risk animals (28). Culling can be logistically intensive and costly, depending on the size of an outbreak. In the event that an outbreak involving domestic swine cannot be controlled using culling, vaccination will potentially be an important strategy to control a large outbreak of ASF in countries that wish to maintain export markets.

Development of an efficacious vaccine against ASF has been challenging. The virus is very large (170–190 kb), complex, and encodes many proteins that evade the host immune response, all of which have complicated vaccine development (29). Additionally, the

key determinants of host protection have been difficult to elucidate (30). Improvements in vaccine development are encouraging, although hurdles remain for the development of a fully licensed DIVA (differentiation of infected from vaccinated animals) compatible vaccine that is available for broadscale use in the U.S. (31). Recently a live-attenuated, DIVA compatible vaccine (ASFV-G-ΔI177L) has been shown to be safe and highly efficacious (32–34). This candidate vaccine is currently being used in Vietnam and the Philippines to control ASF. While this candidate vaccine shows promise, how it will perform during an outbreak to control ASF transmission in the presence of wild suids serving as a source for repeated spillover remains unknown. Thus, in the absence of a commercially available, effective vaccine approved for emergency use in the U.S., virus eradication is the only current strategy for ASFV management.

Another significant challenge for control of ASFV is virus resilience (35, 36). ASFV has been shown to be uniquely resistant to environmental conditions, remaining viable in pork throughout common curing processes and is stable across a broad range of pH levels and temperatures (37). Additionally, the virus is disseminated throughout the body of the host over the course of infection; thus, all secretions, excretions, and tissues contain virus (38). Swill feeding, the practice of feeding food scraps and other waste to swine, is common among smallholder pig operations worldwide and provides an important pathway for ASFV transmission. In fact, contaminated swill has been implicated as an important route of transmission in numerous ASFV outbreaks, globally (39). Garbage feeding is regulated in the U.S. through the Swine Health Protection Act, requiring producers that engage in the practice to obtain a license and adhere to appropriate cooking and handling of garbage feed for swine [(40); (Public Law 96–468)]. Additionally, the Swine Health Protection Act, allows states within the U.S. to further regulate garbage feeding with 23 states fully prohibiting the practice. The capacity for ASFV to be readily transmitted through pork-based products, especially food waste, and resilience to typical curing processes could contribute to the risk of anthropogenic viral movement. In addition to contaminated products containing infectious virus, carcass-based ASFV transmission amongst wild boar and between wild boar and domestic swine (41) also serves as a route of transmission. Disposing of ASFV-infected carcasses is very challenging (42); however, it appears to be important for controlling an outbreak (43).

Challenges of disease control among wild populations

Wild pigs (also commonly referred to as feral swine) are an invasive species that are non-native to North America (44). Widespread and abundant populations of invasive wild pigs, particularly through Texas and the southeastern region of the U.S., could increase the complexity of achieving disease control or elimination in the event of an ASF outbreak. However, achieving control and elimination of ASF in wild pigs is a particularly important objective to limit potential economic consequences. The European experience has demonstrated that once ASF is established in free-living suids (i.e., wild boar in this context), control becomes increasingly difficult (41) and even a small outbreak in wild pigs is expected to incur large economic impacts (45).

Effective surveillance is critical for early detection and subsequent control of a foreign animal disease (FAD) introduction. Delays in detection can result in significant increases in outbreak size, severity, duration, and the likelihood that ASFV persists in wild pigs (46). For example, ASFV was likely circulating in wild boar in Asia well before it was detected (47). These factors have prompted proactive ASF surveillance in wild pigs in the U.S. to shorten time-to-detection (48).

Unique risk posed by ASF establishment in wild pigs

In the U.S., wild pigs are characterized as any free-living suid regardless of whether the ancestral origins of an individual pig are that of domestic swine or Eurasian wild boar; however, genetic analysis has demonstrated that the vast majority of animals removed from invasive populations are hybrids of domestic and wild lineages (49, 50). The invasive potential of wild pigs is well established as they are a highly adaptable, generalist species with uniquely high reproductive rates given their body size (51)—all attributes that contribute to wild pigs being characterized as among the worst invasive species in the world (52). Wild pigs are broadly distributed with self-sustaining populations established across many U.S. states and territories [Figure 1; (53)]. Further, environmental and climatic models indicate that much of the U.S. is suitable habitat and, thus, susceptible to wild pig invasion (54). The broad distribution of invasive wild pigs would increase the complexity of achieving disease control or elimination in the event of a ASF outbreak—reflective of the European experience in which ASF has become established among native wild boar—with abundant populations of a free ranging suids serving as a source for ASFV and representing a persistent spillover threat. Although ecologically similar, management of native wild boar as compared to invasive wild pigs have some innate differences in that a stated management objective of elimination may be socially acceptable for invasive species (55). Accordingly, to protect domestic herds from ASFV introduction and/or establishment, an integrated plan is needed that considers the importance of managing ASF among wild populations.

Managing wild populations for control of a foreign animal disease (FAD) such as ASF is fundamentally different than other wildlife disease control programs in North America, which have focused on managing risks associated with chronic endemic diseases (e.g., brucellosis, bovine tuberculosis, rabies, or chronic wasting disease). Containing and controlling an ASF outbreak at the landscape scale first requires identifying the presence of ASFV, through active or passive surveillance of wild pigs, as early detection is essential for rapid disease control. However, disease detection can be particularly difficult among wild populations (47). Implementing wildlife surveillance at a national scale is highly complex and the resulting data can pose challenges for inferring epidemiological parameters (e.g., prevalence) (56). To help mitigate these issues, an adaptive risk-based surveillance approach has been adopted for FAD surveillance in wild pigs in the U.S. (48). As a component of ongoing population control efforts conducted throughout the extent of the invaded range, samples for ASFV surveillance are being collected from apparently healthy wild pigs that are lethally removed by USDA APHIS Wildlife Services and from federally inspected slaughterhouses prior to commercial sale of pork from wild pigs into national and international markets (57). This targeted approach is responsive to changes in perceived risk over

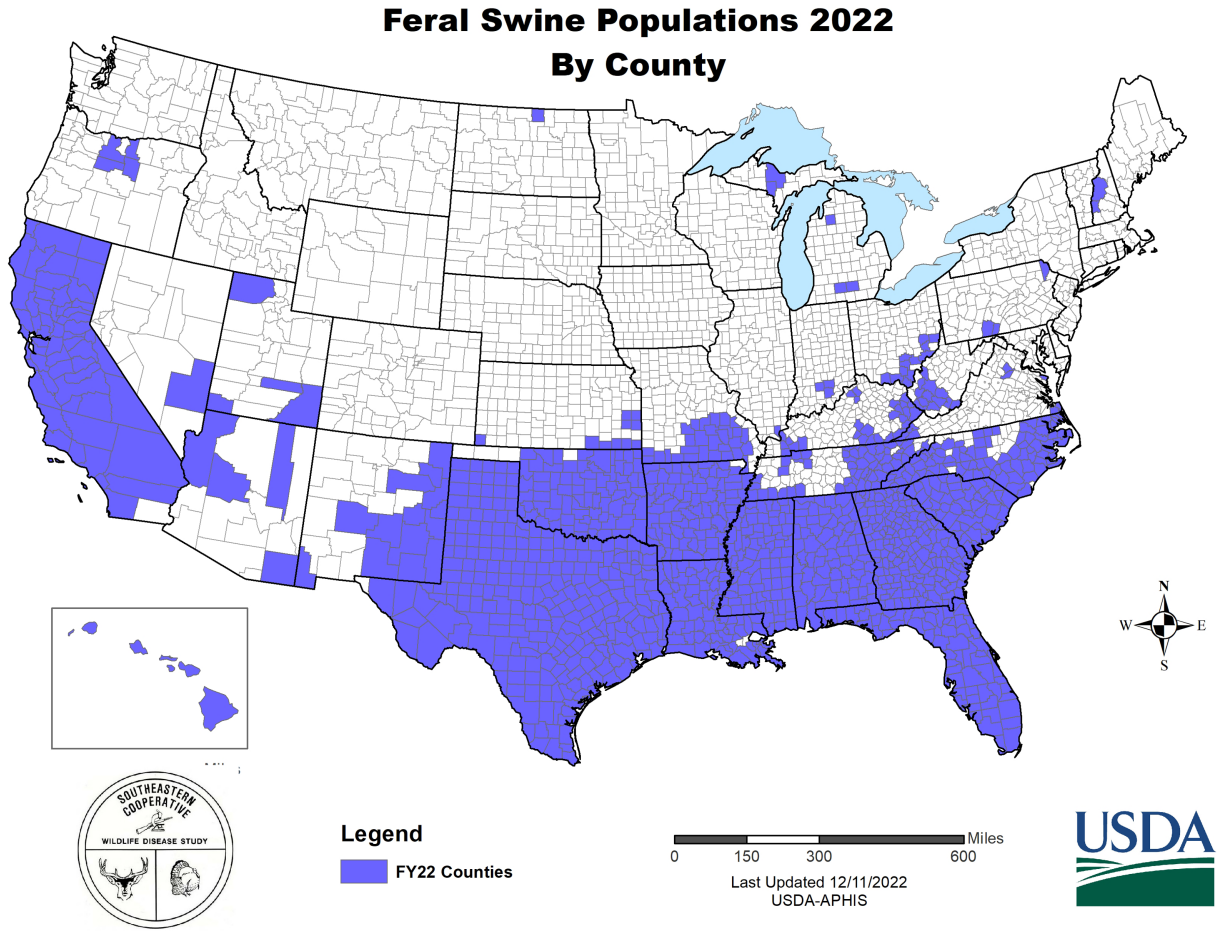

FIGURE 1
Spatial extent of wild pigs in the United States in 2022.

time, as surveillance effort is reallocated annually to reflect a dynamic risk landscape and prioritize those areas deemed to be at the greatest risk of ASFV introduction.

Many of the globally circulating ASFV strains are highly virulent and result in high lethality rates within a week of infection. Therefore, it is likely that there would be epidemiological impacts to infected wild pigs, such as altering movement patterns and social behaviors (58), during both the latency and infectious periods (59, 60). Detecting sick wildlife or their carcasses is extremely difficult due to stoicism (61) and decomposition rates (62), respectively, thus elevating the importance of proactive surveillance. If ASFV were to be detected among wild pigs, expanded surveillance would be conducted to determine the geographic extent of the outbreak (23). Properly balancing what is appropriate, necessary, and feasible within the context of an initial response requires a robust understanding of ASFV epidemiology, wild pig ecology, and logistical constraints of an operational response.

In response to a potential ASF detection among wild pigs, policy developed as a component of FAD preparedness specifies the delineation of a control area comprised of an infected zone (inner most zone that immediately surrounds infected wild pigs) and a buffer zone (zone that immediately surrounds an infected zone). The surveillance zone (zone outside and along the border of a control area) is part of the free area (i.e., areas not included in any control area) (24). These control areas would be defined based on radii surrounding the area where the positive animal(s) was detected. The control area would be adaptive, such that it would be expanded by the same distance to encompass additional detections of infected animals. As general guidance, the established response plan recommends a minimum 3 km radius for the infected zone, 2 km for the buffer zone, and 5 km for the surveillance zone, for a total radius of 10 km from the detection location. Radii defining the control area were determined based on observations of wild pig movement distances (63), wild pig contact distances (64, 65), and domestic swine disease response policies. However, as evidenced from animal movement studies conducted across a breadth of invaded ecosystems (63–65), it is likely that the most effective radii differ based on local population attributes and ecological factors (66).

Upon pathogen introduction, the spatial spread of infectious disease in any host population is driven by contact rates among hosts and the pathway of pathogen exposure. To predict ASF dynamics among wild pigs, it is necessary to consider the social structure of the species and movement patterns as the underlying mechanism that would dictate rates of disease spread (67, 68). Wild swine are highly social with populations organized into matrilineal family groups called sounders (69, 70). Sounders generally consist of one to several

adult females and their offspring, with adult males moving among sounders (71). Understanding of the hierarchical structure of local populations and the concomitant contact rates within versus between social groups is, thus, crucial for accurately predicting spatial transmission dynamics of ASFV. Contact rates between sounders is influenced by the home range characteristics and movement patterns of wild pigs. Wild pig movement patterns exhibit two distinct movement processes: (1) short-term, day-to-day movements characterized by a local home range centroid and (2) infrequent long-distance directional movements, well beyond established home ranges, that can occur when resource conditions change or social structure is disrupted, particularly when populations are at low densities (66, 72). Home range attributes and daily movement rates are influenced by population densities and resource availability, which complicates scaling predicted rates of disease spread across the diversity of ecosystems invaded by wild pigs in the U.S. For example, wild pigs require water for thermoregulation, and previous work has shown that wild pigs establish larger home ranges in more arid environments. Thus, a model for predicting local movement behavior and home range centroid shifts over fine temporal scales (i.e., weekly) from factors such as habitat, ecoregion, time of the year, and local density is needed to predict spatial spread of ASFV over a time scale that is relevant to response efforts.

In addition to the natural movements of wild pigs that drive epidemiological dynamics within and between social groups in hierarchically structured populations, genetic analyses have repeatedly demonstrated high frequency of human-mediated translocation for this species, with the potential for translocation to amplify rates of disease spread (50, 73, 74). For example, Tabak et al. (74) and Hernandez et al. (73) leveraged population genetic analyses to delineate genetically cohesive populations and map the movement of wild pigs among those populations in California and Florida, respectively. Tabak et al. (74) identified informative sociological factors associated with both domestic pig production and recreational hunting that were informative in predicting rates of wild pig translocation into and out of California counties. Hernandez et al. (73) determined that holding facilities—intermediate facilities in which live-trapped wild pigs are temporarily held before animals are moved to slaughter—serve as foci in local patterns of translocation, presumably with animals either escaping or being released from these facilities. Smyser et al. (50), working across the invaded range within the contiguous U.S., identified numerous anecdotes in which emergent populations were attributable to long-distance translocations from established invasive population as opposed to the escape or release of domestic pigs. The concern with high rates of human-mediated translocation, regardless of whether the movement is within state boundaries (73, 74) or over much greater distances (50), is that this process could greatly accelerate the rate of disease spread beyond what could be expected from epidemiological processes informed by natural movement patterns and contact rates alone.

As a tool to integrate ecosystem-and population-specific knowledge of movement patterns and contact rates into an ASF response, a spatial disease transmission model was developed based on the epidemiological characteristics of genotype II virus circulating in Europe (66). This epidemiological model was used to evaluate the potential impacts of control area size under different ecological conditions and management intensities. The radial distance delineating the control area was optimized to minimize outbreak duration and distance of spatial spread given reasonable management constraints (e.g., control intensity, local movement and contact ecology, and time of the introduction relative to initial detection). Several different optimal radii were identified depending on local wild pig movement patterns and contact rates, suggesting that understanding how these parameters vary among invaded ecosystems is needed to define the appropriate size of the control area given landscape-and population-specific attributes. Under most conditions, radii of >14 km were needed to rapidly contain an outbreak when initial detection occurred 4 months after introduction, but smaller radii were effective under early detection (<8 weeks after introduction) when high culling intensities (>15% weekly) could be implemented. Disease elimination was generally possible within 22 weeks across the conditions examined, but high control intensities (>10% weekly) were needed to achieve elimination within a year when wild pig movement and contact rates were high.

Modeling efforts highlighted uncertainties in parameters that could improve confidence in predictions of the epidemic duration and spatial spread under different response strategies (66). In particular, feasible rates of removal can vary dramatically depending on local conditions such as ecosystem attributes (e.g., vegetation density or terrain ruggedness), road access, and landownership with potential restrictions for accessing private property. These factors would affect both removal rates and carcass recovery rates. Little information exists to understand realistic removal rates for intense, continuous control within a large control area across different habitats. Relatedly, removal rates may decline as density is reduced as animals may become more difficult to locate at low densities or could increase their daily movement rates (i.e., home range size). Field studies to understand the relationship between density and removal rates could help to reduce uncertainty in elimination time. Also, as it is likely that elimination of ASF would occur before complete elimination of wild pigs in the control area as wild pig abundance falls below a level that can sustain ongoing transmission. Understanding which field-based measures provide the earliest evidence of ASF elimination is needed for efficient determination that an outbreak among wild pigs has been controlled.

In addition to understanding the epidemiological and ecological underpinnings of ASF, human activities are recognized as playing an important role in disease dynamics (75). As such, public outreach and stakeholder engagement are fundamental to any successful management response (76, 77). Drawing from previous experiences of disease outbreaks in wildlife such as with highly pathogenic avian influenza and chronic wasting disease, it is imperative to identify stakeholders and communicate risk prior to an outbreak event (78, 79). Garnering awareness and sociopolitical support in advance of a crisis, allows for a smoother and more rapid transition from preparedness, prior to detection, to a management response following detection. Clear, sustained communication is paramount through all stages of an outbreak, whether eradication is achievable or the response objective is minimizing economic or ecological costs as the disease transitions to endemic status (76).

Knowledge gaps and management needs

The task for those working on ASF preparedness is to integrate the best available knowledge in the formulation of a response plan that

will ensure disease containment and, ultimately, elimination. However, much of the available knowledge pertaining to wild pigs has been collected from routine population control and damage management efforts, which are distinct from intense, continuous removal efforts that would be mobilized in the event of a disease outbreak response. Because mobilizing a simulated FAD response is logistically challenging and very expensive, uncertainty persists in logistical, ecological, and epidemiological aspects of an ASF response.

Various logistical challenges could delay or prolong the elimination of ASF once established among wild pig populations. Landscapes invaded by wild pigs vary in the extent and accessibility of road networks. Road infrastructure differentially influences the feasibility of various control techniques. For example, whole sounder removal efforts implemented with the deployment of large traps is more dependent on road networks in that it is difficult to haul large traps into remote habitats, whereas aerial gunning is far less dependent on road infrastructure. Landownership could represent another logistical constraint in that private properties with potentially infected wild pigs may not be accessible for control activities due to limitations regarding owner permission. Modeling efforts, as described above, could help quantify the epidemiological consequences of heterogeneous land access, at least during the initial stages of a response while permission to access private land would be sought as a component of the integrated and unified response effort. Thus, the operational response to ASF detection will need to be adaptive, tailored for the landscape in which the introduction occurs based on logistical constraints and resources available for control.

An ASF outbreak, with expected high mortality rates and an ensuing management response, in which wild pigs would be removed from the infected zone through intensive culling efforts, would represent an ecological perturbation with uncertainty in the behavioral response of wild pigs. One knowledge gap in the described response plan is how wild pig movement patterns may change in response to rapidly decreasing abundance within the infected zone and/or increased human activity and culling pressure. Boundaries of the delineated control areas and surveillance zones [infected (0–3 km) and buffer (3–5 km) representing the control area, and 5 km for the surveillance zone] are only conceptual for free-living wild pigs unless physical structures are built for containment. Thus, research is needed to quantify the behavioral response of wild pigs to the combination of intense control and potential disease die-offs to elucidate the frequency and distance of animal movements within the control areas. For example, disruptions to social groups due to disease-loss or control efforts could stimulate long-distance dispersal, thus breaching the infected zone (72, 80). Similarly, animals could disperse from the infected zone, fleeing the increased human activity associated with carcass recovery and pressure exerted during control efforts (81, 82). Conversely, wild pigs from surrounding habitats may enter the control area as a result of lower population densities and potentially increased availability of resources, which could increase the burden of culling efforts or rates of disease transmission with increased contact. Integrating understanding of the movement response into a disease spread modeling is needed to inform whether fencing or other similar barriers are crucial for disease elimination in wild pigs.

Identifying and removing carcasses of wild pigs that have succumbed to ASF is another important component of disease control (83) and distinct from routine population control activities that have been used to inform ASF response scenarios. Carcass ground searches—response personnel walking transects through the control area—is labor-intensive and diverts mobilized personnel from other potential response activities. Accordingly, additional tools are needed (e.g., drones, carcass detection dogs) that can be used to efficiently locate carcasses over potentially large control areas. Further, the efficacy (i.e., detection rates) and resources required to implement carcass discovery, regardless of whether those efforts are represented by ground searches or the use of alternative tools, would be expected to vary among ecosystems (in response to vegetation characteristics and topography) and with wild pig densities. Thus, field studies are needed to quantify resources needed and detection rates of carcasses distributed across diverse landscapes in a manner that simulates an ASF outbreak. However, the frequency in which wild pigs contact carcasses (thus posing a transmission risk) throughout the decay process [e.g., (84)] and understanding how contact rates vary across environmental conditions and wildlife communities are elusive. Field studies to resolve these processes help identify effective response strategies for a disease system in which carcasses contribute to transmission. Results of these field studies can then be used to improve disease spread modeling scenarios and evaluate whether the resources invested in carcass removal positively contribute to disease containment and elimination or whether those response resources would be better allocated to other activities (e.g., population control or fencing).

In addition to those wild pigs that may be succumbing to ASF, population control efforts represent a second source of carcasses that will require management, as some animals removed through culling efforts may be infected with ASF. Established methods for carcass disposal in response to mass culling are largely based on production animal settings where animals are concentrated at a single location (e.g., from a single production barn). In the context of an ASFV response among wild pigs, the animals culled as a part of control efforts will be distributed throughout the control area (e.g., 5 km radius surrounding all positive detections). Further, some removal techniques, such as aerial gunning, do not involve direct contact with the animals and would require additional effort for carcass recovery. Thus, additional consideration will need to be given to carcass management of those animals removed from the control area through culling.

As an ASF response progresses, the stated goal is to contain and ultimately eliminate the disease from the affected population, yet substantiating disease freedom poses a distinct challenge. Further, substantiating the absence of disease after an outbreak has been controlled is vitally important for reestablishing export markets and resolving impacts to markets affected by an outbreak. Typical approaches for substantiating disease freedom rely on sampling sufficient numbers of animals to provide high levels of confidence (e.g., 95% certainty) that the disease, if present, is below a given prevalence (e.g., 1% infection rate). This is complicated by spatial dynamics of wild pig populations that are likely to have heterogeneous densities across space and may demonstrate increased and perhaps unpredictable movement patterns after large reductions in abundance. These challenges for substantiating disease freedom in wild populations, using approaches typically applied in domestic animals, will require the development of novel statistical methods that can integrate multiple lines of evidence to determine when an ASF outbreak has been controlled.

In the U.S., regulation of wild pig-related activities primarily falls under the jurisdiction of the states rather than the federal government. State legislatures and agencies have taken a variety of policy and

management approaches to wild pig populations that range from population elimination to mitigating damage while maintaining recreational hunting opportunity (85, 86), and this has resulted in a diversity of state regulatory approaches (87). States differ, for example, in the extent to which they allow activities such as wild pig hunting, possession, transport, and release of the animals (88). Additionally, the types of regulatory authorities with responsibility for wild pigs also vary by state and may depend in part on how the animals are classified (i.e., "game" or "nuisance species") (86) and in some states, there may even be multiple agencies with limited scopes of authority over wild pigs. This variability among states has resulted in a complex and sometimes difficult-to-decipher regulatory landscape that will impact what agency takes the lead on controlling an ASF outbreak as well as what is permitted when conducting control operations. Thus, defining the regulatory environment on a state-by-state basis is an important, but easily overlooked aspect, of preparedness as a response that spans state borders is plausible while coordination and efficient communication will be essential for the success of the response effort.

Policy as a tool for management/ disease protection

Given that the ASFV can be readily transmitted from direct and indirect contact and available vaccines remain in early stages of development, quarantine and movement restrictions for exposed and infected domestic swine and their products is incumbent for successful ASF management (89, 90). The global ASF epidemic has demonstrated that the involvement of wild suids greatly increases the epidemiological complexity of the outbreak (91). The presence of wild pigs in the U.S. adds an additional layer of regulatory complexity largely due to jurisdictional responsibility that is distributed among various local and federal agencies. The rules governing what can and cannot be done with wild pigs varies on a state-to-state basis as does the entity with jurisdiction over regulatory enforcement. For example, approximately half of U.S. states have "no tolerance" policies when it comes to the transport of wild pigs, prohibiting any and all manner of transport, while most of the remaining states allow transport to approved locations and/or under specified conditions (86). As this relates to ASF-related risk, one may reasonably infer that states with more permissive wild pig transport laws and related policies (e.g., allowance of wild pig hunting preserves and slaughter facilities) and larger wild pig populations would have a relatively greater risk of ASF spatial spread through human-mediated movement of the animals. Among such states, Texas stands out for both the size of its wild pig population and the extent of its wild pig transportation and use networks, as described below.

Nested case study: Texas wild pig movement

Texas has the largest number of wild pigs of any U.S. state, with an estimated population of at least 2.5 million (51). The state also has a deeply entrenched wild pig hunting culture and mature industries (e.g., meat processing and related transportation infrastructure or services associated with recreational hunting or live-capture for slaughter) that profit off the species' abundance (92, 93). Although Texas funds wild pig population control efforts to mitigate damages suffered by agricultural producers and landowners (94), state policies also accommodate certain wild pig-related interests. For example, Texas allows recreational hunting of wild pigs year-round, including at fenced hunting preserves, and it permits limited and regulated holding and transport of live wild pigs (4 Tex. Admin. Code § 55.9). This is in addition to the unknown but possibly large volume of illegal transport of wild pigs by individuals who wish to release them into uninvaded areas or to augment existing populations for recreational hunting (95).

If ASF were to emerge in Texas, the state-sanctioned pathways for holding and transporting wild pigs (referred to herein as "wild pig market chains") would present a risk of ASF spread on account of, among other things, the possibility of escapes and improper carcass disposal. To gain a better understanding of wild pig market chains in Texas, including their regulation, eleven individuals from relevant federal and Texas agencies were interviewed, including the Texas Animal Health Commission (TAHC), the USDA-Food Safety and Inspection Service (FSIS), USDA-APHIS-Veterinary Services (VS), and the Texas Department of State Health Services (DSHS). Additionally, federal and state statutes and regulations that bear upon wild pigs in Texas were analyzed, and relevant news reports and published literature was reviewed.

In Texas, the TAHC is primarily responsible for regulating wild pig market chains. Its regulations permit individuals who capture wild pigs to transport them directly to approved holding facilities, authorized hunting preserves, and recognized slaughter facilities—i.e., facilities that operate under federal or state meat inspection laws and regulations (4 Tex. Admin. Code § 55.9(b)). Holding facilities are numerous and widespread in Texas—as of July 11, 2023, there were 62 publicly listed holding facilities in 55 cities—and they serve as linkages in wild pig market chains. These holding facilities purchase live wild pigs from individuals, and the facilities are permitted to hold animals for up to 7 days before transporting them directly to another holding station, to a recognized slaughter facility, or licensed hunting preserve (also referred to as captive hunt facilities or shooting preserves). Importantly, the TAHC requires wild pig holding facilities and hunting preserves to maintain records of wild pig transactions and to meet specified biosecurity requirements. For example, they must maintain a swine-proof fence, and holding facilities cannot be located within 200 yards of domestic swine (4 Tex. Admin. Code § 55.9(c) and (d)). Holding station operators are also required to remove and properly dispose of carcasses of wild pigs that die of certain communicable diseases (4 Tex. Admin. Code §§ 55.9(c) and 59.12). While these regulations do not require disposal if the animals are not suspected to have died from a communicable disease, all holding facility operators sign an agreement with the TAHC that requires prompt removal and burial of all feral swine carcasses.

With regard to wild pig slaughter facilities, there are three general categories in Texas: (i) custom exempt slaughter facilities that process swine for the use of the owner; (ii) state-inspected slaughter facilities, which process swine intended for sale within Texas; and (iii) federally inspected slaughter facilities, which slaughter and process swine intended for domestic and overseas markets. However, only federally inspected facilities typically accept and slaughter live wild pigs in Texas. Akkina et al. (57) reported that between January 1, 2017 and January 4, 2020, the six federally inspected facilities in Texas slaughtered 239,338 wild pigs, which represented nearly 99% of all

wild pigs slaughtered in the U.S. at federally inspected facilities during that period (57). According to interviewees with direct knowledge, two of the six Texas facilities slaughter and process the vast majority of wild pigs. Given the immense size of Texas and the relatively small number of federally inspected slaughter facilities, wild pigs may be transported over long distances, including entering Texas across state borders. An interviewee familiar with one facility indicated that it regularly receives wild pigs transported from Oklahoma, more than 110 km to the north. With the stress of trapping and transport, it is not uncommon for wild pigs to become sick or expire before they reach slaughter. Another interviewee reported that at one facility, wild pigs often arrive stressed and in poor health, which is reflected in the relatively high rate of condemnation reported by Akkina et al. (57) between 2017 and 2020.

All wild pigs at federally inspected facilities receive an antemortem inspection and a follow-up inspection by a veterinarian for animals labeled "suspect" (57). It is imperative that wild pigs at slaughter facilities are monitored for signs of foreign animal diseases and are part of a comprehensive surveillance program. Wild and domestic swine at slaughter facilities are targeted for surveillance in the U.S. as part of the integrated surveillance plan for swine hemorrhagic fevers (i.e., African and classical swine fever) (96).

As the foregoing suggests, there is a well-developed regulatory and organizational infrastructure in Texas to support a large network of wild pig market chains. Although biosecurity requirements imposed by federal and state regulations mitigate the risk of escapes and other paths of disease transmission, they do not completely eliminate the risk. In 2011, for example, a Dallas-Fort Worth news organization reported that approximately 30 wild pigs escaped from a Fort Worth slaughter facility (97). Moreover, the stress and hardship wild pigs experience prior to reaching their final destination increase the likelihood of mortalities and improper carcass disposal at holding facilities or during transit. These avenues could have severe consequences, including the loss of domestic swine production, if ASF were to emerge in the wild pig population (98) and would make for an extremely challenging on-the-ground disease management scenario.

Discussion

The risk of spillover-spillback of ASF at the wild-domestic interface poses a unique challenge for protecting the U.S. domestic pig herd and limiting economic consequences. These challenges are multifaceted, complicated by the biology of the virus, the widespread distribution of wild pigs that could serve as a source of ongoing disease transmission, and diversity of pork production practices. As the global ASF epizootic continues, viral circulation among domestic and wild populations across Africa, Asia, Europe, and on the island of Hispaniola poses risks for introduction into the U.S. because of an increasingly globalized economy and the uniquely resilient nature of the virus. In the absence of effective treatment, the introduction of ASF into domestic herds would elicit a strategic response structured around disease containment, necessary culling, surveillance, and contact tracing. However, effective containment of ASF among invasive wild pigs would require a far more complex and intensive response given the challenges of disease containment among wild populations (99).

In response to threats posed to the pork industry by ASF, a great deal of resources have been invested in developing response plans for a potential ASF introduction, both in the U.S. and many countries across the globe (24). Plans to respond to ASF outbreaks involving wild pigs have been informed with the best available information drawn from ongoing control efforts to reduce population abundance and damage caused by this invasive species (66). However, the potential scale of an ASF response involving wild pigs could be much larger than current control efforts for population and damage reduction. The distinction between past population control activities and planned response efforts highlights knowledge gaps in our understanding of the biological response within the host-pathogen system. How might wild pig movement patterns and concomitant disease transmission dynamics change in response to intensive culling efforts, decreasing densities attributable to both culling pressures and disease-related mortality, and increased human activity associated with carcasses searches/disposal? How can wild pig population densities be efficiently predicted during control efforts to support effective surveillance design and the declaration of post-outbreak disease freedom? Filling these knowledge gaps will require studies that implement consistent, intense control at the scale of a disease response. Modeling exercises have very effectively integrated the available data while delineating the limits of understanding and identifying where assumptions regarding disease dynamics need to be made to continue response planning (66). However, these analyses have also demonstrated that the response of wild pigs, a uniquely generalist and highly adaptable species, varies with landscape context, thus limiting the capacity to generalize across the breadth of invaded habitats. Similar challenges have been reflected in the European experience of managing ASF among wild boar in that management strategies may not be universally effective due to both biological and sociological differences among countries.

In both developing and conducting an effective ASF disease response, it is imperative to not overlook sociological aspects that may impede control. Public education related to biosecurity is an important tool to reduce the risk of initial introduction. During planning phases and throughout an outbreak, public outreach is a critical component of a successful response as a diverse set of stakeholders will be impacted. Education and outreach are essential for generating support among the general public however policy makers have a critical role in establishing a regulatory landscape conducive for an effective response. The spurious description of wild pigs as simply feral domestic animals further confuses jurisdiction of this invasive species (50, 100). The current state-by-state patchwork of policies that regulate wild pigs will need to be integrated into a unified State-Federal Incident Command in the event that a multi-state ASF response is required. Multiple studies have demonstrated ongoing and frequent human-facilitated movement of wild pigs—even into those states that prohibit the possession, transport, or release of wild pigs (50, 73, 74). These translocations have also been linked to the introduction of endemic diseases (i.e., swine brucellosis and pseudorabies) and similarly could function to amplify the spread of ASF. Given the heightened risk of ASF introduction, there is need for improved regulation of movement of wild pigs. As with the extensive research and operational investment into preparation and planning for an ASF response, developing and implementing education, outreach, and policy solutions also represents a lengthy investment. Accordingly, equal urgency and determination is needed in preparing effective

strategies for managing the sociological aspects of an ASF outbreak as has been given to biological and logistical concerns.

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Conflict of interest

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

Publisher's note

References

1. Ruiz-Saenz J, Diaz A, Bonilla-Aldana DK, Rodriguez-Morales AJ, Martinez-Gutierrez M, Aguilar PV. African swine fever virus: A re-emerging threat to the swine industry and food security in the Americas. *Front Microbiol.* (2022). 13:1011891. doi: 10.3389/fmicb.2022.1011891

2. Brown VR, Miller RS, McKee SC, Ernst KH, Didero NM, Maison RM, et al. Risks of introduction and economic consequences associated with African swine fever, classical swine fever and foot-and-mouth disease: a review of the literature. *Transbound Emerg Dis*. (2020):1–56. doi: 10.1111/tbed.13919

3. Gallardo MC, de la Torre Reoyo A, Fernández-Pinero J, Iglesias I, Muñoz MJ, Arias ML. African swine fever: a global view of the current challenge. *Porcine Health Manag*. (2015) 1:1–14. doi: 10.1186/s40813-015-0013-y

4. Jori F, Vial L, Penrith ML, Perez-Sanchez R, Etter E, Albina E, et al. Review of the sylvatic cycle of African swine fever in sub-Saharan Africa and the Indian Ocean. *Virus Res*. (2013) 173:212–27. doi: 10.1016/j.virusres.2012.10.005

5. Cadenas-Fernandez E, Sanchez-Vizcaino JM, Pintore A, Denurra D, Cherchi M, Jurado C, et al. Free-ranging pig and wild boar interactions in an endemic area of African swine fever. *Front Vet Sci*. (2019) 6:376. doi: 10.3389/fvets.2019.00376

6. Costard S, Wieland B, de Glanville W, Jori F, Rowlands R, Vosloo W, et al. African swine fever: how can global spread be prevented? *Philos Trans R Soc B*. (2009) 364:2683–96. doi: 10.1098/rstb.2009.0098

7. Gaudreault NN, Madden DW, Wilson WC, Trujillo JD, Richt JA. African swine fever virus: an emerging DNA arbovirus. *Front Vet Sci*. (2020) 7:215. doi: 10.3389/fvets.2020.00215

8. Sanchez-Vizcaino JM, Laddomada A, Arias AL. Chapter 25: African swine fever virus In: JJ Zimmerman, LA Karriker, A Ramirez, KJ Schwartz, GW Stevenson and J Zhang, editors. *Diseases of swine*. Hoboken, NJ: John Wiley & Sons, Inc (2019). 443–52.

9. Kebler C, Forth JH, Keil GM, Mettenleiter TC, Blome S, Karger A. The intracellular proteome of African swine fever virus. *Sci Rep*. (2018) 8:14714. doi: 10.1038/s41598-018-32985-z

10. Sanchez-Vizcaino JM, Mur L, Gomez-Villamandos JC, Carrasco L. An update on the epidemiology and pathology of African swine fever. *J Comp Pathol*. (2015) 152:9–21. doi: 10.1016/j.jcpa.2014.09.003

11. Jiang D, Ma T, Hao M, Ding F, Sun K, Wang Q, et al. Quantifying risk factors and potential geographic extent of African swine fever across the world. *PLoS One*. (2022) 17:e0267128. doi: 10.1371/journal.pone.0267128

12. Pikalo J, Schoder M-E, Sehl J, Breithaupt A, Tignon T, Cay AB, et al. The African swine fever virus isolate Belgium 2018/1 shows high virulence in European wild boar. *Transbound Emerg Dis*. (2020) 67:1654–9. doi: 10.1111/tbed.13503

13. European Commission. (2020). African swine fever: Eradication in Belgium confirmed. Available at: https://ec.europa.eu/newsroom/sante/items/694493

14. Sauter-Louis C, Forth JH, Probst C, Staubach C, Hlinak A, Rudovsky A, et al. Joining the club: first detection of African swine fever in wild boar in Germany. *Transbound Emerg Dis*. (2020) 68:1744–52. doi: 10.1111/tbed.13890

15. Iscaro C, Dondo A, Ruocco L, Masoero L, Giammarioli M, Zoppi S, et al. January 2022: index case of new African swine fever incursion in mainland Italy. *Transbound Emerg Dis*. (2022) 69:1707–11. doi: 10.1111/tbed.14584

16. National Veterinary Institute, Sweden. (2023). African swine fever in wild boar outside Fagersta, Sweden. Available at: https://www.sva.se/aktuellt/pressmeddelanden/african-swine-fever-in-wild-boar-outside-fagersta-sweden/.

17. Spinard E, O'Donnell V, Vuono E, Rai A, Davis C, Ramirez-Medina E, et al. Full genome sequence for the African swine fever virus in the Dominican Republic in 1980. *Sci Rep*. (2023) 13:1024. doi: 10.1038/s41598-022-25987-5

18. Kim SW, Gormley A, Jang KB, Duarte ME. Current status of global pig production: an overview and research trends. *Anim Biosci*. (2023). doi: 10.5713/ab.23.0367

19. Carriquiry M., Elobeid A., Hayes D., Zhang W. (2019). Impact of African swine fever on US and world commodity markets. Agricultural policy review. Center for Agricultural and Rural Development, Iowa State University. Available at: https://dr.lib.iastate.edu/server/api/core/bitstreams/57596270-02d8-422f-8d77-d80809e8fd9e/content

20. Pork Checkoff. (2023). U.S. pork exports. Available at: https://www.porkcheckoff.org/markets/us-pork-exports/#:~:text=Exports%20accounted%20for%2027.5%25%20of,for%20every%20U.S.%20hog%20marketed

21. Carriquiry M., Elobeid A., Swenson D., Hayes D. (2020). Impacts of African swine fever in Iowa and the United States. Center for Agricultural and Rural Development, Iowa State University. Available at: https://www.card.iastate.edu/products/publications/pdf/20wp600.pdf

22. Chenais E, Kepner K, Guberti V, Dietze K, Viltrop A, Stahl K. Epidemiological considerations on African swine fever in Europe 2014-2018. *Porcine Health Manag*. (2019) 5:1–10. doi: 10.1186/s40813-018-0109-2

23. USDA APHIS. (2023). African swine fever response. Outbreak in feral swine: Incident playbook. Available at: https://www.aphis.usda.gov/animal_health/downloads/animal_diseases/swine/asf-feral-swine-playbook.pdf.

24. USDA APHIS. (2023). African swine fever response plan: the red book. Available at: https://www.aphis.usda.gov/animal_health/emergency_management/downloads/asf-responseplan.pdf

25. Kinsley AC, Perez AM, Craft ME, Vanderwaal KL. Characterization of swine movements in the United States and implications for disease control. *Prev Vet Med*. (2019) 164:1–9. doi: 10.1016/j.prevetmed.2019.01.001

26. Scheidt AB, Cline TR, Clark LK, Mayrose VB, Van Alstine WG, Diekman MA, et al. The effect of all-in-all-out growing-finishing on the health of pigs. *Swine Heal Prod*. (1995) 3:202–5.

27. Marchant-Forde JN, Boyle LA. COVID-19 effects on livestock production: a one welfare issue. *Front Vet Sci*. (2020) 7:585787. doi: 10.3389/fvets.2020.585787

28. Costard S, Mur L, Lubroth J, Sanchez-Vizcaino JM, Pfeiffer DU. Epidemiology of African swine fever virus. *Virus Res*. (2013) 173:191–7. doi: 10.1016/j.virusres.2012.10.030

29. Teklue T, Sun Y, Abid M, Luo Y, Qiu H-J. Current status and evolving approaches to African swine fever vaccine development. *Transbound Emerg Dis*. (2019) 67:529–42. doi: 10.1111/tbed.13364

30. Rock DL. Challenges for African swine fever vaccine development—"… perhaps the end of the beginning.". *Vet Microbiol*. (2017) 206:52–8. doi: 10.1016/j.vetmic.2016.10.003

31. Urbano AC, Ferreira F. African swine fever control and prevention: an update on vaccine development. *Emerg Microbes Infect*. (2022) 11:2021–33. doi: 10.1080/22221751.2022.2108342

32. Ramirez-Medina E., Vuono E., Silva E., Valladares A.R.A, Pruitt S., Espinoza N., Velazquez-Salinas L., Borca M.V., Gladue D.P. (2022). Evaluation of the deletion of MGF110-5L-6L on swine virulence from the pandemic strain of African swine fever virus and use as a DIVA marker in vaccine candidate ASFV-G-ΔI177L. *J Virol* 96: e00597–e00522. doi: 10.1128/jvi.00597-22

33. Tran XH, Thu Phuong LT, Quang Huy N, Thanh Thuy D, Dung Nguyen V, Hào Quang P, et al. Evaluation of the safety profile of the ASFV vaccine candidate ASFV-G-ΔI177L. *Viruses*. (2022) 14:1–12. doi: 10.3390/v14050896

34. Zhang H, Zhao S, Zhang H, Qin Z, Shan H, Cai X. Vaccines for African swine fever: an update. *Front Microbiol*. (2023) 14:1139494. doi: 10.3389/fmicb.2023.1139494

35. Arzumanyan H, Hakobyan S, Avagyan H, Izmailyan R, Nersisyan N, Karalyan Z. Possibility of long-term survival of African swine fever virus in natural conditions. *Vet World*. (2021) 14:854–9. doi: 10.14202/vetworld.2021.854-859

36. Mazur-Panasuik N, Zmudzki J, Wozniakowski G. African swine fever virus – persistence in different conditions and the possibility of its indirect transmission. *J Vet Res*. (2019) 63:303–10. doi: 10.2478/jvetres-2019-0058

37. Kalmar ID, Cay AB, Tignon M. Sensitivity of African swine fever virus (ASFV) to heat, alkalinity and peroxide treatment in presence or absence of porcine plasma. *Vet Mic*. (2018) 219:144–9. doi: 10.1016/j.vetmic.2018.04.025

38. Guinat C, Gogin A, Blome S, Keil G, Pollin R, Pfeiffer DU, et al. Transmission routes of African swine fever virus to domestic pigs: current knowledge and future research directions. *Vet Rec*. (2016) 178:262–7. doi: 10.1136/vr.103593

39. Nuanualsuwan S, Songkasupa T, Boonpornprasert P, Suwankitwat N, Lohlamoh W, Nuengjamnong C. Therman inactivation of African swine fever virus in swill. *Front Vet Sci*. (2022) 9:906064. doi: 10.3389/fvets.2022.906064

40. U.S. Congress. (1980). Swine health protection act. Public Law 96-468.

41. Sauter-Louis C, Conraths FJ, Probst C, Blohm U, Schulz K, Sehl J, et al. African swine fever in wild boar in Europe: a review. *Viruses*. (2021) 13:1–30. doi: 10.3390/v13091717

42. Bowden CF, Grinolds J, Franckowiak G, McCallister L, Halseth J, Cleland M, et al. Evaluation of the effect of hydrated lime on the scavenging of feral swine (*Sus scrofa*) carcasses and implications for managing carcass-based transmission of African swine fever virus. *J Wildl Dis*. (2023) 59:49–60. doi: 10.7589/JWD-D-22-00061

43. Pepin KM, Golnar A, Abdo Z, Podgorski T. Ecological drivers of the persistence of African swine fever in wild boar populations: insight for control. *Ecol Evol*. (2020) 10:2846–59. doi: 10.1002/ece3.6100

44. Keiter DA, Mayer JJ, Beasley JC. What is in a "common" name? A call for consistent terminology for nonnative *Sus scrofa*. *Wildl Soc Bull*. (2016) 40:384–7. doi: 10.1002/wsb.649

45. Slatyer R., Hafi A., Richards K., Cozens M., Addai D., Cao L., et al. (2023). Potential economic consequences of African swine fever in Australia, ABARES research report 23.07, Canberra, March, CC BY 4.0.

46. East IJ, Martin PAJ, Langstaff I, Iglesias RM, Sergeant ESG, Garner MG. Assessing the delay to detection and the size of the outbreak at the time of detection of incursions of foot and mouth disease in Australia. *Prev Vet Med*. (2016) 123:1–11. doi: 10.1016/j.prevetmed.2015.12.005

47. Vergne T, Guinat C, Pfeiffer DU. Undetected circulation of African swine fever in wild boar, Asia. *Emerg Infect Dis*. (2020) 26:2480–2. doi: 10.3201/eid2610.200608

48. Miller RS, Bevins SN, Cook G, Free R, Pepin KM, Gidlewski T, et al. Adaptive risk-based targeted surveillance for foreign animal diseases at the wildlife-livestock interface. *Transbound Emerg Dis*. (2022) 69:e2329–40. doi: 10.1111/tbed.14576

49. Smyser TJ, Pfaffelhuber P, Giglio RM, DeSaix MG, Davis AJ, Bowden CF, et al. Probabilistic genetic identification of wild boar hybridization to support control of invasive wild pigs (*Sus scrofa*). *Ecosphere*. (2024).

50. Smyser TJ, Tabak MA, Slootmaker C, Robeson MS II, Miller RS, Bosse M, et al. Mixed ancestry from wild and domestic lineages contributes to the rapid expansion of invasive feral swine. *Mol Ecol*. (2020) 29:1103–19. doi: 10.1111/mec.15392

51. Lewis JS, Corn JL, Mayer JJ, Jordan TR, Farnsworth ML, Burdett CL, et al. Historical, current, and potential population size estimates of invasive wild pigs (*Sus scrofa*) in the United States. *Biol Invasions*. (2019) 21:2373–84. doi: 10.1007/s10530-019-01983-1

52. Lowe SM, Browne M, Boudjelas S, de Poorter M. *100 of the world's worst invasive alien species: A selection from the global invasive species database*. Auckland, New Zealand: Invasive Species Specialist Group (2000).

53. USDA APHIS. (2023). History of feral swine in the Americas. Available at: https://www.aphis.usda.gov/aphis/ourfocus/wildlifedamage/operational-activities/feral-swine/feral-swine-history

54. McClure ML, Burdett CL, Farnsworth ML, Lutman MW, Theobald DM, Riggs PD, et al. Modeling and mapping the probability of occurrence of wild pigs across the contiguous United States. *PLoS One*. (2015) 10:e0133771. doi: 10.1371/journal.pone.0133771

55. van Eeden LM, Newsome TM, Crowther MS, Dickman CR, Bruskotter J. Diverse public perceptions of species' status and management align with conflicting conservation frameworks. *Biol Conserv*. (2020) 242:108416. doi: 10.1016/j.biocon.2020.108416

56. Morner T, Obendorf DL, Artois M, Woodford MH. Surveillance and monitoring of wildlife diseases. *Rev Sci Tech*. (2002) 21:67–76. doi: 10.20506/rst.21.1.1321

57. Akkina J, Burkom H, Estberg E, Carpenter L, Hennessey M, Meidenbauer K. Feral swine commercial slaughter and condemnation at federally inspected slaughter establishments in the United States 2017-2019. *Front Vet Sci*. (2021) 8:690346. doi: 10.3389/fvets.2021.690346

58. Morelle K, Barasona JA, Bosch J, Heine G, Daim A, Arnold J, et al. Accelerometer-based detection of African swine fever infection in wild boar. *Proc R Soc B*. (2023) 290:20231396. doi: 10.1098/rspb.2023.1396

59. Hu B, Gonzales JL, Gubbins S. Bayesian inference of epidemiological parameters from transmission experiments. *Sci Rep*. (2017) 7:16774. doi: 10.1038/s41598-017-17174-8

60. Main AR, Halasa T, Olesen AS, Lohse L, Rasmussen TB, Belsham GJ, et al. Estimating transmission dynamics of African swine fever virus from experimental studies. *Transbound Emerg Dis*. (2022) 69:3858–67. doi: 10.1111/tbed.14757

61. Rhyan JC, Spraker TR. Emergence of diseases from wildlife reservoirs. *Vet Pathol*. (2010) 47:34–9. doi: 10.1177/0300985809354466

62. Rietz J, van Beeck Calkoen STS, Ferry N, Schluter J, Wehner H, Schindlatz K-H, et al. Drone-based thermal imaging in the detection of wildlife carcasses and disease management. *Transbound Emerg Dis*. (2023) 2023:1–12. doi: 10.1155/2023/5517000

63. Kay SL, Fischer JW, Monaghan AJ, Beasley JC, Boughton R, Campbell TA, et al. Quantifying drivers of wild pig movement across multiple spatial and temporal scales. *Mov Ecol*. (2017) 5:14. doi: 10.1186/s40462-017-0105-1

64. Pepin KM, Davis AJ, Beasley J, Boughton R, Campbell T, Cooper SM, et al. Contact heterogeneities in feral swine: implications for disease management and future research. *Ecosphere*. (2016) 7:e01230. doi: 10.1002/ecs2.1230

65. Yang A, Schlichting PE, Wight B, Anderson W, Chinn S, Wilber MQ, et al. Effects of social structure and management on risk of disease establishment in wild pigs. *J Anim Ecol*. (2021) 90:820–33. doi: 10.1111/1365-2656.13412

66. Pepin KM, Brown VB, Yang A, Beasley JC, Boughton R, VerCauteren KC, et al. Optimising response to an introduction of African swine fever in wild pigs. *Transbound Emerg Dis*. (2022) 69:e3111–27. doi: 10.1111/tbed.14668

67. Pepin KM, Golnar A, Podgorski T. Social structure defines spatial transmission of African swine fever in wild boar. *J R Soc Interface*. (2021) 18:20200761. doi: 10.1098/rsif.2020.0761

68. Pepin KM, VerCauteren KC. Disease-emergence dynamics and control in a socially-structured wildlife species. *Sci Rep*. (2016) 6:25150. doi: 10.1038/srep25150

69. Maselli V, Rippa D, Russo G, Ligorne R, Soppelsa O, DAniello B, et al. Wild boars' social structure in the Mediterranean habitat. *Ital J Zool*. (2014) 81:610–7. doi: 10.1080/11250003.2014.953220

70. McIlraith J. (2021). Social structure and cohesiveness of GPS tracked wild pigs in the southeastern United States. Electronic Thesis and Dissertation Repository. 8089. Available at: https://ir.lib.uwo.ca/etd/8089.

71. Titus CL, Bowden CF, Smyser TJ, Webb SL, Beasley JC. Genomic tools reveal complex social organization of an invasive large mammal (*Sus scrofa*). *Biol Invasions*. (2022) 24:3199–216. doi: 10.1007/s10530-022-02840-4

72. Clontz LM, Yang A, Chinn SM, Pepin KM, VerCauteren KC, Wittemyer G, et al. Role of social structure in establishment of an invasive large mammal after translocation. *Pest Manag Sci*. (2023) 79:3819–29. doi: 10.1002/ps.7567

73. Hernandez FA, Sayler KA, Bounds C, Milleson MP, Carr AN, Wisely SM. Evidence of pseudorabies virus shedding in feral swine (*Sus scrofa*) populations in Florida, USA. *J Wildl Dis*. (2018) 54:45–53. doi: 10.7589/2017-04-071

74. Tabak MA, Piaggio AJ, Miller RS, Sweitzer RA, Ernest HB. Anthropogenic factors predict movement of an invasive species. *Ecosphere*. (2017) 8:e01844. doi: 10.1002/ecs2.1844

75. Brown VR, Lenoch JB, Bowden CF. African swine fever In: D Jessup and RW Radcliffe, editors. *Wildlife disease/health and conservation*. Baltimore, MD: Johns Hopkins University Press (2023)

76. Jori F, Chenais E, Boinas F, Busauskas P, Dholllander S, Fleischmann L, et al. Application of the world Café method to discuss the efficiency of African swine fever control strategies in European wild boar (*Sus scrofa*) populations. *Prev Vet Med*. (2020) 185:105178. doi: 10.1016/j.prevetmed.2020.105178

77. Jori F, Massei G, Licoppe A, Ruiz-Fons F, Linden A, Václavek P, et al. Management of wild boar populations in the European Union before and during the ASF crisis In: L Iacolina, M-L Penrith, S Bellini, E Chenais, F Jori and M Montoya, et al. editors. *Understanding and combatting African swine fever a European perspective*. Wageningen, The Netherlands: Wageningen Academic Publishers (2021)

78. Thompson NE, Huang MHJ, Christensen SA, Demarais S. Wildlife agency responses to chronic wasting disease in free-ranging cervids. *Wildl Soc Bull*. (2021) 47:e1435. doi: 10.1002/wsb.1435

79. Voss SJ, Malladi S, Sampedro F, Snider T, Goldsmith T, Hueston WD, et al. Incorporating risk communication into highly pathogenic avian influenza preparedness and response efforts. *Avian Dis*. (2012) 56:1049–53. doi: 10.1637/10186-041012-Reg.1

80. Donnelly CA, Woodroffe R, Cox DR, Bourne FJ, Cheeseman CL, Clifton-Hadley RS, et al. Positive and negative effects of widespread badger culling on tuberculosis in cattle. *Nature*. (2006) 439:843–6. doi: 10.1038/nature04454

81. Bastille-Rousseau G, Schlichting PE, Keiter DA, Smith JB, Kilgo J, Wittemyer G, et al. Multi-level movement response of invasive wild pigs (*Sus scrofa*) to removal. *Pest Manag Sci*. (2020) 77:85–95. doi: 10.1002/ps.6029

82. Keuling O, Massei G. Does hunting affect the behavior of wild pigs? *Hum-Wildl Interact*. (2021) 14:1. doi: 10.26077/3a83-9155

83. Probst C, Globig A, Knoll B, Conraths FJ, Depner K. Behaviour of free ranging wild boar towards their dead fellows: potential implications for the transmission of African swine fever. *R Soc Open Sci*. (2017) 4:170054. doi: 10.1098/rsos.170054

84. Leivers S, Campbell TA, Bodenchuk M, Tomecek JM. Behavior of wild pigs toward conspecific carcasses: implications for disease transmission in a hot, semiarid climate. *Transbound Emerg Dis*. (2023) 2023:1–10. doi: 10.1155/2023/4195199

85. Centner TJ, Shuman RM. Governmental provisions to manage and eradicate feral swine in areas of the United States. *Ambio*. (2015) 44:121–30. doi: 10.1007/s13280-014-0532-9

86. Smith AL, Strickland BK, Leopold BD, Cummins JL, Mayer JJ, Street GM. Cultural and regulatory factors influence distribution and trajectory of invasive species in the United States: a wild pig case study. *J Environ Manag*. (2023) 338:117742. doi: 10.1016/j.jenvman.2023.117742

87. USDA NIFA. (2023). Feral hogs: laws and regulations. Available at: https://feralhogs.extension.org/laws-and-regulations/

88. Moritz W.E., Cornicelli L. (2023). Research and analysis of policy and law to prevent translocation of live feral swine. Multistate conservation Grant program project #F22AP00690–00. Available at: https://wildlifemanagement.institute/sites/default/files/2023-07/feral_swine_final_report.pdf.

89. European Food Safety Authority (EFSA)Stahl K, Boklund A, Podgorski T, Vergne T, Abrahantes JC, et al. Scientific report on epidemiological analysis of African swine fever in the European Union during 2022. *EFSA J*. (2023) 21:e08016. doi: 10.2903/j.efsa.2023.8016,

90. Gao X, Liu T, Liu Y, Xiao J, Wang H. Transmission of African swine fever in China through legal trade of live pigs. *Transbound Emerg Dis*. (2020) 68:355–60. doi: 10.1111/tbed.13681

91. Danzetta ML, Marenzoni ML, Iannetti S, Tizzani P, Calistri P, Feliziani F. African swine fever: lessons to learn from past eradication experiences – a systematic review. *Front Vet Sci*. (2020) 7:296. doi: 10.3389/fvets.2020.00296

92. Carlisle KM, McKee S, McLean HE, Jaebker LM, Tomeček JM, Bright AD, et al. Texas hunters' perceptions regarding the acceptability of toxicants to control wild pig populations. *Hum-Wildl Interact*. (2022) 16:399–414. doi: 10.26077/4002-0ce2

93. Connally RL, Frank M, Briers G, Silvy N, Carlisle KM, Tomeček JM. A profile of wild pig hunters in Texas. *Hum-Wildl Interact*. (2021) 15:6–21. doi: 10.26077/d51b-9e40

94. Carlisle KM, Didero N, McKee S, Elser J, Shwiff SA. Towards a more comprehensive understanding of wild pig (*Sus scrofa*) impacts on agricultural producers: insights from a Texas case study. *Crop Prot*. (2021) 150:105793. doi: 10.1016/j.cropro.2021.105793

95. Grady M, Harper EE, Carlisle K, Ernst KH, Shwiff SA. Assessing public support for restrictions on transport of invasive wild pigs (*Sus scrofa*) in the United States. *J Environ Manag*. (2019) 237:488–94. doi: 10.1016/j.jenvman.2019.02.107

96. USDA. Swine hemorrhagic fevers: African and classical swine fevers. *Integrated Surveillance Plan*. (2022). https://www.aphis.usda.gov/animal_health/downloads/animal_diseases/swine/hemorrhagic-fevers-integrated-surveillance-plan.pdf

97. Villafranca O. (2011). Feral hogs escape meat processing plant. NBCDFW. Available at: https://www.nbcdfw.com/news/local/feral-hogs-escape-meat-processing-plant/1908902/#:~:text=Fort%20Worth%20residents%20are%20used%20to%20livestock%20drives%2C,a%20north%20side%20meat%20processing%20plant%20Sunday%20morning

98. Brown VR, Bevins SN. A review of African swine fever and the potential for introduction into the United States and the possibility of subsequent establishment in feral swine and native ticks. *Front Vet Sci*. (2018). 5:11. doi: 10.3389/fvets.2018.00011

99. Palencia P, Blome S, Brook RK, Ferroglio E, Jo YS, Linden A, et al. Tools and opportunities for African swine fever control in wild boar and feral pigs: a review. *Euro J Wildl Res*. (2023) 69:69. doi: 10.1007/s10344-023-01696-w

100. Mayer JJ, Brisbin IL. Wild pigs in the United States: their history, comparative biology, and current status. (1991).

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Use of rhodamine B as a biomarker in a simulated oral vaccine deployment against bovine tuberculosis in white-tailed deer

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Introduction: Free-ranging white-tailed deer (*Odocoileus virginianus*) in northeastern lower Michigan, (United States) are a self-sustaining reservoir for bovine tuberculosis (bTB). Farm mitigation practices, baiting bans, and antlerless deer harvests have been ineffective in eliminating bTB in white-tailed deer and risks to cattle. The apparent prevalence has remained relatively constant in deer, prompting interest among wildlife researchers, managers, and veterinarians for an effective means of vaccinating deer against bTB. The commonly used human vaccine for bTB, Bacillus Calmette Guerin (BCG), is the primary candidate with oral delivery being the logical means for vaccinating deer.

Materials and methods: We developed vaccine delivery units and incorporated the biomarker Rhodamine B before delivering them to deer to assess the level of coverage achievable. Following deployment of Rhodamine B-laden vaccine delivery units on 17 agricultural study sites in Alpena County, MI in Mar/Apr 2016, we sampled deer to detect evidence of Rhodamine B consumption.

Results and discussion: We collected a total of 116 deer and sampled them for vibrissae/rumen marking and found 66.3% (n = 77) of the deer collected exhibited evidence of vaccine delivery unit consumption. Understanding the level of coverage we achieved with oral delivery of a biomarker in vaccine delivery units to deer enables natural resource professionals to forecast expectations of a next step toward further minimizing bTB in deer.

KEYWORDS

biomarker, bovine tuberculosis, rhodamine B, vaccine delivery, white-tailed deer

1 Introduction

Bovine tuberculosis (bTB) is an infectious disease caused by *Mycobacterium bovis* (1) and is maintained in several wildlife reservoirs including European badgers (*Meles meles*) in the United Kingdom (2), France (3), and in the Republic of Ireland (4); brushtail possums (*Trichosurus vulpecula*) in New Zealand (5), cape buffalo (*Syncerus caffer*) in Africa (6); and wild boar (*Sus scrofa*), red deer (*Cervus elaphus*), and fallow deer (*Dama dama*) in Spain (7, 8). In the United States, the wildlife reservoir of bTB is free-ranging white-tailed deer (*Odocoileus virginianus*) (hereafter referred to as 'deer') of northeastern lower Michigan (NELM), United States (9). Transmission of bTB between deer and cattle in NELM is a

primary concern for wildlife managers, the livestock industry, and the public. Transmission can occur through direct cattle-to-deer contact and indirect contact through shared feed and water (10, 11).

Wildlife managers implemented several methods in attempts to decrease the incidence of bTB in deer and decrease potential risks to cattle. Mitigation methods directed at wildlife have included actions such as exclusionary fences (12), increased antlerless harvest, restrictions on baiting (13), and issuing disease control permits to landowners and United States Department of Agriculture-Wildlife Services (USDA-WS) by Michigan Department of Natural Resources (MDNR) to decrease the incidence of bTB and potential for transmission (14, 15). These strategies have had limited success in reducing the apparent prevalence of bTB thus far. The MDNR established Deer Management Unit 452 (DMU 452) and more recently the expanded DMU 487 to encompass the core area of bTB infection in deer and focus disease management activity. Over the past 15 years the apparent prevalence of bTB in DMU 452 has stalled with minimal fluctuation between 1 and 2% (16, 17). The continued transmission of bTB from deer to cattle and the stalled apparent prevalence has given precedent for seeking novel management strategies to combat bTB.

Oral vaccination of wildlife may be a viable strategy for disease management and is becoming more common for protecting wildlife, livestock, and people against disease transmission. For example, the Oral Rabies Vaccination program targeting raccoons (*Procyon lotor*) distributes nearly 10 million vaccine-laden baits across 18 primarily eastern states of United States annually and has been successful at preventing the spread of rabies (18, 19). Ongoing oral vaccination programs for reservoir hosts of bTB are demonstrating success in reducing incidence of bTB or severity of infection in the European badger in Ireland (20, 21) and the Eurasian wild boar in Spain (22). By combining depopulation efforts with oral vaccination, bTB incidence was significantly reduced in Brushtail possums in New Zealand (23). Experimental oral vaccination of red deer is proving effective and vaccine deployment strategies are being refined in Spain (24, 25).

Researchers have shown the bacillus Calmette-Guerin (BCG) vaccine reduces bTB disease severity in penned white-tailed deer which likely equates to decreased potential to transmit disease (26, 27). Deer that were orally vaccinated with BCG then intratonsilarly challenged with virulent *M. bovis* had reduced gross lesions and a BCG persistence of up to 12 months in lymphoid tissues (26, 27). Additionally, there is some evidence that deer can transmit BCG to unvaccinated deer (28, 29). The efficacy of BCG to be administered orally at scale to deer in NELM provides the capacity to make vaccination against bTB a reality. Although capture and vaccination of deer via injection has been deemed an option, it is labor intensive and costly (30).

Given the availability of a vaccine to inoculate deer against bTB, one primary obstacle for successful oral vaccination was the formulation and field delivery method of a species-specific vaccine delivery unit (VDU) that could be distributed and readily consumed by deer. Oral delivery may be the most cost-effective and feasible method to maximize delivery of a vaccine to a deer population (26). Before a bTB vaccination system can be initiated in free-ranging deer in NELM, understanding the potential coverage of delivery to deer must be investigated.

Rhodamine B (RB) has been used as an effective biomarker for several oral vaccination studies due to (1): the utility of RB as a systemic marker in whiskers and claws (2), the rapid absorption of RB into keratinous tissues (3), the ease of detection of fluorescent bands on whiskers using a fluorescence microscope, and (4) it is commercially available and relatively inexpensive (31). Rhodamine B has proven effective in bait uptake studies of European badgers (32), black-tailed prairie dogs (*Cynomys ludovicianus*) (33), raccoons (34), mountain beavers (*Aplodontia rufa*) (35), stoats (*Mustela ermine*) (36) and wild pigs (*Sus scrofa*) (37).

With current deer harvest rates and the baiting ban in NELM, eradication of bTB is predicted unlikely in the next 30 years. Even with a 100% compliance rate of the baiting ban there is only an 8% chance of reducing the incidence of bTB without implementation of additional strategies (14). However, models have demonstrated a vaccine coverage of 50% in the deer of DMU 452 could achieve an 86% probability of bTB eradication within 30 years (14). Thus, if further reduction or eradication of bTB in NELM is truly desired, additional management strategies must be explored and implemented. By distributing biomarker-laden VDUs to free-ranging deer in NELM it was possible to investigate the potential coverage of vaccination to combat bTB. The primary objective of our evaluation was to quantify the potential coverage of delivering pharmaceuticals orally to free-ranging deer by quantifying uptake of RB in customized VDUs.

2 Materials and methods

2.1 Study location

We implemented our RB-VDU trial from 7 February 2016 to 26 May 2016 in Alpena County of northeastern lower Michigan, United States Alpena County (439,000 ha) is the northeast county of DMU 452 (147,629 ha), the endemic bTB area with the highest apparent prevalence of bTB in Michigan deer (38). To date, bTB has been identified in 82 cattle herds in the area (39). At the time of the study, there were 189 cattle farms in Alpena County with a total of 8,838 head of cattle (40). One-hundred and eleven (58.7%) of these farms were primarily beef cattle operations and another 37 farms (19.6%) contained mostly dairy cows. Average farm size in Alpena County, United States was 61.1 ha with a total of 458 farms (40). Primary crops produced in Alpena County were hay and grass silage (8,030 ha), soybeans (2,258 ha), corn (2,146 ha) and wheat (1,152 ha) (40). We distributed VDUs on 25 agriculture fields consisting of crops including corn, wheat, alfalfa, or soybeans.

Alpena County consisted of forested land and agriculture lands with deer densities ranging from 10–14 deer/km^2 (41). Historically, deer density in this area has been as high as 18 deer/km^2 (42). Average annual temperature in the area was 6.6° C with annual rain and snowfall of 72.5 cm and 175.0 cm, respectively Huey. Elevation ranged from 150-390–m above sea level (43). Well-drained, sandy loam soils comprised much of the landscape and supported a variety of deciduous trees such as aspen (*Populus* spp.) and maple (*Acer* spp.) (44). Lowland conifer stands comprised of conifers such as northern white cedar (*Thuja occidentalis*) and balsam fir (*Abies balsamea*) were an important resource providing deer with thermal cover during winter (44).

Approximately 58% of the deer in this region of Michigan are migratory; most migratory deer (>80%) typically leave winter ranges by 1 May (45). During spring migration, migratory deer typically move to heavily forested areas and away from open-agriculture lands; however, as many as 45% of deer may establish summer ranges near

agriculture areas (45). Non-migratory deer in this area tend to establish home ranges in agriculture areas of NELM. Alfalfa fields are an important food resource for deer during the spring, contributing to significant crop loss within 90 m of field edges (46).

2.2 Vaccine delivery unit development

Based on previous work in developing VDUs for deer, we determined that an alfalfa and molasses-based matrix would maximize our potential for targeted delivery to deer, while minimizing consumption by non-target species (47). We combined the alfalfa and molasses-based livestock feed (Chaffhaye® Dell City, TX, United States) with Xanthan gum and water in a ribbon mixer to produce a coarse material that could be easily molded. We hand molded each VDU into 17–20-g "bite size" portions to adequately encase the RB-containing capsule while minimizing the potential for spillage which could encourage visitation by nontargets. Using a manual capsule filling machine (CN-100CL, CapsulCN International CO. LTD, Ruian, Zhejiang, China), we inserted 475 mg of RB (7 mg/kg dose for 67.8 kg deer) into 00 size gel capsules (1.17 cm x 2.02 cm), kept in sealed bags at room temperature until needed. This quantity of RB would provide sufficient marking in white-tailed deer, minimize any taste aversion, and was the highest quantity of RB that would physically fit into 00 size capsules. Once in the field, we inserted a single RB capsule into each VDU as we deployed them on agriculture fields. Ingestion of RB-laden VDU by deer causes two staining events (1); the oral (mouth, tongue) and internal cavity (rumen, intestine, and digestive tract) of deer are stained fluorescent pink for 24–36 h after consumption and (2) a fluorescent band appears on deer vibrissae and remains for at least 5 weeks post-consumption (48). The presence of oral, internal, or vibrissae staining allowed us to calculate the percentage of deer that consumed at least one VDU. We recorded total time (min) and cost ($ United States; adjusted to 2023 $) to construct VDUs for the entire process.

2.3 Vaccine delivery unit distribution and consumption

We distributed VDUs on 30 agriculture fields on 17 privately owned properties from 6 March 2016 to 26 May 2016. Specific VDU sites were selected using data from road surveys conducted in 2014 by USDA-Wildlife Services during which concentrations of deer were recorded (P. Ryan, Wildlife Biologist, USDA APHIS WS, personal communication). Specific agriculture fields were chosen based on (1), the type of crop grown during the previous year, and (2) anticipated deer activity from conversations with landowners and proximity to vegetation types that would provide deer habitat components. Before VDUs were distributed, all fields considered were monitored with trail cameras (Reconyx, RC60, Holmen, WI, United States) for thawing of snow cover and deer use and abundance from 7 February 2016–6 March 2016. A thawing event was defined as patches of soil and residual crops being exposed in otherwise snow-covered fields resulting from an increase in temperature and exposure to sun. We deployed VDUs when the first thawing event was observed on our VDU grids which coincided with increased deer use.

We established VDU grids on agricultural fields previously planted to wheat, soybean, alfalfa, or corn, which retained residual crop left after harvest. We determined previously that selecting lowland conifer stands maximized potential for visitation by multiple deer at this time of year (47), thus situated grids adjacent to lowland conifer stands when possible. Each VDU grid consisted of 52.5-m x 12.5-m plots with 100 VDUs spaced 2.5-m apart in grid format (Figure 1). We deployed VDUs for 4–9 consecutive nights with each night that VDUs were distributed being considered a VDU night and used for comparisons of visitation. During the first three VDU nights, we distributed VDUs that did not contain RB to accustom deer to visit grids and consume our VDUs. We checked grids once every 24 h and recorded all VDUs that were missing, assumed eaten and replaced. We recorded the number of VDUs deployed and consumed, paying close attention to whether RB capsules were consumed or left in the field (assumed detected and spit out).

2.4 White-tailed deer and non-target visitation

We installed three trail cameras focused on VDU grids from adjacent field edges and captured motion-activated and time-lapse imagery (1 image every 15 min). Images with the highest number of deer and non-target species in a single frame during a 24-h period were used to determine minimum number of individuals visiting VDU grids (Figure 2). Grid visits were recorded for deer and all non-target species raccoons, skunks (*Mephitis mephitis*), squirrels (*Sciurus* spp.), turkeys (*Meleagris gallopavo*), and eastern cottontail rabbits (*Sylvilagus floridanus*). We compared visitation using trail camera data and the percent of VDU grid nights visited by deer and non-target species.

2.5 Biomarker analysis

As early as the last night of VDU distribution, USDA-Wildlife Services began lethally sampling deer on each Rb VDU grid under the direction of MDNR Disease Control Permits. We targeted selection of 10 individual deer per site, but the final number of deer collected was dependent on landowner discretion and success rate. Deer collections were continued each night until our target number of deer was met, or opportunities no longer existed. All deer sampled were first necropsied and visually examined for internal staining of their digestive tract (primarily oral cavity and rumen), confirming RB uptake. Additionally, we collected six maxillary vibrissae (three tactile hairs or "whiskers" from each side of the mouth) from each deer using tweezers and immediately placed into a #7-coin envelope to be evaluated later for detection of fluorescent markings under ultraviolet light (49, 50).

We conducted vibrissae analyses at the USDA/APHIS/Wildlife Services – National Wildlife Research Center (NWRC, Fort Collins, CO, U.S.A.). Vibrissae were mounted on a 75 mm x 25 mm microscope slide (three vibrissae on each slide) using a fluoromount™ aqueous mounting medium. We used a fluorescent microscope (TRITC, Leica, Germany) with a 100 W mercury bulb and RB filter block to identify fluorescent bands on each vibrissae indicating consumption of an RB-laden VDU (Figure 3). All VDU

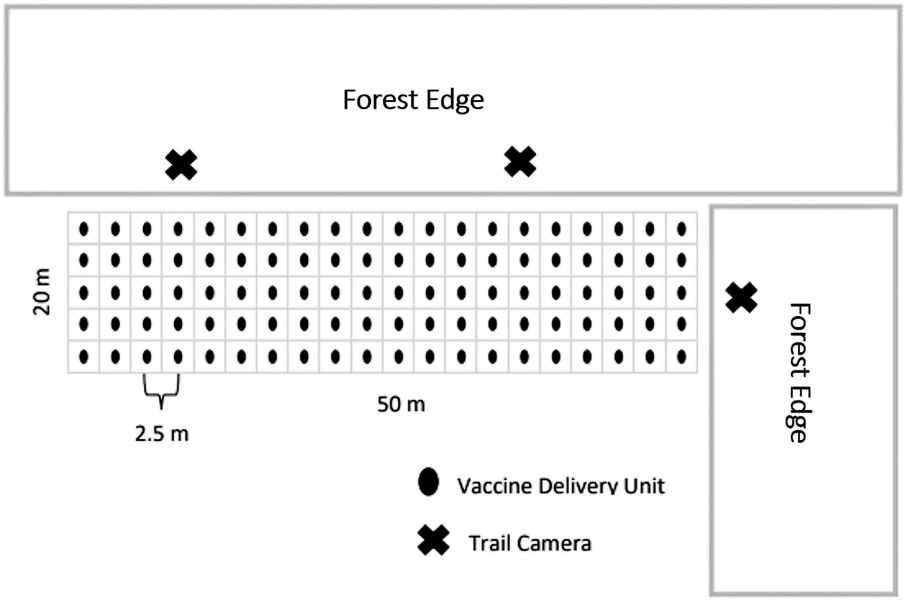

FIGURE 1
Layout of the 50-m by 20-m, 100-vaccine delivery unit grids placed on agriculture fields next to forest edges in 2016 simulated vaccine deployment against bovine tuberculosis in white-tailed deer (*Odocoileus virginianus*) in northeastern lower Michigan, United States.

FIGURE 2
Natural congregation of white-tailed deer (*Odocoileus virginianus*) on a thawed patch of an agricultural field during 2016 simulated vaccine deployment against bovine tuberculosis in white-tailed deer in northeastern lower Michigan, United States.

development, deployment, and data collection were reviewed and approved by the Michigan State University Animal Care and Use Committee (AUF # 05/15–084-00; 29 April 2015; Amended 4 January 2016).

2.6 Statistical analysis

We examined whether the probability of being marked with RB was influenced by sex of deer using a binomial generalized

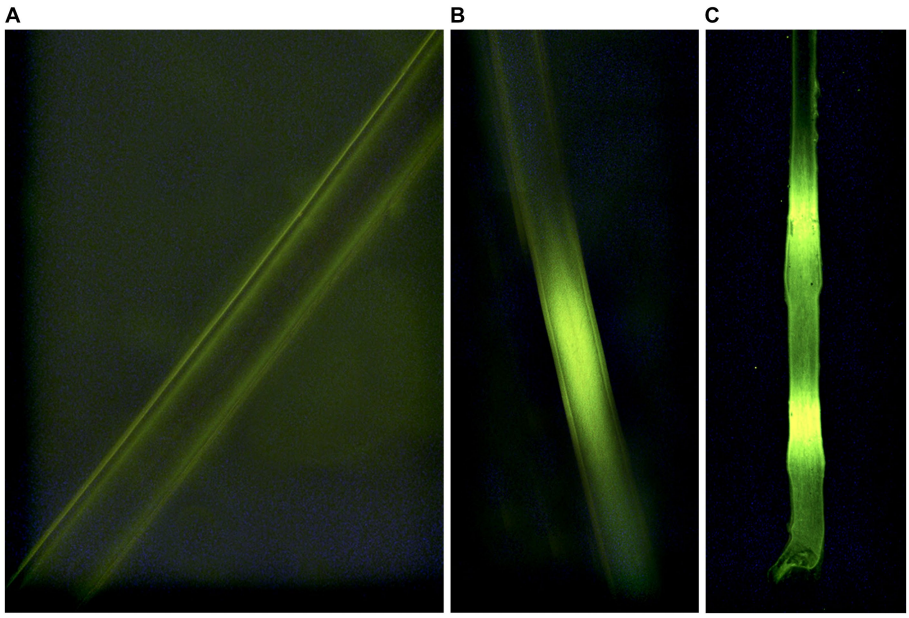

FIGURE 3
Indications of consumption (**A**-negative; **B**,**C**-positive) of Rhodamine b in whiskers sampled from white-tailed deer (*Odocoileus virginianus*) during 2016 simulated vaccine deployment against bovine tuberculosis in white-tailed deer in northeastern lower Michigan, United States.

linear model with the lme4 package (51) in Program R (v 4.2.0, The R Foundation for Statistical Computing). We considered site ID as a random effect to account for site-site variation. We evaluated the parameter estimates and 95% confidence intervals (CIs) of those estimates for non-overlap of zero to indicate statistical and biological differences. We also calculated the model predicted values for the response variables and their 95% CIs for making inferences. We presented the average number of each wildlife species visiting VDU grids and examined for non-overlap of standard errors, suggesting statistical difference in overall visitation.

3 Results

3.1 Vaccine delivery unit distribution and consumption

We distributed a total of 7,080 VDUs to free-ranging deer in NELM across 30 VDU grids on 17 sites during the 2016 field season. Overall, 3,279 non-RB VDUs were distributed and 1,878 (57.2%) were consumed. A total of 3,801 VDUs containing RB were distributed of which 2,101 (55.3%) were consumed. However, deer rejected 34.64% of the RB capsules; evidenced by the consumption of the VDU and not the RB capsule.

3.2 White-tailed deer and non-target visitation

With 113 VDU nights recorded from 6 March to 28 April, we calculated a minimum average of 11.03 (SE = 0.78) deer visiting sites per 24-h (Figure 4), though the highest number of deer per 24-h photographed on a single site was 45 deer on 5 April 2016. Turkeys and raccoons were the second and third most prevalent species visiting sites though averaged only 0.55 (SE = 0.26) and 0.30 (SE = 0.05) per night, respectively. Documented visitation by turkeys was limited to 30% (5 of 17) of sites with 87% (54 of 62) observed on one site with a flock of as many as 22 birds. Visitation by raccoons was more widespread across 76% (13 of 17) of sites, though were lower in number with a maximum of 5 observations on three sites. Skunks, squirrels, and rabbits were observed, though very rarely, on VDU grids.

3.3 Biomarker analysis

Overall, we sampled 116 deer from 17 sites. The number of deer sampled per site ranged from 1 to 13. We observed that 77 (66.3%) of the deer sampled were marked with RB (range = 0–100%). Of the 77 deer marked, 6 were identified RB positive by internal staining and 71 were identified RB positive by vibrissae marking. Although when excluding sites with ≤3 deer sampled, the percent marked ranged from 20–100%. We found there was no difference in the probability of being marked between males and females ($\beta = 0.02$, 95% CI = −0.80–0.87). Model predictions indicated that males had a 0.66 probability (95% CI = 0.50–0.80), and females had a 0.66 (95% CI = 0.55–0.76) probability of being marked.

3.4 Vaccine delivery unit development and cost

The total time to produce 800 VDUs (average VDUs produced from a single 22.68 kg bag of dry product) with RB was 200 min. Time estimates included encapsulating RB, mixing ingredients, and forming

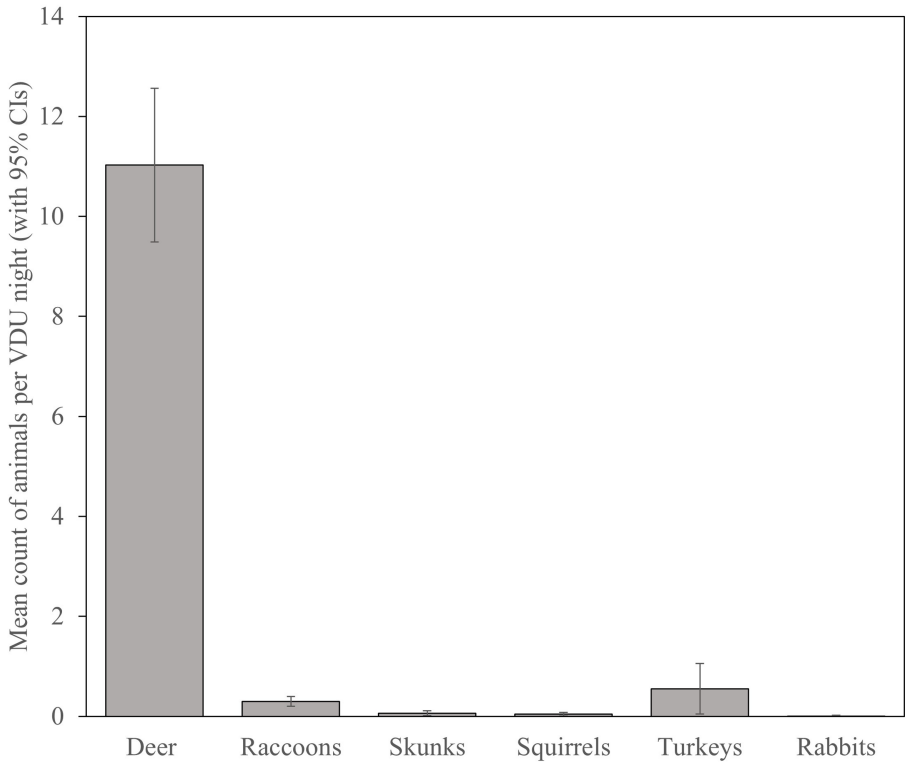

FIGURE 4
Mean count (with 95% CIs) of white-tailed deer (*Odocoileus virginianus*) and non-target species visiting simulated vaccination sites and potentially consuming vaccination delivery units during 2016 evaluation of oral vaccine deployment against bovine tuberculosis in white-tailed deer in northeastern lower Michigan, United States.

VDUs by hand. Based on average consumption across sites, the overall cost of producing and deploying VDUs was $654/site.

4 Discussion

We demonstrated that it was possible to deliver pharmaceuticals to the majority of free-ranging white-tailed deer visiting our selected agricultural fields in late winter/early spring in NELM. Specifically, we found the alfalfa/molasses VDUs we developed were sufficiently palatable to be sought out and readily consumed by deer. Our distribution strategy utilizing single VDUs dispersed across an elongated rectangular grid design facilitated delivery to individuals within groups of deer while minimizing nose-to-nose contact and associated potential for disease transmission. By locating our grids in agricultural fields and adjacent to lowland conifer stands, deer appeared to encounter them during daily movements typical of late-winter and early spring. Using RB, we successfully confirmed consumption of ≥1 RB-laden VDUs in 66.3% of the 116 deer sampled. This is 16.3% above the 50% vaccination rate in simulation models needed to achieve an 86% probability of eradication of bTB in 30 years if used in conjunction with other ongoing management strategies (14).

By timing the initiation of our VDU deployment during initial thawing events and winter break-up (6 March 2016), we benefitted from seasonal concentrations of deer. Deer in Michigan demonstrate high site fidelity to yarding areas associated with lowland conifer stands (52, 53) and as environmental conditions permit, (i.e., decrease in snow cover and depth) deer leave their associated yarding areas to search for spring foods (53). As such, an increase in deer abundance on agriculture fields occurs during this time in NELM and may be a condition of the proximity of agriculture lands to lowland conifer stands (45). Deer metabolism also begins to increase with the initiation of spring (March and April) (54), resulting in dispersal to feed on agriculture waste grain and alternative agricultural foods that provide needed nutritional components. By deploying VDUs early in the winter break-up period (March and April), as opposed to May and June, we observed relatively more deer on our VDU grids compared to a 2015 trial (47). Later, deer disperse, targeting newly sprouting vegetation, especially in aspen/birch stands and upland mixed forest stands to meet their spring and summer life requisites (44, 55). These seasonal dispersals may suggest the appropriate time to cease targeted oral vaccinations, as fewer deer will encounter VDU grids, and food preferences and demands will likely have changed.

The timing of our simulated vaccination also benefitted from seasonally reduced activity and visitation by most non-target species except for occasional turkeys (47). Consumption of VDUs intended for deer has the potential to hinder the delivery of VDUs to all visiting deer, though complete consumption in a single night was never an issue. Ongoing monitoring with cameras throughout the deployment process could inform the number of VDUs needed to maximize coverage of deer visiting. Additionally, delivery of vaccine-laden VDUs over multiple nights, with monitoring between nights would alert VDU deployment crews to situations in which all VDUs were

consumed, suggesting an insufficient number of VDUs and need to increase numbers being delivered.

Wildlife managers must take into consideration the efficacy of the methods and the cost associated with an oral vaccination of deer in NELM. The alfalfa/molasses VDU we developed and used was relatively inexpensive to produce. With an average cost to produce and deploy alfalfa/molasses VDUs (without vaccine) of $654 per site (for 6 days), the use of this oral vaccination strategy on the entirety of DMU 452 is a real possibility. The cost of expanding this oral vaccination across DMU 452 (1,476 km^2) would need to take into consideration the number of VDUs to distribute, the cost of the BCG vaccine, the spatial scale at which distribution would occur and the cost of specialized training needed to handle the BCG vaccine. The cost of an oral vaccination across DMU 452 would likely be substantially lower than the estimated cost for other proposed management strategies of vaccine delivery (i.e., trap/vaccinate methods, $1.5 million annually) (30). We are aware that the cost estimate may increase when BCG is added to the VDUs but may still be cost effective at 0.36 to 0.67 cents/dose (42) (M. Palmer, Veterinary Medical Officer, USDA ARS National Animal Disease Center).

The relatively low cost of production, relatively high consumption rates by deer, and minimal non-target visitation makes the alfalfa/molasses VDU a suitable candidate to deliver the BCG vaccine to free-ranging deer adjacent to lowland conifer, then shifting to aspen/birch stands during winter break-up in NELM. With the use of a biomarker (RB) we demonstrated that by targeting deer on agriculture fields during winter break-up, it may be possible to vaccinate the targeted ≥50% of deer on the landscape. It is also possible for wildlife managers and others to expedite the development of VDUs and the deployment strategy. By mixing larger quantities of ingredients and with aid of off-road vehicles and mechanical feeders, managers may be able to decrease the time needed to distribute VDUs. Further research should evaluate the efficacy of BCG vaccine insertion into these VDUs and the viability of distributing BCG to deer of NELM. Developing this vaccination strategy has shown it may be a cost-effective strategy to vaccinate when compared to other labor-intensive strategies i.e., trap and vaccinate (30); and could be a significant contribution to ongoing wildlife disease mitigation strategies implemented in the area.

5 Conclusion

The development of our alfalfa/molasses VDU and associated delivery strategy may be the most scalable and effective method for vaccinating deer in NELM against bTB. Initiating an oral vaccination program during winter break-up would help maximize the number of deer that encounter and consume VDUs. Further, initial vaccination efforts should target using agriculture fields adjacent to lowland conifer stands at the end of winter-early spring (March). If efforts extend into late spring (May), a shift toward agriculture fields near aspen/birch stands would follow shifts in habitat use by deer. This continuous and adaptive strategy would allow the vaccination effort to target those deer with high site fidelity to lowland conifer stands and migratory deer moving to spring food resources in late spring. Bovine tuberculosis is a pervasive issue in NELM and the continued spillover into cattle poses great economic and social consequences for many stakeholders. The 66.3% coverage of free-ranging deer that we achieved exceeds the previously stated vaccination rate of 50% needed to maximize the probability of eradication of bTB in 30 years. Our proposed vaccination strategy could be an additional management tool to combat bTB in NELM and further progress toward eradicating the disease.

Data availability statement

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

Ethics statement

The animal study was approved by Michigan State University Animal Care and Use Committee. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

DD: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. KV: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. ML: Conceptualization, Investigation, Methodology, Writing – review & editing. NS: Formal analysis, Writing – review & editing. HC: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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products or companies does not represent an endorsement by the United States government.

Conflict of interest

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

Publisher's note

References

1. Palmer M. *Mycobacterium bovis*: characteristics of wildlife reservoir hosts. *Transbound Emerg Dis*. (2013) 60:1–13. doi: 10.1111/tbed.12115

2. Delahay RJ, Cheeseman CL, Clifton-Hadley RS. Wildlife disease reservoirs: the epidemiology of *Mycobacterium bovis* infection in the European badger (*Meles meles*) and other British mammals. *Tuberculosis*. (2001) 81:43–9. doi: 10.1054/tube.2000.0266

3. Payne A, Ruette S, Jacquier M, Richomme C, Lesellier S, Middleton S, et al. Estimation of bait uptake by badgers, using non-invasive methods, in the perspective of Oral vaccination against bovine tuberculosis in a French infected area. *Front Vet Sci*. (2022) 9:787932. doi: 10.3389/fvets.2022.787932

4. Byrne A, Kenny K, Fogarty U, O'Keeffe J, More S, McGrath G, et al. Spatial and temporal analyses of metrics of tuberculosis infection in badgers (*Meles meles*) from the Republic of Ireland: trends in apparent prevalence. *Prev Vet Med*. (2015) 122:345–54. doi: 10.1016/j.prevetmed.2015.10.013

5. Nugent G, Buddle B, Knowles G. Epidemiology and control of *Mycobacterium bovis* infection in brushtail possums (*Trichosurus vulpecula*), the primary wildlife host of bovine tuberculosis in New Zealand. *N Z Vet J*. (2015) 63:28–41. doi: 10.1080/00480169.2014.963791

6. Wielgus E, Caron A, Bennitt E, De Garine-Wichatitsky M, Cain B, Fritz H, et al. Inter-group social behavior, contact patterns and risk for pathogen transmission in cape buffalo populations. *J Wildl Manag*. (2021) 85:1574–90. doi: 10.1002/jwmg.22116

7. Naranjo V, Gortazar C, Vicente J, de la Fuente J. Evidence of the role of European wild boar as a reservoir of *Mycobacterium tuberculosis* complex. *Vet Microbiol*. (2008) 127:1–9. doi: 10.1016/j.vetmic.2007.10.002

8. Aranaz A, de Juan L, Montero N, Sanchez C, Galka M, Delso C, et al. Bovine tuberculosis (*Mycobacterium bovis*) in wildlife in Spain. *J Clin Microbiol*. (2004) 42:2602–8. doi: 10.1128/JCM.42.6.2602-2608.2004

9. O'Brien DJ, Schmitt SM, Fitzgerald SD, Berry DE, Hickling GJ. Managing the wildlife reservoir of *Mycobacterium bovis*: the Michigan, USA, experience. *Vet Microbiol*. (2006) 112:313–23. doi: 10.1016/j.vetmic.2005.11.014

10. Palmer MV, Kanipe C, Lombard JE, Boggiatto PM. Bovine tuberculosis at the Interface of cattle, wildlife, and humans. *Tuberculosis*. (2023) 2023:829–46. doi: 10.1007/978-3-031-15955-8_40

11. Lavelle MJ, Kay SL, Pepin KM, Grear DA, Campa H, VerCauteren KC. Evaluating wildlife-cattle contact rates to improve the understanding of dynamics of bovine tuberculosis transmission in Michigan, USA. *Prev Vet Med*. (2016) 135:28–36. doi: 10.1016/j.prevetmed.2016.10.009

12. Lavelle MJ, Campa HI, LeDoux K, Ryan PJ, Fischer JW, Pepin KM, et al. Deer response to exclusion from stored cattle feed in Michigan, USA. *Prev Vet Med*. (2015) 121:159–64. doi: 10.1016/j.prevetmed.2015.06.015

13. Cosgrove MK, O'Brien DJ, Ramsey DS. Baiting and feeding revisited: modeling factors influencing transmission of tuberculosis among deer and to cattle. *Front. Vet. Sci.* (2018) 5:306. doi: 10.3389/fvets.2018.00306

14. Ramsey DSL, O'Brien DJ, Cosgrove MK, Rudolph BA, Locher AB, Schmitt SM. Forecasting eradication of bovine tuberculosis in Michigan white-tailed deer. *J Wildl Manag*. (2014) 78:240–54. doi: 10.1002/jwmg.656

15. VerCauteren KC, Lavelle MJ, Campa H 3rd. Persistent spillback of bovine tuberculosis from White-tailed deer to cattle in Michigan, USA: status, strategies, and needs. *Front. Vet. Sci.* (2018) 5:301. doi: 10.3389/fvets.2018.00301

16. O'Brien DJ, Thacker TC, Salvador LC, Duffiney AG, Robbe-Austerman S, Camacho MS, et al. The devil you know and the devil you don't: current status and challenges of bovine tuberculosis eradication in the United States. *Ir Vet J*. (2023) 76:16. doi: 10.1186/s13620-023-00247-8

17. Okafor CC, Grooms DL, Bruning-Fann CS, Averill JJ, Kaneene JB. Descriptive epidemiology of bovine tuberculosis in Michigan (1975–2010): lessons learned. *Vet. Med. Int.* (2011) 2011:874924. doi: 10.4061/2011/874924

18. Chipman RB, Gilbert AT, Slate D. Wildlife rabies Management in the new World: prevention, control and elimination in Mesocarnivores In: CE Rupprecht, editor. *History of rabies in the Americas: From the pre-Columbian to the present, volume I: Insights to specific Cross-cutting aspects of the disease in the Americas*. Berlin: Springer (2023). 143–98.

19. Slate D, Algeo TP, Nelson KM, Chipman RB, Donovan D, Blanton JD, et al. Oral rabies vaccination in North America: opportunities, complexities, and challenges. *PLoS Negl Trop Dis*. (2009) 3:e549. doi: 10.1371/journal.pntd.0000549

20. Gormley E, Ní Bhuachalla D, Fitzsimons T, O'Keeffe J, McGrath G, Madden JM, et al. Protective immunity against tuberculosis in a free-living badger population vaccinated orally with *Mycobacterium bovis* Bacille Calmette–Guérin. *Transbound Emerg Dis*. (2022) 69:e10–9. doi: 10.1111/tbed.14254

21. Gormley E, Corner LA. Pathogenesis of *Mycobacterium bovis* infection: the badger model as a paradigm for understanding tuberculosis in animals. *Front. Vet. Sci.* (2018) 4:247. doi: 10.3389/fvets.2017.00247

22. Díez-Delgado I, Sevilla IA, Romero B, Tanner E, Barasona JA, White AR, et al. Impact of piglet oral vaccination against tuberculosis in endemic free-ranging wild boar populations. *Prev Vet Med*. (2018) 155:11–20. doi: 10.1016/j.prevetmed.2018.04.002

23. Nugent G, Yockney IJ, Whitford EJ, Cross ML, Aldwell FE, Buddle BM. Field trial of an aerially-distributed tuberculosis vaccine in a low-density wildlife population of brushtail possums (*Trichosurus vulpecula*). *PLoS One*. (2016) 11:e0167144. doi: 10.1371/journal.pone.0167144

24. Thomas J, Risalde MÁ, Serrano M, Sevilla I, Geijo M, Ortíz JA, et al. The response of red deer to oral administration of heat-inactivated Mycobacterium bovis and challenge with a field strain. *Vet Microbiol*. (2017) 208:195–202. doi: 10.1016/j.vetmic.2017.08.007

25. Martinez-Guijosa J, Casades-Marti L, González-Barrio D, Aranaz A, Fierro Y, Gortázar C, et al. Tuning oral-bait delivery strategies for red deer in Mediterranean ecosystems. *Eur J Wildl Res*. (2020) 66:51. doi: 10.1007/s10344-020-01389-8

26. Palmer MV, Thacker TC. Use of the human vaccine, *Mycobacterium bovis* bacillus calmette guérin in deer. *Front. Vet. Sci.* (2018) 5:244. doi: 10.3389/fvets.2018.00244

27. Palmer MV, Thacker TC, Waters WR, Robbe-Austerman S. Oral vaccination of white-tailed deer (*Odocoileus virginianus*) with *Mycobacterium bovis* Bacillus Calmette-Guerin (BCG). *PLoS One*. (2014) 9:e97031. doi: 10.1371/journal.pone.0097031

28. Nol P, Rhyan JC, Robbe-Austerman S, McCollum MP, Rigg TD, Saklou NT, et al. The potential for transmission of BCG from orally vaccinated white-tailed deer (*Odocoileus virginianus*) to cattle (*Bos taurus*) through a contaminated environment: experimental findings. *PLoS One*. (2013) 8:e60257. doi: 10.1371/journal.pone.0060257

29. Palmer MV, Thacker TC, Waters WR. Vaccination of white-tailed deer (*Odocoileus virginianus*) with *Mycobacterium bovis* bacillus Calmette Guerin. *Vaccine*. (2007) 25:6589–97. doi: 10.1016/j.vaccine.2007.06.056

30. Cosgrove MK, Campa H, Schmitt SM, Marks DR, Wilson AS, O'Brien DJ. Live-trapping and bovine tuberculosis testing of free-ranging white-tailed deer for targeted removal. *Wildl Res*. (2012) 39:104. doi: 10.1071/WR11147

31. Fisher P. Review of rhodamine B as a marker for wildlife studies. *Wildl Soc Bull*. (1999) 27:318–29.

32. Southey A, Sleeman D, Gormley E. Sulfadimethoxine and rhodamine B as oral biomarkers for European badgers (*Meles meles*). *J Wildl Dis*. (2002) 38:378–84. doi: 10.7589/0090-3558-38.2.378

33. Fernandez JR-R, Rocke TE. Use of rhodamine B as a biomarker for oral plague vaccination of prairie dogs. *J Wildl Dis*. (2011) 47:765–8. doi: 10.7589/0090-3558-47.3.765

34. Fry TL, Atwood T, Dunbar M. Evaluation of rhodamine B as a biomarker for raccoons. Human-wildlife. *Interactions*. (2010) 4:14. doi: 10.26077/t3rz-dh25

35. Lindsey GD. Rhodamine B: a systemic fluorescent marker for studying mountain beavers (*Aplodontia rufa*) and other animals. *Northwest Sci*. (1983) 57:16–21.

36. Spurr E. Rhodamine B as a systemic hair marker for assessment of bait acceptance by stoats (*Mustela erminea*). *N Z J Zool*. (2002) 29:187–94. doi: 10.1080/03014223.2002.9518302

37. Webster SC, Cunningham FL, Kilgo JC, Vukovich M, Rhodes OE Jr, Beasley JC. Effective dose and persistence of rhodamine-B in wild pig vibrissae. *Wildl Soc Bull*. (2017) 41:764–9. doi: 10.1002/wsb.834

38. Carstensen M, O'Brien DJ, Schmitt SM. Public acceptance as a determinant of management strategies for bovine tuberculosis in free-ranging U.S. wildlife. *Vet Microbiol*. (2011) 151:200–4. doi: 10.1016/j.vetmic.2011.02.046

39. MDARD. *Bovine tuberculosis eradication program quarterly update in: Development MDoAaR*. (2023).

40. Agriculture USDo. *National Agricultural Statistics Service*. (2002). Available at: http://wwwnassusdagov/Data_and_Statistics/Quick_Stats/indexasp Accessed May 18, 2007).

41. O'Brien DJ, Schmitt SM, Rudolph BA, Nugent G. Recent advances in the management of bovine tuberculosis in free-ranging wildlife. *Vet Microbiol*. (2011) 151:23–33. doi: 10.1016/j.vetmic.2011.02.022

42. Cosgrove MK, Campa H, Ramsey DSL, Schmitt SM, O'Brien DJ. Modeling vaccination and targeted removal of white-tailed deer in Michigan for bovine tuberculosis control. *Wildl Soc Bull*. (2012) 36:676–84. doi: 10.1002/wsb.217

43. Hughey BD. *Are there "hot spots" of bovine tuberculosis in the free-ranging white-tailed deer (Odocoileus virginianus) herd of northeastern Michigan?* East Lansing: Michigan State University (2003).

44. Felix AB, Campa H, Millenbah KF, Winterstein SR, Moritz WE. Development of landscape-scale habitat-potential models for forest wildlife planning and management. *Wildl Soc Bull*. (2004) 32:795–806. doi: 10.2193/0091-7648(2004)032%5B0795:DOLHMF%5D2.0.CO;2

45. Sitar K. *Seasonal movements, habitat use patterns, and population dynamics of white-tailed deer in an agricultural region of northern lower Michigan: MS thesis*. East Lansing, Michigan, USA: Michigan State University (1996).

46. Braun KF. *Ecological factors influencing white-tailed deer damage to agricultural crops in northern lower*. Michigan: Michigan State University. Department of Fisheries and Wildlife (1996).

47. Dressel D. *Development of strategies to orally deliver vaccine for bovine tuberculosis to White-tailed deer of northeastern lower*. Michigan: Michigan State University (2017).

48. Phillips GE, Goldade DA, KC VerCauteren, Ott TL, Wagner DC. *Iophenoxic acid and rhodamine B as biomarkers of bovine tuberculosis vaccine bait uptake by white-tailed deer*. Proceedings of the vertebrate Pest conference (2014).

49. Fisher P, Algar D, Sinagra J. Use of rhodamine B as a systemic bait marker for feral cats (*Felis catus*). *Wildl Res*. (1999) 26:281–5. doi: 10.1071/WR98041

50. Weerakoon MK, Price CJ, Banks PB. Hair type, intake, and detection method influence rhodamine B detectability. *J Wildl Manag*. (2013) 77:306–12. doi: 10.1002/jwmg.459

51. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *arXiv*. (2014) 2014:14065823. doi: 10.48550/arXiv.1406.5823

52. Ozoga JJ. Variations in microclimate in a conifer swamp deeryard in northern Michigan. *J Wildl Manag*. (1968) 32:574. doi: 10.2307/3798938

53. Verme LJ. Movements of white-tailed deer in upper Michigan. *J Wildl Manag*. (1973) 37:545–52. doi: 10.2307/3800320

54. Moen AN. Seasonal changes in heart rates, activity, metabolism, and forage intake of white-tailed deer. *J Wildl Manag*. (1978) 42:715–38. doi: 10.2307/3800763

55. Kohn BE, Mooty JJ. Summer habitat of white-tailed deer in north-Central Minnesota. *J Wildl Manag*. (1971) 35:476–87. doi: 10.2307/3799701

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Seasonal changes in bird communities on poultry farms and house sparrow—wild bird contacts revealed by camera trapping

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Introduction: Wild birds are considered reservoirs of poultry pathogens although transmission routes have not been conclusively established. Here we use camera trapping to study wild bird communities on commercial layer and red-legged partridge farms over a one-year timeframe. We also analyze direct and indirect interactions of other bird species with the house sparrow (*Passer domesticus*), a potential bridge host.

Methods: We conducted camera trapping events between January 2018 and October 2019, in two caged layer farms, one free-range layer farm, and two red-legged partridge farms in South-Central Spain.

Results and Discussion: We observed wild bird visits on all types of farms, with the significantly highest occurrence on red-legged partridge farms where food and water are more easily accessible, followed by commercial caged layer farms, and free-range chicken farms. The house sparrow (*Passer domesticus*) followed by spotless starlings (*Sturnus unicolor*) was the most encountered species on all farms, with the highest frequency in caged layer farms. On partridge farms, the house sparrow accounted for 58% of the wild bird detections, while on the free-range chicken farm, it made up 11% of the detections. Notably, the breeding season, when food and water are scarce in Mediterranean climates, saw the highest number of wild bird visits to the farms. Our findings confirm that the house sparrow, is in direct and indirect contact with layers and red-legged partridges and other wild birds independent of the type of farm. Contacts between house sparrows and other bird species were most frequent during the breeding season followed by the spring migration period. The species most frequently involved in interactions with the house sparrow belonged to the order Passeriformes. The study provides a comparative description of the composition and seasonal variations of bird communities in different types of layer/ poultry farms in Southern Spain i.e. a Mediterranean climate. It confirms the effectiveness of biosecurity measures that restrict access to feed and water. Additionally, it underscores the importance of synanthropic species, particularly the house sparrow, as potential bridge vector of avian pathogens.

KEYWORDS

synanthropic birds, indirect contact, shared diseases, poultry farms, bridge species, biosecurity

1 Introduction

In recent years, in large parts of the Northern hemisphere there has been an increasing focus on enhancing animal welfare within the agricultural sector, encompassing poultry production (1, 2). To address these concerns, new production systems have been designed and implemented, providing animals with the opportunity to reside in environments that are more like their natural habitats and less restrictive. However, these innovative production systems, especially those employed in the poultry industry, can result in greater interaction between domestic and wild birds, including their feces (3–5).

Various species of wild birds, known as synanthropic birds, belonging to the families Columbidae, Corvidae, and Passeridae, have demonstrated a remarkable adaptation to exploit resources generated by human activities, such as food, water and shelter (6). Examples from these families that include the house sparrow (*Passer domesticus*), the tree sparrow (*Passer montanus*), European starlings (*Sturnus vulgaris*), and feral pigeons (*Columba livia*), can inhabit diverse environments created by humans, from urban areas to isolated farms. Some of these birds, especially the house sparrow easily enter production facilities, through small gaps in exterior walls often even when protected by bird nets (6). Thus, if individuals of such species are in close contact with poultry on one hand and wild bird species such as waterfowl that not usually enter enclosures or barns on the other hand, they could act as so-called bridge hosts in the transmission of pathogens.

In terms of risks in addition to abundance of farm birds, composition of the farm bird community could be important (7). The dilution effect hypothesis postulates that a higher biodiversity is linked to a lower prevalence of pathogens, as species-rich communities harbor individuals in which a specific pathogen cannot multiply to sufficient levels to transmit infection to new susceptible individuals. This reduces the overall success of pathogen transmission and, consequently, the prevalence of pathogens (8).

In the context of pathogens transmitted by wild birds, it is expected that in places where birds congregate in farms, the presence of many different species with diverse susceptibilities would make it more difficult for a pathogen to persist and spread, especially if a single species is the key reservoir for this pathogen. Meanwhile, the presence of species that are migratory on farms could increase the likelihood of the introduction of pathogens that these birds may have encountered on their migratory route. Finally, the risk of pathogen spillback from poultry to wild birds may also vary considerably with the species of wild bird encountering poultry or its feces.

Poultry farms attract wild birds due to water (puddles, canals, ditches) or food resources (spilled feed, drying feed, insects in manure, carcasses). These factors could increase the contacts between wild birds and bridge bird species, as well as increase the abundance of the latter and thus also contact between wild birds and domestic poultry (chickens, turkeys, game birds) (9). This contact can occur directly or indirectly through contamination of resources, thereby increasing the risk of transmission and spillback of avian pathogens, such as avian influenza viruses (AIV), *Salmonella* sp., and avian coronaviruses (10) among others. In this context, European starlings for example are a high-priority species for avian pathogen exposure detection studies as they can form large flocks in livestock feeders during the winter and autumn seasons, representing a potential risk of pathogen incursion into poultry farms, especially during the breeding season (11).

The unforeseen and unprecedented spread and change in the epidemiology of the highly pathogenic avian influenza virus (HPAIV) H5N1 of clade 2.3.4.4b, now fatally affecting new species, new continents, during all seasons, is decimating wild and domestic bird populations in much of the world, especially in the European and American continents (12). In contrast to other HPAIV it shows self-sustained prolonged transmission in wild birds and has already affected many poultry operations globally (12). This increases concerns regarding the potential transmission pathways of AIV by synanthropic bridge species. Migratory waterfowl, considered the main reservoirs for AIV (13, 14), play a key role in the introduction of many AIV subtypes through asymptomatic shedding, exerting a significant factor in the redistribution and transmission of these subtypes to domestic poultry (15). Several studies on the movements of migratory waterfowl have demonstrated their involvement in the large-scale spread of the virus (16). However, it should be noted that due to their ecological needs these wild birds rarely come into direct contact with poultry (17). In this scenario, synanthropic birds such as the house sparrow or the European starling are perceived as potential carriers and transmitters of AIV (18, 19), and could act as bridge both after exposure through direct contact with infected waterfowl, or contaminated environment in shared habitat (20).

Biosecurity protocols on farms rarely comprehensively assess how the virus enters the farm and which farm animals may be carriers of AIV. Despite some experimental evidence of the potential for synanthropic bird species to transmit AIV, there are very few studies dedicated to quantifying wild bird interactions with poultry farms. These studies include research in Australia using camera traps to monitor wild birds on different types of layer and meat chicken farms (21). Another study in the Netherlands quantified wild bird access to a free-range commercial laying hen farm by installing video cameras at a critical point for avian influenza (4). In southwestern France, a study used individual direct observations of wild birds on a free-range duck farm (5). Additionally, a recent study in northwestern Italy employed direct observations and camera traps on turkey and broiler duck farms, as well as laying hen farms (22). A study using satellite transmitter data from radio-marked waterfowl, showing occasional but regular incursions of marked birds onto poultry farm premises (23).

Collectively, these studies evaluate the accessibility of poultry farms for wild birds and identify the house sparrow as one of the most common species on farms due to its resident and sedentary nature. However, knowledge gaps exist regarding the frequency of farm visits by other species, seasonal changes in wild bird communities and characterization of sparrow interactions with other wild birds or even poultry on poultry farms.

The goal of this study was to generate data on seasonal changes of wild bird communities on different types of poultry farms and to investigate contacts of a key bridge species with other bird species that are non-residents on poultry premises and that could potentially lead to the acquisition and transmission of pathogens on to poultry. The latter is based upon the fact that in initial visits we observed a significant presence of house sparrows on poultry farms, commonly sighting them in barns and surrounding crop areas, even entering the barns where layers were housed/flight cages of red-legged partridges. Considering the persistence of AIV in bird feces and the environment

(20) it has been confirmed that, under favorable conditions of high humidity and low temperature, AIV can persist in feces for extended periods, even in dry manure (24, 25). We hypothesize that in environments where house sparrows and other birds share resources such as food, water, or resting areas, contact could occur through shared surfaces contaminated by feces. If this occurs the house sparrow could become a potential vector for AIV as well as other pathogens.

For our purpose, we conducted camera trapping on the premises of various commercial layer and red-legged partridge farms in the Castilla-La Mancha region, in south central Spain at different time-points throughout the year corresponding to phenological events in wild bird ecology such as the breeding and wintering season as well as the periods during which migratory species conduct their spring and fall migration. We characterized the wild bird communities observed and used the house sparrow, which is the most abundant resident species, and the species most likely to also enter the layer barns/enclosures/flight cages as a potential bridge species. Hence, we analyzed the direct and indirect contacts of the house sparrow with other wild bird species observed in the camera traps. The collected data were used to quantify wild bird visits and their interactions with the house sparrow.

2 Materials and methods

2.1 Study area

Our study was conducted in three commercial layer farms and two red-legged partridge farms between January 2018 and October 2019 in south-central continental Spain (Figure 1). The predominant climate in this region is Southern Plateau Continental Mediterranean or according to the Köppen classification Hot summer Mediterranean (26) characterized by mean annual rainfall (mm) from 350 to 550 mm. Mean annual temperature fall between 12 and 15 (°C) and an annual mean temperature range spanning from 18 to 20.5 (°C) (27). The farms under study are not close to large wetlands however the area has a collection of inland temporary wetlands (mostly dry in summer) known as the "Mancha humeda," which play a crucial role in winter and in the spring and fall migration of wild birds from northern and central Europe to Africa. Below is a brief description of the farms included in the study.

1 Commercial layer farms: We included three different layer farms (A, B, C). Two of these are in the south-central part of the provinces of Toledo (39.450527, −3.628713) and Cuenca (39.542977, −1.934466), designated as sites A and B and house 50,000 and 600,000 layers in cages indoors, respectively. The surroundings of these poultry farms primarily consist of fields of non-irrigated crops, including vineyards, barley fields, and almond trees. Additionally, the farms are situated near or include small water sources such as temporary ponds or streams. The third farm designated as site C (39.455741, −2.015767), holds free- range layers and is surrounded by vineyards, barley fields and open pine tree forest. On the premises used by the chickens are almond trees.
2 Red-Legged Partridge Farms: We sampled two different red-legged partridge farms in the north of Ciudad Real (39.232715, −3.602193) and Albacete (38.937303, −2.556022) provinces, designated as sites D and E, respectively. Both farms are situated on the outskirts of a village alongside other agricultural operations. The red-legged partridges raised on these farms are intended for release in hunting estates for recreational hunting and later use in the game meat industry. The entire production cycle, except for the first month of chick rearing, occurs outdoors. This includes housing juvenile partridges in large groups in flight cages and of the breeders in pairs in elevated breeding cages. Like the layer farms, the arable

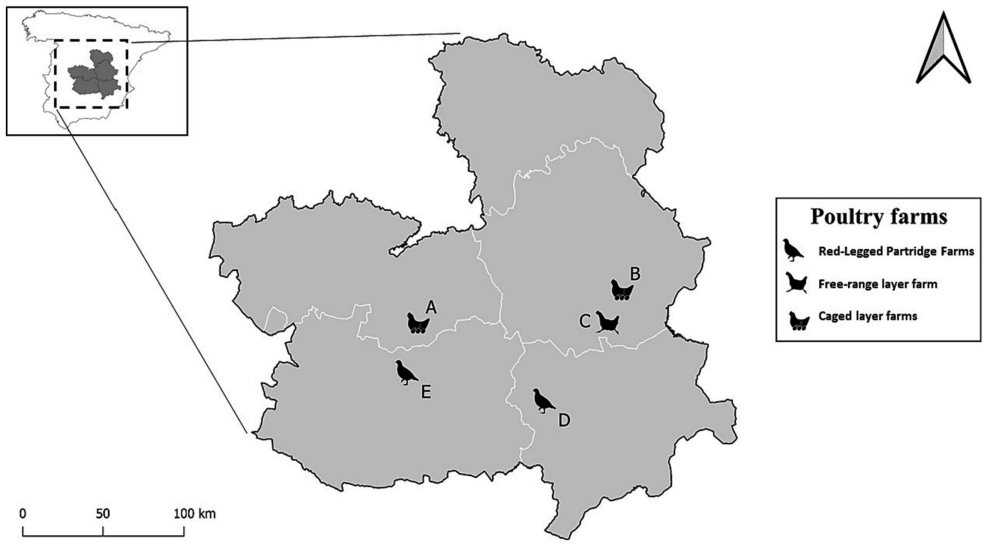

FIGURE 1
Location of the five farms within the Castilla-La Mancha region in Central Spain, categorized by species and type. The letters (A, B) represent the caged layer farms, the letter C denotes the Free-range layer farm, and the letters (E, D) indicate the red-legged partridge farms.

fields surrounding these farms predominantly consist of non-irrigated crops, such as vineyards, cereal plots, almond and olive trees, and open pine forest.

2.2 Camera trapping design

We used camera traps Little Acorn CT cameras (Ltl 5310 Series LED IR Invisible) on each of the study farms, to cover at least one of each phenological periods (spring and fall migration, breeding, and wintering) in locations representative of the study farms, particularly in places attractive to birds, such as silos, water points, temporary ponds, as well as at the entrances of poultry houses/poultry enclosures and feeding and watering areas. The number of cameras employed on each farm varied with the size of the farm and camera availability between 5 and 10 cameras. Cameras were deployed to obtain a similar number of days ($n = 7$) of camera trapping on each farm for each phenological period. However due to logistical reasons (distance of farms, camera failures) the number of trapping events varied between farms and phenological periods and the data was corrected according to camera trapping effort. Sampling involved the use of 5–10 cameras that remained active for an average of 18 days on commercial layer farms (Farm A; camera activity range = 3–69 days, total sampling effort = 349 camera-days. Farm B; camera activity range = 6–14 days, total sampling effort = 92 camera-days). The cameras on the pasture-based Farm C were active for an average of 9 days (camera activity range = 6–14 days, total sampling effort = 45 camera-days). On red-legged partridge farms, the cameras on Farm D were active for 6 days (camera activity range = 1–10 days, total sampling effort = 18 camera-days), while on Farm E, the cameras were operational for 9 days (camera activity range = 2–13 days, total sampling effort = 34 camera-days) (Supplementary Table S1).

The camera traps were set up in photo mode with passive motion sensors, capturing three consecutive images every 10 min whenever the motion sensor detected movement within the camera's field of view. The camera traps were positioned 30–50 cm above ground level with no apparent vegetation obstructions to avoid false detections caused by natural movements such as wind or vegetation. To capture the movement of all birds, regardless of their size, the sensitivity of all cameras was set to high. The cameras operated throughout the day and used infrared flash at night. Each image automatically recorded the date and time. All cameras collected data on SD cards, which were periodically transferred to 4 TB hard drives for storage.

2.3 Data management and analysis

All camera trapping (CT) images were examined individually. Only pictures containing birds or other wildlife were included and classified by species. Data extracted from each picture included the following categories: camera location, CET time (day, month, year, hours, minutes), species names, number of visits, taxonomic category order, and migration phenology (spring and fall migration, breeding, and wintering).

We estimated the species richness of wild birds in each study farm using four non-parametric estimators (Abundance-based coverage estimator ACE, incidence based coverage estimator ICE, Chao2, and Bootstrap) with EstimateS v.9.1.0 (28) to assess the species visiting the farms. Two estimators have been used that rely on abundance data and are based on the statistical concept of sampling coverage (ACE and ICE). It refers to the sum of the probabilities of finding observed species within the total of present but unobserved species (29). The ACE estimator makes its estimations considering 10 or fewer individuals per sample, while the ICE utilizes species found in 10 or fewer samples (30). The Chao2 richness estimator combines presence/absence data for a species in a given sample, such as those obtained with camera traps, to estimate whether the species is present and how many times that species is present in the sample set. Finally The Bootstrap estimator was used to assess the variability of the sample. This method involves generating new observations by obtaining multiple samples with replacement from the original sample. Its significance lies in its ability to consistently estimate the sampling distribution of a statistic and to accurately estimate its variance (31).

We used the average of these estimators to calculate the proportion of species documented on the farms, dividing the number of observed species by the mean of the estimators. Additionally, the percentage of registered species is presented as a measure of sampling completeness (%) (Supplementary Table S2). Individual rarefaction curves were calculated using 95% confidence intervals from the estimator (32). To estimate the number of visits by individual wild birds, we classified pictures according to O'Brien et al. (33) into dependent and independent events (Supplementary Figure S2). We designated events as independent when there was a time gap of more than 30 min between two consecutive photos of the same species, or at least two different species were present in the three consecutive images (as illustrated in Supplementary Figure S2A). On the other hand, events were classified as dependent when all three images featured birds of the same species, making it impossible to determine whether the same or a different individual was present in the picture, and when time between two consecutive photos of the same species was less than 30 min (as shown in Supplementary Figure S2B).

To account for the hypothesis of the house sparrow as a bridge species, we investigated the interaction of house sparrows with other species through camera traps. For this we recorded any interaction of the house sparrow, whether direct or indirect, with any other wild bird species. We defined a direct contact as the presence of one or more house sparrow and any other bird species together in the same picture (see Supplementary Figure S2C). Additionally, we considered any image that showed a bird species different from the house sparrow within a period of less than 24 h before capturing an image with house sparrows in the same location as an "indirect contact" (see Supplementary Figure S2D).

Using the data obtained from the camera traps we analyzed factors that modulate bird communities and wild bird visits to poultry farms, as well as direct and indirect contacts between house sparrows and other wild bird species. Specifically, we included explanatory variables such as the type of poultry farm, bird order, phenology, and migratory behavior (Table 1).

For this analysis, we constructed three generalized linear models (GLM) with a binomial distribution and a logit link function. The first model was used to explore the effect of explanatory variables on wild bird visits, while the second and third models examined their effect on the observation of direct and indirect contacts of other wild bird species with house sparrows. The dependent variable was defined as the count of independent events involving wild birds in the images,

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TABLE 1 Predictor categories defined for the models used.

Predictor	Description
Poultry farms	Caged layer farms
	Free- range layer farms
	Red legged partridge farms
Bird order	Anseriformes
	Bucerotiformes
	Charadriiformes
	Columbiformes
	Passeriformes
	Pelecaniformes
Migration	Spring migration (February–April)
	Breeding (May–July)
	Fall migration (August–October)
	Wintering (November–January)
Behavior	Resident wild birds
	Migratory wild birds
	Partial migrants

TABLE 2 Results of the GLM used to evaluate the number of visits of wild birds to type of poultry farms, bird order, migration phenology and migratory behavior.

Predictor		*B*	S. Error	*p*-value
Intercept		1.713	0.3285	**<0.001**
Poultry farms	Caged layer farms	–	–	–
	Free-range layer farms	−1.393	0.2061	**0.001**
	Red legged partridge farms	0.902	0.1234	**<0.001**
Bird order	Anseriformes	−0.777	0.5699	0.151
	Bucerotiformes	−2.833	1.1298	0.012
	Charadriiformes	0.822	0.4808	0.087
	Columbiformes	1.586	0.3673	**<0.001**
	Passeriformes	1.539	0.3450	**<0.001**
	Pelecaniformes	–	–	–
Migration	Spring migration	–	–	–
	Breeding	0.733	0.1383	**<0.001**
	Fall migration	−1.505	0.1479	**0.000**
Migratory behavior	Wintering	−1.419	0.1357	**0.000**
	Migratory	−3.836	0.2881	**0.000**
	Partially migratory	−1.769	0.1893	**<0.001**
	Resident	–	–	–

Statistically significant results are highlighted in bold.

encompassing both direct and indirect contacts of wild birds with house sparrows. Model construction followed a stepwise forward Akaike selection (34). Statistical analyses were carried out using SPSS 28.0 (Statistical Package for Social Sciences Inc.), with statistical significance set at $p < 0.05$.

3 Results

A total of 139,246 images were captured with the camera traps in the years 2018–2019. Among these, 78,779 images were taken on commercial layer farms, 30,187 on free-range layer farms, and 29,280 on red-legged partridge farms. A total of 31,816 birds belonging to 33 species, 21 families, and seven different orders were observed on the five farms. Out of these, 18 were resident bird species (55%), 11 were partially migratory birds (33%), and three were migratory bird species (12%). Most resident species belonged to the order Passeriformes (72%), such as the house sparrow, tree sparrow, and spotless starling (*Sturnus unicolor*) (see Supplementary Table S3).

The non-parametric estimators calculated 96.4% ± 3.6 and 95.2% ± 4.8% of the total observed species richness on Farms D and E, respectively. However, the non-parametric estimators suggest that species richness is higher on the remaining farms: Farm A (83.8% ± 16.2%), Farm B (69.2% ± 30.8%), and Farm C (55.4% ± 44.6%) (see Supplementary Figure S1, Supplementary Table S2).

We detected a significantly higher frequency of visits by wild birds on red-legged partridge farms as compared to caged layer farms and free-range layer farms (see Table 2, Figure 2). Visits detected on caged layer farms were less numerous, but from a much larger variety of species (Supplementary Table S3, Supplementary Figure S3). Species in the Columbiformes and Passeriformes order were significantly more likely to be detected (Table 2). Also, the number of bird visits detected was significantly higher during the breeding season (see Table 2, Supplementary Figure S3).

Resident bird species visited farms significantly more than partially migratory or migratory species (Table 2). Additionally, resident species had significantly more direct and indirect contacts with house sparrows as compared to migratory species (Table 3).

The house sparrow was the most frequently captured species in photographs, on all five farms and during all phenological periods. Spotless starlings were observed on four farms, being more abundant in the red-legged partridge farms, but not on the free-range layer farm (Supplementary Figure S4). In three of the farms, the camera traps recorded species such as the white wagtail (*Motacilla alba*), crested lark (*Galerida cristata*), and rock pigeon (*Columba livia*) (Supplementary Table S3). On the free-range layer farm, the house sparrow was less common (11%), and the Eurasian magpie (*Pica pica*) was the most frequently observed species (53%) (Supplementary Figure S4). Notably although anecdotical, waterbirds (mallard *Anas platyrhinchos*, black winged stilt *Himantopus himantopus*, ring-necked plover *Charadrius hiatus*) were detected on at least three of the farms (Supplementary Table S3).

Direct contacts between house sparrows and other species were significantly more likely during the breeding season and with resident bird species (see Table 3, Figure 2). The need to seek food and water, especially in juvenile birds, increases interactions with other species, particularly with the house sparrow. Indirect contacts with house sparrows (use of the same location by house sparrows within a time span of 24 h and thus potential of exposure to fecal contamination) was significantly more likely during the breeding season and least likely between house sparrows and Anseriformes and on the free-ranger layer farm (Table 4). Species that interacted more frequently with the house sparrow belonged to the order Passeriformes, being almost twice as common as the second most common type, the Columbiformes. Birds in the Charadriiformes

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order had fewer contacts with the house sparrow (see Supplementary Figure S4).

4 Discussion

Our study applies camera trapping technology to the poultry farm environment to comparatively describe the composition and seasonal changes of farm bird communities on different types of layer/gamebird farms. This is to the best of the authors knowledge the first time such a study is carried out in Spain. Previous work and data collected in the present study provide evidence of the house sparrow as key species that enters layer buildings and flight cages of partridges and other bird species which has led to its designation, together with several other synanthropic bird species as potential bridge hosts (6, 9, 35) (Supplementary Figure S5). However, although bidirectional exchange of pathogens at the interface has been demonstrated and the potential of bridge hosts is generally accepted (36) little information exists yet on the frequency of contact and potential of contamination of resident farm birds by visiting migratory birds. For this reason we used the pictures obtained to also study the contact of wild birds that visit farm premises, but are unlikely to enter the buildings and enclosures, with the house sparrow (4, 5, 21).

The farms studied here are not directly connected to any wetland, which makes them theoretically unattractive to wild waterfowl (13), however satellite telemetry data has recently shown that waterfowl occasionally does make incursions onto poultry farm premises (23) and in fact our data shows, that even the studied farm premises are occasionally visited by waterbirds, either at open water tanks or temporary pools after heavy rains (Supplementary Figure S5).

The camera traps used for this study covered locations at the external fencing of the farms, aggregation hotspots such as water and food sources and possible entrances to the farm buildings. Actual farm bird diversity is certainly greater than that observed in this study. Camera traps do not always capture the total number of bird species visiting the farm, as they are positioned and focused on specific points, making it challenging to obtain a complete picture of the bird population. Additionally, even with increased sampling effort, as observed in farms A and B ($n = 349$ days; $n = 92$ days), we notice that the observed richness

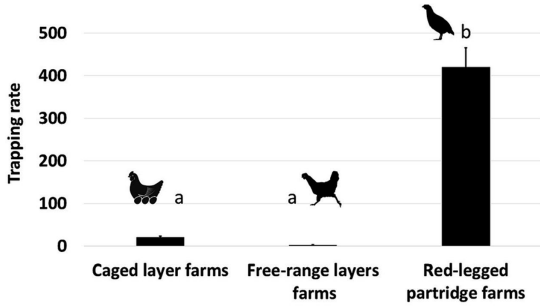

FIGURE 2
Wild bird trapping rate in each poultry farm (Caged layer farms, free-range layers farms, red-legged partridge farms).

TABLE 3 Results of the GLM used to evaluate the number of the direct contacts of wild birds with house sparrows according to type of poultry farm, bird order, migration phenology and migratory behavior.

Predictor		*B*	S.Error	*p*-value
Intercept		−0.63	0.6410	0.325
Poultry farms	Caged layer farms	–	–	–
	Free-range layers farms	−29.639	399222.1547	1.000
	Red legged partridge farms	−0.188	0.1762	0.286
Bird order	Anseriformes	−1.235	0.9792	0.207
	Bucerotiformes	–	–	–
	Charadriiformes	−0.270	0.7456	0.717
	Columbiformes	0.869	0.6521	0.183
	Passeriformes	0.759	0.6239	0.224
	Pelecaniformes	–	–	–
Migration	Spring migration	–	–	–
	Breeding	0.498	0.1796	**< 0.05**
	Fall migration	−1.683	0.2794	**< 0.01**
Migratory behavior	Wintering	−1.441	0.2605	**< 0.01**
	Migratory	−2.546	0.6183	**<0.001**
	Partially migratory	−0.315	0.2881	0.274
	Resident	–	–	–

Statistically significant results are highlighted in bold.

TABLE 4 Results of the GLM used to evaluate the number of the indirect contact of wild birds with house sparrows in relation to type of poultry farm, bird order, migration phenology and migratory behavior.

Predictor		*B*	S.Error	*p*-value
Intercept		−0.459	0.5376	0.393
Poultry farms	Caged layer farms	–	–	–
	Free-range layers farms	−2.392	0.4451	**<0.001**
	Red legged partridge farms	−0.611	0.1786	<0.001
Bird order	Anseriformes	−2.528	1.1509	**0.022**
	Bucerotiformes	–	–	–
	Charadriiformes	−1.042	0.6605	0.115
	Columbiformes	0.638	0.5506	0.246
	Passeriformes	0.471	0.5181	0.363
	Pelecaniformes	–	–	–
Migration	Spring migration	–	–	–
	Breeding	0.176	0.1791	0.326
	Fall migration	−1.363	0.2325	**<0.001**
Migratory behavior	Wintering	−1.700	0.2540	**<0.001**
	Migratory	−1.884	0.4288	**<0.001**
	Partially migratory	−0.035	0.2487	0.889
	Resident	–	–	–

Statistically significant results are highlighted in bold.

does not align with the studied estimators, indicating the need to extend our sampling period (Supplementary Table S2, Supplementary Figure S1). Logistical issues such as farm size, the number of cameras used, or limitations in data storage due to an abundance of individuals in a single location hinder obtaining the actual number of species. This is due to the size of the poultry farms, surrounding vegetation and habitats (various crops, buildings) (24) and changes in these (crop harvest, sowing etc.). However as the camera trap distribution design was similar for all studied farms and data was corrected for trapping effort, we can at least to some extend compare the collected information (21). Our results show that red-legged partridge farms attracted the highest number of visits, followed by caged and free-range layer farms. Most of the observed species were passerines. Possible reasons why wild birds were most attracted to partridge farms are the relatively easy access to food and water as both breeders and juvenile partridges are raised outdoors in batteries of breeding cages or large flight cages, respectively. Previous studies have shown that wild birds are not particularly attracted to free-range chicken farms, potentially as the grazing areas are rapidly degraded by the chickens while the large numbers of chickens also appear to intimidate most wild birds (24).

Among the species identified, the house sparrow is the most frequently observed and interacts directly and indirectly with a large variety of other species and a considerable number of individuals from which they could acquire pathogens including AIV as sparrows have been shown to be susceptible to AIV infection (18, 37, 38). The adaptation of the sparrow to human modified habitats, gregarious behavior and obligate commensalism drives their potential as bridge host (39). Other frequently detected species included the spotless starling and the rock dove. Both species are known to be frequently exposed to and carriers of pathogens such as Salmonella spp. and antibiotic resistance mechanism carrying *Escherichia coli* among others (10). Also, European starlings, a species closely related to the spotless starling have been experimentally shown to be able to transmit avian influenza virus to poultry (40). Species detected in free-range layer farms such as the white wagtail are consistent with the species detected in a study of free-living birds in enclosures of duck farms in France (5), while other species observed on the duck farms, such as cattle egrets, were observed less frequently.

Direct but also indirect contacts are often the main risk factor in pathogen transmission between wild and domestic birds (41). Contacts of other birds with house sparrows were observed generally in association to food and/or water and less frequently roosting space. Our results show that direct and indirect contacts of sparrows with other bird species on farms occur significantly more frequently with resident species than with partially or fully migratory species (Figure 3). If, in addition to direct contacts, the possibility of indirect contamination through secreta and feces is considered, with a maximum residence time of approximately 24 h, the potential for contamination of a sparrow by AIV or other pathogens doubles (Figure 3).

The highest number of direct and indirect contacts were recorded during the breeding season, followed by the spring and fall migration periods. For the wintering season, hardly any direct and indirect contacts were recorded even though the highest number of visits occurred at this time of year. As our farms are situated in a Mediterranean continental climate where food and water in natural habitats are more restricted in summer than in winter, likely during this period the availability of food and water was less important than other functions of the farm environment. More frequent farm visits during the breeding season, may be linked to the high number of juvenile individuals that rely on easily accessible resources. The high number of juvenile individuals increases the number of contacts with likely a higher number of naïve, more susceptible individuals thus increasing the probability of pathogen transmission (42). Also, during the breeding and post-breeding periods, the food requirements of breeding adult sparrows are at the highest, while it is under the Mediterranean continental climate the period with less food and water resources increasing the attraction to farm premises considerably. The lower number of direct and indirect contacts during fall migration is probably due to the abundance of food (cereal and fruits) in the season.

Both migratory and resident birds can be carriers of pathogens either directly or by exposure in contaminated environments and by Borie et al. (43) by contaminating resources, such as water or feed, with their droppings. Here we have detected few migratory or partially migratory birds on farm visits which in turn underlines the potential bridge host role of sparrows (18). While contact restriction measures are generally focused on the protection of poultry they also need to take into account the risk of environmental transmission from poultry to wildlife, by sewage, feathers, dust and aerosols from that can represent a major source of contamination for synanthropic wild birds such as the house sparrow that could than contaminate non-resident visiting birds during direct or indirect contacts (44).

5 Conclusion

Camera trap-based characterization allowed to describe composition and fluctuation of wild bird communities in different farm type environments across seasons and to identify species that could represent bridge hosts. Identifying the house sparrow as key species with resident populations on farms we evidence its connection to other bird species that visit the farm environment. Our results indicate that general biosecurity measures such as restriction o access to food and water are highly effective as the number of bird visits on red-legged partridge farms were significantly more frequent, than on other farms.

Data availability statement

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

Author contributions

AS-C: Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. M-CC: Methodology,

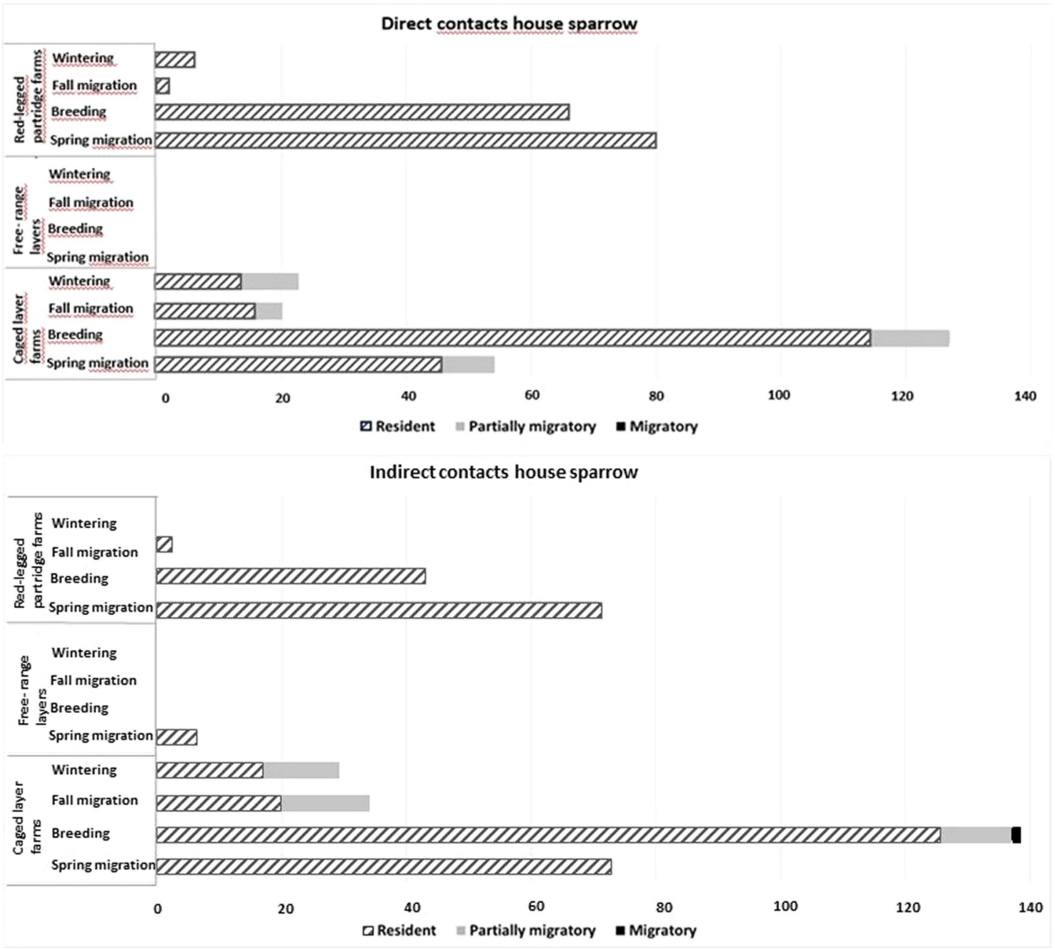

FIGURE 3
Total number of direct (in black) and indirect (in gray) contacts of visiting wild birds with house sparrows according to their migratory behavior (resident, partially migratory, migratory).

Resources, Writing – review & editing. YR: Methodology, Resources, Writing – review & editing. TC: Methodology, Resources, Writing – review & editing. UH: Conceptualization, Methodology, Resources, Writing – original draft, Writing – review & editing.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2024.1369779/full#supplementary-material

References

1. Broom DM, Galindo FA, Murgueitio E. Sustainable, efficient livestock production with high biodiversity and good welfare for animals. *Proc R Soc B Biol Sci*. (2013) 280:20132025. doi: 10.1098/rspb.2013.2025

2. Bessei W. Impact of animal welfare on worldwide poultry production. *Worlds Poult Sci J*. (2018) 74:211–24. doi: 10.1017/S0043933918000028

3. Cappelle J, Gaidet N, Iverson SA, Takekawa JY, Newman SH, Fofana B, et al. Characterizing the interface between wild ducks and poultry to evaluate the potential of transmission of avian pathogens. *Int J Health Geogr*. (2011) 10:60. doi: 10.1186/1476-072X-10-60

4. Elbers ARW, Gonzales JL. Quantification of visits of wild fauna to a commercial free-range layer farm in the Netherlands located in an avian influenza hot-spot area assessed by video-camera monitoring. *Transbound Emerg Dis*. (2020) 67:661–77. doi: 10.1111/tbed.13382

5. Le Gall-Ladevèze C, Guinat C, Fievet P, Vollot B, Guérin J-L, Cappelle J, et al. Quantification and characterisation of commensal wild birds and their interactions with domestic ducks on a free-range farm in Southwest France. *Sci Rep*. (2022) 12:9764. doi: 10.1038/s41598-022-13846-2

6. Shriner SA, Root JJ. A review of avian influenza a virus associations in Synanthropic birds. *Viruses*. (2020) 12:1209. doi: 10.3390/v12111209

7. Gilbert M, Xiao X, Pfeiffer DU, Epprecht M, Boles S, Czarnecki C, et al. Mapping H5N1 highly pathogenic avian influenza risk in Southeast Asia. *Proc Natl Acad Sci*. (2008) 105:4769–74. doi: 10.1073/pnas.0710581105

8. Keesing F, Holt RD, Ostfeld RS. Effects of species diversity on disease risk. *Ecol Lett*. (2006) 9:485–98. doi: 10.1111/j.1461-0248.2006.00885.x

9. Caron A, Cappelle J, Cumming GS, De Garine-Wichatitsky M, Gaidet N. Bridge hosts, a missing link for disease ecology in multi-host systems. *Vet Res*. (2015) 46:83. doi: 10.1186/s13567-015-0217-9

10. Franklin AB, Bevins SN, Shriner SA. Pathogens from wild birds at the wildlife–agriculture Interface In: JC Owen, DM Hawley and KP Huyvaert, editors. *Infectious disease ecology of wild birds*. Oxford: Oxford University Press (2021). 207–28.

11. Cabe PR. European starlings (*Sturnus vulgaris*) as vectors and reservoirs of pathogens affecting humans and domestic livestock. *Animals*. (2021) 11:466. doi: 10.3390/ani11020466

12. Youk S, Torchetti MK, Lantz K, Lenoch JB, Killian ML, Leyson C, et al. H5N1 highly pathogenic avian influenza clade 2.3.4.4b in wild and domestic birds: introductions into the United States and reassortments, December 2021–April 2022. *Virology*. (2023) 587:109860. doi: 10.1016/j.virol.2023.109860

13. Olsen B, Munster VJ, Wallensten A, Waldenström J, Osterhaus ADME, Fouchier RAM. Global patterns of influenza a virus in wild birds. *Science*. (2006) 312:384–8. doi: 10.1126/science.1122438

14. Caron A, Cappelle J, Gaidet N. Challenging the conceptual framework of maintenance hosts for influenza a viruses in wild birds. *J Appl Ecol*. (2017) 54:681–90. doi: 10.1111/1365-2664.12839

15. Slusher MJ, Wilcox BR, Lutrell MP, Poulson RL, Brown JD, Yabsley MJ, et al. Are passerine birds reservoirs for influenza a viruses? *J Wildl Dis*. (2014) 50:792–809. doi: 10.7589/2014-02-043

16. Gaidet N, Cappelle J, Takekawa JY, Prosser DJ, Iverson SA, Douglas DC, et al. Potential spread of highly pathogenic avian influenza H5N1 by wildfowl: dispersal ranges and rates determined from large-scale satellite telemetry. *J Appl Ecol*. (2010) 47:1147–57. doi: 10.1111/j.1365-2664.2010.01845.x

17. Kleyheeg E, Van Dijk JGB, Tsopoglou-Gkina D, Woud TY, Boonstra DK, Nolet BA, et al. Movement patterns of a keystone waterbird species are highly predictable from landscape configuration. *Mov Ecol*. (2017) 5:2. doi: 10.1186/s40462-016-0092-7

18. Yamamoto Y, Nakamura K, Yamada M, Mase M. Pathogenesis in Eurasian tree sparrows inoculated with H5N1 highly pathogenic avian influenza virus and experimental virus transmission from tree sparrows to chickens. *Avian Dis*. (2013) 57:205–13. doi: 10.1637/10415-101012-Reg.1

19. Ellis JW, Root JJ, McCurdy LM, Bentler KT, Barrett NL, VanDalen KK, et al. Avian influenza a virus susceptibility, infection, transmission, and antibody kinetics in European starlings. *PLoS Pathog*. (2021) 17:e1009879. doi: 10.1371/journal.ppat.1009879

20. Blagodatski A, Trutneva K, Glazova O, Mityaeva O, Shevkova L, Kegeles E, et al. Avian influenza in wild birds and poultry: dissemination pathways, monitoring methods, and virus ecology. *Pathogens*. (2021) 10:630. doi: 10.3390/pathogens10050630

21. Scott AB, Phalen D, Hernandez-Jover M, Singh M, Groves P, Toribio J-ALML. Wildlife presence and interactions with chickens on Australian commercial chicken farms assessed by camera traps. *Avian Dis*. (2018) 62:65–72. doi: 10.1637/11761-101917-Reg.1

22. Martelli L, Fornasiero D, Scarton F, Spada A, Scolamacchia F, Manca G, et al. Study of the Interface between wild bird populations and poultry and their potential role in the spread of avian influenza. *Microorganisms*. (2023) 11:2601. doi: 10.3390/microorganisms11102601

23. McDuie F, Matchett EL, Prosser DJ, Takekawa JY, Pitesky ME, Lorenz AA, et al. Pathways for avian influenza virus spread: GPS reveals wild waterfowl in commercial livestock facilities and connectivity with the natural wetland landscape. *Transbound Emerg Dis*. (2022) 69:2898–912. doi: 10.1111/tbed.14445

24. Hauck R, Crossley B, Rejmanek D, Zhou H, Gallardo RA. Persistence of highly pathogenic and low pathogenic avian influenza viruses in footbaths and poultry manure. *Avian Dis*. (2017) 61:64–9. doi: 10.1637/11495-091916-Reg

25. Figueroa A, Derksen T, Biswas S, Nazmi A, Rejmanek D, Crossley B, et al. Persistence of low and highly pathogenic avian influenza virus in reused poultry litter, effects of litter amendment use, and composting temperatures. *J Appl Poult Res*. (2021) 30:100096. doi: 10.1016/j.japr.2020.09.011

26. Kottek M, Grieser J, Beck C, Rudolf B, Rubel F. World map of the Köppen-Geiger climate classification updated. *Meteorol Z*. (2006) 15:259–63. doi: 10.1127/0941-2948/2006/0130

27. De Castro M, Martín-Vide J, Alonso S. The climate of Spain: past, present and scenarios for the 21st century In: *Prelim Gen Assess Impacts Spain Due Eff Clim Change Madr Spain Minist Medio Ambiente* (2005). 1–62.

28. Colwell RK. *EstimateS, version 9.1: Statistical estimation of species richness and shared species from samples. Software and user's guide*. Connecticut: University of Connecticut (2013) Available at: http://viceroy.eeb.uconn.edu/estimates.

29. Colwell RK, Mao CX, Chang J. Interpolating, extrapolating, and comparing incidence-based species accumulation curves. *Ecology*. (2004) 85:2717–27. doi: 10.1890/03-0557

30. Lee S-M, Chao A. Estimating population size via sample coverage for closed capture-recapture models. *Biometrics*. (1994) 50:88–97. doi: 10.2307/2533199

31. Chao A, Chiu C. "Nonparametric Estimation and Comparison of Species Richness," *Encyclopedia of Life Sciences*. Wiley (2016). 1–11.

32. Gotelli NJ, Colwell RK. Estimating species richness In: *Biological Diversity: Frontiers in Measurement and Assessment* (2011). 39–54.

33. O'Brien TG, Kinnaird MF, Wibisono HT. Crouching tigers, hidden prey: Sumatran tiger and prey populations in a tropical forest landscape. *Anim Conserv*. (2003) 6:131–9. doi: 10.1017/S1367943003003172

34. Akaike H. A new look at the statistical model identification. *IEEE Trans Autom Control*. (1974) 19:716–23. doi: 10.1109/TAC.1974.1100705

35. Hirschinger J, Vergne T, Corre T, Hingrat Y, Guerin JL, Le Loc'h G. Exposure assessment for avian influenza and Newcastle disease viruses from peridomestic wild birds in a conservation breeding site in the United Arab Emirates. *Transbound Emerg Dis*. (2022) 69:2361–72. doi: 10.1111/tbed.14253

36. Iqbal M, Yaqub T, Mukhtar N, Shabbir MZ, McCauley JW. Infectivity and transmissibility of H9N2 avian influenza virus in chickens and wild terrestrial birds. *Vet Res*. (2013) 44:100. doi: 10.1186/1297-9716-44-100

37. Brown JD, Stallknecht DE, Berghaus RD, Swayne DE. Infectious and lethal doses of H5N1 highly pathogenic avian influenza virus for house sparrows (*Passer Domesticus*) and rock pigeons (*Columbia Livia*). *J Vet Diagn Invest*. (2009) 21:437–45. doi: 10.1177/104063870902100404

38. Forrest HL, Kim J-K, Webster RG. Virus shedding and potential for interspecies waterborne transmission of highly pathogenic H5N1 influenza virus in sparrows and chickens. *J Virol*. (2010) 84:3718–20. doi: 10.1128/JVI.02017-09

39. Shaw LM, Chamberlain D, Evans M. The house sparrow *Passer domesticus* in urban areas: reviewing a possible link between post-decline distribution and human socioeconomic status. *J Ornithol*. (2008) 149:293–9. doi: 10.1007/s10336-008-0285-y

40. Root JJ, Ellis JW, Shriner SA. Strength in numbers: avian influenza a virus transmission to poultry from a flocking passerine. *Transbound Emerg Dis*. (2021) 69:e1153–9. doi: 10.1111/tbed.14397

41. Scott AB, Toribio J-A, Singh M, Groves P, Barnes B, Glass K, et al. Low pathogenic avian influenza exposure risk assessment in Australian commercial chicken farms. *Front Vet Sci*. (2018) 5:68. doi: 10.3389/fvets.2018.00068

42. Caron A, De Garine-Wichatitsky M, Gaidet N, Chiweshe N, Cumming GS. Estimating dynamic risk factors for pathogen transmission using community-level bird census data at the wildlife/domestic Interface. *Ecol Soc*. (2010) 15:art25. doi: 10.5751/ES-03547-150325

43. Borie C, Zurita P, Sánchez M, Rojas V, Santander J, Robeson J. Prevención de la infección por *Salmonella enterica* subespecie enterica serotipo Enteritidis (*Salmonella Enteritidis*) en pollos mediante un bacteriófago. *Arch Med Vet*. (2008) 40:197–201. doi: 10.4067/S0301-732X2008000200013

44. Gaide N, Foret-Lucas C, Figueroa T, Vergne T, Lucas M-N, Robertet L, et al. Viral tropism and detection of clade 2.3.4.4b H5N8 highly pathogenic avian influenza viruses in feathers of ducks and geese. *Sci Rep*. (2021) 11:5928. doi: 10.1038/s41598-021-85109-5

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Role of the World Organisation for Animal Health in global wildlife disease surveillance

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This paper examines the role of the World Organisation for Animal Health (WOAH) in the global surveillance and management of pathogens. Since the creation of WOAH, one of its missions has been to ensure transparency of the global animal health situation. WOAH established a Working Group on Wildlife in 1994 to inform and advise WOAH Members, leadership, and technical teams on issues relating to wildlife health. In 2020 it conducted a consultation with its Members before developing a Wildlife Health Framework to improve global health and wildlife conservation. WOAH Members report diseases in wildlife, but detections are dependent on the surveillance systems in place. As an example of data collected in the most recent years (2019–2023), 154 countries have reported 68,862,973 cases, through alert messages and weekly updates, for 84 diseases. One-hundred and fifty countries have reported 68,672,115 cases in domestic animals and 95 countries have reported 190,858 cases in wild animals. These figures illustrate the performance of the organization in collecting data on wildlife, and provide an indication of the difference in completeness of data collected in domestic animals and wildlife. There are several challenges to wildlife disease surveillance and real figures remain unknown; they depend on the existence, quality and sensitivity of national surveillance. A WOAH-led One Health approach with cross-sectoral collaboration is needed to improve surveillance sensitivity, address the challenges and help safeguard wildlife population health and biodiversity conservation.

KEYWORDS

disease reporting, disease surveillance, One Health, wildlife health, Wildlife Health Framework, WOAH

Introduction

Historical and recent disease threats to animals and humans worldwide have highlighted the need to consider diseases in a global context, as multi-host pathogens do not recognize the boundaries between species or countries. Initially founded in 1924 in response to the international spread of rinderpest, considered the deadliest cattle disease in history, the World Organisation for Animal Health (WOAH, founded as OIE) is an intergovernmental organization with a mission to improve animal health worldwide (1, 2). To this end, WOAH is now the global authority on animal health and focuses, among other objectives, on transparent dissemination of information on prioritized animal diseases (3).

Although WOAH is best known for its work with veterinary authorities on farmed animal diseases, Members of the organization recognize the importance of taking a holistic approach when addressing transboundary animal disease management. Wildlife health has been considered by WOAH and its Members from as early as 1954. In 1994, a Working Group on Wildlife was established to inform and advise WOAH Members, provide leadership, and technical input on issues related to wild animal health (captive, feral or free-ranging). Additionally, nearly all 183 WOAH Members have adopted the approach of nominating a Focal Point for Wildlife – forming a global network responsible for collecting and reporting disease information in wildlife to WOAH. Members have agreed that impact on wildlife is considered in the criteria for listing diseases by WOAH, and that information sharing on wildlife is considered within the mandatory scope of most diseases listed by WOAH (4, 5).

Several diseases have crossed interfaces between humans, livestock and wildlife, and are transboundary between countries. Wildlife and domestic livestock have been affected by shared diseases such as African swine fever, lumpy skin disease or avian influenza (6–11). Wildlife may also be important in the epidemiology of zoonotic diseases, for example, Nipah virus (12). Avian influenza that circulates widely in wildlife (mainly as low pathogenic avian influenza) and has the potential to become pathogenic to people, usually requiring a domestic animal intermediate host (13). Early disease detection and information sharing enable better risk management of disease transmission within populations and spillover to other species (including humans), often with significant financial benefits (14, 15). Acknowledging the importance of disease surveillance in wildlife, WOAH Members committed to report detection of diseases listed by WOAH in wild animals, through the World Animal Health Information System (WAHIS) (16); other reporting channels and modalities are also currently under review.

In 2020, in light of lessons learned from the COVID-19 pandemic, WOAH launched an extensive stakeholders' consultation, leading to the development of a comprehensive Wildlife Health Framework (Figure 1) dedicated to the protection of wildlife health within the One Health context (17). WOAH's historical, present, and prospective future contributions to wildlife disease surveillance are described in this article, to clarify and raise awareness of the organization's role in supporting and sharing information on global wildlife disease surveillance.

Past and current role of WOAH in wildlife disease surveillance

For Members in need of support, WOAH provides guidelines and standards related to animal diseases to establish national "surveillance," which is defined as "the systematic ongoing collection, collation, and analysis of information related to animal health and the timely dissemination of information so that action can be taken" (5, 18).

At its creation, Members mandated WOAH, among other things, to promote research concerning the contagious diseases of livestock for which international collaboration is deemed desirable. As early as 1952, WOAH (then known as the OIE) recommended more research on the wild reservoirs of relevant livestock species diseases. In 1954, the first resolution of the World Assembly of Members on wildlife was adopted (19). In 1965, WOAH highlighted the need for research on bat rabies to safeguard sustainable bat populations while also protecting public health. The need to preserve biological conservation was also raised by WOAH at the joint OIE/ONS/FAO 1967 meeting (20). The establishment of an *ad hoc* group for Wildlife in 1993—which rapidly transformed into the permanent WOAH Working Group on Wildlife in 1994—was a logical concretization of the involvement of WOAH and its Members in the global discussion on wildlife diseases.

At the request of countries, the world-renowned experts of the WOAH Working Group on Wildlife have prepared recommendations and statements, and overseen numerous scientific publications on the surveillance and control of the most important wildlife diseases, while providing technical guidance to manage outbreaks in wild animals for almost three decades. Since 2010, WOAH's action on wildlife health has been organized around the network of Focal Points for Wildlife who undertake professional training on wildlife health surveillance-related topics every 2 years. These Focal Points are generally civil servants, working for the Ministry of Agriculture or Environment (or equivalent); they are responsible for establishing and maintaining national networks of wildlife experts and for submitting wildlife disease information to WOAH. Additionally, WOAH Reference Laboratories are designated to pursue scientific and technical problems for specific diseases, and WOAH Collaborating Centers provide expertise and support, and promote international collaboration for specific topics (21, 22). Several of these Reference Laboratories and Collaborating Centers have experts in topics related to wildlife health (e.g., Collaborating Center on Research, Diagnosis and Surveillance of Wildlife Pathogens (associate) in the USA and Canada, Collaborating Center on Training in Integrated Livestock and Wildlife Health and Management in South Africa, and the Collaborating Center on Wildlife Health Risk Management in Australia).

To take a step further in achieving its mandate, WOAH developed a Wildlife Health Framework – this is WOAH's Global Strategy for Wildlife Health. As part of its 2020 early design phase, a stakeholders' consultation showed that 95% of WOAH Members considered that Veterinary Services should be involved in the epidemiological surveillance of diseases in wildlife at the human–animal–ecosystem interface (23). Iterative contributions from the Working Group on Wildlife, the stakeholders' consultation, WOAH staff worldwide and external partners were used to prepare the WOAH Wildlife Health Framework. This document aligns the WOAH 7th Strategic Plan (2021–2025) which includes consideration of intersectoral issues such as the role of wildlife in disease emergence and spread, and works toward integrating wildlife health into all areas of the organization's activity (24). The two objectives in the framework aim to support Members to improve (i) their ability to reduce, anticipate and manage the risk of pathogen emergence and transmission at the human–animal–ecosystem interface, and (ii) early detection, notification and management of wildlife diseases. The Framework was thus designed with a dual goal of improving global health and wildlife conservation.

A key output of the Framework is "improved quality data [on wildlife health events and potential drivers, especially wildlife trade] collection, reporting, analysis and use." As such, WOAH supports Members, particularly their Veterinary Services, to improve health event surveillance and reporting, as described below. Specifically, activities under the Framework aim to support WOAH Members to improve their ability to manage the risk of health event occurrence

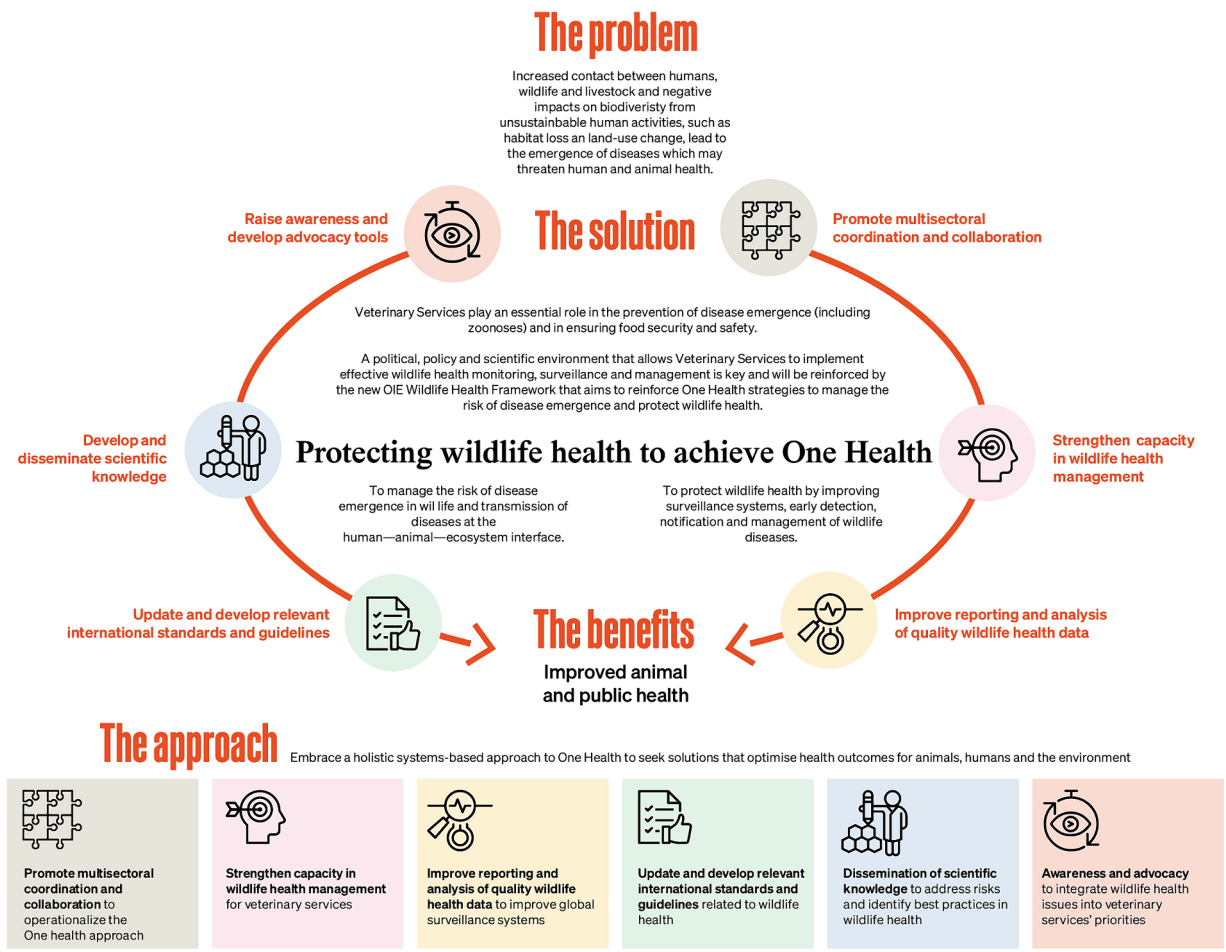

FIGURE 1
The WOAH Wildlife Health Framework was developed to ensure that wildlife health issues are fully integrated and transversally addressed in WOAH's core work such as Standards and guidelines, Performance of Veterinary Services (PVS) pathway, disease notification systems, among others to better support WOAH Members. Domestic animals have been at the center of animal health strategies worldwide, but equal investment in wild animal health is needed to ensure a holistic approach to animal health management, maintain healthy animal populations (both wild and domestic), ensure healthy ecosystems, and contribute to global health.

(including pathogens) in wildlife and transmission at the human-animal-ecosystem interface, while considering the protection of wildlife. The Framework recognizes the costs associated with appropriate wildlife health surveillance systems, but also highlights that the costs and risks to public health and animal health of not investing are greater. Additionally, Members receive technical and structural support from WOAH and its network of Reference Laboratories and Collaborating Centers to improve surveillance systems, early detection, notification and management of wildlife health events. The Framework has been converted into an action-oriented program with a 5-year implementation plan. In 2021, at the 88th General Session, the World Assembly of WOAH Delegates adopted Resolution No. 31 on "How WOAH can support Veterinary Services to achieve One Health resilience" (25). This Resolution further recognizes the key role of wild animals in global disease management and strengthens the inclusion of wildlife health in the organization's work.

The stocktaking and baseline assessment of the Wildlife Health Framework consisted in a set of consultations and surveys (in 2022 and 2023) to better understand country-level surveillance as well as Members' capacity, needs and challenges for wildlife disease surveillance. The most challenging task identified by Focal Points for Wildlife to fulfill their role was the integration of wildlife health into national animal health strategies. A survey revealed that Veterinary Services were involved either alone (43% of respondents) or in association with other sectors (43% of respondents) in management of wildlife health events (26). However, important needs regarding investigation of wildlife outbreaks were highlighted, with 63% of Members reporting impediments to collecting, handling or transporting wildlife samples (26). The stocktaking step also revealed that a high level of wildlife health disease recording (69%) used unreliable recording systems (paper records or local computer recording systems); this underscores the need for reinforced capacity on wildlife health information management (27).

The steps of WOAH and the evolution of its role in data collection, surveillance and reporting of wildlife diseases are summarized in Figure 2.

Indeed, when it comes to animal health data reporting, WOAH has developed processes to organize reporting priorities with its Members and experts, allowing consensus and engagement. WOAH provides a common global tool for Members to report both livestock and wildlife health events – the World Animal Health Information System (WAHIS) (28). To support data reporting from Members, WOAH shares definitions and standards, as well as guidelines, and conducts regional trainings or one-on-one sessions, as needed, ensuring useful and accurate reporting. WOAH Standards include disease and diagnostic test definitions (5, 18, 29, 30). In addition, WOAH conducts "web scraping "of disease signals using event-based surveillance tools (web-based systems that allow detection and collection of relevant news on diseases based on pre-determined search algorithms), to coordinate with Members and support them in their reporting obligations. WOAH also works closely with global and regional partners from various sectors to share information on disease events at the human-animal-ecosystem interface, and to support Members in their disease surveillance, early warning and preparedness efforts. Despite these efforts, engaging WOAH Members in voluntary wildlife disease reporting was deemed challenging for various reasons such as the lack of information related to wildlife health reaching WOAH Members, a non-fit for purpose reporting system, and wildlife health often not falling under the Veterinary Services's realm of action. After identifying the source of the challenges, WOAH has taken steps to set up corrective actions and is currently working on a new initiative regarding early warning and enhanced information on these non-WOAH-listed diseases that will be better adapted to the needs of WOAH Members and their partners working with wildlife. Meanwhile, an interim system is in place to collect information on non-WOAH-listed diseases on a voluntary basis.

To support improved capacities for wildlife surveillance in line with the Framework, WOAH is embracing the One Health approach and integrating wildlife health more fully into existing established programs such as the Performance of Veterinary Services (PVS) Pathway evaluations and Laboratory Twinning activities (31). An example of a Laboratory Twinning activity is represented by the US-Thailand Wildlife Health Twinning Project, based on expertise sharing on wildlife disease risk assessment and improvement of the national wildlife disease surveillance system (32). Online e-learning materials relevant to wildlife health are being developed based on WOAH Standards and Guidelines, and digital or printable communication tools are also made available (see, e.g., Wildlife health is everyone's health). In addition, all material developed by WOAH for training its Focal Points for wildlife is made available online (33). The International Health Regulations (IHR) – PVS National Bridging Workshops (NBWs) assist countries to prepare for and respond to prioritized health threats, and have begun to involve more participants

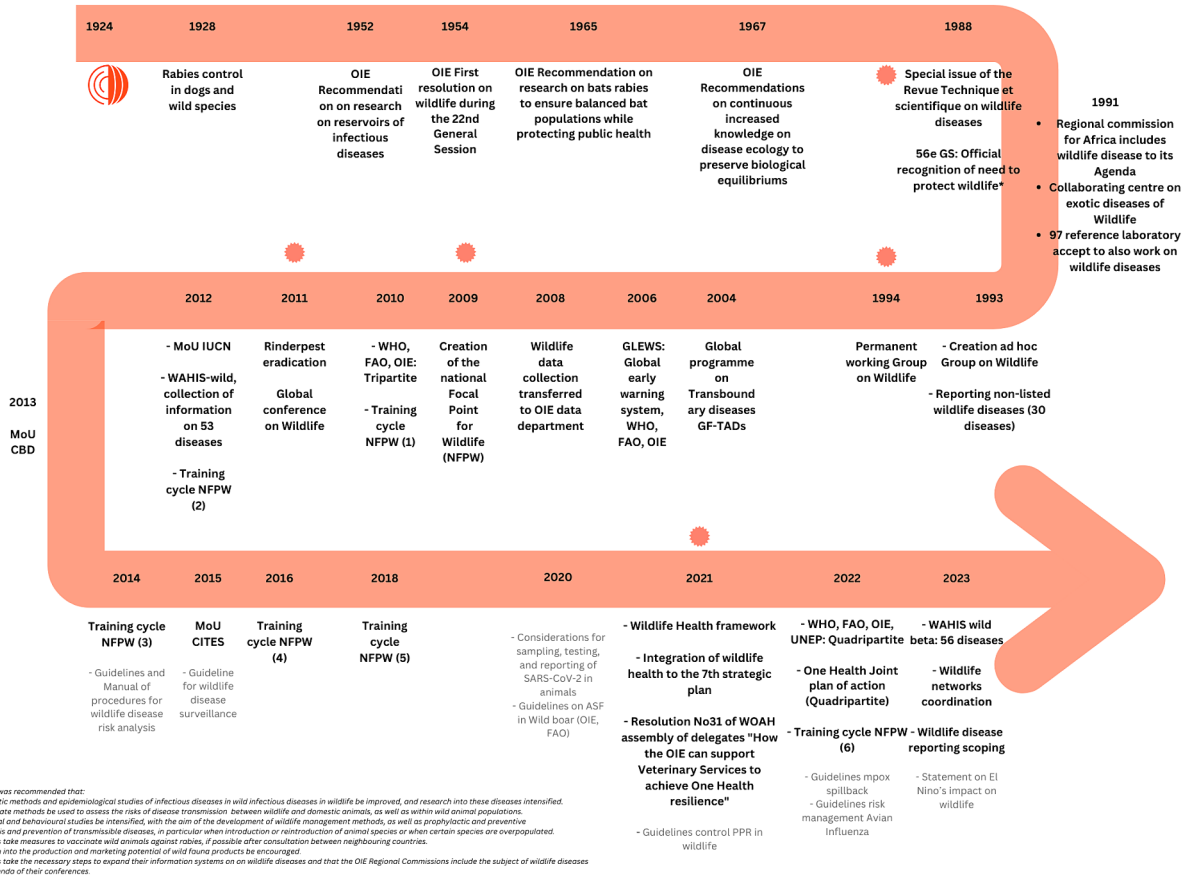

FIGURE 2
Timeline showing WOAH's evolving role in data collection, surveillance and reporting of wildlife diseases since 1924 and as of 2023.

from environmental sectors including wildlife health representatives (34). WOAH is also working with other global partners on initiatives which strive to promote and normalize multi-sectoral collaborations on health issues, such as the Nature for Health Initiative (35). In this context, it is worth highlighting the recent integration (March 2022) of the United Nations Environment Program (UNEP) into the Tripartite collaboration (comprising the Food and Agriculture Organization of the United Nations (FAO), World Health Organization (WHO) and WOAH) to accelerate a coordinated strategy on human, animal and ecosystem health. With this, the Tripartite has formally become the Quadripartite. The work of the newly expanded alliance is focused on the One Health Joint Plan of Action (OH JPA), with six main action tracks: (i) enhancing countries' capacity to strengthen health systems through a One Health approach; (ii) reducing the risks of emerging or re-emerging zoonotic epidemics and pandemics; (iii) controlling and eliminating endemic zoonotic, neglected tropical or vector-borne diseases; (iv) strengthening food safety risk assessment, management and communication; (v) stemming the silent pandemic of antimicrobial resistance (AMR); and (vi) better integrating the environment into the One Health approach (36).

Wildlife disease reporting to WOAH

Since the creation of WOAH, one of its main missions has been to ensure transparency of the global animal health situation and improve knowledge of animal diseases, including those transmissible to humans (i.e., zoonoses). To this purpose, WOAH Members have committed and are required to notify relevant information on their animal health situation in domestic animals and, when relevant, in wildlife, in compliance with the provisions of WOAH's Terrestrial Animal Health Code (Terrestrial Code) and Aquatic Animal Health Code (Aquatic Code) (37, 38). This has two main objectives: (i) sharing information on the known situations of diseases prioritized by WOAH Members (called "listed diseases") and (ii) early information sharing on unusual animal health events both for such priority listed diseases and for emerging diseases. To ensure engagement of countries, the criteria for disease prioritization, list of prioritized diseases and early information sharing scope are subject to Members' review and adoption by voting.

Animal disease information submitted from countries to WOAH is verified through an internal process and made publicly available through WAHIS. The system therefore comprises highly specific data (based on validated diagnosis tests and validated by competent authorities and WOAH) (5, 18, 39–41). Wildlife data are collected on about 100 listed diseases from 183 WOAH Members, and a few non-Members. However, countries and territories have different capacities in terms of disease surveillance, detection, and diagnosis. Moreover, test validations for many wild species and diseases may not be available, rendering submission to WAHIS unworkable for some wildlife disease information. In fact, the occurrence of some listed diseases in wild host species does not fit the WOAH Terrestrial Animal Health Code definition and does not fall under mandatory notification. Collecting these data in a standardized and coordinated manner therefore represents one of the main challenges for a global surveillance system.

For each listed disease, countries are requested to provide information through various reporting streams. For unusual events (such as the first occurrence of a disease in a country, or occurrence in an unexpected species), countries are asked to provide an immediate "alert" report, followed by weekly updates until the event is resolved or stabilized. Conversely, for more stable situations (such as when the disease is considered endemic), they should update the situation for each listed disease on a six-monthly basis. Each semester, they should provide at least the disease situation in the country ("presence," "absence" or "no information collected"). Where possible, countries are asked to complement this with information on surveillance and control measures implemented, and quantitative data on diseases present including number of outbreaks, cases and deaths.

Since the WOAH Working Group on Wildlife was established, the coordinated collection of data on wildlife health has been extended to cover a further 50 or so disease groups (with each group including one or more pathogens) deemed to be a priority by experts, mainly for conservation purposes. To support countries' notification and clarify reporting boundaries, Technical Disease Cards have been published on the WOAH website (42). Although Members are encouraged to contribute to this additional effort, they are not legally obliged to do so. As alluded to above, data on wildlife diseases are mostly neglected with variable degrees of surveillance systems in place.

In addition, when addressing wildlife diseases, it is important to account for the historical, cultural, political, economic, and sociological context in countries and territories, as the perceived value of wild species might vary depending on these factors (43–45). For this reason, WOAH is placing increased importance on its epidemic intelligence framework - evaluating, assessing and integrating information derived from official data collected from WOAH's experts & partner network, as well as data from unofficial sources (e.g., using an information system for automatized collection of information such as the Epidemic Intelligence from Open Source (EIOS) initiative) that presents useful sources to assist in better evaluating the real occurrence of disease (46). These complementary sources also support Members in their reporting activities, and in risk assessment and communication. To minimize the number of unreported events, WOAH has been actively searching for non-official information, rumors and signals relating to animal health and veterinary public health events around the world since 2002. As a result of this activity and the incorporation of a web-based system for the automatic detection of relevant news, WOAH is able to review approximately 120,000 news items each year. Consequently, on average, about 10–14% of events reported through immediate "alert" are additionally submitted to WAHIS each year. This value represents the potential of epidemic intelligence activities to increase mandatory reporting. In the past year, special efforts have been made to ensure increased sensitivity for detecting disease events in wildlife, including potential new and emerging threats, through the development of specific search algorithms in several languages.

Despite bias associated with wildlife disease reporting by Members, some figures are provided in this section to illustrate data collected by the system in recent years (since 2019), compared with the same figures for domestic animal diseases. The purpose of these numbers is not to present any in-depth analysis of the information collected by WOAH, but to provide some data for reflection on the role of the organization. Between 1 January 2019 and 2 November 2023, 154 countries reported 68,862,973 cases through alert messages and weekly updates to WAHIS, for 84 different diseases. One-hundred and fifty countries have reported 68,672,115 cases in domestic animals

and 95 countries have reported 190,858 cases in wild animals. These simple figures indicate the difference in completeness of data collected by WAHIS for domestic animals and wildlife. The percentage of countries being able to detect and report information on exceptional epidemiological situations through WAHIS is lower for diseases in wildlife than for domestic animals. The diseases for which the highest number of countries reported presence in wildlife through this alert channel were: avian influenza of high pathogenicity ($N = 78$ countries), African swine fever ($N = 26$ countries), SARS-CoV-2 in animals ($N = 18$ countries), rabbit hemorrhagic disease ($N = 5$ countries), and West Nile fever ($N = 5$ countries).

Despite being incomplete, the data collected on diseases in wildlife can be useful for Members and general users. They can provide an idea of the global situation of several diseases, and their evolution in time and space. They can be used for risk assessment, to assist decision-making, and to assess the impact of diseases on biodiversity and conservation. As part of the conservation objective, it is important to present the data collected in context. Indeed, while wildlife can represent a reservoir in certain situations (thereby increasing the risk of transmission of pathogens to livestock and humans), it can also be infected through contamination by livestock and humans. These spillover and spillback phenomena have been widely described in the literature, and it is important to consider wildlife from a health point of view not just as a potential source of disease but also as a potential victim (47–50). WOAH regularly uses this information to produce situation reports on selected diseases to provide easy and "digestible" access to data. This is done regularly for diseases such as African swine fever, highly pathogenic avian influenza and SARS-CoV-2 which are considered relevant for both domestic animals and wildlife (51–53). In addition, a specific situation report on wildlife disease reporting is produced monthly to assess the importance of reported cases in wildlife for animal health, public health and biodiversity conservation (54). Official data reported for aquatic animals (including wildlife) are also periodically presented, acknowledging associated surveillance gaps (55).

WOAH's future role in wildlife health

Since its creation, 183 Members have progressively adhered to the principles and rules of WOAH by joining the organization. Driven by the needs expressed by Members and to adapt to the changing global animal health situation, WOAH has continuously built and enriched its activities and contributions to the surveillance and management of diseases and welfare in wild and domestic, terrestrial and aquatic animals. WOAH's implementation of the Wildlife Health Framework now positions the organization to support its Members worldwide in strengthening their wildlife disease surveillance efforts. WOAH, as an international organization responsible for ensuring transparency on animal diseases and with a well-structured network of veterinary services—is in a unique position to collect information on wildlife disease distribution and surveillance activities at the global level. The scope of this information gathering is gradually evolving, in constant consultation with scientific experts, and, first and foremost, its Members.

To minimize the burden of data collection on Members, WOAH is regularly consulting its 70-plus partner international organizations, some of which are already collecting key data for the global surveillance and epidemic intelligence effort (among them, UNEP, IUCN, CITES, and Interpol). Acknowledging that wildlife disease surveillance is by substance a collaborative and multisectoral activity, WOAH encourages Members to take part or lead national networks of public and private stakeholders collecting wildlife health information in the field (56).

The challenges ahead will most probably include continuing to reflect on the synergies between existing information systems, adopting the most adapted technologies, and making good use of data of all kinds and in all formats in an integrated effort to provide Members with the best possible support in their understanding of the global situation and risks. This will involve not only consideration of technological advances but also workflow and responsibility for reporting.

These efforts will need to address the various challenges and gaps highlighted in this paper. In particular, they will address the relative lack of surveillance and resources dedicated to the detection of diseases in wildlife in many countries, as well as the lack of communication between different health sectors which results in poor information sharing at the international level.

To move toward earlier risk assessment and communication, it is necessary to develop international efforts to monitor and analyze drivers and unusual morbidity/mortality events, as well as non-infectious causes of wildlife mortality in addition to pathogen surveillance. WOAH, other members of the Quadripartite, and international partners in general have a duty to lead by example, by coordinating their exchanges of information for risk analysis and communication more effectively. This process of reflection has already begun and must continue in the years to come (57).

Enhanced monitoring of the implementation of WOAH's various standards and guidance documents is required to more effectively tailor the guidance to the Members' needs. The organization has launched a transversal program, the Observatory, that provides an overview of the uptake of international standards by Members. It provides valuable feedback on implementation and effects of Standards, contributing to the progressive improvement of their implementation as well as to the constant assessment of WOAH's corporate initiatives. The Observatory program will help WOAH adjust its activities to Members' needs, including those relating to wildlife (58). The development of new guidance or statements on wildlife health will necessarily adapt to topicalities (e.g., Considerations for emergency vaccination of wild birds against high pathogenicity avian influenza in specific situations) but also intensively use foresight (e.g., Early warning and early action – the coming El Niño Southern Oscillation phenomenon and health impacts) (59, 60). Recommendations for increased awareness of wildlife disease and ecosystem balance from WOAH occurred long before wildlife diseases and conservation of biodiversity became topical. Nevertheless, tangible actions were delayed, and more decisive measures and engagement are yet to come - for instance, the incorporation of the One Health principle in its core mission statement and the inclusive definition of animals in its Code and Manual. By identifying and addressing old and new challenges, fostering international collaboration, and embracing a One Health approach in alignment with the Quadripartite collaboration, WOAH will contribute to a safer and more secure future for both animals and humans in the face of evolving global health challenges. Continued support from WOAH and its initiatives is imperative as we navigate the complexities of wildlife health on a global scale.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found at: https://wahis.woah.org/#/home.

Author contributions

LT: Conceptualization, Supervision, Validation, Writing – original draft, Writing – review & editing. CC: Writing – original draft, Writing – review & editing. LA: Writing – original draft, Writing – review & editing. SM: Writing – original draft, Writing – review & editing. DS: Writing – original draft, Writing – review & editing. JW: Writing – review & editing. PT: Conceptualization, Supervision, Validation, Writing – original draft, Writing – review & editing.

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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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References

1. World Organisation for Animal Health (1925). International agreement for the creation of an office international des epizooties. Available at: (https://www.woah.org/en/who-we-are/structure/framework/basic-texts/international-agreement-for-the-creation-of-an-office-international-des-epizooties/).

2. World Organisation for Animal Health (1925). Organic statutes of the office international des epizooties. Available at: (https://www.woah.org/en/who-we-are/structure/framework/basic-texts/international-agreement-for-the-creation-of-an-office-international-des-epizooties/).

3. World Organisation for Animal Health (2002). Fight against animal diseases: resolution no. XXIII. Available at: (https://www.woah.org/en/who-we-are/structure/framework/basic-texts/fight-against-animal-diseases/).

4. World Organisation for Animal Health (WOAH) (2023). Criteria for the inclusion of diseases, infections and infestations in the WOAH list, terrestrial animal health code. Available at: (https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmfile=chapitre_criteria_diseases.htm).

5. World Organisation for Animal Health (WOAH) (2023). WOAH - world organisation for animal health. Available at: (https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/).

6. Blome S, Gabriel C, Beer M. Pathogenesis of African swine fever in domestic pigs and European wild boar. *Virus Res*. (2013) 173:122–30. doi: 10.1016/j.virusres.2012.10.026

7. Fisher MC, Garner TWJ, Walker SF. Global emergence of Batrachochytrium dendrobatidis and amphibian chytridiomycosis in space, time, and host. *Ann Rev Microbiol*. (2009) 63:291–310. doi: 10.1146/annurev.micro.091208.073435

8. Gamarra-Toledo V, Plaza PI, Gutiérrez R, Luyo P, Hernani L, Angulo F, et al. Avian flu threatens Neotropical birds. *Science*. (2023) 379:246. doi: 10.1126/science.adg2271

9. Luskin MS, Meijaard E, Surya S, Walzer C, Linkie M. African swine fever threatens Southeast Asia 11 endemic wild pig species. *Conserv Lett*. (2021) 14:e12784. doi: 10.1111/conl.12784

10. Namazi F, Khodakaram Tafti A. Lumpy skin disease, an emerging transboundary viral disease: a review. *Vet Med Sci*. (2021) 7:888–96. doi: 10.1002/vms3.434

11. Puryear W, et al. Outbreak of highly pathogenic avian influenza H5N1 in New England seals. *bioRxiv*. (2022) 2022:1155. doi: 10.1101/2022.07.29.501155

12. Parashar UD, Sunn LM, Ong F, Mounts AW, Arif MT, Ksiazek TG, et al. Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998-1999 outbreak of severe encephalitis in Malaysia. *J Infect Dis*. (2000) 181:1755–9. doi: 10.1086/315457

13. van Kerkhove M, Mumford E, Mounts AW, Bresee J, Ly S, Bridges CB, et al. Highly pathogenic avian influenza (H5N1): pathways of exposure at the animal-human interface, a systematic review. *PLoS One*. (2011) 6. doi: 10.1371/journal.pone.0014582

14. Dobson AP, Pimm SL, Hannah L, Kaufman L, Ahumada JA, Ando AW, et al. Ecology and economics for pandemic prevention: investments to prevent tropical deforestation and to limit wildlife trade will protect against future zoonosis outbreaks. *Science*. (2020) 369:379–81. doi: 10.1126/science.abc3189

15. Karesh WB, Dobson A, Lloyd-Smith JO, Lubroth J, Dixon MA, Bennett M, et al. Ecology of zoonoses: natural and unnatural histories. *Lancet*. (2012) 380:1936–45. doi: 10.1016/S0140-6736(12)61678-X

16. Cāceres P, Tizzani P, Ntsama F, Mora R. The world organisation for animal health: notification of animal diseases. *Rev Sci Tech*. (2020) 39:289–97. doi: 10.20506/rst.39.1.3082

17. OIE Wildlife Health Framework. *World organisation for animal health (WOAH)*. Paris: OIE Wildlife Health Framework (2020).

18. World Organisation for Animal Health (WOAH) (2023). Aquatic animal health code. Available at: (https://www.woah.org/en/what-we-do/standards/codes-and-manuals/aquatic-code-online-access/).

19. OIE World Organization for Animal Health. Resolutions adopted by the 33rd General Conference of the Committee of the Office International des Epizooties, Paris. *Bull Off Int Epizoot*. (1954) 64:1517–20.

20. Blancou J. Role of the Office International des Epizooties in protecting the health of free-ranging mammals. *Rev Sci Tech*. (1992) 11:1019–24.

21. World Organisation for Animal Health (WOAH). (2023). Collaborating centres: terms of reference, WOAH global website. Available at: (https://www.woah.org/en/what-we-offer/expertise-network/collaborating-centres/).

22. World Organisation for Animal Health (WOAH) (2023). Reference laboratories: terms of reference, WOAH global website. Available at: (https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#searchform-header).

23. WOAH ex-OIE (2021) Wildlife health survey report - online report. Available at:https://www.woah.org/app/.

24. WOAH ex-OIE. *OIE seventh strategic plan for the period 2021–2025 (88 SG/14)*. Paris: World Organization for Animal Health (2021).

25. World Organisation for Animal Health (WOAH). *RESOLUTIONS adopted by the world assembly of OIE delegates during their 88th general session 24–28 may 2021*. Paris: World Organization for Animal Health (2021).

26. WOAH. *In-country wildlife disease surveillance survey*. Paris: World Organization for Animal Health (2021).

27. WOAH (2023). Dashboard: 2023-in-country wildlife data management survey. Available at: (https://app.powerbi.com/view?r=eyJrIjoiMWRjMTBkNmUtNzA5OC00ZTY4LWI3ZmItOGQ2OGI4MDliZDUzIiwidCI6ImYxZmFmNTYzLWIwNmQtNGMzNS04NzM5LTM0Y2NjMjgwZGNhZiIsImMiOjh9&pageName=ReportSectionc23ecdac85d480b9de31).

28. World Organisation for Animal Health (WOAH) (2023). WAHIS: world animal health information system. Available at: (https://wahis.woah.org/#/home).

29. World Organisation for Animal Health (WOAH) (2023). Manual of diagnostic tests and vaccines for terrestrial animals. Available at: (https://www.woah.org/en/what-we-do/standards/codes-and-manuals/#ui-id-2).

30. World Organisation for Animal Health (WOAH) (2023). Manual of diagnostic tests for aquatic animals. Available at: (https://www.woah.org/en/what-we-do/standards/codes-and-manuals/#ui-id-4).

31. Ulaankhuu A, Bazarragchaa E, Okamatsu M, Hiono T, Bodisaikhan K, Amartuvshin T, et al. Genetic and antigenic characterization of H5 and H7 avian influenza viruses isolated from migratory waterfowl in Mongolia from 2017 to 2019. *Virus Genes*. (2020) 56:472–9. doi: 10.1007/s11262-020-01764-2

32. George SE, Smink M, Sangkachai N, Wiratsudakul A, Sakcamduang W, Suwanpakdee S, et al. Stakeholder attitudes and perspectives on wildlife disease surveillance as a component of a one health approach in Thailand. *One Health*. (2023) 17:100600. doi: 10.1016/j.onehlt.2023.100600

33. World Organisation for Animal Health (WOAH) (2023). Wildlife health. Available at: (https://www.woah.org/en/what-we-do/animal-health-and-welfare/wildlife-health/#ui-id-5).

34. Tripartite (2023). IHR-PVS national bridging workshop program. Available at: (https://rr-asia.woah.org/wp-content/uploads/2023/03/nbw-factsheet-2022-2-pages.pdf).

35. Nature for Health (2023). What is N4H? Available at: (https://nature4health.org/about).

36. Quadripartite (2022). One health joint plan of action (2022-2026): working together for the health of humans, animals, plants and the environment. Available at: (https://www.who.int/publications/i/item/9789240059139).

37. World Organisation for Animal Health (2023). Notification of diseases, and provision of epidemiological information. Available at: (https://www.woah.org/en/what-we-do/standards/codes-and-manuals/aquatic-code-online-access/?id=169&L=1&htmfile=chapitre_notification.htm).

38. World Organisation for Animal Health (WOAH) (2023). Notification of diseases and provision of epidemiological information. Terrestrial animal health code. Available at: (https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmfile=chapitre_notification.htm).

39. Angot J-L. *Surveillance et contrôle des risques d'importation des maladies infectieuses animales: le rôle de l'OIE et des services vétérinaires*. Paris: Académie Nationale de Médecine (2009).

40. Jebara KB, et al. WAHIS-wild and its interface: the OIE worldwide monitoring system for wild animal diseases. *Vet Ital*. (2016) 52:91–100. doi: 10.12834/VetIt.235.779.3

41. Vallat B, Thiermann A, Ben Jebara K, Dehove A. Notification of animal and human diseases: the global legal basis. *Rev Sci Tech*. (2013) 32:331–5. doi: 10.20506/rst.32.2.2234

42. World Organisation for Animal Health (WOAH) (2023). Animal diseases: non-listed diseases affecting wildlife. Available at: (https://www.woah.org/en/what-we-do/animal-health-and-welfare/animal-diseases/?_tax_diseases=non-listed-affecting-wildlife).

43. Lawson B, Neimanis A, Lavazza A, López-Olvera JR, Tavernier P, Billinis C, et al. How to start up a National Wildlife Health Surveillance Programme. *Animals*. (2021) 11:1–12. doi: 10.3390/ani11092543

44. Ryser-Degiorgis M-P. Wildlife health investigations: needs, challenges and recommendations. *BMC Vet Res*. (2013) 9:223. doi: 10.1186/1746-6148-9-223

45. Wobeser GA. *Diseases in wild animals: Investigation and management*. Cham: Springer (2007).

46. Fanelli A, Schnitzler JC, de Nardi M, Donachie A, Capua I, Lanave G, et al. Epidemic intelligence data of Crimean-Congo haemorrhagic fever, European region, 2012 to 2022: a new opportunity for risk mapping of neglected diseases. *Eur Secur*. (2023) 28:542. doi: 10.2807/1560-7917.ES.2023.28.16.2200542

47. Alexander K.A., Carlson C.J., Lewis B.L., Getz W.M., Marathe M.V., Eubank S.G., (2018) *The ecology of pathogen spillover and disease emergence at the human-wildlife-environment interface: in the connections between ecology and infectious disease*. Cham: Springer International Publishing. 267–298.

48. Fagre AC, Cohen LE, Eskew EA, Farrell M, Glennon E, Joseph MB, et al. Assessing the risk of human-to-wildlife pathogen transmission for conservation and public health. *Ecol Lett*. (2022) 25:1534–49. doi: 10.1111/ele.14003

49. Nugent G. Maintenance, spillover and spillback transmission of bovine tuberculosis in multi-host wildlife complexes: a New Zealand case study. *Vet Microbiol*. (2011) 151:34–42. doi: 10.1016/j.vetmic.2011.02.023

50. Thompson RA. Parasite zoonoses and wildlife: one health, spillover and human activity. *Int J Parasitol*. (2013) 43:1079–88. doi: 10.1016/j.ijpara.2013.06.007

51. World Organisation for Animal Health (WOAH) (2023). Listed disease: African swine fever. Available at: (https://www.woah.org/en/disease/african-swine-fever/#ui-id-2).

52. World Organisation for Animal Health (WOAH) (2023). Listed disease: avian influenza. Available at: (https://www.woah.org/en/disease/avian-influenza/#ui-id-2).

53. World Organisation for Animal Health (WOAH) (2023). SARS-CoV-2. Available at:https://www.woah.org/en/disease/sars-cov-2/#ui-id-2.

54. World Organisation for Animal Health (WOAH) (2023). Wildlife health: situation on non-listed disease in wildlife. Available at: (https://www.woah.org/en/what-we-do/animal-health-and-welfare/wildlife-health/#ui-id-3).

55. World Organisation for Animal Health (WOAH) (2023). Aquatic animal health strategy. Available at: (https://www.woah.org/en/what-we-do/animal-health-and-welfare/aquatic-animals).

56. OIE (2021). Terms of reference for the OIE National Focal Point on wildlife. Available at: (https://rr-europe.woah.org/wp-content/uploads/2019/10/wildlife.pdf).

57. Quadripartite (2022). Quadripartite one health intelligence scoping study. Available at: (https://cdn.who.int/media/docs/default-source/one-health/quadripartitie-one-health-intelligence-scoping-study-aug2022.pdf?sfvrsn=d7a771e2_3&download=true).

58. World Organisation for Animal Health (WOAH) (2023). Observatory: overview. Available at: (https://www.woah.org/en/what-we-do/standards/observatory/).

59. WOAH Working Group on Wildlife (2023). Early warning and early action – the coming El Niño southern oscillation phenomenon and health impacts. Available at: (https://www.woah.org/en/early-warning-and-early-action-the-coming-el-nino-southern-oscillation-phenomenon-and-health-impacts/).

60. World Organisation for Animal Health (2023). Considerations for emergency vaccination of wild birds against high pathogenicity avian influenza in specific situations. Available at: (https://www.woah.org/app/uploads/2024/01/vaccination-wild-birds-hpai-outbreak-dec2023.pdf).

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A combined methodological approach to characterize pig farming and its influence on the occurrence of interactions between wild boars and domestic pigs in Corsican micro-regions

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The pig sector in Corsica is based by a wide range of farming systems, mainly characterized on traditional extensive practices, which favor contacts between domestic and wild individuals. These contacts are suspected to influence the maintenance and the transmission of shared infectious diseases between both populations. Therefore, it is important to develop methods that allow to understand and anticipate their occurrence. Modeling these interactions requires accurate data on the presence, location and use of land on pig farms and farming practices, but such data are often unavailable, incomplete or outdated. In this study, we suggest a method to collect and analyze pig farming information that combines approaches from social sciences and epidemiology and enables a spatial representation of an index of potential interaction (IPI) between wild and domestic pigs at municipality level in the Corsican territory. As a first step of the process, interviews were conducted to gather information from 103 pig farms. Then, using hierarchical clustering, we identified five different clusters of pig farming practices which were evaluated and validated by local experts using participatory tools. The five pig farming clusters with their respective estimated levels of direct and indirect interactions with wild boars were combined in a linear equation with pig density to estimate a hypothetical index of potential interaction (IPI) in 155 municipalities. Our results revealed the diversity of pig farming practices across the island of Corsica and pointed out potential hotspots of interaction. Our method proved to be an effective way to collect and update information on the presence and typology of pig farms which has the potential to update official livestock production statistics. The spatial representation of an IPI between wild boars and domestic pigs in the Corsican territory could help design regional disease management strategies and policies to improve the control of certain shared pig pathogens in pig farms from Corsica.

KEYWORDS

biosecurity, incursion, outdoor farming, pig production, *Sus scrofa*, typology, wildlife

1 Introduction

Recent episodes of emergence, re-emergence or persistence of animal infectious diseases has drawn scientific attention to the wildlife-livestock interfaces as a key factor to improve our understanding of shared pathogen dynamics (1–3). The interest on interactions between wild boars and domestic pigs has particularly grown with the global spread of African swine fever across the world (4–7). However, other diseases shared between wild boars and domestic pigs jeopardize disease eradication efforts in the pig sector, while affecting the health of wild boar populations of and representing a potential public health risk (8–13). Moreover, the recent increase of consumer demand for outdoor farming products in developed economies has raised concerns about the biosecurity of open production systems in general, and about potential interactions between domestic and wild/feral pigs (2, 9, 14–17). Understanding the different drivers of interactions between populations of wild and domestic pigs requires analysis of the infectious interface using approaches from different disciplines (3). Such approaches often include ecological, epidemiological or sociological methodologies focused on a farm perimeter, water points, or the edge of a protected area, whereas fewer studies have addressed the risk of wildlife-livestock interactions and pathogen spill-over at a larger geographical scale (18–21).

Because of their ancestral tradition of outdoor pig farming, Mediterranean habitats are particularly prone to interactions between domestic pigs, feral swine and wild boars. For instance, free ranging farming systems in Sardinia have been held accountable for the persistence of African swine fever for decades (5, 22, 23), while in the Iberian Peninsula, the co-existence of Iberian pigs with a large wild boar population in extensive estates is considered as a risk for the re-emergence of Aujeszky's disease or the maintenance of bovine tuberculosis (18, 20, 24). The French Island, Corsica, is an example of specific socio-ecological context favoring different types of direct and indirect sexual, trophic and agonistic interactions between wild and domestic pigs and the resulting dissemination of shared porcine pathogens among these populations (16, 25). These include endemic diseases and re-emerging or recent diseases that can have a serious impact on livestock productivity and public health such as classical swine fever (26), Aujeszky's disease (15), trichinellosis (27), toxoplasmosis (28) or hepatitis E virus (8).

Several authors have characterized the type, frequency, intensity and location of interactions between wild boar and domestic pigs, which are significantly influenced by hunting and farming practices (8, 15). However, the whole range of outdoor farming systems and the potential impact of their spatial distribution on the probability of interaction with wild boars has not been well characterized in Corsica to date. Given the variety of landscapes and the distribution of resources in Corsica, we hypothesize that some regions of the island with specific ecological features or forms of land use are prone to certain types of pig management practices that facilitate these interactions. Nevertheless, studying such complex interface at a territorial scale is challenging as data on farming practices and specific locations of farming systems is often inaccurate. A possible approach to address this challenge is to rely on local knowledge and expertise (29, 30), with the implementation participatory epidemiology methods (31, 32). As several epidemiological and zootechnical information was already available in Corsica from previous studies (15–17, 25), we decided to combine different geographical, epidemiological and zootechnical approaches to conduct a spatial analysis of farming systems that could be used as an indicator of spatial potential interaction patterns.

The specific purpose of our work was to explore new methodologies, combining participatory approaches and analysis of zootechnical data, to represent the distribution of pig management practices at the scale of some Corsican micro-regions and their potential risk of interactions with wild boar based on pig farming.

2 Materials and methods

2.1 Study area and context

The island of Corsica is located in the Mediterranean Sea off the coast of the South of France and covers 8,722 square kilometers. Its altitude (ranging from 0 to 2,706 m and 568 m on average) and landscape characteristics are emblematic of Corsican identity (33). The variability of soils and topography in the Island enables the adoption of a diversity of crop and livestock production systems (34). In 2015, the French Ministry of Agriculture and Food defined 16 micro-regions resulting from the aggregation of the 30 small natural regions originally defined in the 1979 agricultural census (35) and based on homogeneity and natural limits criteria (see Aggregated Small Natural Regions, Supplementary material S1). Based on these criteria, we defined the term micro-region as a division of the territory based on certain homogenous geographical characteristics that influenced its land use and agricultural production practices and used this classification throughout this article.

In the past, pig farming was widespread in Corsica. The traditional Corsican pig farming system, which consisted fundamentally on free-ranging systems of backyard animals for family consumption, is based on the exploitation of sylvo-pastoral resources by a local breed of slow growing pigs. Pigs aged 18–24 months are slaughtered in winter after a period of free ranging in autumn and winter to finish their fattening with acorns and chestnuts (36). In some areas, farmers also keep their pigs in mountain pastures in summer (17, 25, 37). The Corsican pig sector consequently has a strong link with certain micro-regions featuring particular ecological landscapes such as mountain pastures or chestnut forests. Today, especially thanks to PDO (Protected Designation of Origin) certification and use of the "Nustrale" breed, the production of Corsican dry cured meat (*charcuterie*) is prized for both its quality and flavor.

In Corsica, domestic and wild swine populations are largely represented in terms of their distribution and suspected abundance (38, 39) encompassing an interesting genetic diversity composed of different domestic pigs' breeds, feral pigs, wild boars and cross-bred individuals. Although the proportion of cross-bred animals in this population has not been accurately quantified, it was estimated to reach 55% in some regions during the 1980's (11, 40).

2.2 Study design

Given the diversity and heterogeneity of farming practices, we hypothesized that the potential contribution of pig farming to the probability of occurrence of interactions with wild boar was

multi-factorial (41). Based on previous work in Corsica (16) and other pig farming locations (42–44), we first identified key zootechnical practices involved in the occurrence of different types of interaction and defined a method based on the clustering of farming practices. The main steps to comply with this process were the following: (i) Implementation of interviews with key informants in order to collect regional data on formal and informal pig production; (ii) Creation of a reliable database combining information collected through interviews with existing data and technical knowledge; (iii) Hierarchical clustering on principal components (HCPC) based on multiple correspondence analysis (MCA) to identify a preliminary typology of farming systems (clusters) based on the use of distinct pig farming practices; (iv) Adjustment of our preliminary farm typology by a group of local experts (38) to determine the main factors that describe the clusters and determination of the IPI associated to each cluster; (v) Validation of a new classification of the clusters undertaken with local stakeholders and a classification tree method; (vi) Evaluation and mapping of IPI related to pig farming at the municipal scale, using pig density and the IPI in each cluster. A summary of the methodology is shown in Figure 1.

2.2.1 Data collection

Data collection was organized on semi-structured interviews with key informants (30, 45) in two different periods: the first from February to April 2019 and the second from September 2019 to February 2020. The questionnaire was designed to gather regional information on formal and informal data on pig production farms from local key informants, including pig density, land area occupied by pigs, and the main pig farming practices. All the semi-structured interviews were conducted by the same interviewer at the place where the key informants lived or worked. Key informants were selected on the basis of their experience in livestock farming, their involvement in local farming organizations, and on the recommendation of other informants or stakeholders in pig farming. The interviews allowed to compile a final list of 176 farms (106 farms during the first period and 70 farms during the second). After the first period, only 103 of those farms had sufficient quantity and quality of data to perform the HCPC analysis, including 84 farms from the first data collection period and 19 farms from data available from a previous study (16) were retained (Figure 1).

2.2.2 Selection of farming practices

We focused our selection of factors on free-range pig farming, the permeability of fences and management of feed and waste taking into account direct and indirect wild-domestic pig interactions (25). Because direct interactions are often driven by sexual and agonistic behavior, we focused on farming practices linked with reproductive management, such as castration or spaying of pigs not intended for breeding.

For the MCA analysis, we selected 16 categorical factors among the farming practices hypothesized to have an influence on interactions with wild boars (9, 12, 15, 46). The selection criteria and categorical factors used for these variables are provided in Supplementary material S2.

2.2.3 Study area and spatial scales

From an administrative point of view, Corsica is a region divided into two departments, "Haute-Corse" (Northern Corsica) and "Corse du Sud" (Southern Corsica). We collected data in two micro-regions in Southern Corsica, including "Haute Gravone" and "Secteur Ajaccio" and six micro-regions in Northern Corsica including "Cap Corse," "Nebbiu," "Balagne," "Haute Corse Intérieure," "Castagniccia" and "Plaine Orientale" (Supplementary material S1). The choice of these micro-regions was not only based on the possibility of collecting information from different geographic locations but also, on the possibility to test our approach on a representative and diverse sample of farming systems, land uses and vegetation types of the island.

In France, municipalities represent the smallest administrative level. Although not ideal because the land used by pigs does not always coincide with administrative boundaries, we considered the municipal scale to be the most practical and appropriate to represent the distribution of the pig population. The data collected on each pig farm was converted to the municipality scale on the basis of the ratio of the extent of land used by each herd in each municipality to the

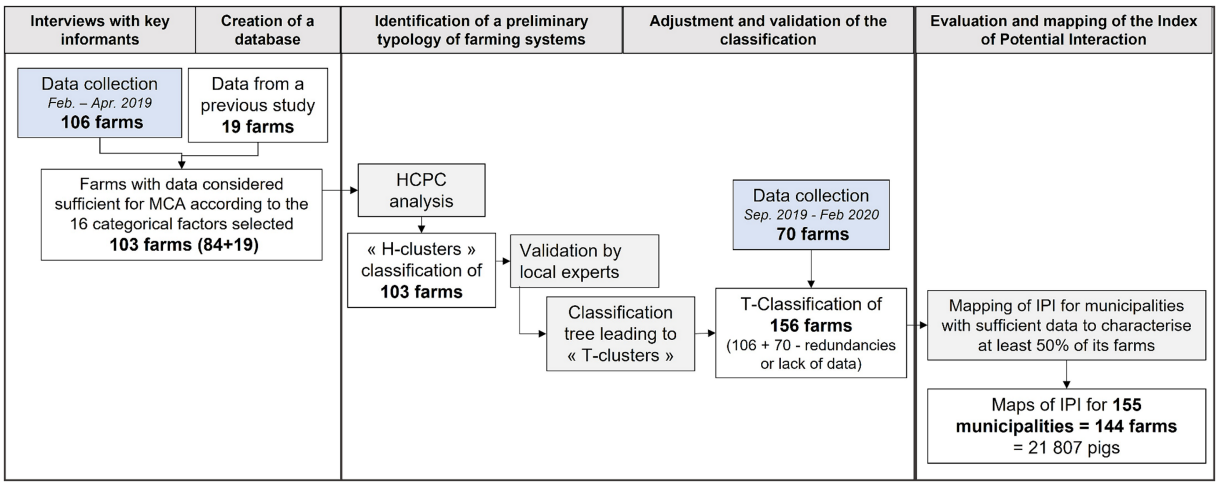

FIGURE 1
Summary of the methodology used and the number of farms covered.

total area of land used by each herd. By combining this ratio with the size of the herd, we calculated the number of pigs in each herd in each municipality.

2.3 Exploring the diversity of pig farming systems in Corsica

The diversity of farming system based on reported practices was explored following three steps.

2.3.1 Identification of clusters

The MCA was performed on 16 categorical factors (Supplementary material S2) describing the farming systems' practices to summarize the information in a lower-dimensional Euclidean space (five dimensions) where distances represent similarity (16). Next, using Ward's method, hierarchical clustering was performed of the MCA results to identify groups of farmers who use similar practices, subsequently termed "H-clusters." Both operations were performed in R version 3.5.3 using the package FactoMineR for MCA (47) and HCPC (48). In the hierarchical clustering process, we considered the inertia gain ratio as the parameter determining the variance gain when the number of clusters increased.

2.3.2 Validation and ranking of the clusters by local experts

A meeting was organized with a group of nine pig farming experts from different micro-regions to present our methodology and results and validate the conformity of our farm classification. The group was made up of four breeders and five technicians from pig farming -related organizations with a solid background knowledge of the Corsican pig sector, based on their activity, experience and training. During the meeting, we combined three types of participatory exercises selected for their complementarity (49) and their ability to capture and leverage local knowledge and expertise (50): focus-group discussion (51), cluster ranking, and proportional piling (32). The details on these participatory methods can be found in Supplementary material S3.

2.3.3 Classification tree for cluster classification

We performed a classification tree (52), called "T-cluster," with the Rpart R-package (53) to classify individuals not included in the initial HCPC analysis and farms identified in other data collection into specific clusters. We considered tree combinations of the different factors mentioned by experts as having the strongest impact on interactions and compared their percentage of correspondence with the HCPC attribution (called "H-cluster"). In this way, we selected trees with the least divergence between the "H-cluster" and "T-cluster" classifications.

2.4 Quantification of an index of potential interaction (IPI) per municipality

We selected the 155 municipalities for which we had sufficient data to characterize at least 50% of the farms, accounting for 144 farms and 21,807 pigs. To quantify the index of potential interaction (Y) due to pig farming based on its presence and practices in each municipality, we used a weighted average of the five cluster-specific pig-densities (X_1 to X_5):

$$Y = \sum w_i X_i$$

where the set of weights $w_1, \ldots, w_5$, verify $\sum w_i = 1$ determined by experts depended on whether the interaction Y is direct or indirect. The IPI could thus be interpreted as an effective number of animals at risk, and used to compare municipalities with different distributions of farming systems.

2.5 Characterization of municipalities

A principal component analysis (PCA) was performed on the number and density of pigs in each of the five clusters and on the surface area of the 155 municipalities included in the study (11 variables in total), to identify their main patterns of variation. The PCA provided a reduced representation of the profiles of municipalities on a two-dimensional Euclidean space, and allowed us to explore and investigate cases with different characteristics but similar levels of risk of interaction between domestic pigs and wild boars.

3 Results

3.1 Pig farm typology

Based on the results of the MCA, the distribution of dimensions explained 38.9% of the total variance with each dimension contributing to at least 5% of this value. In our case, evaluation of the inter-cluster inertia gain revealed that the best cut-off values were three and five. Based on this statistical evaluation and on our field observations, we chose five clusters (Supplementary material S4). All the variables in the analysis were identified as significant (p-value $<10^{-3}$) except the period of domestic boar castration ($p = 0.0068$). Among the 103 farms studied, 16 were in H-cluster 1, 21 in H-cluster 2, 20 in H-cluster 3, 39 in H-cluster 4, and seven in H-cluster 5.

Table 1 summarizes the main characteristics of the five farm types identified. A detailed description is available in Supplementary material S5. Clusters 1 to 3 represented farms where pigs were allowed to free-range all or part of the year. Conversely, clusters 4 and 5 represented farms where pigs were fenced in all year round, either outdoors for cluster 4 or in a building for cluster 5. The main differences found between clusters 1 and 3 were in terms of free-ranging time, partial for cluster 3, and reproduction management, which was more controlled in cluster 2 than in cluster 1.

3.2 Use of local knowledge for validation of our farm typology and assessment of interactions between wild and domestic pigs

3.2.1 Validation of farm typology using local knowledge

During focus group sessions, experts readily agreed on the definition and representativity of the five farming clusters of pig farming systems occurring in Corsica. When talking about the H-clusters and factors of interest, experts tended to focus on direct sexually driven interactions, but when considering a wider range of

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TABLE 1 Summary of the five types of farming practices used in the typology.

Cluster	Main breed	PDO	Farrow	Free-ranging	Fencing	Material used for fencing	Waste left outdoor	Feed supplement supplied	Spaying of sows	Mating in fenced area	Enclosure used for farrowing	Frequency of hybrid litters	Interactions with other pigs
1	Mixed or population	No	Yes	All year round	No fences or partial	Wire grid	Yes	No rules	No	No	No or yes	Regular	Yes
2	Nustrale	Yes	Yes	All year round	No fences or partial	Wire grid	Yes	Seasonal, some regularly	Yes or no	Yes	Yes	50% never, 50% sometimes	Yes
3	None	No	Yes	Seasonal (Autumn/ Winter +/− Summer)	Some fully fenced, some partial	Wire grid	Yes	Regularly	No or yes	Yes	Yes	Sometimes	Yes
4	Nustrale or mixed	Yes or No	Yes	Never	Fully fenced	Steel mesh or wire grid	50% yes 50% no	Regularly	No	Yes	Yes	Never	No
5	Other (LW, Duroc)	No	No	Never	Fully fenced	Construction	50% yes 50% no	Regularly	No	Yes	NC	NC	No

interactions, they drew their attention to three major factors, including sow reproduction, fence and carcass management:

- Reproductive Management: The key factor reported in order to minimize sexually driven interactions was the availability of receptive reproductive females in free ranging systems. The experts considered spatial compartmentalization of reproductive females and surgical neutering of animals not intended for reproduction as the two major strategies likely to have a positive impact in reducing the attraction of wild boars toward farmed sows and hence potential interactions.
- Fence management: the experts emphasized the fencing material used and its maintenance were the two major limiting factors for impeding wild boar incursions. In their opinion, only building welded mesh and electric fences, although not perfect, under regular maintenance could potentially contain wild boar incursions in the farm and prevent interactions with their domestic pigs. The use of adequate and well-maintained materials was considered instrumental to avoid spaying females non-targeted for reproduction. In all other cases, the absence of spaying necessarily led to incursions and subsequent interactions, particularly sexually driven ones.
- Management of carcasses and offal: experts regretted in Corsica this aspect was overlooked and becoming an increasing concern because some parts of pig carcasses are no longer processed and wild boar meat is less frequently consumed by hunters.

Concerning free ranging systems, experts identified a strong influence of the season during which animals were widely kept free ranging (autumn) in the number and length of interactions.

This local knowledge and expertise enabled us to refine our classification and agree on a final cluster typology concerning zootechnical practices.

3.2.2 Final T-cluster classification

The selected tree based on the above-mentioned criteria included 19 results that diverged from the results of the HCPC of the total 103 farms (Figure 2). Such rate was considered to be acceptable given that the number of divergences most frequently observed for trees obtained with Rpart was 15 out of 103. Moreover, unlike the original tree (9/15 cases), our designed tree respected the precautionary principle in 17 out of 19 cases, meaning that, in the event of divergence, the farms were classified in a higher-risk T-cluster than the original H-cluster. Hereafter, all the results presented are based on the T-cluster classification.

As shown in Figure 3, cluster 4 is the biggest, representing 37% of the pig population, whereas cluster 5 only represents 2%; clusters 2 and 3 represent equivalent proportions (23 and 22%) and cluster 1 represented 16% of the pig population. Although not all the municipalities in the selected micro-regions were accounted for, coverage of the main breeding micro-regions of our selection, Castagniccia and Haute-Gravone, was almost complete.

3.2.3 Weighting of the index of potential interaction (IPI) for each cluster

A consensus on the ranking of H-clusters was easily reached by the focus groups on the case of direct interactions. However, the concept of indirect interactions required more discussion to reach a consensus on ranking results. The results of the focus group on the

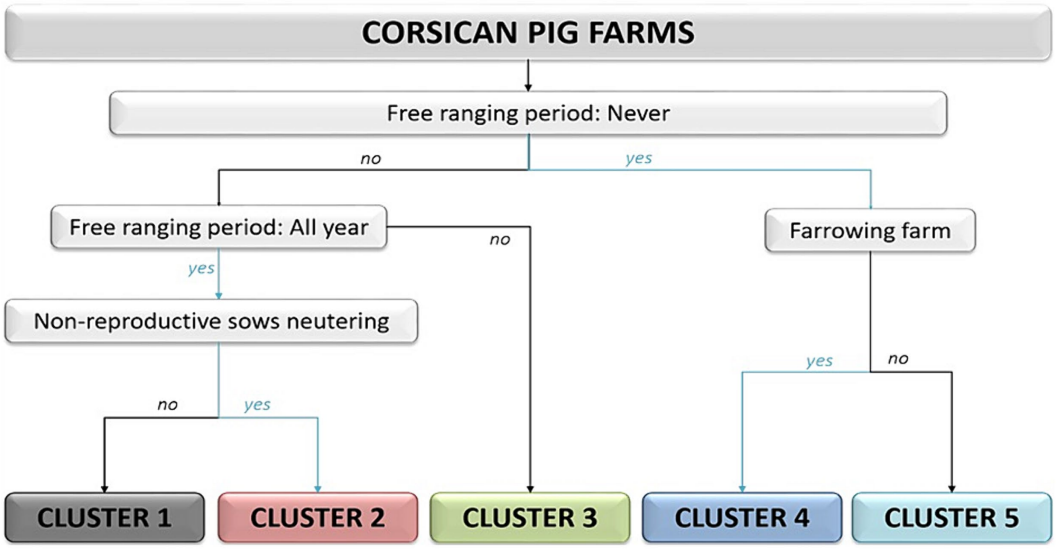

FIGURE 2
Final cluster classification combining typology and validation of local experts. These five clusters represent the T clusters.

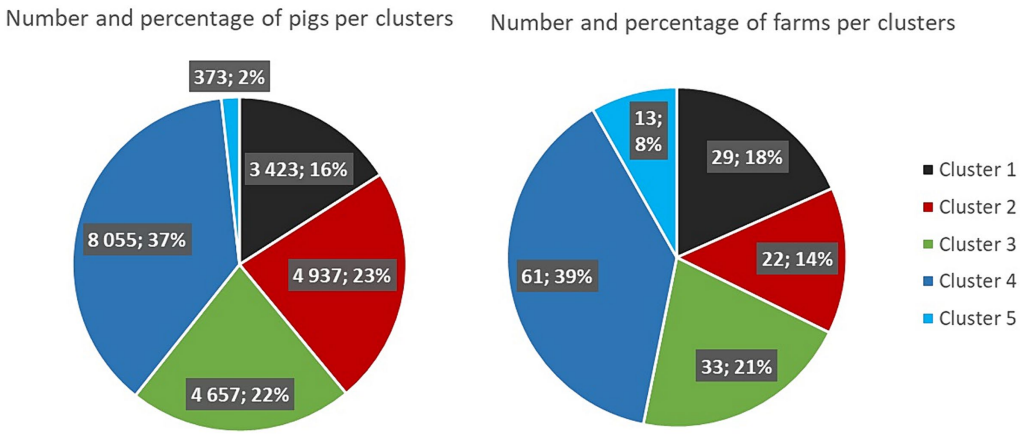

FIGURE 3
Distribution of number of pigs and farms in each cluster (T Clusters). Clusters 1, 3 and 4 show a certain homogeneity between the number of farms and the number of pigs in each cluster. The difference in percentage between number of farms and number of pigs for clusters 2 and 5 indicates a higher average farm size than clusters 1, 3, and 4.

main factors defining clusters (see 1.2) were subsequently confirmed in the discussions concerning ranking.

Concerning direct interactions (Table 2), higher numerical values were revealed for clusters 1 and 3 than for the other clusters. The main factors that influenced ranking and piling by experts were reproductive management, spaying of sows, and seasonality of free ranging animals. Moreover, although cluster 3 had less probability of interactions occurring during the free-ranging period than cluster 2, experts agreed that spaying of sows non targeted for reproduction was the most important factor influencing direct interactions, which explains why cluster 3 was considered to have more direct interactions than cluster 2.

Concerning indirect interactions (Table 2), two experts disagreed with the order proposed in the proportional piling ranking exercise. In their opinion, clusters 1 and 2 had the same weight because free ranging and waste management practices had a similar impact on interactions. However, they all agreed that free ranging facilitated the sharing of food resources such as pastures (but also carcasses or offal) and water points. This explained the lower value for cluster 3 and the similar values for clusters 4 and 5.

3.3 Data visualization

3.3.1 The spatial distribution of pigs and types of practices

Densities of pigs and absolute numbers were closely correlated and independently of the area in the first PCA factorial plane, meaning that high densities tend to be explained by large numbers rather than by small surface areas. In contrast, the

TABLE 2 Weighting and ranking of clusters by local experts based on the occurrence of direct and indirect interactions in pig farms.

Rank (1 = highest)	Cluster	Main arguments for ranking (compared with a lower-ranked cluster)	Numerical weight of the IPI (mean of expert's results)
Direct interactions			
1	Cluster 1	No management of reproduction Spaying of sows not intended for reproduction: none Pigs allowed to range free all year round	43.7
2	Cluster 3	Management of reproduction Spaying rate of sows not intended for reproduction: 35.0% Free ranging part of the year	29.5
3	Cluster 2	Spaying rate of sows not intended for reproduction: 57.1%	16.5
4	Cluster 4	No free ranging	7.4
5	Cluster 5	Type of fencing	2.9
Indirect interactions			
1	Cluster 1	Free ranging all year round Carcasses and leftovers left on the ground outdoors	36.8
2	Cluster 2	Better management of reproduction: reduced attraction of wild boars	31.2
3	Cluster 3	Free ranging part of the year	19.6
4	Cluster 4	No free ranging = very few resources shared	7.4
5	Cluster 5	Better management of carcasses and leftovers	5

density of pigs in cluster 3 was explained to a large extent by smaller areas. Density thus enabled an accurate representation of the number of pigs at the scale of the island. A number of municipalities could be distinguished by the number of pigs, especially in the case of Castagniccia micro-region (Figures 4A,B).

The different types of farming practices were distributed across the island, even if some municipalities stood out among the dominant clusters. Clusters 1 and 2, characterized by year-round free ranging, were more frequent in mountain and piedmont municipalities (Castagniccia, Plaine Orientale), while Clusters 3 and 4 were found in most regions, but specially in municipalities with high pig densities, such as Castagniccia and Haute-Gravone. Clusters 3 and 4 were dominant in the Haute-Gravone, whereas fewer farms mainly corresponding to 4 and 5 cluster types, were located in Cap Corse.

The PCA results showed that 42% of the variability of densities and number of pigs in each cluster and the surface area of municipalities can be explained in two dimensions. The first principal component (PC1) measured volume (i.e., number and density of pigs regardless of the cluster), while the second component (PC2) measured specificity (i.e., concentration either in clusters 1 and 2 or in clusters 4 and 5). The municipalities could be reasonably well characterized using only two quantitative variables, such as the densities of pigs in clusters 1–2 and 4–5, or equivalently, principal components one and two. The different colors in Figure 4C identified municipalities with similar profiles in terms of cluster distribution. The visible contrast between different aggregations of municipalities suggests a neighborhood effect or the use of similar practices depending on the characteristics of the area, such as valleys as opposed to mountains. This effect is particularly relevant in Haute-Gravone and in the western part of Castagniccia where clusters 4 and 5 were predominant, while in the central part of Castagniccia, profiles of municipalities were primarily composed of clusters 1 and 2.

3.3.2 The spatial distribution of the IPI based on pig farming practices

In general, direct and indirect IPI co-evolved within municipalities as shown in Figure 5. The spatial projection (logarithmic scale) of these indexes per municipality made it possible to qualify them according to their IPI and to distinguish hotspots of potential interaction between domestic pigs and wild boars.

These hotspots mainly concern municipalities or groups of municipalities with both a high density of farms and number of pigs raised in them. A major hotspot was located in the north central part of Castagniccia, which also showed a more variable IPI than other more homogeneous microregions such as Haute-Gravone.

3.3.3 Heterogeneous farm profiles despite similar IPI

In our results IPI seemed to correlate roughly with pig density. However, farming practices, through cluster attribution, could have had a significant influence in IPI values. For example, the municipality of Lano encompassed 315 pigs raised on a cluster 4 farm and although the density of pigs was high (61.3 pigs/km^2), the IPI remained relatively low (9.04).

Figure 5 showed that interactions varied from one municipality to another. In this case, IPI alone was not sufficient to qualify the interaction as some municipalities had similar IPI values, but different configuration in terms of clusters and thus, of practices (Supplementary material S6). Thus, the map shown in Figure 5 allowed the identification of hotspots of high interaction risk in pig farms despite the municipality cluster distribution profile concerned provided a better understanding of the parameters that could influence interactions.

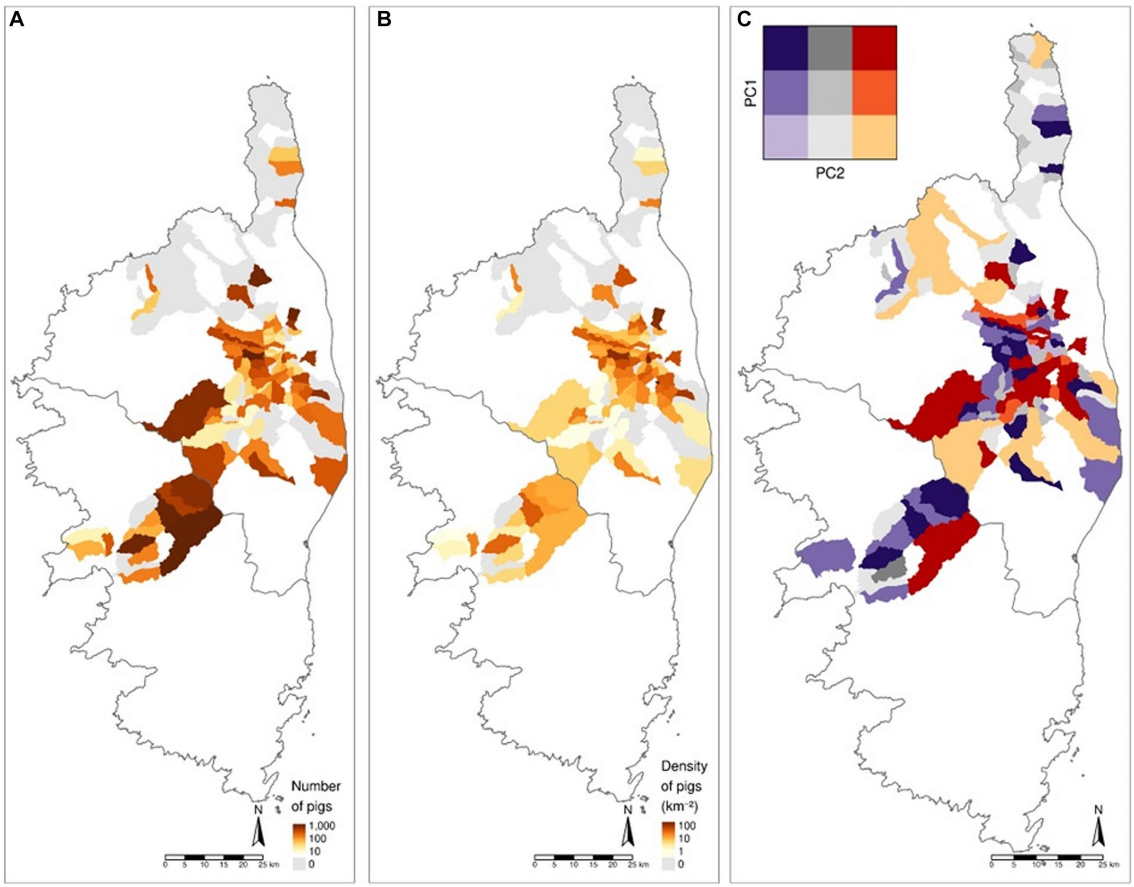

FIGURE 4
Maps of the number **(A)** and density **(B)** of pigs per municipality investigated. There are higher concentrations of pigs in the Haute Gravone and Castagniccia micro-regions. **(C)** Map of profiles of municipalities according to the principal components 1 and 2. Purple shades show a predominance of cluster 4 or 5 farms, while red shades show a predominance of cluster 1 or 2 farms.

4 Discussion

4.1 Outlining the diversity of farmers' practices to assess the interaction

Research to understand wildlife-livestock interfaces has gained increasing attention in the last decades (54). Such interfaces represent complex and dynamic socio-ecological systems influenced by several components including pathogens, hosts and human behavior. Considering that the environment and land use can influence human activities and that pig farming practices can have a major impact on wild boar-domestic pig interactions, our work proposes a territorial large-scale approach to provide an index of the risk of interactions based on predominant pig farming practices. The advantage of our approach is that it allows the development of different options for the management of infectious interactions from a regional policy perspective (identification of hotspots), as well as from a farming system perspective (major drivers of interactions in pig farming practices). Finally, the spatialization of our results required making choices at the administrative level (municipality scale) that can be useful for decision making in the management of shared infectious diseases affecting the pig industry or public health.

As based on previous literature (29, 41), a farming practice is not merely a simple technical choice disconnected from the farmer's overall logic. A specific practice is often linked to the implementation of other practices used to pursue the same goal. For example, in our study, farmers who wanted to avoid wild boar intrusions in their farm could choose between improving biosecurity by building a fence to, spaying all young sows not intended for reproduction, or a combination of both methods. The selected clusters managed to capture this diversity of practices targeting the same goal rather than a series of disconnected practices. However, in our analysis, the division of practices in clusters shaped by key practices could have hidden the overall logic of farmers' choices. Farmers' logic is better considered using the "systems of practice" concept highlighted by some pioneering work on rural sociology from last century (55). More recent publications suggested that the "systems of practice" approach allows a better understanding of farms complexity in a region as it connects the object of the study (here biosecurity) to other dimensions of farming systems and to farmers' overall logics (56). Hence in our study, farmers identified seasonal feed resources and reproduction as major drivers toward which their practices should be targeted to manage individual risk. One possible explanation is that in autumn, the availability of abundant resources such as chestnuts and acorns, coincides with the rutting period of adult wild boars, and the period

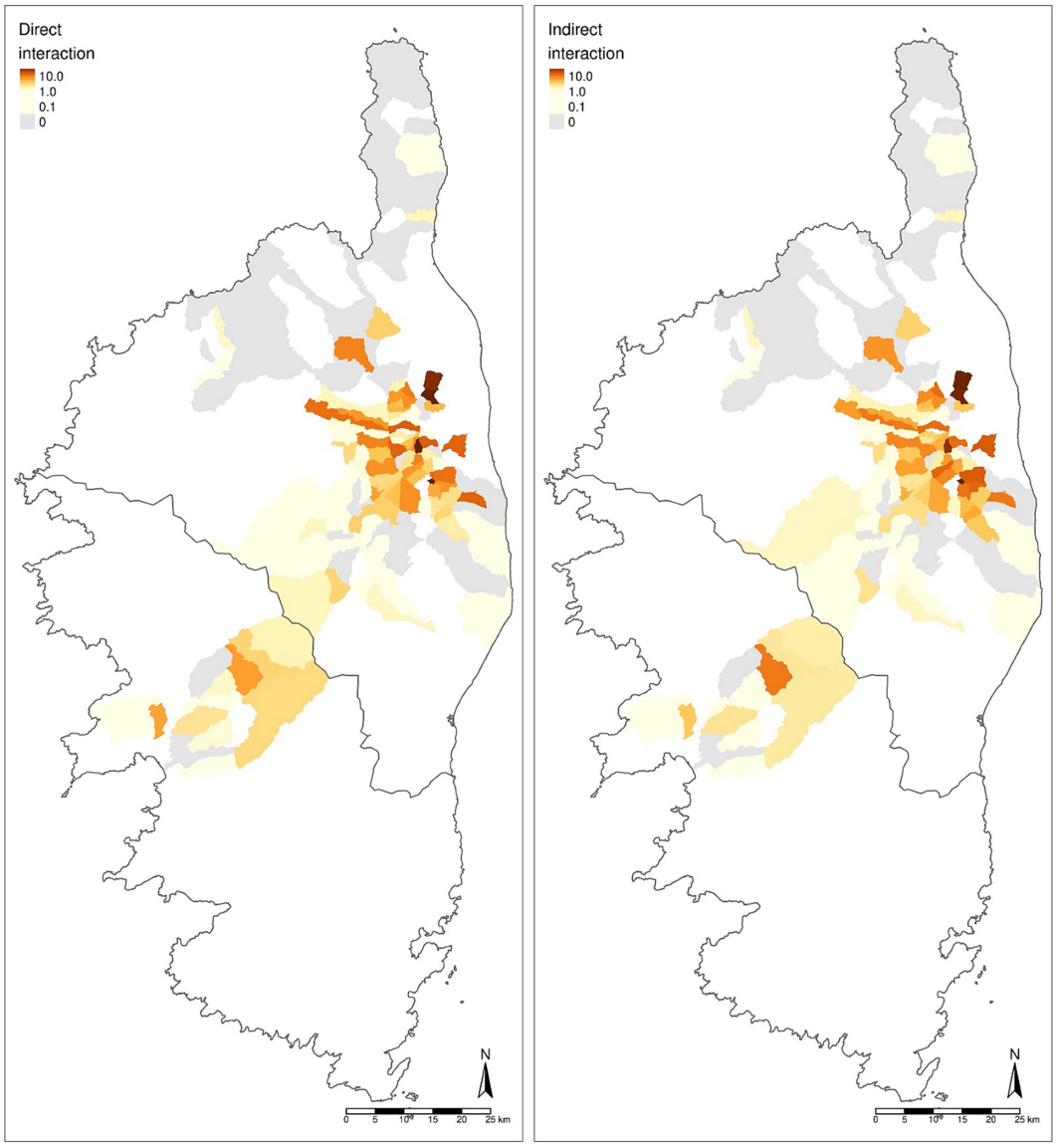

FIGURE 5
Map of IPI based on potential direct and indirect interactions as estimated by the custom weighted averages of cluster densities.

of oestrus period sows. Autumn thus represents the most suitable moment of the year for the occurrence of direct interaction. This may explain the importance of reproduction practices underlined by pig farmers in contrast to observations reported in drier Mediterranean areas, where sources of water in spring and summer appear as predominant drivers of interaction (18). An originality of our approach is that the quantification of our proxy of interaction (IPI) is not based on biosecurity measures and standards as in other studies, but rather on the farmers' perception of the local drivers and available methods to address the problem. In this case, spaying sows non-targeted for reproduction, despite being discouraged by animal health professionals and questionable in terms of animal welfare, is frequently practiced in Corsica and perceived as an important factor for mitigating sexually driven interactions with wild boars (16, 17, 25).

Several authors have underlined the advantage of using clustering to process local knowledge collected by experts (57, 58) as well as organizing the information in systems based on their statements and/or opposing perceptions (59). Cluster analysis is always a representation of reality in response to a specific research question (in this case pig farming practices). Other attempts to analyze and classify pig farming in Corsica produced different representations of the same reality based on different zootechnical, sanitary or socio-economic perspectives (16, 60, 61), the typology of Relun et al. being the closest to our results. Comparable pig farming adaptations can be found in other Mediterranean islands or regions (61) with in some cases, an accepted level of risk, as illustrated by regular outbreaks of African swine fever reported for decades in neighboring Sardinia (5, 22, 62). Clusters 2 and 3 can be distinguished from cluster 1 by their implementation of more restrictive practices in terms of domestic pig-related behavior and its interactions with wild boar. In the opinion of the local experts, cluster classification could be influenced by the priority given by each farmer to avoid direct (for cluster 2) or indirect (for cluster 3) interactions. These practices were common, and often associated with the occupation of agriculturally abandoned areas.

Finally, the remaining typologies included more standard clusters in which, the spatial behavior of animals was moderately (cluster 4) or strongly (cluster 5) constrained. Cluster 4 allowed complete control of the herd, avoiding conflicts between neighbors, making better use of feed resources, while being compatible with origin certification (PDO). It also permitted the combination of biosecurity measures (control of contact with wild boars) with a semi-extensive free ranging system.

4.2 Methodological contributions and limitations

The choice of participatory methods allowed us to deal with two main challenges. On one hand, the information gathered compensated for the lack of an official exhaustive and updated list of farmers, a situation which is quite common in some regions or countries and represents a constraint. On the other hand, the contribution of key informants compensated for other difficulties in the data collection process such as access to isolated farms, the absence of a reliable and complete list of available farmers, missing data or the potential lack of farmers willing to participate. Although it is possibly not exhaustive, the list of farmers we were able to compile through the implementation of our methods was considered by several extension agents and health services to be the most complete list of farmers obtained to date. Moreover, our approach made it possible to cross-reference the information gathered from different informants in the same area. In addition to their own zootechnical know-how, key informant farmers provided information about their immediate neighborhood as represented by their informal network of local stakeholders. Despite there was a risk of subjectivity in the information provided by key informants this bias was compensated by triangulating information from different farms in the same region. Moreover, the way we collected our data shed some light on the problems experienced by local actors in their respective situations (63). The role of local experts as additional providers of local knowledge, represents another innovative aspect of our approach and more generally, demonstrates the relevance of applying participatory methods in such contexts (64). Such an approach fell somewhere between two standardized methods such as the expert elicitation and stakeholder opinion survey. As an expert elicitation process, their choice could be considered subjective because it was mainly based on the local social recognition based on their pig farming experience. As social stakeholder survey, the sample size ($n = 9$), was below the minimum required threshold. Nevertheless, despite these recognized methodology flaws, the combined adaptation of these two approaches succeeded in collecting relevant information for the purpose of the study.

A strong assumption made in our approach was that the probability of interaction was mainly driven by pig farming practices rather than by the distribution and abundance of wild boar populations. The local abundance of wild boar populations is likely to influence the occurrence and frequency of wild boar incursions into low biosecurity farms and thus, the occurrence of interactions with domestic pigs (9). However, since information on wild boar abundance at the scale of the island of Corsica is not available, our spatial representation of the IPIs only considered the pig farming perspective and not the influence of wild boar population abundance. Therefore, the resulting map is provisional first assessment to this topic and needs to be completed with information on wild boar estimated densities and compared with other field data such as genetic introgression of wild boar populations (65) or the distribution of shared swine pathogens (8, 11, 15). Spatializing our index in order to successfully link the information obtained through our participative approach with the development of strategies to manage disease risks in our study sites is a major challenge and goal in the Corsican context that relatively few studies have addressed in the literature to date (18, 44). One of the first difficulties encountered when addressing this challenge was the choice of an adequate spatial scale to map the risk of interaction. By choosing the municipality scale, we sacrificed precision and representativeness. However, neighborhood effects between farms represented an unavoidable bias. In addition, in the case of small-sized municipalities, farming estates could exceed the boundaries of a single municipality and often, the pigs were not equally distributed but rather concentrated in parts of the municipality which were more resource-abundant. Another source of bias occurred when the surface or perimeter of a farm was located between two municipalities or in a different administrative division from the one it was registered.

The spatial distribution of clusters contributed to identify contrasted micro-regions that could be informative from the risk management perspective. Indeed, calculating a proxy of wild-domestic pig interaction in pig farming areas such as IPI enables the identification of regional "hotspots," and the municipalities profile in terms of cluster distribution provides key information to target pig farming development and biosecurity efforts. Last but not least, our equation to calculate the IPI overemphasized pig density in detriment of farmers practices. This limitation needs to be addressed in future work, particularly when considering municipalities that host a wide diversity of farming systems.

5 Conclusion

Our work proposes an original methodology to collect zootechnical information and classify pig farms in order to spatially represent and compare a proxy of interaction with wild boars among 8 pig farming micro-regions from the Corsican territory. Our approach was particularly successful to identify some micro-regions particularly prone to extensive pig farming, as potential hot spots of interaction with wild boars. The method is based in the combination of approaches from different disciplinary fields including social sciences, epidemiology, animal husbandry, geography and ecology. This preliminary information could help to identify priority areas for the implementation of regionally-adapted management strategies of porcine disease shared with wild or feral pigs. Our approach is particularly applicable regions prone to extensive livestock farming where information on farming practices is lacking. Our method has the potential to be improved and implemented at a larger territorial scale not only in Corsica but also in other regions confronted with similar types of extensive animal production, exposed to interactions with wildlife and challenges of disease transmission risks.

Data availability statement

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

Author contributions

FJ, FCh, FCa, ML, and LD contributed to conception of the study. LD designed method, implemented data collection, organized the database and performed the statistical analysis, and wrote the first draft of the manuscript. FM contributed to the statistical analysis. FJ and FCh contributed to the supervision of the study. FJ, FCa, BT, and FCh completed and improved different sections of the manuscript. All authors contributed to the manuscript revision, read, and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2024.1253060/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Map of the micro-regions of Corsica.

SUPPLEMENTARY FIGURE 2
Factor maps of the 5 clusters from the MCA.

SUPPLEMENTARY FIGURE 3
Comparison of profiles of villages with the same IPI values.

References

1. Dohna HZ, Peck DE, Johnson BK, Reeves A, Schumaker BA. Wildlife–livestock interactions in a western rangeland setting: quantifying disease-relevant contacts. *Prev Vet Med*. (2014) 113:447–56. doi: 10.1016/j.prevetmed.2013.12.004

2. Jori F, Hernandez-Jover M, Magouras I, Dürr S, Brookes VJ. Wildlife–livestock interactions in animal production systems: what are the biosecurity and health implications? *Anim Front*. (2021) 11:8–19. doi: 10.1093/af/vfab045

3. Triguero-Ocaña R, Vicente J, Lavelle M, Acevedo P. Collecting data to assess the interactions between livestock and wildlife In: J Vicente, KC Vercauteren and C Gortázar, editors. *Diseases at the wildlife - livestock interface, research and perspectives in a changing world*. Switzerland: Springer Nature. (2021). p. 307–38.

4. Bergmann H, Schulz K, Conraths FJ, Sauter-Louis CM. A review of environmental risk factors for African swine fever in European wild boar. *Animals*. (2021) 11:2692. doi: 10.3390/ani11092692

5. Cappai S, Rolesu S, Coccollone A, Laddomada A, Loi F. Evaluation of biological and socio-economic factors related to persistence of African swine fever in Sardinia. *Preven Vet Med*. (2018) 152:1–11. doi: 10.1016/j.prevetmed.2018.01.004

6. Dixon LK, Stahl K, Jori F, Vial L, Pfeiffer DU. African swine fever epidemiology and control. *Ann Rev Anim Biosci*. (2020) 8:221–46. doi: 10.1146/annurev-animal-021419-083741

7. Scollo A, Valentini F, Franceschini G, Rusinà A, Calò S, Cappa V, et al. Semi-quantitative risk assessment of African swine fever virus introduction in pig farms. *Front Vet Sci*. (2023) 10:10. doi: 10.3389/fvets.2023.1017001

8. Jori F, Laval M, Maestrini O, Casabianca F, Charrier F, Pavio N. Assessment of domestic pigs, wild boars and feral hybrid pigs as reservoirs of hepatitis E virus in Corsica, France. *Viruses*. (2016) 8:8. doi: 10.3390/v8080236

9. Jori F, Petit G, Civil N, Decors A, Charrier F, Casabianca F, et al. A questionnaire survey for the assessment of wild-domestic pig interactions in a context oedema disease outbreaks among wild boars (*Sus scrofa*) in South-Eastern France. *Transboundary Emerg Dis*. (2022) 69:4009–15. doi: 10.1111/tbed.14704

10. Meng XJ, Lindsay DS, Sriranganathan N. Wild boars as sources for infectious diseases in livestock and humans. *Philos Trans R Soc Lond B Ser Biol Sci*. (2009) 364:2697–707. doi: 10.1098/rstb.2009.0086

11. Richomme C. *Epidemiology of zoonosis in wild boar (Sus scrofa) from an insular Mediterranean area, Corsica*. [PhD thesis]. Lyon, France: ENVL (2009). p. 320.

12. Wu N, Abril C, Thomann A, Grosclaude E, Doherr MG, Boujon P, et al. Risk factors for contacts between wild boar and outdoor pigs in Switzerland and investigations on potential *Brucella suis* spill-over. *BMC Vet Res*. (2012) 8:116. doi: 10.1186/1746-6148-8-116

13. Hars J, Garin-Bastuji B, Richomme C, Payne A, Rossi S. De l'éradication à la réapparition des maladies infectieuses animales. Les dangers de la faune sauvage: contexte et outils de gestion. *Epidémiologie et Santé Animale*. (2013) 64:57–69.

14. Maxime D, Pol F, Dufour B, Rose N, Fablet C. Pig farming in alternative systems: strengths and challenges in terms of animal welfare, biosecurity, animal health and pork safety. *Agriculture*. (2020) 20:261.

15. Charrier F, Rossi S, Jori F, Maestrini O, Richomme C, Casabianca F, et al. Aujeszky's disease and hepatitis E viruses transmission between domestic pigs and wild boars in Corsica: evaluating the importance of wild/domestic interactions and the efficacy of management measures. *Front Vet Sci*. (2018) 5:1. doi: 10.3389/fvets.2018.00001

16. Relun A, Charrier F, Trabucco B, Maestrini O, Molia S, Chavernac D, et al. Multivariate analysis of traditional pig management practices and their potential impact on the spread of infectious diseases in Corsica. *Prev Vet Med*. (2015) 121:246–56. doi: 10.1016/j.prevetmed.2015.07.004

17. Trabucco B, Charrier F, Jori F, Maestrini O, Etter E, Molia S, et al. Stakeholder's practices and representations of contacts between domestic and wild pigs: a new approach for disease risk assessment? *Acta Agric Slovenica*. (2013) 4:119–22.

18. Jiménez-Ruiz S, Laguna E, Vicente J, García-Bocanegra I, Martínez-Guijosa J, Cano-Terriza D, et al. Characterization and management of interaction risks between livestock and wild ungulates on outdoor pig farms in Spain. *Porcine Health Manag*. (2022) 8:2. doi: 10.1186/s40813-021-00246-7

19. Laguna E, Barasona JA, Carpio AJ, Vicente J, Acevedo P. Permeability of artificial barriers (fences) for wild boar (*Sus scrofa*) in Mediterranean mixed landscapes. *Pest Manag Sci*. (2022) 78:2277–86. doi: 10.1002/ps.6853

20. Martínez-Guijosa J, Lima-Barbero JF, Acevedo P, Cano-Terriza D, Jiménez-Ruiz S, Barasona J, et al. Description and implementation of an on-farm wildlife risk mitigation protocol at the wildlife-livestock interface: tuberculosis in Mediterranean environments. *Prev Vet Med*. (2021) 191:105346. doi: 10.1016/j.prevetmed.2021.105346

21. Triguero-Ocaña R, Laguna E, Jiménez-Ruiz S, Fernández-López J, García-Bocanegra I, Barasona J, et al. The wildlife-livestock interface on extensive free-ranging pig farms in Central Spain during the "montanera" period. *Transbound Emerg Dis*. (2021) 68:2066–78. doi: 10.1111/tbed.13854

22. Jurado C, Fernández-Carrión E, Mur L, Rolesu S, Laddomada A, Sánchez-Vizcaíno JM. Why is African swine fever still present in Sardinia? *Transbound Emerg Dis*. (2017) 65:557–66. doi: 10.1111/tbed.12740

23. Loi F, Laddomada A, Coccollone A, Marrocu E, Piseddu T, Masala G, et al. Socio-economic factors as indicators for various animal diseases in Sardinia. *PLoS One*. (2019) 14:e0217367. doi: 10.1371/journal.pone.0217367

24. Vicente J, Ruiz-Fons F, Vidal D, Höfle U, Acevedo P, Villanúa D, et al. Serosurvey of Aujeszky's disease virus infection in European wild boar in Spain. *Vet Rec*. (2005) 156:408–12. doi: 10.1136/vr.156.13.408

25. Jori F, Relun A, Trabucco B, Charrier F, Maestrini O, Chavernac D, et al. Questionnaire-based assessment of wild boar/domestic pig interactions and implications for disease risk management in Corsica. *Front Vet Sci*. (2017) 4:198. doi: 10.3389/fvets.2017.00198

26. Casabianca F, Picard P, Sapin JM, Gautier JF, Vallée M. *Contribution à L'épidémiologie des Maladies Virales en Elevage Porcin Extensif. Application à la Lutte Contre la Maladie D'Aujeszky en Région Corse*. Paris: Journées de Recherche Porcine (1989).

27. Richomme C, Lacour SA, Ducrot C, Gilot-Fromont E, Casabianca F, Maestrini O, et al. Epidemiological survey of trichinellosis in wild boar (*Sus scrofa*) and fox (*Vulpes vulpes*) in a French insular region, Corsica. *Vet Parasitol*. (2010) 172:150–4. doi: 10.1016/j.vetpar.2010.04.026

28. Richomme C, Afonso E, Tolon V, Ducrot C, Halos L, Alliot A, et al. Seroprevalence and factors associated with toxoplasma gondii infection in wild boar (*Sus scrofa*) in a Mediterranean island. *Epidemiol Infect*. (2010) 138:1257–66. doi: 10.1017/S0950268810000117

29. Darré P, Mathieu A, Lasseur J. *Le sens des pratiques: Conceptions d'agriculteurs et modèles d'agronomes*. Versailles, France: INRA edition. (2006). p. 320.

30. Tremblay M. The key informant technique: a nonethnographic application. *Am Anthropol*. (1957) 59:688–701. doi: 10.1525/aa.1957.59.4.02a00100

31. Mariner J, Hendrickx S, Pfeiffer D, Costard S, Knopf L, Okuthe S, et al. Integration of participatory approaches into surveillance systems. *Revue Scientifique et Technique*. (2011) 30:653–9. doi: 10.20506/rst.30.3.2065

32. Mariner JC, Paskin R. *Manual on participatory epidemiology: methods for the collection of action-oriented epidemiological intelligence, UN food and agriculture organisation*. Rome: Italy (2000). 87 p.

33. Fabiani J. Sociologie de la Corse. *Editions La Découverte, Paris*. (2018):128. doi: 10.3917/dec.fabia.2018.01

34. PDRC. *Programme de développement rural de la corse 2007–2013. Version 3* Collectivité Territoriale de Corse (2009).

35. Agreste. Atlas Agricole Corse. résultats durecensement Agricole. (2010). Available at: https://www.corse-du-sud.gouv.fr/IMG/pdf/Atlas_Corse_2015_premiere_partie.pdf.

36. De Sainte Marie C, Casabianca F, Poggi M. *Les activités d'élevage porcin en Corse. Des difficultés de la transition entre économie souterraine et économie formelle. Corsica Isola problema tra Europa e Mediterraneo*. Salerno: Salerno University, Italy. (1994). p. 263–286.

37. INAO. Décret n° 2012–444 du 2 avril 2012 relatif à l'appellation d'origine contrôlée « Coppa de Corse » ou « Coppa de Corse – Coppa di Corsica. (2012). Available at: https://www.inao.gouv.fr/show_texte/3133.

38. Agreste. Chiffres clés de l'agriculture corse. Bilan de campagne 2017 2018. (2018). Available at: https://draaf.corse.agriculture.gouv.fr/chiffres-cles-de-l-agriculture-corse-edition-2018-bilan-de-campagne-2017-a1062.html.

39. ONCFS. Tableaux de Chasse Ongulés Sauvages. saison 2018–2019. Suplément Faune Sauvage. (2019). Available at: http://www.oncfs.gouv.fr/IMG/file/mammiferes/ongules/tableau/FS296_tableaux_chasse_ongules.pdf.

40. Franceschi P. Quelques caractéristiques de la population de sangliers de Corse et analyses de ses échanges avec le porc domestique. *Bulletin Mensuel de l'O.N.C.* (1984). 85:25–35.

41. Commandeur M, Grossetti Q. Caractériser les « styles d'élevage » dans l'espace d'information de l'éleveur porcin. *Journées de la Recherche Porcine*. (2005) 37:397–406.

42. Costard S, Porphyre V, Messad S, Rakotondrahanta S, Vidon H, Roger F, et al. Multivariate analysis of management and biosecurity practices in smallholder pig farms in Madagascar. *Prev Vet Med*. (2009) 92:199–209. doi: 10.1016/j.prevetmed.2009.08.010

43. Beltrán-Alcrudo D, Kukielka EA, de Groot N, Dietze K, Sokhadze M, Martínez-López B. Descriptive and multivariate analysis of the pig sector in Georgia and its implications for disease transmission. *PLoS One*. (2018) 13:e0202800. doi: 10.1371/journal.pone.0202800

44. O'Hara KC, Beltrán-Alcrudo D, Hovari M, Tabakovski B, Martínez-López B. Descriptive and multivariate analysis of the pig sector in North Macedonia and its implications for African swine fever transmission. *Front Vet Sci*. (2021) 8:733157. doi: 10.3389/fvets.2021.733157

45. Marshall M. The key informant technique. *Fam Pract*. (1996) 13:92–7. doi: 10.1093/fampra/13.1.92

46. Martin C, Pastoret PP, Brochier B, Humblet MF, Saegerman C. A survey of the transmission of infectious diseases/infections between wild and domestic ungulates in Europe. *Vet Res*. (2011) 42:70. doi: 10.1186/1297-9716-42-70

47. Lê S, Josse J, Husson F. FactoMineR: an R package for multivariate analysis. *J Stat Softw*. (2008) 25:1–18. doi: 10.18637/jss.v025.i01

48. Husson F, Josse J, Pagès J. *Principal component methods hierarchical clustering partitional clustering: why would we need to choose for visualizing data?* Technical Report – Agrocampus (2010) Contract No. 17.

49. Pretty JN. Participatory learning for sustainable agriculture. *World Dev*. (1995) 23:1247–63. doi: 10.1016/0305-750X(95)00046-F

50. Catley A, Alders RG, Wood JLN. Participatory epidemiology: approaches, methods, experiences. *Vet J*. (2012) 191:151–60. doi: 10.1016/j.tvjl.2011.03.010

51. Wong LP. Focus group discussion: a tool for health and medical research. *Singapore Med J*. (2008) 49:3.

52. STATISTICA. Techniques exploratoires multivariées: arbres de classification. (2019). Available at: https://www.statsoft.fr/concepts-statistiques/arbres-de-classification/arbres-de-classification.php.

53. Vidhya A. A Complete Tutorial on Tree Based Modeling from Scratch (in R & Python). (2016). Available at: https://www.analyticsvidhya.com/blog/2016/04/complete-tutorial-tree-based-modeling-scratch-in-python/

54. Wiethoelter AK, Beltrán-Alcrudo D, Kock R, Mor SM. Global trends in infectious diseases at the wildlife–livestock interface. *Proc Natl Acad Sci*. (2015) 112:9662–7. doi: 10.1073/pnas.1422741112

55. Cristofini B, Deffontaines J-P, Raichon C, de Verneuil B. Pratiques d'élevage en Castagniccia: Exploration d'un milieu naturel et social en Corse. *Études Rurales*. (1978) 71:89–109. doi: 10.3406/rural.1978.2423

56. Vanwindekens F, Baret P, Stilmant D. A new approach for comparing and categorizing farmers' systems of practice based on cognitive mapping and graph theory indicators. *Ecol Model*. (2014) 274:1–11. doi: 10.1016/j.ecolmodel.2013.11.026

57. Perrot C. Typologie d'exploitations construite par agrégation autour de pôles définis à dire d'experts: Proposition méthodologique et premiers résultats obtenus en Haute-Marne. *INRAE Prod Anim*. (1990) 3:51–66. doi: 10.20870/productions-animales.1990.3.1.4360

58. Landais E. Modelling farm diversity: new approaches to typology building in France. *Agric Syst*. (1998) 58:505–27. doi: 10.1016/S0308-521X(98)00065-1

59. Denzin N. *The research act: a theoretical introduction to sociological methods*. New York: Transaction Publishers (2017). 368 p.

60. DRAAF. Compte rendu du Conseil Régional d'Orientation de la Politique Sanitaire Animale et Végétale (CROPSAV) – 2 mai. (2017). Available at: https://draaf.corse.agriculture.gouv.fr/IMG/pdf/20170502_CR_CROPSAV_animal__cle09ef47.pdf

61. Molenat M, Casabianca F. *Contribution a la maîtrise de l'élevage porcin extensif en*, vol. *32*. Corse: INRAE Jouy-en-Josas (1979).

62. Mur L, Atzeni M, Martínez-López B, Feliziani F, Rolesu S, Sanchez-Vizcaino JM. Thirty-five-year presence of African swine fever in Sardinia: history, evolution and risk factors for disease maintenance. *Transbound Emerg Dis*. (2014) 63:e165–77. doi: 10.1111/tbed.12264

63. Girard N, Duru M, Hazard L, Magda D. Categorising farming practices to design sustainable land-use management in mountain areas. *Agron Sustain Dev*. (2008) 28:333–43. doi: 10.1051/agro:2007046

64. Calba C, Antoine-Moussiaux N, Charrier F, Hendrikx P, Saegerman C, Peyre M, et al. Applying participatory approaches in the evaluation of surveillance systems: a pilot study on African swine fever surveillance in Corsica. *Preven Vet Med*. (2015) 122:389–98. doi: 10.1016/j.prevetmed.2015.10.001

65. Schleimer A, Richart L, Drygala F, Casabianca F, Maestrini O, Weigand H, et al. Introgressive hybridisation between domestic pigs (*Sus scrofa domesticus*) and endemic Corsican wild boars (*S. s. meridionalis*): effects of human-mediated interventions. *Heredity*. (2022) 128:279–90. doi: 10.1038/s41437-022-00517-1

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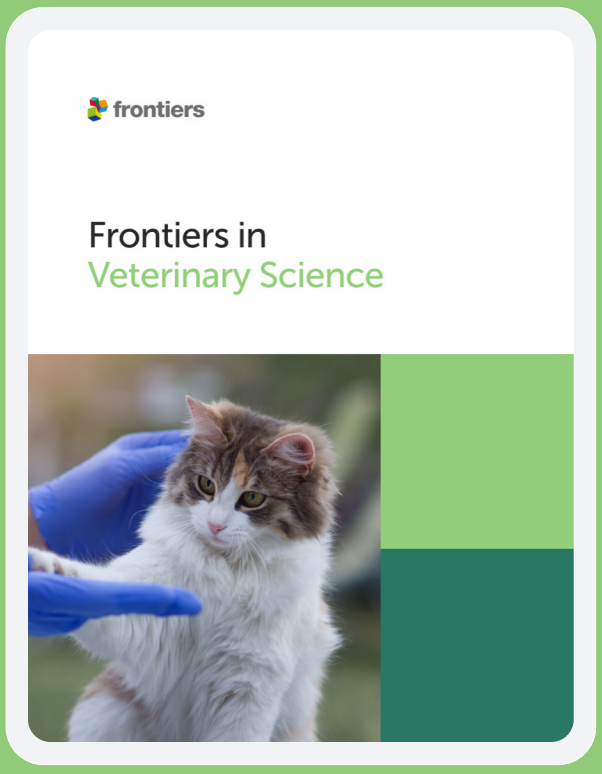

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