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Interdisciplinary approaches in veterinary sciences after COVID-19

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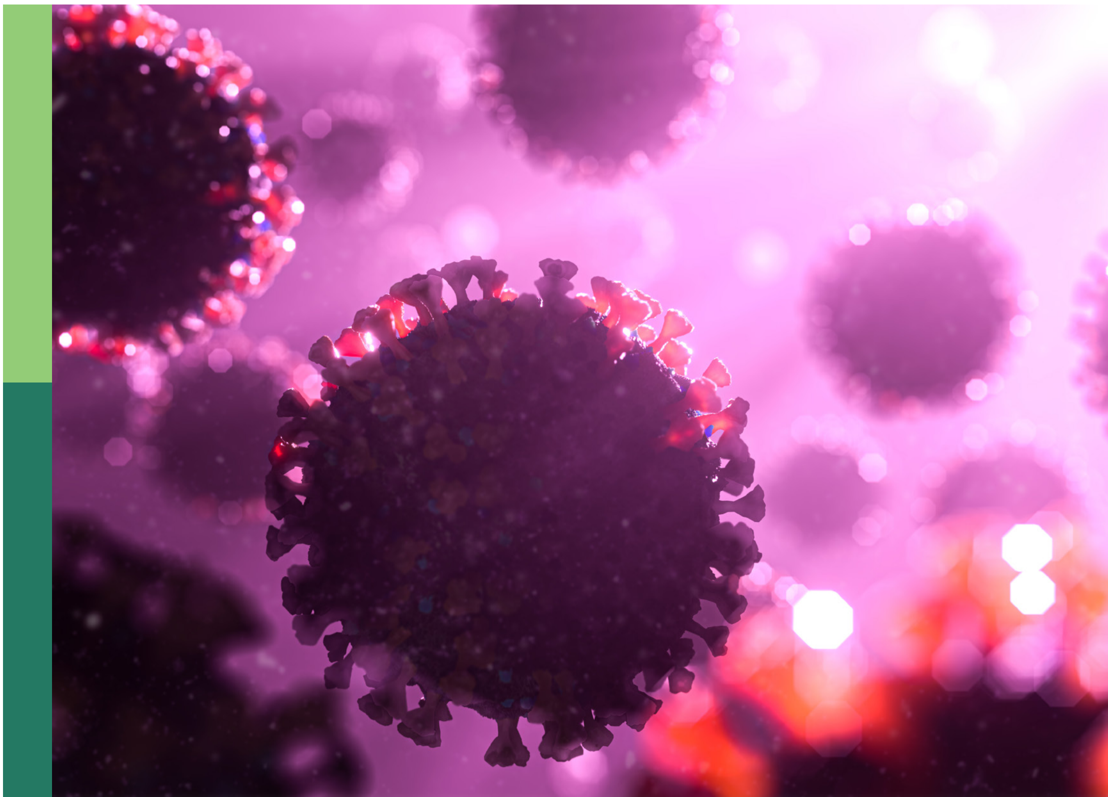

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Interdisciplinary approaches in veterinary sciences after COVID-19

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Table of contents

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Editorial: Interdisciplinary approaches in veterinary sciences after COVID-19

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KEYWORDS

infectious diseases, new therapies, zoonosis, medical geography, decision-making

Editorial on the Research Topic
Interdisciplinary approaches in veterinary sciences after COVID-19

At the end of December, 2023, the *Knowledge of Science*^{TM} reported at least 657,000 publications that refer to COVID 19. It has been claimed that never in the history of science was so much published so fast on a single topic (1). Such an unprecedented development has generated numerous new challenges that can be synthesized with one question: how to summarize a vast and diverse body of published science into a publication that gives a synoptic view?

To answer that question, two different research styles should be reconciled, which involve specialized (uni-disciplinary) and inter-/transdisciplinary knowledge, respectively (2). Other problems affecting knowledge integration include: (a) reporting specialized research that is not necessarily accessible to readers unfamiliar with the specific field; (b) the fact that any selection of specialized studies is bound to be long and, therefore, difficult to be read; and (c) the local relevance of topics, which may apply to one place but not necessarily everywhere. While hundreds of systematic reviews on the COVID-19 related literature have been published (3) and many of them have been conducted within the context of veterinary medicine (4), the prompt applicability of such research, within Veterinary Medicine, remains unexplored. Given such challenges, this Research Topic Issue was designed to (i) generate a concise list of studies, (ii) follow a logic and structure that integrated two or more topics, disciplines, professions and/or technologies; and (iii) provide global perspectives.

Accordingly, ten studies are reported here, which involve authors and reviewers from more than twenty countries. These studies attempt to provide information potentially complementary and applicable. This Research Topic covers four focus areas, which refer to: zoonoses, new tests and therapies, geography and epidemiology, and social-biological sciences.

Zoonoses

Focusing on potential reservoirs of infectious (zoonotic or epizootic) diseases, three studies conducted in the United States, the Netherlands and Georgia investigate domestic carnivores (Hecht et al.; Fischer et al.)

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and ruminants (Rivas et al.) with or without human data. The overall purpose of these studies is to expand the target of veterinary practices to address not only diseased individuals and populations but interactions that may include other species.

New tests and therapies

To better detect and/or treat infectious diseases, new molecular tests or therapies (including acoustic pulse technology) are described by Indian and Israeli researchers (Das et al.; Blum et al.). The focus of these studies is to illustrate expanded or alternative approaches that may detect infectious diseases earlier or treat such diseases more effectively.

Medical geography

Geography and epidemiology are integrated in two studies that include authors from Mexico, Chile, Spain, United States and Uruguay (Hoogesteyn et al.; Picasso-Risso et al.). These studies shed light on how the properties of geographical data may influence disease dissemination.

The educational/policy-making continuum

The abundance and complexity of novel research may require new educational programs that explicitly include economic (cost/benefit-oriented) decision-making. That is conveyed from the perspective of German, Chinese, and Kenyan viewpoints (Becker et al.), economics (Yu et al.), and policy-making (Sitawa et al.).

The previous description of these studies is only partial because these works refer to many fields, theories or applications. For example, Picasso-Risso et al. use geographical data within the context of Network Theory. Addressing a topic of global relevance in policy-making (antimicrobial resistance), the possible use of therapies that do not utilize antibiotics is explored by Blum et al.. An example of an anticipatory study of wildlife species that may host potential vectors of emergent diseases (such as malaria induced by parasites previously found in non-human species) is offered by Das et al.. Integrating economics with control of zoonoses, Rivas et al. compare the cost-effectiveness of two approaches that utilize geo-referenced data. The fact that the prompt dissemination of these topics and technologies may require new educational configurations–including non-presential formats–is explored and evaluated by Becker et al..

Together, it is expected that this material may provide a guide to consider when developing new educational and research programs. The editors thank the participation of all authors and reviewers.

Author contributions

AR: Conceptualization, Writing – original draft, Writing – review & editing. SS: Conceptualization, Writing – original draft, Writing – review & editing.

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A novel multiplex qPCR assay for clinical diagnosis of non-human malaria parasites-*Plasmodium knowlesi* and *Plasmodium cynomolgi*

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Introduction: The imminent risk of zoonoses of non-human malaria parasites is not far from reality in India, as has been observed in the case of Plasmodium knowlesi (Pk), and so is possible with *P. cynomolgi* (Pc), already reported from South East Asian countries. Therefore, a novel multiplex qPCR assay was developed and evaluated for detection of non-human malaria parasites- Pk and Pc in populations at risk.

Methods: The qPCR primers were designed in-house with fluorescence labeled probes (HEX for Pk and FAM for Pc). DNA samples of Pk and Pc were used as templates and further the qPCR assay was evaluated in 250 symptomatic and asymptomatic suspected human blood samples from malaria endemic areas of North Eastern states of India.

Results: The qPCR assay successfully amplified the target 18S rRNA gene segment from Pk and Pc and was highly specific for Pk and Pc parasites only, as no cross reactivity was observed with *P. falciparum* (Pf), *P. vivax* (Pv), *P. malariae* (Pm), and *P. ovale* (Po). Standard curves were generated to estimate the limit of detection (LOD) of Pk and Pc parasites DNA (0.00275 & 0.075 ng/μl, respectively). Due to COVID-19 pandemic situation during 2020–21, the sample accessibility was difficult, however, we managed to collect 250 samples. The samples were tested for Pf and Pv using conventional PCR- 14 Pf and 11 Pv infections were observed, but no Pk and Pc infections were detected. For Pk infections, previously reported conventional PCR was also performed, but no Pk infection was detected.

Discussion: The multiplex qPCR assay was observed to be robust, quick, cost-effective and highly sensitive as compared to the currently available conventional PCR methods. Further validation of the multiplex qPCR assay in field setting is desirable, especially from the high-risk populations. We anticipate that the multiplex qPCR assay would prove to be a useful tool in mass screening and surveillance programs for detection of non-human malaria parasites toward the control and elimination of malaria from India by 2030.

KEYWORDS

***Plasmodium knowlesi*, *Plasmodium cynomolgi*, diagnostics, multiplex qPCR, non-human malaria**

Introduction

In Indian context, major human malaria parasites are *P. falciparum* (Pf), *P. vivax* (Pv), *P. malariae* (Pm), *P. ovale* (Po) and *P. knowlesi* (Pk) (1–7). Moreover, non-human malaria causing *Plasmodium* species- *P. cynomolgi* (Pc) with established zoonosis in humans has become a cause for concern, at least in the South East Asian countries (8). The risk of expansion of non-human malaria in humans is increasing gradually, majorly due to deforestation, substantial changes in the ecology, host & vector availability and adaptive changes in the parasites (9). It would not be surprising that non-human primate *Plasmodium* parasites- Pk and Pc might be in circulation in the Indian populations, but they are rendered elusive owing to the probable misdiagnosis by routine microscopy and lack of robust diagnostic tools to detect sparsely distributed Pk and Pc infections (10, 11). There are reports of more than 30 species of non-human primate *Plasmodium* spp. and seven of them including Pk, Pc and others- *P. brasilianum, P. eylesi* (Pe*), P. inui* (Pi), *P. schwetzi* (Ps), and *P. simium* had been observed as transmissible to humans (10, 12–18). The major hosts of Pk, Pc, Pi*, P. fieldi* and *P. coatneyi* are the non-human primates *Macaca fascicularis* (long-tailed macaques), *Macaca nemestrina* (the pig-tailed macaque), *Trachypithecus obscuras* (dusky leaf monkey or spectacled langur) and *Presbytis melalophus* (banded leaf monkey) (19, 20). These non-human primate species are prevalent in South East Asia and India as well (21).

The mosquito vectors of human and non-human primate malaria are *Anopheles minimus*, *An. dirus, An. sundaicus, An. sinensis* and *An. maculates* are commonly found in geographically specific regions of India (1, 5, 22–25). Natural infections of Pk and Pc have been previously reported in macaque monkeys and humans from Malaysia (26, 27). North-Eastern states of India are in proximity to such regions where non-human malaria parasites might be in circulation and humans frequently travel on both sides. India has all the suitable vectors and hosts for non-human *Plasmodium* species; the geographical and climatic conditions are also conducive for the proliferation of Pk and Pc (28).

Pc is phenotypically and phylogenetically similar to Pv; thereby making the identification of Pv and Pc quite difficult in blood slides during routine microscopy. Often, routine microscopy of Pk, Pc and Pm can lead to misdiagnosis by the microscopists in primary health centers of the remote areas. In these circumstances, it becomes imperative to accurately estimate and understand the burden and transmission dynamics of non-human *Plasmodium* spp.- especially Pk and Pc in India human populations.

The current study presents in-house development of a rapid, sensitive and species-specific multiplex qPCR assay targeting the 18S rRNA, for detection of Pk and Pc. Multiplexing for multiple *Plasmodium* parasites (Pk and Pc) in single tube would be efficient to save resources during any kind of mass screening programmes and would also save on precious biological samples. The qPCR assay for Pk was also compared to well-known established PCR assays to detect Pk infections in humans (29).

Materials and methods

Design of in-house multiplex qPCR assay

The gene sequences of Pk and Pc 18S rRNA gene were extracted from Reference GenBank accession numbers (Pk-LC483580.1 and Pc-KU708868.1) for design of in-house multiplex qPCR forward and reverse primers and the fluorescence labeled probes (HEX for Pk and FAM for Pc) using online tool (https://biosearchtech.com) (Table 1). The probes were BLAST searched (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and observed to be species-specific for Pk and Pc. The oligonucleotides and probes were commercially synthesized from GCC Biotech (I) Pvt. Ltd., India. The final reaction volume (20 µl) constituted of 4 µl of genomic DNA, 500 nM of each species-specific primers and 400 nM of each probe, 10 µl of 2X Platinum multiplex PCR master mix (Applied Biosystems; Thermo Fisher Scientific, USA). The qPCR conditions were- initial denaturation 95°C for 5 min. followed by 40 cycles of denaturation at-95°C for 15 s. and primer annealing-extension at 53°C for 1 min. The amplifications were performed in BioRad CFX96 Connect Real-Time PCR System, USA.

TABLE 1 List of qPCR primers and respective probes for amplification of Pk and Pc 18S rRNA target regions.

Primers	Sequences (5′-3′)
18S PkF	TGCCGACTAGGCTTTGGATG
18S PkR	GGCACTGAAGGAAGCAATCTAAG
Pk Probe	HEX-CTTTTCTCTCCGGAGA-HEX-BHQ1
18S PcF	GCGGTCGCAAATAATGAAGATC
18S PcR	GGGAACAGAAGGAGCGAGAATA
Pc Probe	FAM-TGCTTTTCACGTCAGTGTTTCCAAGA- FAM-BHQ1

Sensitivity and specificity of qPCR assay

The DNA samples of Pk and Pc parasites were obtained from CSIR-Central Drug Research Institute, Lucknow, India and the yields were observed to be 27 and 7.5 ng/µl, respectively (30). Further, the Pk and Pc genomic DNAs were 10-fold serially diluted four times to estimate the limit of detection (LOD), and each dilution was tested in triplicate. To rule out the cross-reactivity with other species of *Plasmodium* (Pf, Pv, Pm, Po) the assay was validated in 250 blood samples collected from suspected endemic areas of North Eastern States of India. The specificity of Pk was also cross checked using a conventional reported PCR method (29).

Human blood sample collection and DNA isolation

During 2020-21, the COVID-19 pandemic severely hampered the sample collection. However, a total of 250 samples were collected from north eastern state of India from mass surveys to identify symptomatic and asymptomatic-suspected malaria patients with ethical approval from the Institutional ethics committee (IEC No. ECR/NIMR/EC/ 2019/332). Blood smears were made and blood samples were also collected on Whatman 3MM filter paper for detection of non-human Pk and Pc parasites by microscopy and molecular methods. DNA was isolated from the punched spots using QIAGEN kit as per manufacturer's instructions. All the 250 blood samples were tested for detection of Pf and Pv using nested PCR methods (31).

Results

The qPCR assay was designed for detection of 18S rRNA gene segment of the Pk and Pc parasites. The in-house designed primers and probes were used for the amplification of the 18S rRNA gene

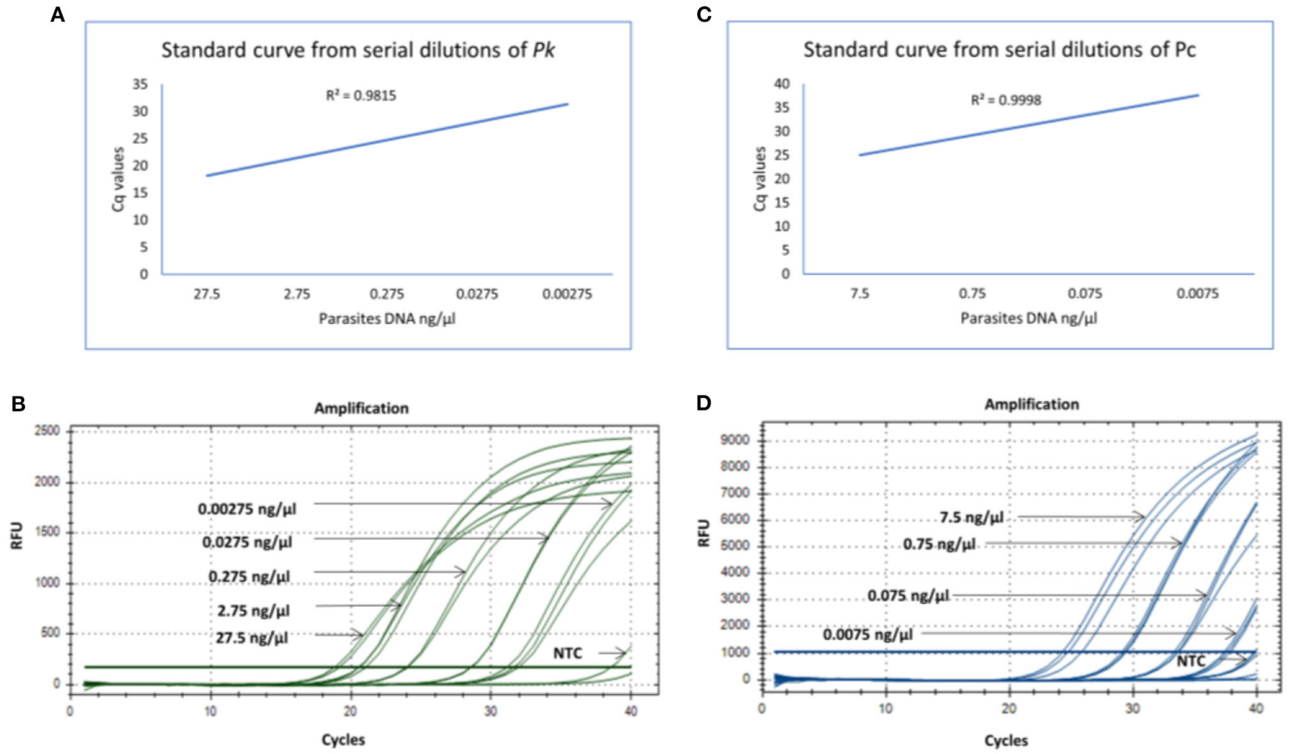

FIGURE 1
(A) Standard curve generated from 10-fold serial dilutions from known concentrations of parasites DNA (Pk) in the qPCR assay. **(B)** qPCR amplification cycles for Pk serial dilutions with fluoroscent probe HEX (green). **(C)** Standard curve generated from 10-fold serial dilutions from known concentrations of parasites DNA (Pc) in the qPCR assay. **(D)** qPCR amplification cycles for Pc serial dilutions with fluoroscent probe FAM (blue).

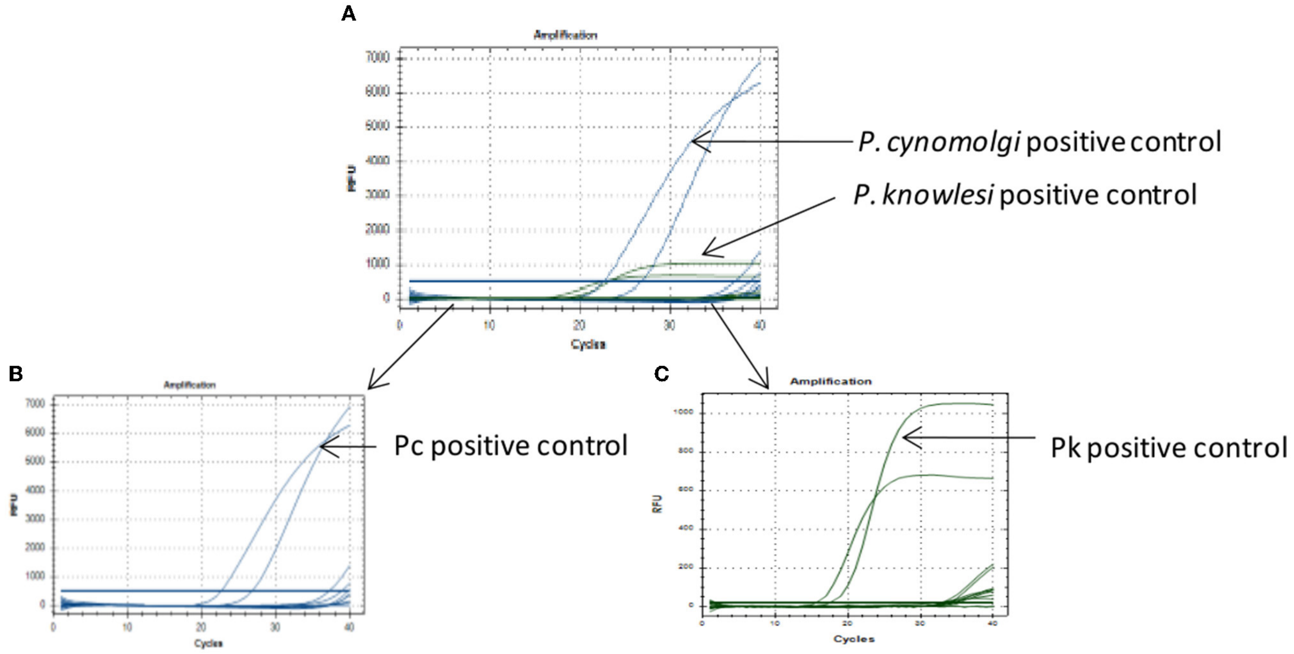

FIGURE 2
Specificity of the qPCR assay. **(A)** Multiplex qPCR assay for detection of Pk and Pc parasites DNA as positive controls, while templates from Pf, Pv, Pm and Po parasites DNA did not amplify. **(B)** Selected fluorophore probe FAM (blue) for *P.cynomolgi* showed good amplification but no amplification for Pf, Pv, Pm, and Po. **(C)** Selected fluorophore probe HEX (green) for *P. knowlesi* showed good amplification but no amplification for Pf, Pv, Pm, and Po.

TABLE 2 Age-group wise infections of Pf and Pv in symptomatic/asymptomatic patients.

Age groups (years)	No. of samples	Males	Females	Febrile (Symptomatic)	Febrile cases		Afebrile cases	
					Pf	Pv	Pf	Pv
01–09	82	41	41	17	4	5	0	0
10–19	51	29	22	3	1	2	0	2
20–29	48	23	25	0	0	0	5	0
30–39	24	13	11	2	0	0	0	1
40–49	23	11	12	0	0	0	3	1
50–59	11	4	7	5	1	0	0	0
>60	11	6	5	0	0	0	0	0
Total	250	127	123	27	6	7	8	4

segments of the Pk and Pc, respectively. Standard curves from the serial dilutions were generated for each parasite (Pk and Pc) with estimated R^2 values of 0.98 and 0.99, respectively (Figures 1A, C). To assess the limit of detection (LOD) of parasites DNA, the Pk and Pc DNA were serially diluted and subjected to qPCR assay. The LOD of parasites DNA in multiplex qPCR assays were observed to be 0.00275 and 0.075 ng/µl of Pk and Pc genomic DNA, respectively (Figures 1B, D). The associated C_q values corresponding to the minimal detection limits were 28.50 and 31.5 for Pk and Pc, respectively (Figures 1B, D). The in-house designed qPCR primers and probes were observed to species-specific for Pk and Pc in the multiplex format, as the 18S rRNA primers and probes specific for Pk and Pc did not amplify any of the human malaria parasites DNA (Pf, Pv, Pm and Po) (Figure 2); thus, validating the multiplex qPCR assay to be species-specific for Pk and Pc.

Microscopy was used for detection of malaria parasites and further, the multiplex qPCR primers and probes were evaluated with 250 human blood samples collected from suspected areas from North Eastern states of India. We did not observe any Pk and Pc positive infection from these samples. However, we did observe Pf and Pv infections using microscopy and previously reported primers for detection of Pf and Pv parasites (31) in these samples as listed in Table 2. 27/250 samples were febrile (symptomatic) at the time of sample collection; males and females were in approximately equal proportion of the total number of samples. 14/250 samples were found to be positive for Pf infections; while 11/250 were found positive for Pv infections. However, none of the samples were observed positive for Pk and Pc parasites, either by the in-house developed qPCR assay as well as with established conventional PCR primers described previously for detection of Pk (29). In the absence of any positive sample for Pk at the least even by the conventional PCR primers reported previously, the qPCR primers for Pk would be considered highly sensitive, however further validation of the primers shall be assessed with Pk positive samples from other sites in future.

Discussion

The non-human *Plasmodium* parasites Pk, Pc, *P. fieldi* and *Pi* have been reported from Malaysia; while Pk and Pc were also reported from India as well (4–8). The non-human malaria parasite Pk in the human host and vector have been reported from different states of India such as Bihar, Delhi, Andaman & Nicobar Islands and Uttar Pradesh (1, 2, 5, 7). In this study, we developed in-house multiplex qPCR assay for detection of non-human malaria parasite species Pk and Pc. This diagnostic tool shall prove to be critical in detection, identification, surveillance and monitoring of the non-human malaria parasite Pk and Pc, ultimately contributing toward the control and elimination of malaria by 2030 from India.

The lowest limit of detection from genomic DNA of Pk and Pc parasites DNA was found to be 0.00275 and 0.075 ng/µl, respectively (Figures 1B, D). The probes were also BLAST searched and found to species-specific for Pk and Pc only. Further, no cross-reactivity was observed with any of the human malaria parasites- Pf, Pv, Po and Pm, proving them to be species-specific (Figure 2). Therefore, the in-house developed species-specific multiplex qPCR assay is highly specific and sensitive for detection of Pk and Pc. The assay was further evaluated for detection of Pk and Pc in 250 human blood samples collected from highly malaria endemic areas of North Eastern states of India and yielded no positive infection for Pk and Pc.

We anticipate that the qPCR assay would prove to be a useful tool for detection of Pk and Pc infections in the vector mosquitoes also (*An. dirus*, *An. minimus, An. sundaicus, An. sinensis* and *An. maculates*) as well as in their natural hosts- non-human primates (1, 5, 21, 23–25, 28). The multiplex qPCR assay for detection of Pk and Pc parasites is robust, quick, cost-effective, sensitive and species-specific to undertake investigations particularly focused on the transmission dynamics of these non-human malaria parasites in India. However, further studies with larger number of samples are needed to validate the usefulness of the qPCR assay for Pk and Pc.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Ethics Committee ICMR-National Institute of Malaria Research, Delhi, India (IEC No. ECR/NIMR/EC/2019/332). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

RD conceived the study, designed and performed the experiments, analyzed the data, and wrote the manuscript. KV analyzed the data and wrote the manuscript. KP analyzed the data and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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Veterinarians' perspective on telemedicine in Germany

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Introduction: Information on the use of telemedical approaches in the context of veterinary medicine is evolving. As in human medicine, veterinary medicine is subject to an increasing digitalization effort. The aim of the current study was to investigate the perspective of German veterinarians regarding their awareness and usage of telemedical approaches. Furthermore, the degree of implementation of different digital approaches in the context of German veterinary medicine was elaborated.

Methods: A literature review, that also aimed to address the necessary framework or standardization of these digitalization efforts and potential barriers such as legal or infrastructural aspects, provided information for the empirical research. Using a quantitative research approach, the perspective of German veterinarians was surveyed.

Results: In total, responses from 169 veterinarians were analyzed. The results show that digital approaches were used by veterinarians and the usage was enhanced by the COVID-19 crisis.

Discussion: However, the lack of a clear legal framework may be a significant barrier for further implementation. This survey provides a basis for a critical discussion on the use of veterinary telemedicine in Germany. The results may contribute to future strategies for the implementation and development of necessary policies, training, and service applications within Germany, which may be transferable for the profession in other countries.

KEYWORDS

telemedicine, veterinary telemedicine, teleconsultation, teleradiology, digitalization

1. Introduction

The German Medical Association presents a basic definition of telemedicine: "*Telemedicine is a collective term for various medical care concepts that have in common the principle approach of providing medical services for the health care of the population in the areas of diagnostics, therapy and rehabilitation as well as medical decision-making advice over spatial distances. Information and communication technologies are used for this purpose.*" (1). This means, for example, that a follow-up call after surgery also falls under this definition of telemedicine.

Telemedicine may be important in the future for everyday work in veterinary medicine (2). It offers various advantages for veterinarians, the patients and their owners, as patient information can be passed from one specialist to another much more easily, allowing faster or possibly more accurate diagnosis and more efficient treatment. Ideally, telemedicine can fill in gaps and provide better care and treatment for patients without owners or veterinarians having to make a time-consuming and often arduous journey to the city or far into the countryside. Therefore, telemedicine and the underlying concept of bringing information digitally to where it is needed are among the most groundbreaking developments in the field of medicine (3). Veterinary telemedicine can be a success factor by helping to reduce error rates, perform more procedures and improve the overall client-veterinarian relationship when applied appropriately (3). As an example, the authors Papageorges and Tilley cite the sharing of "radiograph, sonogram, and cytology to radiologists, cardiologists, and pathologists," while other activities are carried out. Discussion with or reports by board-certified specialists allow for more comprehensive advice, diagnosis and a treatment plan even in remote areas (3, 25). However, examples listed are mainly individual and subjective opinions without statistical evidence or empirical research have been described. Video visits, remote patient monitoring and telecare are methods that have already found their way into human medicine and will increasingly influence veterinary medicine in years to come (2). Some veterinarians already offer their clients the possibility of tele-consultation, and presumably clients' demands will enhance the importance of telemedicine in the field of veterinary medicine in the medium term (2), while teleradiology is already established in daily work and companies offering teleradiology services exist (3–5). Teleradiological services include primary interpretation of images, systems to directly or indirectly send data to specialists, on-call coverage or second opinion consultations, as well as image storage and archival or database banking (5). Increasing pressure for innovations in the field of digital technologies and telemedicine are obvious (6–8). Smartphones, apps and camera systems are constantly improving and systems are being adapted and implemented also within veterinary medicine. IHS Technology suggested in their report that the number of patients using veterinary telemedicine services globally would grow from 350,000 in 2013 to 7 million in 2018 (7, 8). In the field of animal health, the innovation debate primarily revolves around veterinarians themselves who are considered to be innovation-friendly and open (6). Telemedicine has been described as one key development in 2018 by the Royal College of Veterinary Surgeons (RVCS) survey, which included more than one thousand participating British veterinarians. In that survey, a large proportion of participants rated telemedicine as a topic worth discussing, but concerns were also expressed (9). Those concerns about risks included "risk of error due to incomplete information, a lack of a physical examination, limitations to technology and issues relating to owner trust" (9). The survey also revealed uncertainty about the extent to which telemedicine services can and should be included in the Code to Professional Conduct for vets. However, some veterinary medicines (such as antiparasiticides) that could be prescribed remotely were endorsed.

Thus, the development of veterinary medicine seems to be in line with developments in human medicine where the widespread use of telemedicine approaches has been discussed with greater detail (10). Telemedicine and telehealth as a means of caring for hard-to-reach patients are attractive in many ways (10). These approaches can save time and money and reduce the inconvenience of travel for healthcare professionals as well as patients and clients. They can also make rare expertise more widely available by enabling better networking among colleagues and greater international availability of expert knowledge (10). In addition, telemedicine approaches can also help patients who would otherwise go untreated (10). At the same time, however, these developments are met with a high degree of mistrust, which seems to be based on several uncertainties, for example, regarding the issue of responsibility or cost factors (10). Due to lack of healthcare infrastructures and an increased risk of zoonotic diseases, telecommunication technologies, such as smartphones and digital cameras, are particularly useful in developing countries (11). Telehealth equipment can support early diagnosis, surveillance, warning or reporting and the prevention of zoonotic diseases, thereby playing an important role in One Health approaches (11).

The only exception here is teleradiology, which is an established branch in the field of telemedicine. The possibilities for increased productivity and expansion of diagnostic options due to digitalization, particularly in diagnostic imaging, were described as a pioneering future trend as early as 2001 (12). Numerous successful business models have been developed in the field of teleradiology, also in veterinary medicine. Corresponding teleradiology solutions also play an important role with regard to the increasing desire for teleworking and telecommuting in the context of the COVID-19 crisis (13, 14).

Veterinary medicine and animal husbandry are described as highly specialized fields encompassing the management and health care of various animal species: "disease diagnosis, treatment, and prevention; quality assessment of meat and food, including milk and dairy products; quarantine procedures; animal welfare; feed formulation and testing; technology dissemination; and teaching, training, innovation, generation, and transfer of knowledge or technologies for end users as well as administrators" (15). Accordingly, the critical role of veterinarians in meeting these growing responsibilities is complex. Despite this complexity, however, telemedicine, with the exception of teleradiology, still seems to be comparatively underutilized and, above all, insufficiently researched in the field of veterinary medicine (15).

Telemedicine seems to be rapidly gaining relevance, especially in the context of the COVID-19 crisis (16). As in other areas of social and economic life, the pandemic and the measures taken by governments led to increased demand and support for digitalization efforts (17). While home office or telework/telecommuting models increased in other sectors, there was also a trend in medicine to minimize avoidable physical interactions (18, 19). More fundamentally, many governments did not adequately explain the critical role of veterinary medicine. While medical staff in the human health care sector were clearly defined as key personnel who were accordingly exempt from lockdown measures, this was only partially clarified in veterinary medicine and related fields (20). Overall, telemedicine appears to be an emerging subfield in veterinary medicine as well, despite or even as a result of the COVID-19 pandemic (21).

While telemedicine in human medicine is already considered comparatively well researched, there seems to be a research gap in the field of veterinary medicine (15, 22). This lack of research in the field of veterinary telemedicine has already been criticized (23). Mars and Auer (2006) pointed out that telemedicine approaches have been used in veterinary medicine for a long time but are rarely discussed and researched. Modern telemedicine should be evaluated in detail and the billing of animal owners should be better clarified (23). In addition to the fundamental research gap on the question of the extent to which telemedicine has also found its way into the field of veterinary medicine and which approaches are used in practice in this context, the topic of the effects of the COVID-19 crisis also emerges: society as a whole seems to have experienced a digitalization surge in the last 2 years, which has also led to an increased use of remote communication, for example in the fields of medicine, education or business (24, 25).

In the context of this study, the extent to which veterinary medicine itself has been exposed to this digitalization push is elaborated, as the study was carried out during the pandemic as were the corresponding countermeasures. Therefore, the aim of this study was to investigate German veterinarians' perception of digital technologies and potential change in usage with regard to infrastructural, technical and legal frameworks using an online survey. Furthermore, it was investigated whether the attitude to telemedicine has an influence on the use of telemedicine and whether the COVID-19 pandemic has served as a catalyst for a change in awareness. Results shall inform about the developing of or improvement to guidelines and regulations to introduce and implement telemedical approaches in a more standardized manner in veterinary medical practices.

2. Method

An online survey (LimeSurvey®) was created on the basis of existing instruments such as the "Telehealth Practice Survey" (26), "a survey of knowledge and use of telehealth among veterinarians" (27) or the survey "Digitalisierungsreport" ["digitalization report," (28)] to evaluate the attitude toward and the implementation of telemedical services among individuals in veterinary practice. Before publishing the current survey, it was pre-validated and piloted by expert staff from the University of Veterinary Medicine in Hannover as well as veterinary colleagues as potential respondents (29).

The survey consisted of four question groups:

1. Sociodemographic questions and general questions about the participating veterinary practice. Questions about age, gender, type and localization of the practice (rural or urban environment) and role in the clinic/practice are supplemented by questions about mainly treated species.
2. The perception and basic attitudes of the participating practitioners regarding digitalization in relation to the veterinary professional ethos were assessed using rating questions with a rating scale from "1 = strongly agree" to "4 = strongly disagree."

Fifteen questions evaluated communication-related aspects. The participants rated communications tools using a scale that ranged from "1-mostly used" to "5-not specified." The questions were separated into two main parts. The focus of the first parts was the communication management of a veterinarian in daily work life. The options for "communication" were "telephone," "physically (which means "in the practice")," "video chat," "E-mail," "messenger" (like WhatsApp, Facebook, SMS), "fax" and "letter." In the second part it was asked how communication took place with new and existing customers.

3. General questions about the status of information and wishes for a legal framework regarding telemedicine in veterinary practices. With the aim to draw conclusions as to whether knowledge of the legal framework is a limiting or motivating factor for the use of telemedical solutions. To verify a possible uncertainty more precisely, the question was asked whether the participant believes that telemedicine is already sufficiently regulated in the professional code of conduct. The last question in this section dealt with suggestions from veterinarians regarding education and information from professional organizations.
4. COVID-19-related questions including questions about the influence of COVID-19 as a catalyst regarding the awareness and the use of telemedicine.

The survey was sent to veterinarian practitioners *via* social media in veterinarian expert groups (e.g., closed veterinary Facebook groups) and *via* newsletters by the local Veterinary Chamber of Lower Saxony between May 2020 and August 2020. Participants were asked to respond as honestly and applicably as possible based on their own experiences. It was pointed out that personal data would be collected, stored and analyzed respecting the General Data Protection Regulations (GDPR) and anonymity of all respondents. Results were evaluated anonymously and participants were free to discontinue participation in the survey at any time, which would result in the deletion of the data collected up to that point. All questions were asked only optionally, which meant that questions could be skipped and no answer was forced in order to continue processing the questionnaire. In addition, the answer option "no answer" was offered for each question to allow for abstention.

Raw data from the survey were first transferred to Microsoft Excel® (2010 Microsoft Corporation). Sorting algorithms were used and questionnaires that were not completed to the end were filtered out. These were deleted and, based on the convenience sample, a descriptive analysis of the results was carried out and presented in tabular and graphical forms using Microsoft Excel® (2010 Microsoft Corporation).

This study was conducted in accordance with the ethical standards of the University of Veterinary Medicine Hannover, Foundation. The Doctoral Thesis Committee of the university validated the project in accordance with the ethical guidelines regarding research with human participants and approved the study. The university's Data Protection Officer approved the project. The voluntarily participating persons consented to the processing of their data in accordance with the EU General Data Protection Regulation of 2018 (General Data Protection Regulation Art. 6 I 1 lit. e i.V.m. 89 and Lower Saxony Data Protection Act § 3 I 1 No. 1 NHG, § 13). Data processing was performed anonymously in accordance with the Data Protection Regulation of the university.

3. Results

3.1. Socio-demographic data

A total of 300 veterinarians started to answer the survey. However, only 169 respondents completed the entire survey and were included in further analyses. The gender ratio within the sample was unbalanced, with 114 females (67.5 %) and 55 males (32.5 %). Participating veterinarians were on average 45.19 years old (SD = 11.96 years). Figure 1 shows the distribution of respondents in different areas of population density.

A large proportion of respondents reported working in a large city, while the rest were distributed among medium-sized cities, small towns and rural areas (Figure 1).

Figure 2 shows that the majority of participating veterinary practices treated small animals (76 %), but participants also came from the livestock (45 %), equine (22 %) and small pet sectors (34 %). Specialty practices (fish and birds) were also among the participants (15 %).

The majority of participants worked in individual practices (*n* = 87, 51.5 %), followed by university (*n* = 29, 17.2 %), group practices

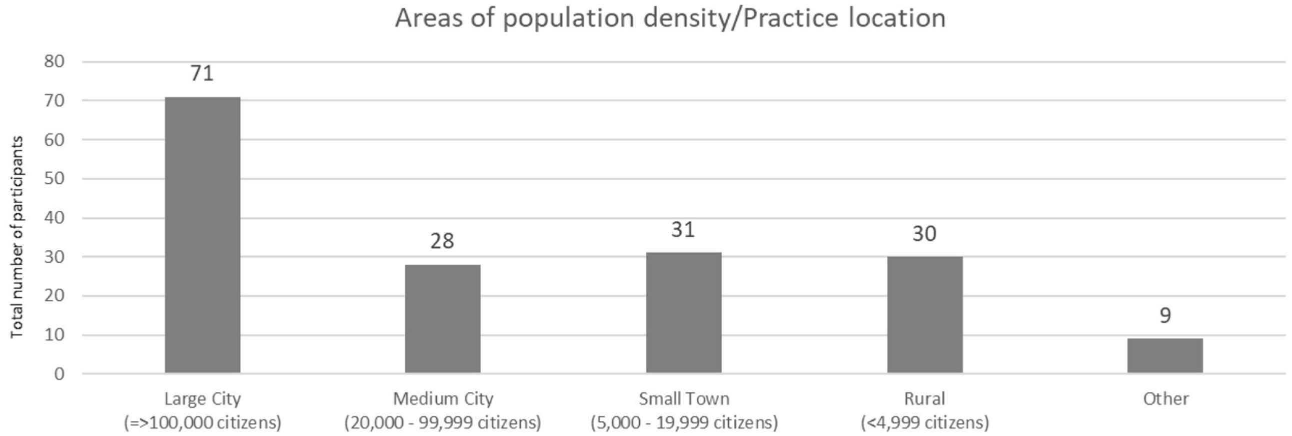

FIGURE 1
Distribution of respondents in different areas of population density ($N = 169$).

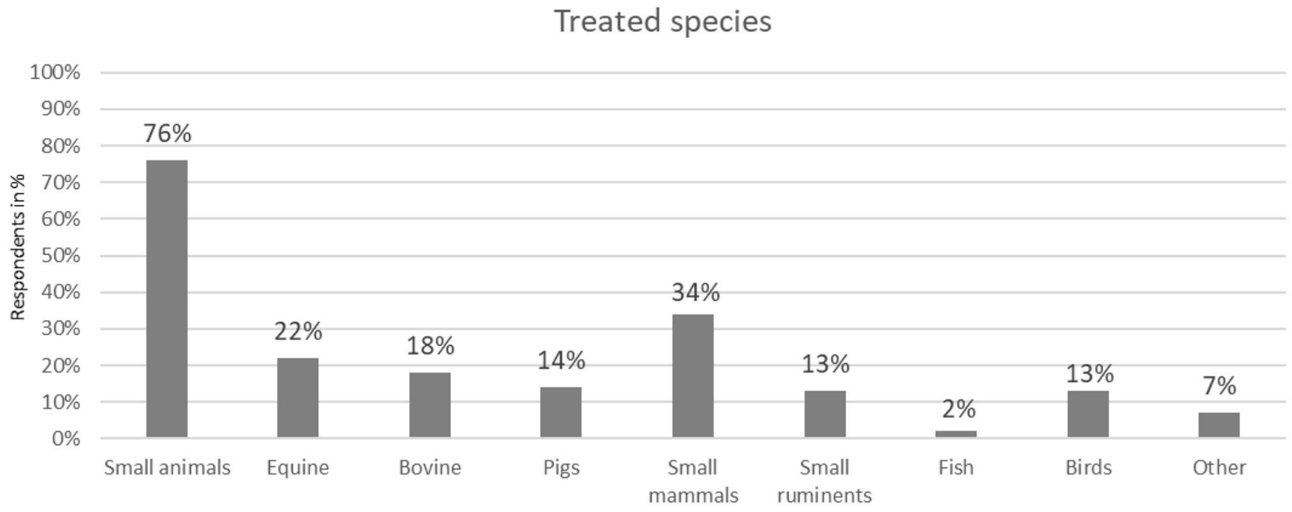

FIGURE 2
Animals treated in practices by respondents ($N = 169$, multiple answers possible).

($n = 22$, 13 %) and clinics ($n = 22$, 13 %) and a small number did not work in any practice ($n = 4$, 2.4 %) (Figure 3).

The majority of respondents were practice owners ($n = 75$, 44.4 %), followed by employed physicians ($n = 38$, 22.5 %) and those on parental leave ($n = 15$, 8.9 %) (Figure 4).

3.2. Questions about communication with the animal owners

As shown in Figure 5, face-to-face communication was used in most cases, followed by telephone consultations. E-mails and various forms of messengers were used less frequently. Video telephony was rarely used, fax and letter were not common anymore.

Figure 6 shows again that face-to-face communication was used in most cases, followed by telephone consultations, E-mails and various forms of messengers being used less frequently.

3.3. Questions about legal aspects and information by the authorities

Figure 7 displays the respondents' thoughts about the legally applicable extent of telemedicine. In the analysis, attention was also paid to whether the telemedicine consultation referred to an already existing or to a new doctor-patient relationship.

The majority of study respondents were of the opinion that telemedical care may be used primarily for existing patients. Interestingly, some of the participants thought that both telemedical diagnosis and telemedical treatment of patients were allowed. A significantly large proportion of the respondents were unsure what was legally permitted and which actions were not permitted.

Figure 8 highlights that only a small percentage of the respondents ($n = 11$, 6.5 %) held the opinion that telemedicine was sufficiently included in the legal framework for veterinary practices, but the majority rated that telemedicine was insufficiently included ($n = 158$, 93.5 %) in the professional regulations.

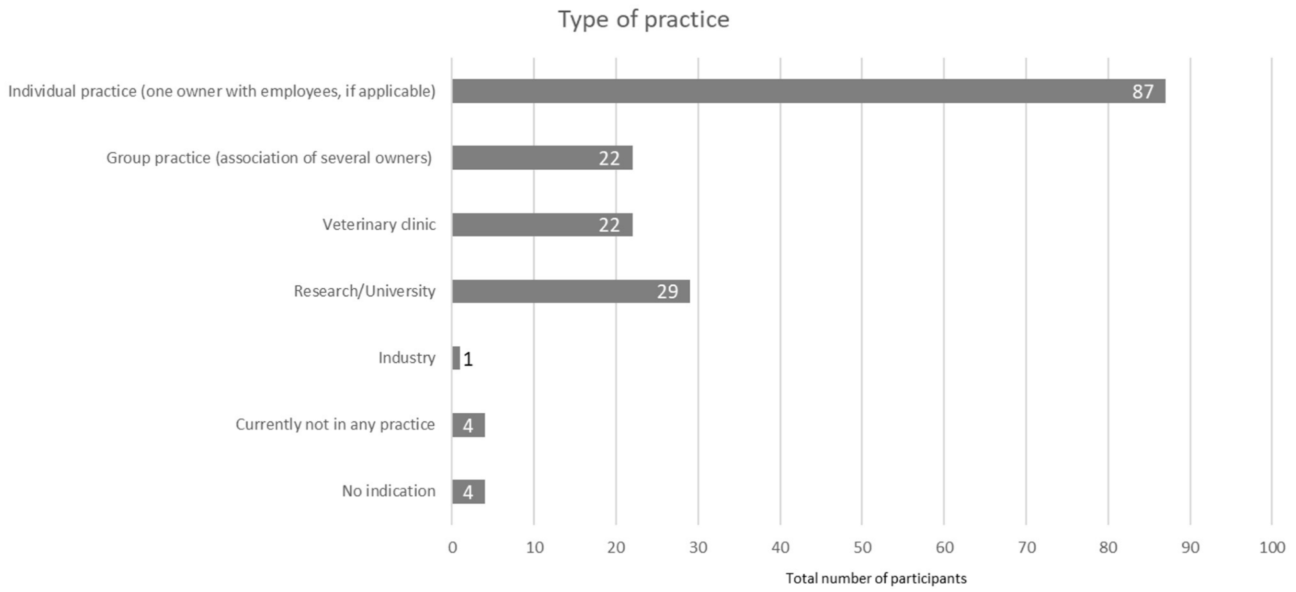

FIGURE 3
Type of practice in which respondents worked ($N = 169$).

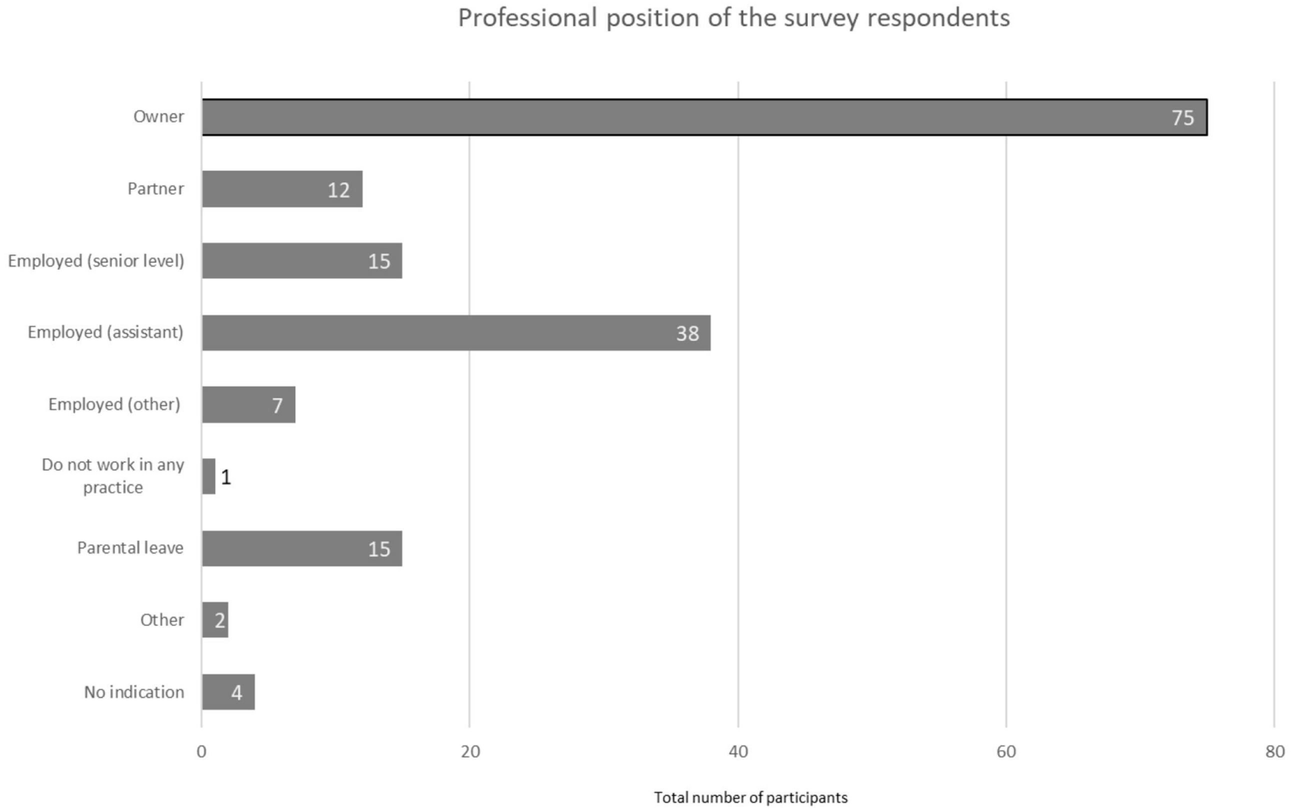

FIGURE 4
Professional position of the survey respondents ($N = 169$).

Also, the majority of respondents ($n = 131$, 77.5 %) do not feel sufficiently informed about current telemedicine opportunities for veterinarians (Figure 9).

Most participants were not aware of the Guidelines_of_the_Ad-hoc-AG-Telemedizin by the German Veterinary Chamber (1) ($n = 122$, 72.19 %) (Figure 10), which was released just one month before the survey was sent.

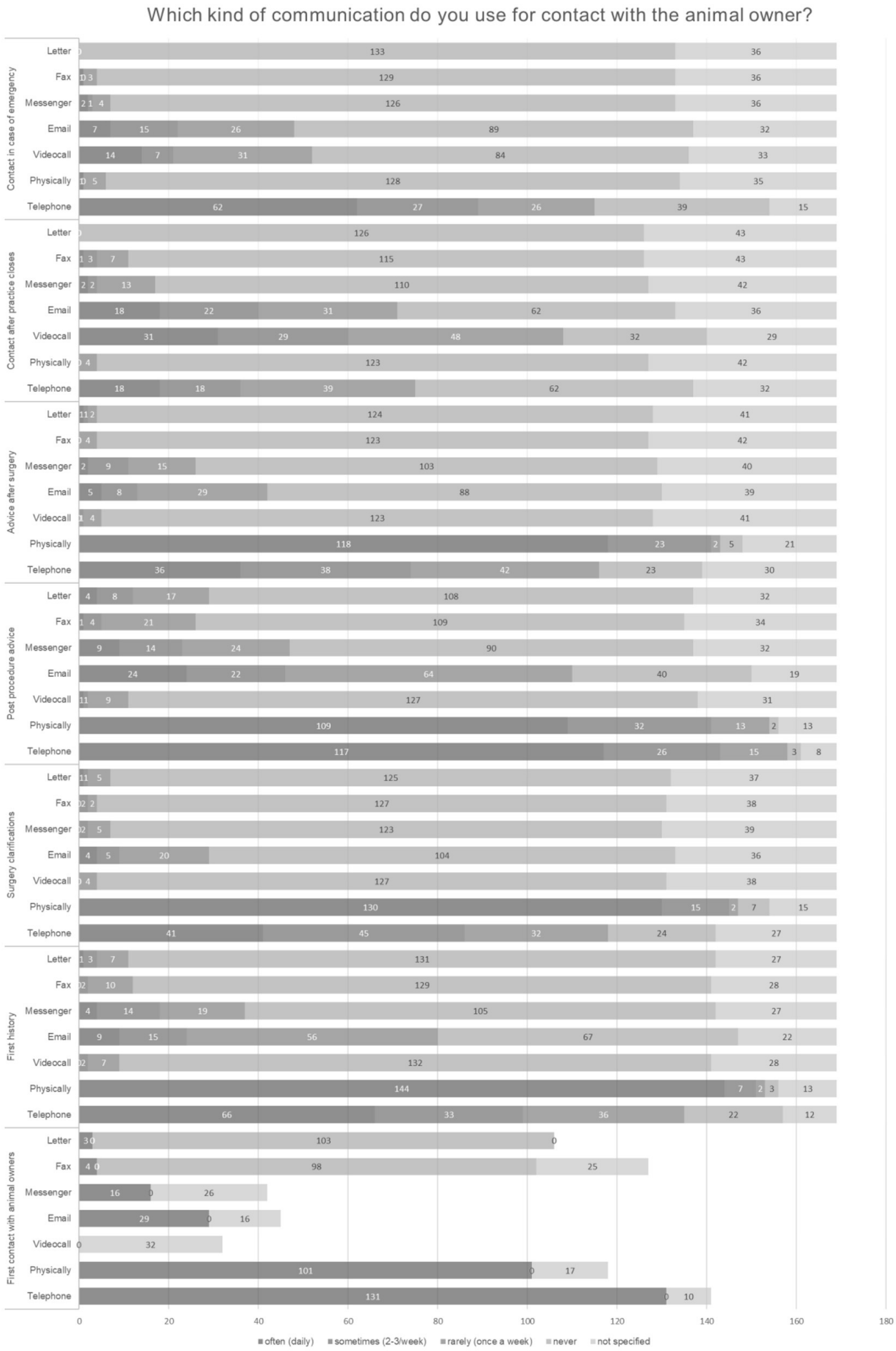

FIGURE 5
Kind of communication with the owner ($N = 169$).

In addition to more information about the legally protected possibilities in relation to telemedicine, the majority of participants would also like to see clear remaining guidelines for using telemedicine in practice. Technical assistance and support were less in demand (Figure 11).

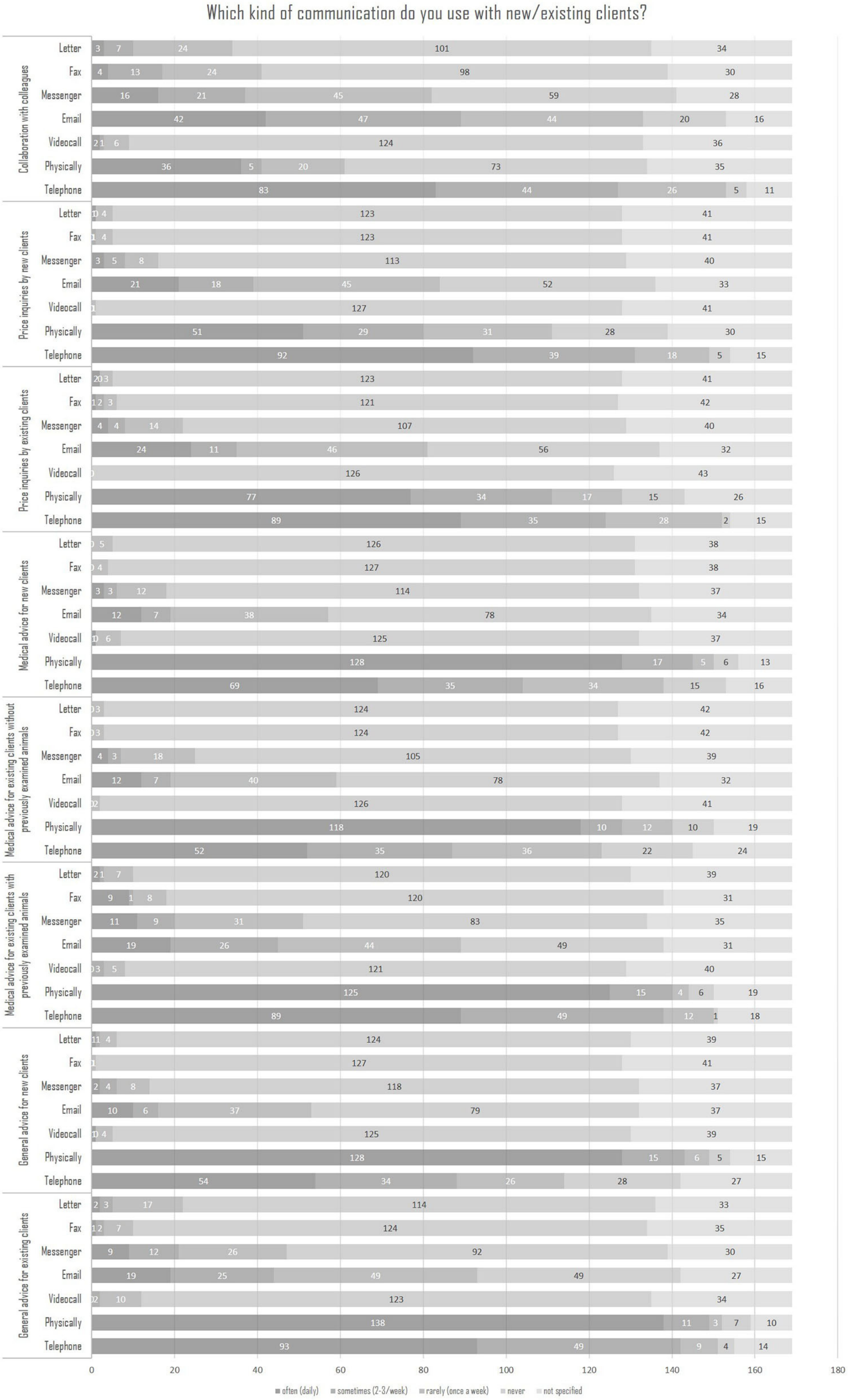

FIGURE 6
Kind of communication regarding new or existing customers (*N* = 169).

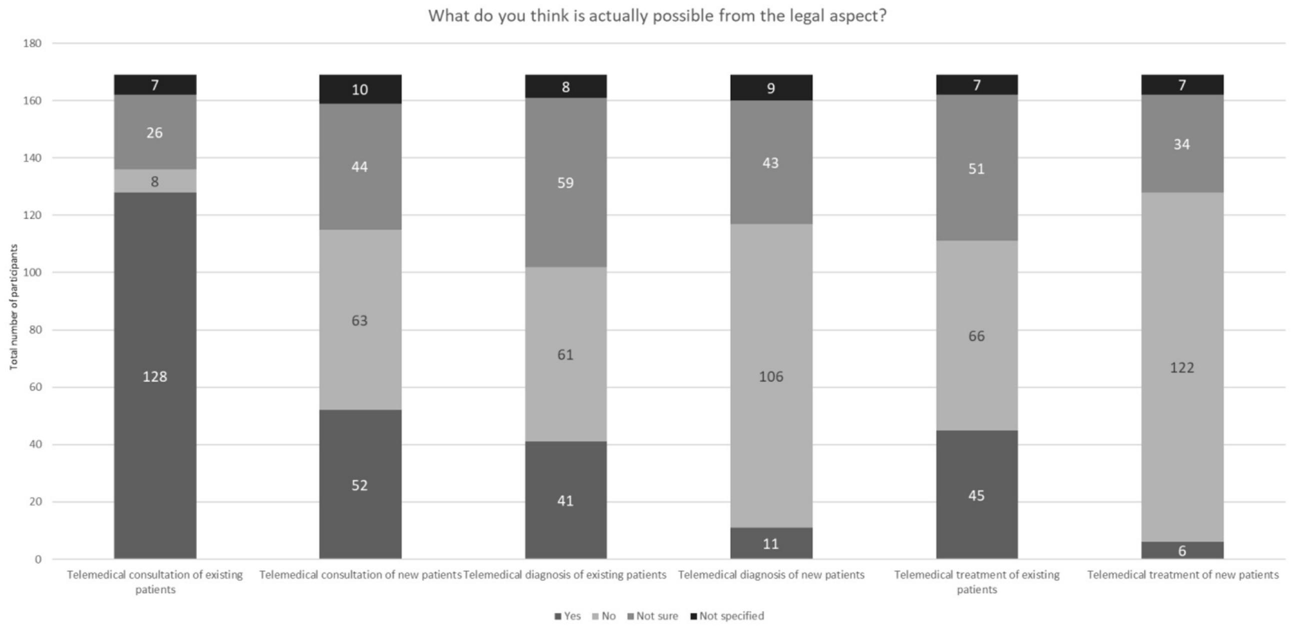

FIGURE 7
Legal possibilities in veterinary medicine in terms of telemedicine ($N = 169$).

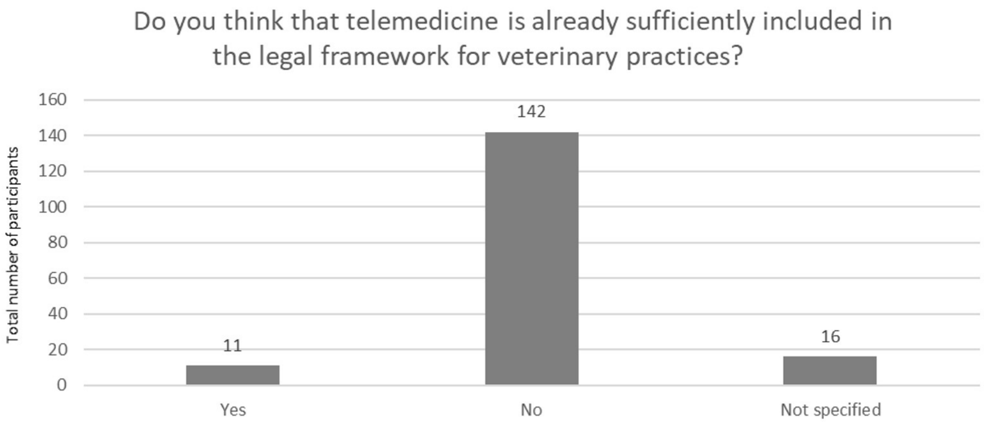

FIGURE 8
Inclusion of veterinary telemedicine in the legal framework for veterinary practices ($N = 169$).

3.4. Questions related to COVID-19 as an influencing factor

The majority of respondents did not want to or intend to use telemedicine applications during their consultations, control- or follow-up-appointments or case discussions only because of the COVID-19 pandemic (Figure 12).

4. Discussion

The current study investigated the usage and awareness of telemedicine and its legal framework among German veterinarians. According to socio-demographic aspects, mostly women who worked in a small animal practice or ran one in a large city were interested in telemedicine in general. It is also interesting that veterinarians who were on parental leave were interested in working with telemedicine. Presumably, these veterinarians want to explore alternative employment models and telemedicine could be at least one of the possibilities.

According to the respondents' answers, digital/telemedical solutions were used already, but did not prominently feature in the daily veterinary medical practice. In contrast, face-to-face and telephone consultations were still the most frequently used communication channels with animal owners.

The only exception was teleradiology, which is well established in the area of doctor-to-doctor communication and has been used for more than 30 years (4, 5). Here, the technical and legal framework seems to be more clearly defined and can presumably be implemented

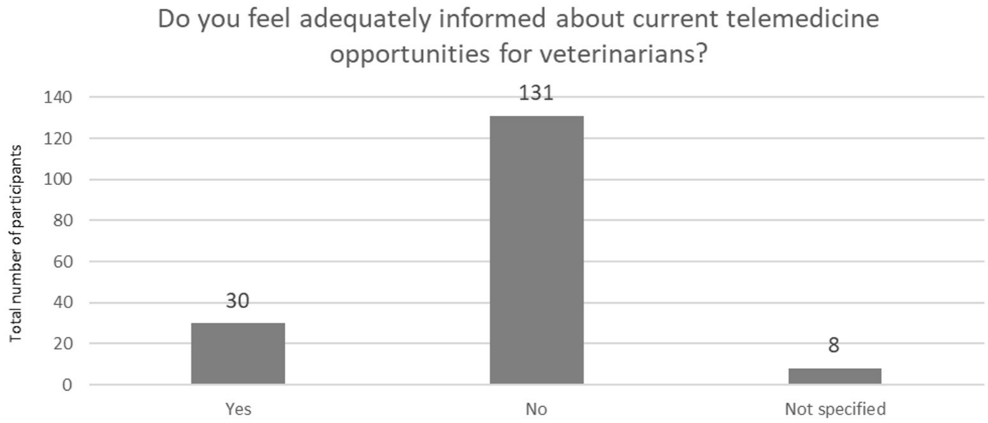

FIGURE 9
Information about current telemedicine opportunities for veterinarians (*N* = 169).

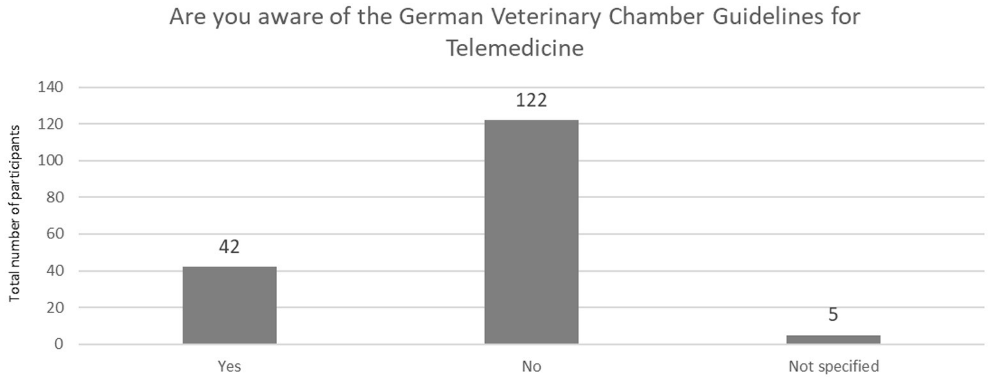

FIGURE 10
Awareness of the German veterinary chamber guidelines for telemedicine (*N* = 169).

well in existing practice structures due to more uniform technical approaches (5).

With regard to the legal framework, which was also a central component of the survey, transparency and further communication are expected and needed. As other authors have shown in relation to initial developments during the COVID-19 crisis, there is significantly less clarity concerning the legal framework in veterinary medicine than in other fields (21). Legal clarification for the authorization of digital procedures for different veterinary medical situations is still lacking and seems to impose a corresponding constraint on practitioners (30). While preparing the survey, first guidelines were discussed and published by the authorities (1) so that this could be the reason for the low awareness of them, as the survey was conducted close to the publication date.

With regard to the implementation of veterinary telehealth and telemedicine research, further research is needed (21). While detailed analyses are available in human medicine (9, 16), in veterinary medicine the topic seems to be less developed. However, at the same time at least in practice, telemedicine approaches are becoming increasingly important (21). Scientific research in this regard is mainly limited to overview articles or forecasts and therefore do not offer any empirically robust statements about the actual implementation status or acceptance of corresponding solutions (16). Only teleradiology, as a technically oriented solution, is an exception. Nevertheless, especially the implementation of corresponding solutions also depends on legal, social and societal framework conditions (12, 13).

In addition, a recent analysis for identifying and analyzing strengths, weaknesses, opportunities and threats (SWOT analysis) performed by a group of experts from the European Coordinating Committee on Veterinary Training identified the urgency to "develop policies and regulations to ensure efficient, safe, ethic and legal use of digital technologies and artificial intelligence in veterinary medicine" (17).

It remains unclear to what extent these framework conditions are also related to different remits and approaches in terms of location (urban vs. rural) and orientation of practices (large/small animals). For example, it can be argued that veterinary practices in rural areas dominated by livestock farming, sometimes differ significantly from those in urban areas (16). It is therefore clear that the approaches to telemedicine taken by practitioners in these two areas should also differ. Approaches in One Health, animal disease control or zoonoses are also relevant areas for which recommendations and standards should be developed (11).

One limitation of the study may be the empirical consideration of the legal framework. While the literature on telemedicine and

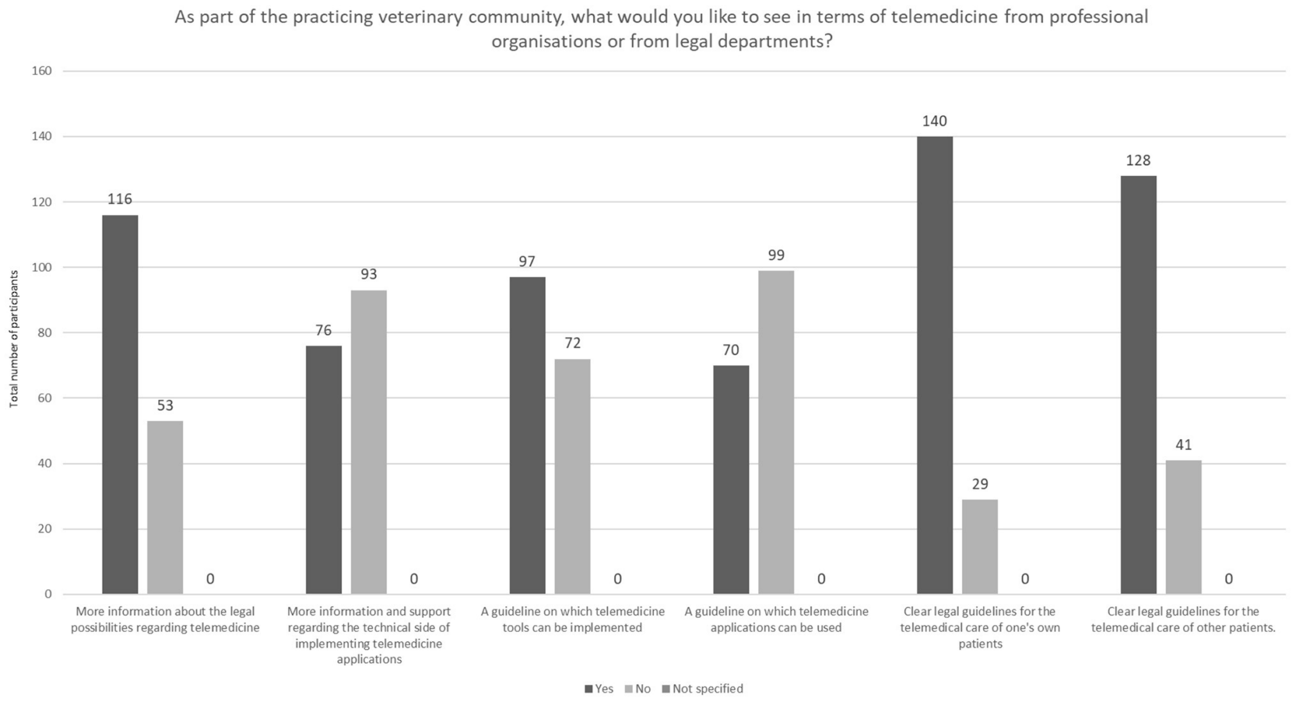

FIGURE 11
Needed support and information by legal departments ($N = 169$).

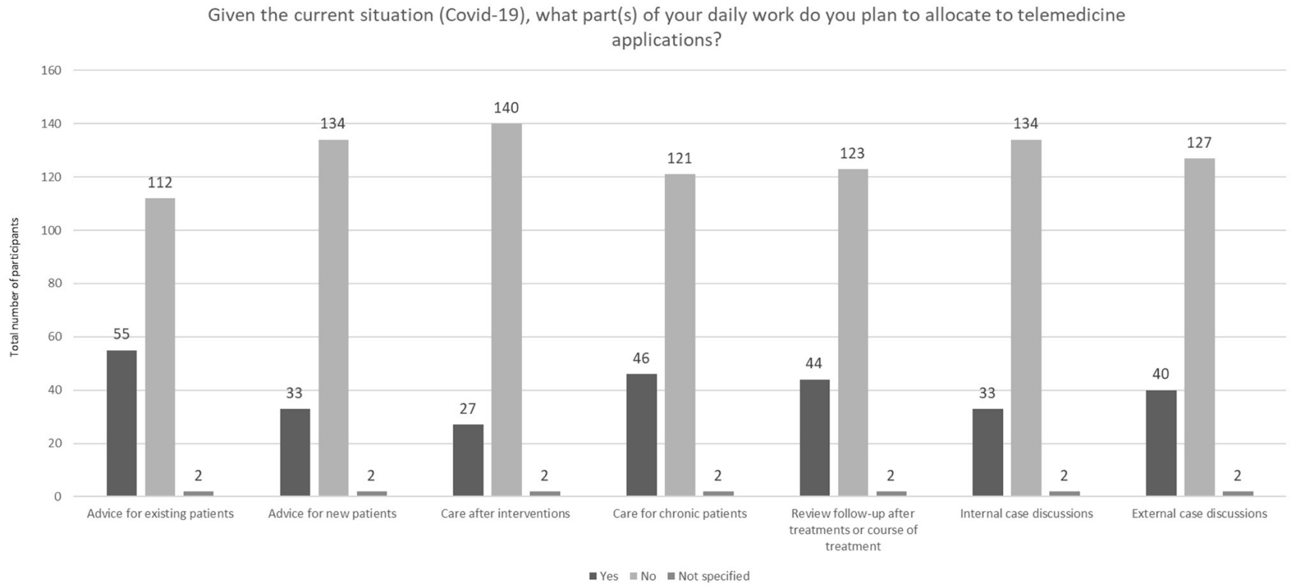

FIGURE 12
Allocation of daily work procedures to telemedical applications given the current situation (COVID-19) ($N = 169$).

veterinary telemedicine states that the legal framework and clarity concerning legal and technical aspects are of fundamental importance for the implementation status, the empirical findings presented here only confirm this to some extent (4, 9). The actual legal framework conditions are not well publicized. Also, a global comparative study is advisable, which not only examines the internal national differences in perception and interpretation of the framework situation, but also the actual differences with regard to legislation, comparing these with the implementation status of telemedicine solutions.

In particular, it is advisable that future research in the field focuses on the perspective of clients. The present study showed the implementation status and the related expectations from the perspective of the practicing veterinarians. For a successful implementation of telemedical solutions, it is relevant to include the perspective of the animal owners, which has corresponding implications especially for the practical work. A deeper understanding of this perspective and the needs of this important group of stakeholders should be taken into account in order to be able to develop telemedical solutions tailored to their wishes.

To establish a telemedical and digital mindset in the veterinarian eco-system, it is recommended to create a clear framework as a guideline for veterinarians.

The guideline may include the following topics:

1. Reference to transparent and well-defined legal basis and definitions with a clear differentiation between teleconsultation, teletreatment and telediagnosis.
2. Recommendation regarding the selection and transformation of previous analog processes in digital or digital/telemedicine-supported processes.
3. Building awareness regarding telemedicine, its possibilities and its advantages.
4. Expanding available guidelines regarding telemedicine.
5. Creating billing regulations for veterinarians.

At the same time, it seems reasonable to formulate additional concrete questions for future surveys, since ignorance about telemedicine, skepticism or unclear understanding *per se* may have an influence on the answers.

In addition to skepticism about digitalized veterinary medicine, the research results also indicate adherence to "old" or analog forms of communication. A recently published study "Digitalized Veterinary Medicine 2030" also shows clear tendencies and results in this regard (27). It states: "Currently, there is a digital divide between practitioners and industry representatives in veterinary medicine. The study results also show that there is a considerable gap in the learning strategies used and positions taken on digitalization within the groups of practitioners studied. Dealing with the opportunities and risks of digitalization in veterinary medicine, however, requires a willingness to learn, curiosity and a willingness to experiment from many more actors. For the future viability of veterinary medicine, a "digital mobilization" of practices and clinics therefore appears to be important." (27). It continues: "In this context, the animal health industry and a still relatively small number of practitioners with a high level of digital competence in the sector are in a pioneering position. These actors have an important potential to support practices, clinics and teaching and to promote the transfer of digital knowledge. But the strategic and political role of professional associations is also important in terms of targeted development of digital competence." (27).

This means that currently only a few innovative players with smaller satellite ideas are actively trying to advance digitalization and telemedicine in veterinary medicine and there is a lack of large-scale solutions. Professional associations such as the BTK (German Veterinary Association) or the BPT (German Association of Practicing Veterinarians) have taken the first steps and published a guideline on the use of telemedicine in the veterinary profession. In addition, more digital competence must be taught and the use of telemedical tools must be encouraged in the field of education and training.

In summary, telemedicine comprises only a small part of the veterinary service, namely the provision of medical information when doctors and patients are physically distant and the exchange of medical information occurs across distances. In view of the general digitalization of many processes in medicine, telemedicine should be considered as an essential but not the only part of this development.

Functioning, effective telemedical services can only be used adequately and in a result-oriented manner in a digital and professional environment. Corresponding tools are already available on the market and are constantly being further developed.

Integration of digital technologies can improve many parts of the veterinarian work in all kinds of practices. At the same time, however, the willingness of veterinarians and their clients to use these tools must be promoted (17).

Many new opportunities are already given, such as artificial intelligence and wearable home diagnostic devices. If the framework is clear and easy to adapt, telemedicine is a huge option rather than a threat, as it can establish a state-of-the-art connection between doctors, animal owners and animals to raise the overall health of animals. "Ignoring it would be deleterious for the future of the veterinary profession" (17).

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by Universities Ethics Committee and approved prior to investigation. The participants provided informed consent to participate in this study. This study was conducted according to the ethical standards of the Stiftung Tierärztliche Hochschule Hanover, Germany. The Doctoral Committee of the University, validated the project according to the ethical guidelines for research with human participants and approved the study. The university's data protection officer approved the project. The voluntary participants consented to the processing of their data according to the EU General Data Protection Regulation of 2018 (General Data Protection Regulation Art. 6 I 1 lit. e i.V.m. 89 and Lower Saxony Data Protection Act § 3 I 1 No. 1 NHG, § 13). Data processing was carried out anonymously in accordance with the University's data protection regulations.. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

BB designed and conducted the study, performed formal analysis, and prepared the original draft manuscript. AT, JE, and CK reviewed the study design. CK supervised the study and validated the analysis.

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Evaluation of acoustic pulse technology as a non-antibiotic therapy for bovine intramammary infections: Assessing bacterial cure vs. recovery from inflammation

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Introduction: The spread of antimicrobial resistance (AMR) is a major threat to human and animal health. Therefore, new solutions are needed to prevent returning to a world without effective antibiotics. Mastitis in dairy cows is a major reason for antimicrobial use in food animal production, and mastitis-causing bacteria have the potential to develop AMR. In this study, acoustic pulse technology (APT) was explored as an alternative to antimicrobials for the treatment of mastitis in dairy cows. APT involves the local transmission of mechanical energy through soundwaves which stimulate anti-inflammatory and angiogenic responses in the udder. These responses promote udder recovery and enhance resistance to bacterial infections.

Methods: We examined 129 Israeli dairy cows with mastitis in this prospective, controlled study to assess the efficiency of APT treatment on *cure* and *recovery* rates. An accurate diagnosis of suspected or confirmed infectious mastitis was made from cows having clinical signs of mastitis and/or somatic cell count (SCC) of above 400,000 cells/mL. The cows were divided into three groups: Group 1 ($n = 29$), cows with no bacterial findings (NBF); Group 2 ($n = 82$), cows with clinical signs of mastitis or SCC >400,000 cells/mL in the most recent test; and Group 3 ($n = 18$), cows with chronic mastitis (two or more tests with SCC >400,000 cells/mL within 3 months). All the cows received APT treatment, which involved 400 pulses on two sides of the infected quarter, delivered in three phases over 3 days. The *cure* for the mammary gland was indicated by the absence of bacterial growth in post-treatment cultures and *recovery* by a decrease in SCC to <250,000 cells/mL in two of three post-treatment tests.

Results and discussion: In Group 2, cure and recovery rates were 67.1 and 64.6%, respectively, and were not significantly different between Gram-negative and Gram-positive infections. A similar recovery rate was found in NBF cows. However, in cows with chronic mastitis, both the cure and recovery rates were significantly lower (22.2 and 27.8%, respectively). These results have important implications for dairy farmers, as APT treatment could lead to substantial savings of up to

$15,106/year in a 100-cow herd, considering the national estimated prevalence of mastitis and the cost of individual treatment. APT should be further investigated as a viable and sustainable alternative to antimicrobial therapy for mastitis, offering economic benefits to dairy producers and the possibility of preventing AMR.

KEYWORDS

acoustic pulse technology, mastitis, bacteriology, cure, recovery, dairy cow, intra-mammary infection, antibiotic alternatives

1. Introduction

Mastitis is a common disease among dairy cows and a major problem in dairy production worldwide. It is the leading cause of antibiotic use in cows, and the high prevalence of mastitis may contribute to the development of antibiotic resistance (1, 2). The main cause of mastitis in dairy cows is an intramammary bacterial infection. While various bacterial species may cause mastitis in dairy cows, most cases involve a single species. Clearing the infecting bacteria from the mammary gland, either naturally or through treatment, is key to the healing process. The term "cure" is often used to address bacterial clearance in mastitis, i.e., no bacteria were isolated in the follow-up tests after the infecting pathogen had been identified in the first test. It is used mainly to assess the success of antibiotic treatments. However, tissue damage caused by either the infecting bacteria or the inflammation process, as well as the detrimental effects of mastitis on udder function and milk quality, may persist long after bacterial clearance. Therefore, *cure* and actual *recovery* of the mammary gland to its physiological status prior to mastitis are distinct outcomes. In many cases, the mammary gland remains inflamed, with reduced milk yield and altered milk quality regardless of bacterial cure (3). Continuous decreases in milk yield and quality can often result in the farmer choosing to dry off the affected gland or even cull the animal for economic losses.

Acoustic pulse technology (APT) has been recently adapted specifically for the treatment of mastitis in dairy cows (4). APT uses repeated projectile collision with an anvil connected to the treatment head to produce low-incidence shockwaves (acoustic pulses), which are transferred non-invasively to the affected mammary gland tissues. The main goal of APT treatment is to trigger the body's self-healing mechanisms to repair damaged tissues (5). In terms of mastitis, APT mainly aims to promote the *recovery* of the mammary gland, that is, a decrease in somatic cell count (SCC) back to the normal level (<250,000/mL) within days or weeks, without a major decrease (<10%) in milk yield (for at least two of three follow-up monthly milk measurements) following treatment. APT also promotes increased activation of the immune system (6). Hence, a secondary goal of APT treatment is to facilitate bacterial clearance or cure. (7). Currently, antibiotics are becoming less available to farmers due to consumers' growing awareness of animal welfare and the spread of bacteria with antimicrobial resistance (AMR) (8, 9). Therefore, APT could be an alternative to antibiotic treatment in bovine to treat mastitis, especially during lactation, when antibiotics must be evidence-based to encourage their judicious use (10) or when their cost-effectiveness is limited due to the retrieval time.

The present study was built upon a previous large-scale retrospective analysis that examined data from the Israeli Dairy Herd Book, which included 123,985 cows from 650 herds. The previous study evaluated two types of mastitis recovery (i) "spontaneous recovery" from mastitis (inflammation) and (ii) recovery from mastitis following antibiotic use (11). With the SCC threshold set at <100,000 cells/mL, only 10% of the cases showed spontaneous recovery, while with a threshold of <250,000 cells/mL, spontaneous recovery was observed in approximately 25% of cases; no significant difference was found between the rates of "spontaneous recovery" and recovery following antibiotic treatment. In addition, a retrospective analysis was performed that compared the effectiveness of APT treatment against no treatment in the cows with subclinical mastitis in commercial dairy farms. The analysis evaluated various factors, such as cure and recovery rates, culling rates, milk yield, and economic impact. It was found that the cows treated with APT had significantly higher cure and recovery rates [Lavon et al., (11) submitted]. However, the latter study had a major limitation regarding the assessment of cure rates due to a partial lack of follow-up tests. Specifically, the cows with a decrease in SCC and no sign of mastitis were not tested by bacteriological culture for the cure. This limitation was addressed in the present study, which aimed to assess, in a prospective trial, the effect of APT treatment in cows with clinical and subclinical mastitis on the cure and recovery rates and the correlation between them.

2. Materials and methods

2.1. Study layout

The study involved 129 Israeli Holstein cows from three commercial dairy herds. These cows had clinical and subclinical mastitis, with an average of 111 days in milk and 2.8 in lactation numbers, and no differences were observed between the three herds. Herd 1 had 700 lactating cows (Hafer dairy farm, Kibbutz Haogen, Israel), herd 2 had 1,200 lactating cows (Haemek dairy farm, Kibbutz Yifat, Israel), and herd 3 had 700 lactating cows (Nofim dairy farm, Kibbutz Gazit, Israel) (farm names mentioned with consent). The cows were housed in open sheds, fed a total mixed ration of 16.8% crude protein and 1.75 Mcal/kg dry matter, and milked three times daily, yielding approximately 12,500 L of milk at 305 days of lactation. The cows were selected for the study when they showed clinical signs of mastitis or subclinical mastitis identified by increased SCC (>400,000 cells/mL) in the routine monthly milk recording of the Dairy Herd Improvement

Program (DHI). The California Mastitis Test was used to identify the specifically affected quarters with subclinical mastitis. Milk samples were aseptically collected by trained farm personnel and tested at the "mastitis control" laboratory of the Israeli Dairy Board (Caesarea, Israel) and the bacteriology laboratory at the Kimron Veterinary Institute (Bet Dagan, Israel) for the presence and identification of the infecting bacteria. The cows were treated with APT and re-sampled for bacteriological culture after 20–25 days. The APT protocol involved adding 400 pulses on two sides of the infected quarter, delivered in three phases for 3 days at 2–3 day intervals (4, 12).

The analyzed data included information about the lactation number, days in milk (DIM), daily milk yield, SCC at the time of treatment, SCC and daily milk yield of the two previous and up to three monthly DHI milk tests after treatment (Herd Book of the Israeli Cattle Breeders Association, Caesarea, Israel), and management decisions regarding dry-off of infected glands or culling of cows. The cows were divided into three groups based on bacteriology results and mastitis type: Group 1 ($n = 29$), cows with clinical or recent subclinical mastitis (i.e., increased SCC in the last DHI test prior to treatment) and no bacterial finding (NBF) before treatment; Group 2 ($n = 89$), cows with clinical or recent subclinical mastitis and a bacterial finding; Group 3 ($n = 18$), cows with chronic mastitis (i.e., increased SCC in more than one DHI monthly milk test before the study). The groups were first analyzed separately to assess the *recovery* rate for each. Then, a second analysis was performed on Groups 2 and 3 to assess bacterial *cure* rates and their correlation with *recovery*. Bacterial species were grouped as either Gram-positive or Gram-negative. The *recovery* rate was calculated based on SCC at up to 3 months post-treatment, using SCC <250,000 cells/mL as the threshold. A cow was considered "recovered" if its SCC was lower than this threshold on two out of the three test days post-treatment. The *cure* rate was defined as the absence of the bacterial species found in the milk sample before APT treatment in the same quarter, but for a more stringent analysis, even the growth of a different bacterial species was not considered a cure.

2.2. Statistical analysis

All statistical analyses were performed using the GLIMMIX procedure of SAS (SAS Institute, version 9.1, 2009). The analyses were conducted separately to calculate cure and recovery rates. To calculate recovery rates for all three groups ($n = 129$), we used the general form: recovery = herd + group + parity + DIM + error, where herd = the three different dairy farms, Group = 1 to 3 as described above, parity = 1st, 2nd, or 3rd and more lactations, and DIM = days in milk as a continuous effect. To calculate cure rates in Groups 2 and 3 (n = 100), we used the general form: cured = herd + Group + parity + DIM + error, where: herd = the three different dairy farms, Group = 2, 3 only, parity = 1st, 2nd, or 3rd and more lactations, and DIM = days in milk as a continuous effect. The third analysis tested cure rates according to bacterial type (Gram-negative, $n = 23$, or Gram-positive, $n = 77$) with the model: cured = bacterial type + parity + DIM + error, where bacterial type = Gram-positive or Gram-negative, parity = 1st, 2nd or 3rd, and more lactations, and DIM = days in milk as a continuous effect. Linear correlations between cured and recovered according to the bacterial type were conducted with the PROC CORR procedure of SAS (SAS Institute, version 9.1, 2009), and the data were presented as means and SEM.

3. Results

Neither the herd nor parity or DIM impacted cure or recovery rates. Before APT treatment, 100 cows were identified with an intramammary bacterial infection. Of these, 82 cows had clinical or recent subclinical mastitis (Group 2), 18 cows had chronic subclinical mastitis (Group 3), and 29 cows with clinical or recent subclinical mastitis had no bacterial findings (Group 1).

The bacterial cure rate at 20–25 days after APT treatment and mastitis recovery rates, including Groups 2 and 3 ($n = 100$ cows), are summarized according to bacterial species in Table 1. The overall cure rate was 59%, with a higher rate for Gram-negative bacteria (77.7%) compared to Gram-positive bacteria (40.0%) ($P < 0.02$). The overall recovery rate was similar to the cure rate, showing the same trend, with a higher rate for Gram-negative bacteria (81.0%) compared to Gram-positive bacteria (35.2%). The relationship between bacterial cure and mastitis recovery was weak, with cure and recovery occurring simultaneously in only 44% of the cows.

The second analysis examined each group separately because there was an interaction between Groups 2 and 3 and Gram-negative and Gram-positive findings. The overall cure and recovery rates for Group 2 were 67.1 and 64.6%, respectively, exhibiting a weak relationship (0.35, Table 2). The cure rates for Gram-negative and Gram-positive bacteria (85.7 and 60.7%, respectively) were not statistically significant ($P > 0.05$), and the same was true for the recovery rates (76.2 and 60.7%, respectively). The results for recovery in Group 1 (NBF) were similar, with a rate of 72.4%. The cure rate could not be determined due to the absence of bacterial isolation prior to treatment. In contrast, the cure and recovery rates for Group 3 (chronic subclinical mastitis) were significantly lower, at 22.2 and 27.8%, respectively, with a correlation of 0.56. Only Gram-positive bacteria were isolated from the cows in Group 3.

The trends in SCC are depicted in Figure 1. The mean SCC before treatment in Group 2 and Group 1 was <log 5, increasing to >log 6 and returning to log <5.5 (i.e., <250,000 cells/mL) for the three DHI monthly milk recordings. Milk yield in these groups increased after treatment within 2 months following treatment (Figure 2). In contrast, the trend in SCC and milk yield was different for Group 3 (chronic subclinical mastitis). The mean SCC in the 2 months before treatment was >log 6; on average, SCC levels were similar after and before treatment. However, daily milk yield gradually decreased after treatment.

4. Discussion

The main factors affecting the successful treatment of mastitis are pathogen species, host factors such as genetics, the immune and physiological status that affect the host's ability to cope with the infecting pathogen, and the time from infection establishment

TABLE 1 Bacterial species found in Groups 2 and 3 ($n = 100$) before APT treatment, rates of same or different findings following treatment, respective cures at 20–25 days, and recovery rates up to 3 months.

Bacteriology finding	Pretreatment (n)	Post-treatment		Cure[a]	Recovery
		Same species isolated[c]	Different species isolated[c]		
Escherichia coli	22	4.5% (1)	13.6% (3)	81%	
Pseudomonas aeruginosa	1	100% (1)		0%	
Total Gram-negative	**23**			**78.3% (18)**	**82.6% (19)**
Streptococcus dysgalactiae	15	26.7% (4)		73.3%	
Streptococcus uberis	15	46.7% (7)	13.3% (2)	40%	
Enterococcus spp.	7	85.7% (6)		14.3%	
Total *Streptococcus* spp.	**37**			**48.6% (18)**	**43.2% (16)**
Staphylococcus haemolyticus	14	57.1% (8)		42.8%	
Staphylococcus chromogens	10	30% (3)	50% (5)	20%	
Other coag-neg. *Staphylococcus*[b]	14	14.3% (2)		85.7%	
Total *Staphylococcus* spp.	**38**			**52.6% (20)**	**57.9% (22)**
Trueperella pyogenes	2		100% (2)	0%	
Total Gram-positive	**77**			**49.4% (38)**	**49.3% (38)**
Total	**100**			**56% (56)**	**57% (57)**

[a] Cure = No bacteria were isolated following treatment.

[b] *Staphlococcus epidermidis* (3), *Staphlococcus hyicus* (1), *Staphlococcus simulans* (1), *Staphlococcus microti* (1), and unidentified coagulase-negative staphylococci (8).

[c] Percentage and number of cases in which the same bacterial species listed in "Bacteriolgy finding" was isolated after treatment or a different species.

Bold values are a total for the corresponding pathogen group.

to treatment initiation. "Spontaneous cure"—when the pathogen is eliminated by the mammary gland immune system without external intervention—occurs only in ∼20% of cases (11), but this rate varies greatly depending on the infecting bacteria. Antibiotic therapy is the most common method of eliminating intramammary infections (1, 2, 13) with various degrees of success. However, the extensive use of antibiotics in treating and preventing intramammary infections may contribute to the development of AMR (14). With increasing consumer awareness and regulatory action aimed at preventing the spread of AMR bacteria, such as the European Union's strategies and action plans for reducing antibiotic overuse (15), antibiotics are becoming less available for use in livestock production, including mastitis therapy (9, 16).

Moreover, due to the high cost of antibiotic treatment during lactation (due to the need to discard milk for the retention period), many cows without a life-threatening infection are treated only at dry-off, thus becoming chronically infected. In these chronically infected cows, tissue damage worsens over time, resulting in lower milk yield and quality (17–20). These cows are the hardest to treat, and even antibiotic therapy often fails to clear the infection.

In the present study, we emphasized the difference between bacterial ***cure*** and ***recovery*** from mastitis. Cure relates to the clearance of infection, often measured as a lack of bacterial isolation in follow-up tests, for example, after treatment. Cure rates reported following antibiotic therapy differ greatly (from 4 to 92%) depending on the pathogen species, the antibiotics studied, and the methods used to detect and follow-up intramammary infections (and their cure), such as the number of samplings and the laboratory technique used for the isolation and identification of pathogens (21). When the cause of mastitis was Gram-negative bacteria, antimicrobial treatment did not improve the outcome of the infection (22, 23).

Healing (recovery) is usually less studied. In the context of mastitis, recovery can be measured by inflammation parameters, with SCC being the most common and well-established one. Because SCC is also used as a milk quality parameter, it has a direct economic impact on the farm. The mammary gland often remains inflamed for a long time following mastitis, regardless of the presence of the infecting bacteria that triggered the inflammation in the first place (24, 25). This, in turn, leads to prolonged lower milk yield and quality and the eventual need to dry off the affected quarter or even cull the cow, based on the farmer's cost-benefit judgment (26–28). In a review by Ruegg (29), it was concluded that "associations between bacteriological cure and clinical outcomes are very weak" and "evaluation of continued decline in quarter-level SCC appears to be the most reliable indicator of success." If the mammary gland tissue has suffered extensive destruction or damage caused directly by the pathogen or the inflammation, milk production and its quality can be affected for a long time despite a bacterial cure. Thus, even though bacterial clearance from the mammary gland is key to healing, it does not ensure the total recovery of the gland's physiological status.

The effectiveness of using APT to treat mastitis in dairy cows has been recently explored (4, 7, 12, 11, submitted), with the major goal of promoting recovery from inflammation rather than curing an intramammary infection. The results in these studies showed 65–75% recovery of cows, which is similar to the results of the present study for Groups 1 and 2 (clinical and recent subclinical mastitis, either with or without detected infection). Although cure was attained in most cows in Group

TABLE 2 The results of the generalized mixed model used to estimate the effect of APT treatment on cure and recovery rates in the cows ($n = 129$, 3 herds) with clinical or subclinical mastitis caused by Gram-negative or Gram-positive bacteria or without bacterial findings.

			Cure[a]				Recovery[b]			
Variable	Level	Cows #	LSM diff[c]	REF level[d]	*P*-value	SE	LSM diff	REF level	*P*-value	SE
Herd	1	18	-	0.667	-	-	-	0.709	-	-
	2	41	−0.188		0.967	0.136	−0.107		0.685	0.1
	3	41	−0.009		1	0.13	−0.174		1	0.095
Parity	1	25	-	0.554	-	-	-	0.694	-	-
	2	19	0.104		1	0.16	−0.08		1	0.117
	3+	56	−0.043		1	0.114	−0.152		0.559	0.082
Bact_BT[e]	NBF	-	-	-	-	-	-	0.724	-	-
	Gram+	78	-	0.4	-	-	−0.186		0.54	0.057
	Gram-	22	0.377		0.019	0.039	0.003		0.99	0.097
Group	1	-	-	-	-	-	-	0.724	-	-
	2	82	-	0.81	-	-	−0.078		0.954	0.053
	3	18	−0.458		0.005	0.181	−0.446		0.05	0.109

[a] Cure: No bacteria were isolated following treatment.

[b] Recovery: Decrease in SCC in 2 out of 3 monthly tests post-treatment to <250,000 cells/mL.

[c] Values in the Least Square Means (LSM) column are deviations from the reference levels.

[d] Reference levels for comparison of cure or recovery rates.

[e] Bact_BT: bacteriological finding before treatment.

Subclinical mastitis was determined by the pattern and level of SCC with a threshold of 400,000 cells/mL.

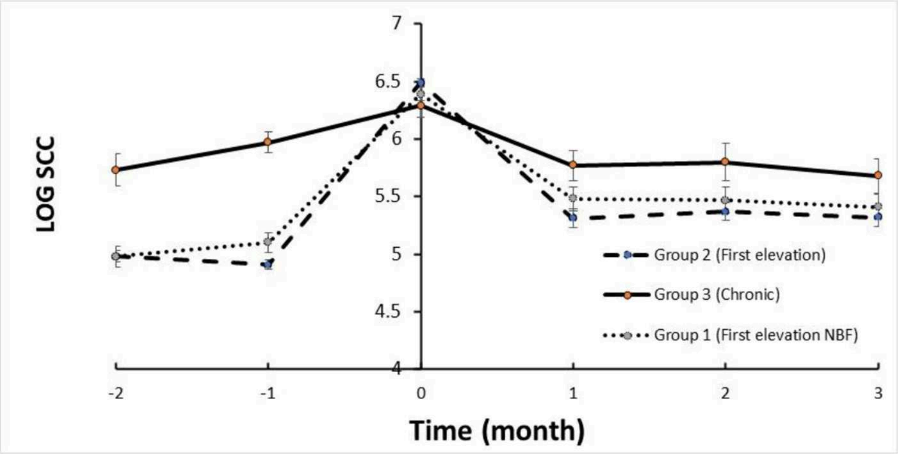

FIGURE 1
Mean log of SCC relative to APT treatment according to groups.

2 (67%), we found a low correlation with actual recovery (0.35). In ∼14% of the cows in this group, bacterial infection was still detected, regardless of the recovery of the gland (based on the parameters of udder health used here), whereas in approximately 14% of the cows, no infection was further detected, but mammary gland health did not return to normal. In this study, the importance of the timing of treatment is emphasized by the results observed in Group 3, which included cows with chronic subclinical mastitis for at least 3 months. In these cows, both the cure and recovery rates were significantly lower (about 27%). Moreover, the significant difference in cure rates in cases caused by Gram-positive bacteria between Groups 2 and 3 (i.e., between clinical or recent subclinical and chronic mastitis) highlights the notion that timing of mastitis identification and prompt treatment may be more important factors than bacterial type.

The results of the present study indicate that APT not only promotes tissue recovery after inflammation but also aids in bacterial clearance, helping to decrease the extent of tissue damage in the mammary gland and limit the spread of pathogens between cows and into the milk tank. A bacterial cure may be due to activation of the immune response, increased vascularization in

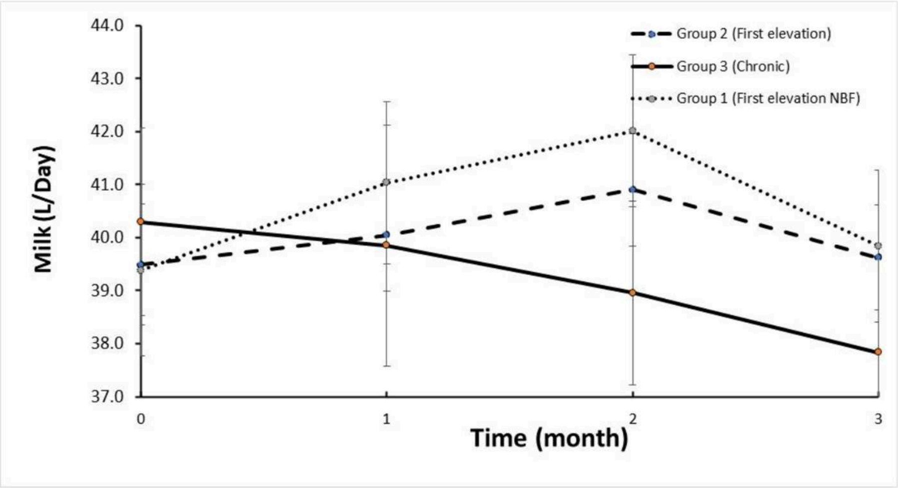

FIGURE 2
Average daily milk yield (L/day) relative to APT treatment according to groups.

the mammary gland, or both. Thus, APT could be of value to the farms' current mastitis control programs and improve milk quality. For instance, an economic cost-benefit analysis was performed for treating subclinical mastitis with APT compared to no treatment [Lavon et al., (11) submitted]. For this estimate, the milk yield of a regular Israeli herd was estimated at 40 L/day on average, or 12,200 L/305 days, with an 18% prevalence of subclinical mastitis. The milk price was fixed at USD$ 0.32/L, and the price for a replacement heifer was fixed at USD$ 1,500. Under these parameters, the present findings suggest that APT could save up to USD$ 15,106/year in mastitis control costs in a 100-cow herd.

The results of the present study highlight the importance of timely treatment of mastitis in dairy cows, as delays between detection and treatment, can significantly impact the success rate of the treatment. As APT is non-invasive and does not involve antibiotics, treatment can be performed during lactation soon after the detection of mastitis, thus potentially increasing the likelihood of success. Timely treatment can reduce the risk of antibiotic residues in milk and the need to dispose of waste milk. Additionally, early treatment can help reduce economic losses due to elevated SCC levels in bulk milk and improve animal welfare throughout the lactation period. Furthermore, early mastitis treatment can decrease the need for antibiotics later on during dry-off therapy, reducing the overall use of antibiotics in dairy production.

In addition to milk quality and safety for human consumption, the use of antibiotics to treat mastitis can affect the development and shedding of antibiotic-resistant bacteria in a herd. Previous research suggests that dry-cow therapy may increase the levels of antibiotic-resistant fecal bacteria in dairy cows (30). Furthermore, it is a common practice to feed calves waste milk, including milk that had to be discarded due to antibiotic treatment during lactation. Existing evidence shows that this can lead to an increase in the shedding of ESBL-producing *Escherichia coli* in calves (31). Hence, the risk of increasing the burden of antimicrobial resistance (AMR) due to antibiotic use for mastitis is multifaceted. Non-antibiotic alternatives to treat mastitis in dairy cows that reduce antibiotics in dairy products are beneficial in combating AMR from a broader "One Health" perspective. Selective dry-off therapy, which involves administering antibiotics only to infected mammary glands at the end of lactation, is one tool that has been proven to decrease the overall use of antibiotics in dairy farms (32). APT may have similar benefits. However, as APT is not an antibiotic, it does not necessarily require bacterial culture results for application. Therefore, APT should be explored further as an alternative therapy for mastitis in dairy cows.

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 Kimron Veterinary Institute institutional committee for ethical animal experimentation. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

Study design, data analysis, and manuscript writing: SB and GL. Field work: AZ, YH, OG, DG, and TS. Laboratory work: OK, LW, and MF. Statistical analysis and graphics design: YL. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The APT apparatus and protocol used in this study were developed and provided by Armenta, Ra'anana, Israel. DG, TS, and GL are employees at Armenta. YL was employed by the company Israel Cattle Breeders Association. AZ was employed by the company Gal-Yam Dairy Farm. YH was employed by the company Nofim Dairy Farm. OG was employed by the company Haemek Dairy Farm.

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

Publisher's note

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“Sniffing” out SARS-CoV-2 in Arizona working dogs: an exploratory serosurvey

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Susceptibility to and infection with SARS-CoV-2 in companion animals has been well-documented throughout the COVID-19 pandemic. Surveillance for the virus in dogs has largely been focused on household pets; however, other canine populations may also be impacted. We partnered with a local veterinary hospital with a high working dog patient volume to conduct viral and neutralizing antibody testing in working dogs and identify potential risk factors in the dog’s work and home environments. Surveillance of SARS-CoV-2 in law enforcement and security working dogs in Arizona found 24.81% (32/129) of dogs to be seropositive. Thirteen dogs presenting with clinical signs or with reported exposure to COVID-19 in the 30days prior to sample collection were also tested by PCR; all samples were negative. 90.7% (n=117) of dogs were reported to be asymptomatic or have no change in performance at the time of sampling. Two dogs (1.6%) had suspected anosmia as reported by their handlers; one of which was seropositive. Known exposure to the dog’s COVID-19 positive handler or household member was identified as a significant risk factor. Demographics factors including sex, altered status, and type of work were not associated with canine seropositivity. Further work is warranted to understand the impact of SARS-CoV-2 and other infectious diseases in working dogs.

KEYWORDS

SARS-CoV-2, working dogs, neutralizing antibodies, surveillance, detection dog, police dog

Introduction

Working dogs play a critical role in supporting the health and safety of the public. These dogs are trained to perform specific tasks and are employed in meaningful work, including protection, detection, and therapy (1). Protection dogs are employed in roles including police patrol and hospital security, while detection dogs are trained specifically in narcotic or explosives detection, and search and rescue. Dogs are also involved in therapeutic roles such as crisis response. Working dogs often go through initial training with their handler, who is then responsible for their continued training (2). Though some live in agency-run kennels, many working dogs live with their handler and often interact with members of the household.

During the COVID-19 pandemic, susceptibility to and infection with SARS-CoV-2 was well-documented in companion animals, including dogs (3, 4). Evidence continues to show that the risk of companion animals spreading the virus to people is low, but that dogs can become exposed through close contact with infected people (5–7). Dogs can have asymptomatic SARS-CoV-2 infection or develop a range of respiratory and gastrointestinal signs. The full extent of SARS-CoV-2 clinical presentation in dogs is still unclear, including whether they experience a loss in their sense of smell, a hallmark sign of COVID-19 in people specifically associated with the Alpha and Delta variants (8). While scent detection abilities may not be as important for pet dogs, working dogs rely on this function to carry out their daily duties (e.g., detecting illicit substances or locating missing persons).

Surveillance for SARS-CoV-2 in dogs has largely been focused on household pets. While surveillance has been conducted for a variety of infectious diseases, such as bartonellosis and Chagas in working dogs (9, 10), efforts to understand SARS-CoV-2 in this population have been minimal. The main objectives of the study were to (1) conduct viral and neutralizing antibody testing in working dogs to determine how the SARS-CoV-2 virus impacts this population, and (2) identify potential risk factors associated with exposure to the virus during interactions in their work and home environments. Additional aims included identifying whether a change in overall performance, including scent detection was observed, in dogs exposed to SARS-CoV-2, and understanding the short and long-term implications.

Methods

Recruitment and sample collection

Dogs were recruited from a veterinary hospital in Maricopa County, Arizona, which experiences a high working dog patient volume. Approval for this work was granted by TGen's Animal Care and Use Committee (#20163) and the ADHS's Human Subjects Review Board (#20-0017). Written consent was received from the handlers of the dogs sampled.

Working dogs presenting to the veterinary hospital for routine examinations, vaccinations, or clinical illness were recruited by the veterinary hospital staff. Serum collection via venipuncture was performed on all dogs; these samples were initially centrifuged and stored in the refrigerator for 48–72 h. Working dogs with known exposure to COVID-19 within the past 30 days or dogs exhibiting signs compatible with viral infection also had nasal and rectal swabs collected. Swab samples were placed in conical vials with 2mls of sterile phosphate-buffered saline and frozen until shipment. A short questionnaire was administered to collect demographic information (e.g., dog breed, age, and sex), type of work the dog was involved in, COVID-19 exposure status, and any signs or symptoms observed by the handler.

SARS-CoV-2 neutralizing antibody and viral testing

Serum was centrifuged at 2,500 rpm for 10 min upon arrival, aliquoted and tested for the presence of viral neutralizing antibodies (vNAbs) using the GenScript cPass™ SARS-CoV-2 Neutralizing Antibody assay per the manufacturer's instructions (11). This is a competitive enzyme-linked immunosorbent assay (ELISA); presence of vNAbs will prevent the binding of the receptor-binding domain to the angiotensin-converting enzyme 2 coated wells, resulting in low optical density (OD). OD values were processed in R to produce inhibition (INH) percentage values (12). A sample was deemed positive if the INH value was ≥30% (11).

SARS-CoV-2 genomic material was extracted from nasal and rectal swab samples using Zymo DNA/RNA extraction kits. Extracted material was tested using a previously described real-time polymerase-chain reaction (rRT-PCR) assay (4, 13), developed and validated in-house, which targets the N and S protein of the virus. Samples were screened on the Bio-Rad CFX96 instrument after cDNA synthesis and denaturation; cycle threshold (Ct) values were generated to determine qualitative results. A sample was considered positive if Ct values for both targets were 38 or below.

Descriptive statistics were determined for breed, age, sex, altered status, type of work, health status, COVID-19 exposure status, SARS-CoV-2 serology, and PCR testing. Chi-square analysis was employed to assess the relationships between serology results and COVID-19 exposure status using SAS version 9.4.

Results were reported back to the veterinary hospital and the collaborating veterinarian communicated those to the handlers of the working dogs. If a working dog tested positive, recommendations regarding COVID-19 prevention strategies were provided to the handler. Handlers of seropositive dogs were also invited to participate in a semi-structured interview with project staff to gain a better understanding of canine medical history, household dynamics, and home and duty environments (14, 15).

Results

During November 2021 through June 2022, 129 working dogs were recruited into the study. Serum samples were collected from all 129 enrolled dogs. Paired nasal and rectal swabs were collected on 13/129 dogs (10.1%) with known exposure to COVID-19 within the past 30 days or if the dog exhibited signs compatible with viral infection. Repeat sampling was performed on 4/13 dogs (3.1%) due to new exposures to COVID-19 positive people and to confirm serology results.

Table 1 summarizes the demographics of the working dogs enrolled. Dogs ranged in age from 9 months to 10 years (average 4.5 years). Belgian Malinois ($n = 78$) and German Shepherds ($n = 15$) were the most common breeds of dog, comprising 72.1% of the study population, although other breeds were represented. A majority of the dogs ($n = 108$) were intact males (63.6%) or spayed females (20.2%). Police patrol and detection was the most common role (43.4%, $n = 56$), followed by detection only (24.8%, $n = 32$), hospital patrol/security (16.3%, $n = 21$), and police patrol only (12.4%, $n = 16$).

90.7% ($n = 117$) of sampled dogs were reported to be asymptomatic or have no change in performance as noted by their handler at the time of sampling. Twelve dogs (9.3%) had mild clinical signs, including vomiting, diarrhea, coughing, lethargy, and decreased performance. Six of the 12 dogs were symptomatic at the time of sampling and 6/12 dogs had symptoms previous to collection but within the month after handler's reported COVID-19 infection. Two of these dogs were diagnosed with Valley Fever. Of the 12 dogs, 1.6% ($n = 2$) were reported by their handlers to have "missed known finds," indicating potentially compromised scent detection ability (i.e., suspected anosmia). Evidence of neutralizing antibodies was present in 24.8% ($n = 32$) of dogs; one (3.1%) of which had suspected anosmia. All 13 dogs tested by PCR was negative.

TABLE 1 Characteristics and SARS-CoV-2 testing results of working dogs in Arizona (N=129).

Characteristics/testing results	Number (%)
Age	
≤3 years	42 (32.6)
4–6 years	58 (44.9)
7–10 years	29 (22.5)
Breed	
Belgian Malinois (pure or mixed)	78 (60.5)
German Shepherd (pure or mixed)	15 (11.6)
Retriever (Labrador, Golden, or mixed)	21 (16.3)
Other (e.g., Bloodhound, Dutch Shepherd, Pointer)	15 (11.6)
Sex/altered status	
Intact male	82 (63.6)
Intact female	4 (3.1)
Male/neutered	17 (13.2)
Female/spayed *sex/altered status*	26 (20.1)
Type of work	
Police patrol (only)	16 (12.4)
Police patrol and detection	56 (43.4)
Detection* (only)	32 (24.8)
Hospital patrol/security**	21 (16.3)
Other (e.g., emotional support, crisis response)	4 (3.1)
Health status of dog (at time of sampling or after handler's COVID-19 infection)	
Asymptomatic	117 (90.7)
Respiratory (e.g., coughing, difficulty breathing)	4 (3.1)
Gastrointestinal signs (e.g., vomiting, diarrhea)	3 (2.3)
Decreased scent detection	2 (1.6)
Other (e.g., general lethargy, decreased performance)	3 (2.3)
*Known exposure to SARS-Cov-2****	
Yes	69 (53.5)
No/unknown	60 (46.5)
Evidence of SARS-CoV-2 vNAbs	
Yes	32 (24.8)
No	97 (75.2)
SARS-CoV-2 viral PCR detection	
Yes	0 (0.0)
No	13 (10.1)
Not tested by *SARS-CoV-2 PCR*	116 (89.9)

*Includes search and rescue, narcotic, bomb, pest, and cadaver detection.

**Includes two explosive detection dogs in the hospital setting.

***Includes exposure to a person with COVID-19 compatible symptoms or a person with a COVID-19 positive test.

53.5% (69/129) of working dogs had known or suspected exposure to SARS-CoV-2 (e.g., handler had COVID-19), while 46.5% (60/129) had no documented exposures in their home or duty environment (Table 1). Of the 69 dogs with known exposure, 43 (62.3%) had an exposure within the 6 months prior to sampling (range 1–14 months, median 3–6 months between exposure and sampling). Assessment of exposure to SARS-CoV-2 specifically among the 32 seropositive dogs showed that 22 (68.8%) dogs had documented exposure, while 10 dogs (31.3%) did not. Working dogs with known or suspected exposure were more likely to test seropositive compared to those with no reported exposure based on chi-square analysis (68.8% vs. 31.3%, $\chi^2 = 3.9525$, $p < 0.05$), illustrated in Figure 1. Demographics factors including sex, age, altered status, and type of work were not associated with canine seropositivity. The timeframe between known SARS-CoV-2 exposure and sample collection date was also not found to be statistically significant.

Project staff conducted in-depth interviews with handlers for 40.6% (13/32) of seropositive dogs. All 13 dogs lived in the home of their handler; 92.3% were single family homes and 76.9% of homes had additional household members. Documented or suspected exposure to SARS-Cov-2 in the home environment was identified for all 13 (100%) dogs. It was further reported that these dogs, when off duty, had frequent and close interactions with their handler and other household members. While in a duty environment, 69.2% of dogs had regular interaction with their human co-workers (e.g., other law enforcement officers), but less than a quarter (23.1%) of dogs interacted with members of the public (e.g., during community events or in hospitals). Interviews also identified a recent history of valley fever in four (30.8%) dogs.

Discussion

Working dogs bring diverse and critical skill sets to the law enforcement, military, and search and rescue environment, ultimately helping to protect the health and safety of the public (2). Given the nature of their work, these dogs are at risk of potential exposure to diseases and other health threats. Since the beginning of the pandemic, numerous studies have conducted SARS-CoV-2 surveillance in companion animals (3, 4, 6), however, to the authors knowledge, this is the first study to include a working dog cohort.

The authors originally hypothesized that working dogs may have a higher risk of SARS-CoV-2 exposure (and therefore likelihood of infection) due to their unique and expanded interactions in their duty environment compared to pet dogs. However, the results of this study are comparable with previous surveillance studies in pet dogs (3, 6, 13) and continue to support that close contact with people in a home or work environment (in this case COVID-19 positive handlers), are likely the main driver of SARS-CoV-2 exposure in dogs. The type of work and duty environment did not play a significant role in seropositivity of enrolled working dogs, which further disproves the original hypothesis. However, 31.3% (10/32) of dogs that were seropositive did not have a documented exposure. Of these 10 dogs, five were dual purpose (police patrol and detection), three were detention only, one did police patrol work only, and one was engaged in hospital security. This work highlights that working dog exposure to COVID-19 could occur unknowingly. Additionally, the true clinical impact and symptom manifestation, specifically loss of smell

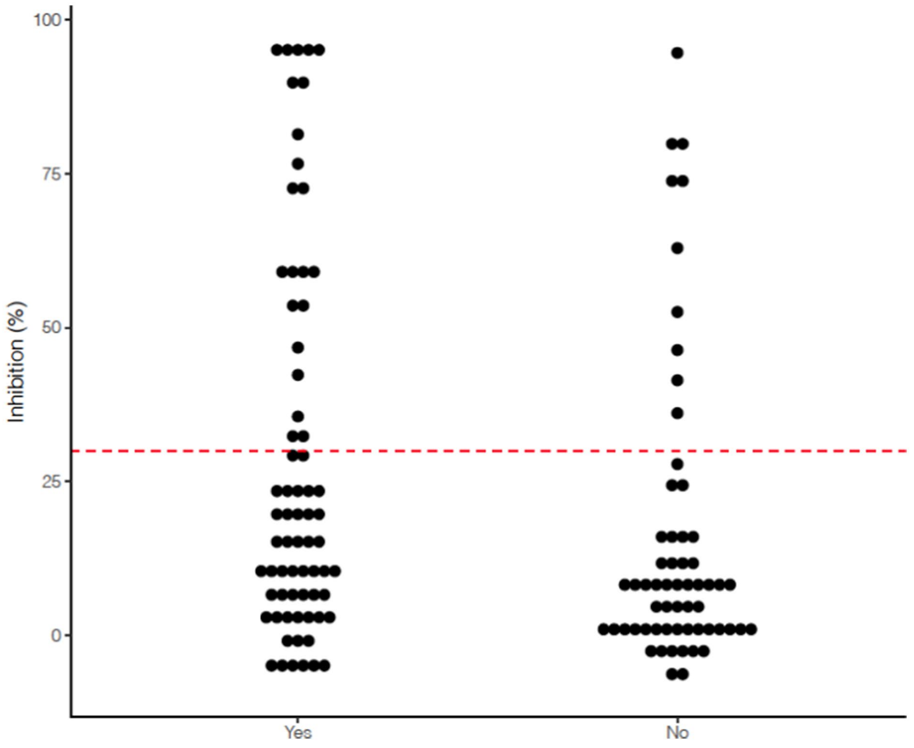

FIGURE 1
Scatterplot indicating spread of % inhibition of serum viral neutralizing antibodies measured using the GenScript cPass™ SARS-CoV-2 Neutralizing Antibody assay among 129 Arizona working dogs based on documented history of exposure to SARS-CoV-2 (Yes indicates known or suspected exposure to a person with COVID-19 compatible symptoms or a person with a COVID-19 positive test; No indicates no known or suspected exposure as reported by handler).

in dogs, has yet to be fully understood, given only two dogs enrolled in this study had reported anosmia.

There are a few noteworthy limitations to report. First, samples were collected from dogs as they visited the veterinary clinic for routine examination, vaccination, and only in some cases onset of clinical illness. The timing of sample collection, therefore, did not specifically correlate with possible SARS-CoV-2 exposure (although this data point was captured), and further explains why only a small number of enrolled dogs had swab samples collected. Second, the single-dilution SARS-CoV-2 neutralizing antibody assay used in this study does provide only a semi-quantitative measurement of antibody response and is not able to differentiate between a viral exposure that occurred more recently (e.g., 3 months) versus an exposure that occurred 6 months or more prior. Therefore, we were unable to pinpoint the exact timeframe of the dog's exposure, unless clearly linked with known human positive cases in the household. An additional limitation is that complete information about potential exposures, work and home environment, and other risk factors were not captured for every dog enrolled. This limitation was recognized by the authors, and the study was designed to capture limited information from the canine handlers since the veterinary clinic was responsible for enrollment, consent, and sample collection. The goal was for the protocol to be minimally burdensome to the staff and fit into their standard operating procedures.

Exposure to varying endemic zoonotic diseases has been previously investigated in working dogs, and continuing this type of surveillance for emerging diseases, like SARS-CoV-2, in this population is important. Although only reported in a limited number of dogs, this study also sheds light on the potential for working dogs to diminish or lose their sense of smell after SARS-CoV-2 exposure. Further investigation in this area is needed to understand the impact that SARS-CoV-2 may have on this population of dogs' work capacity. Agencies that deploy working dogs routinely assess scent detection capabilities during training, however these detection and mitigation plans may need revision. Additionally, this work can inform veterinary practices, policy development (e.g., mandatory SARS-CoV-2 vaccination for working dogs), and guide further management and prevention efforts that may be unique to this canine population (16).

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 TGen's Animal Care and Use Committee (#20163). Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

GH, HY, and HV designed and coordinated the surveillance study with collaborating veterinary hospital. GH, NS, and HY led the preparation of the manuscript, including tables and figures. WS collected samples and data from dogs, as well as provided feedback on the manuscript. NS conducted the laboratory testing of the samples. IR provided guidance on analyses and reviewed manuscript. HM, JA, and DE provided technical support and oversaw the laboratory and surveillance efforts. Additionally, HM, JA, and DE provided important revisions to 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

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Assessing complexity and dynamics in epidemics: geographical barriers and facilitators of foot-and-mouth disease dissemination

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Introduction: Physical and non-physical processes that occur in nature may influence biological processes, such as dissemination of infectious diseases. However, such processes may be hard to detect when they are complex systems. Because complexity is a dynamic and non-linear interaction among numerous elements and structural levels in which specific effects are not necessarily linked to any one specific element, cause-effect connections are rarely or poorly observed.

Methods: To test this hypothesis, the complex and dynamic properties of geo-biological data were explored with high-resolution epidemiological data collected in the 2001 Uruguayan foot-and-mouth disease (FMD) epizootic that mainly affected cattle. County-level data on cases, farm density, road density, river density, and the ratio of road (or river) length/county perimeter were analyzed with an open-ended procedure that identified geographical clustering in the first 11 epidemic weeks. Two questions were asked: (i) do geo-referenced epidemiologic data display complex properties? and (ii) can such properties facilitate or prevent disease dissemination?

Results: Emergent patterns were detected when complex data structures were analyzed, which were not observed when variables were assessed individually. Complex properties–including data circularity–were demonstrated. The emergent patterns helped identify 11 counties as 'disseminators' or 'facilitators' (F) and 264 counties as 'barriers' (B) of epidemic spread. In the early epidemic phase, F and B counties differed in terms of road density and FMD case density. Focusing on non-biological, geographical data, a second analysis indicated that complex relationships may identify B-like counties even before epidemics occur.

Discussion: Geographical barriers and/or promoters of disease dispersal may precede the introduction of emerging pathogens. If corroborated, the analysis of geo-referenced complexity may support anticipatory epidemiological policies.

KEYWORDS

medical geography, complexity analysis, emergence, epidemics, foot-and-mouth disease, movement ecology

Introduction

To occur, epidemics involve more than a pathogen and a susceptible group of hosts. In addition to immunological, microbiological and demographic factors, numerous factors (including but not limited to the geographical environment) may also influence the development and progression of epidemics. To explore such factors, the analysis of variables that can be defined in terms of geographical coordinates (geo-referenced variables) has been proposed (1–5).

Numerous calls have suggested the development of methods that address complexity and dynamics in epidemiology (6, 7). Given its potential relevance in prevention, the study of disease dissemination with geo-referenced data is a topic of particular interest (8).

While some geographical factors (such as the road structure) may facilitate disease dispersal, other factors may act as barriers (1, 9). Yet, geographical factors do not have a single and constant role–they change over time and/or across space. For instance, both low and high road density may prevent disease dissemination. While low road density tends to prevent disease dispersal, high road density may also act as a barrier because, in highly urbanized areas (where road density is invariably high), roads compete against farming for land and, consequently, high road density may inadvertently block dissemination of infections affecting domestic animals (10).

Similarly, bridges may play different roles (11). If used to control epidemics (e.g., as disinfection sites), bridges act as obstacles, complementing the natural barrier effect exerted by rivers and other geographical features, such as mountains. However, in their typical usage—connecting regions separated by rivers—bridges may foster epidemic spread. Consequently, the study of geographical facilitators or non-facilitators of epidemic dissemination is not a discrete and/or static endeavor: it involves the analysis of dynamic interactions among pathogens, hosts, and geography. Rivers on the other hand most likely act as barriers for animal movement, and therefore, prevent infectious diseases. For example, rivers are suggested to shape present-day patterns of ecological and genetic variation among Amazonian species and communities (12).

To study dynamics (interactions that change over time), complexity should be considered. Measuring complexity is not a trivial endeavor because the mathematics (if not also the biology) influencing one scale may differ from the factors that affect other scales (13).

To investigate epidemics, "information" (not just "data") should be generated. Data analysis is not enough–data structuring is needed (14). Structured data may reveal informative data patterns not directly conveyed by simple (non-structured) data.

Data structuring that focuses on *relationships* is not common (15). Much less so is the analysis of dynamic and complex relationships that include but exceed medical expertise (16). The information generated by structured data also depends on the data *format* utilized: for instance, it is not the same to read numbers from a table that lacks relationships than to directly visualize 3D patterns on a map (17–19). The validity and/or informative value of structured data can be objectively determined: it only requires determining whether structured data (complex indicators that include multiple variables) inform more than non-structured (simple) variables (20).

Because 'point predictions' (e.g., the specific number of a specific variable that differentiates two or more specific conditions at a specific time and place) depend on highly variable initial conditions, complexity analysis does not attempt to make long-term predictions. Instead, it focuses on properties (21). Complex systems possess at least three properties: (i) emergence, (ii) irreducibility, and (iii) unpredictability (21–23). *Emergence* (also known as *novelty*) refers to the fact that complex systems are multi-level structures, which reveal *new* features or functions only when the most complex (system-level) structure is assembled. *Irreducibility* means that *emergence* cannot be shown by or reduced to the properties of any one 'simple' (non-structured or low-level) variable. *Unpredictability* refers to the inability to predict emergence when only 'simple' and/or isolated variables are analyzed.

While investigated in infectious diseases, complexity and dynamics have been poorly explored in geo-referenced studies of epidemic dispersal. Yet, several properties of biological complex and dynamic systems are already well known in infectious diseases (24). For example, *data circularity* (data with no beginning and no end) is the essence of *seasonality*—one factor known to influence geo-epidemiology (25). Detecting such properties in epidemics matters because, given the highly combinatorial nature of complex and dynamic systems, numerous informative patterns may be embedded in the data, which may be missed by simple approaches (12, 26). If properties that characterize dynamic complexity were demonstrated in geo-referenced epidemics, it could then be explored whether some geographical factors may act as facilitators or barriers of epidemics.

To that end, data previously analyzed are here re-investigated (10). The reason to re-assess data collected in the 2001 Uruguayan FMD epizootic is because it predominantly affected bovines (a species that displays observable signs when infected by the FMD virus) and, at the time, all bovines in Uruguay were susceptible (no vaccine against FMD had been used in the previous decade). While other (non-bovine) species do not always reveal clinical signs when affected by the FMD virus (27), both the geographical location of the onset and the geo-temporal progression of the 2001 Uruguayan FMD epizootic were unambiguously recorded. While the purpose of this study is not to explore how FMD epizootics can disseminate or how the 2001 Uruguayan episode took place [such questions have been addressed in numerous, earlier studies (10, 28, 29)], data collected in that epizootic are used to ask two questions: (i) do epidemics reveal properties typical of complex systems? and (ii) if so demonstrated, could such properties distinguish geographical factors that may act as facilitators or barriers of epidemic spread?

Materials and methods

Materials

Two hundred and seventy-five counties of Uruguay were investigated. County-level geographical variables were combined with epidemic data collected in the first 11 weeks of the 2001 FMD Uruguayan epidemic (10, 29). These data were complemented with non-epidemiologic, geo-referenced data on area- and line-based structures (counties, rivers and roads, as reported in https://srvgisportal.igm.gub.uy/portal/apps/webappviewer/index.html?id=26d59683d5cb475fa70e8223fa0da173; Supplementary Table 1). Seven variables were investigated: county area (sq. km), FMD case density

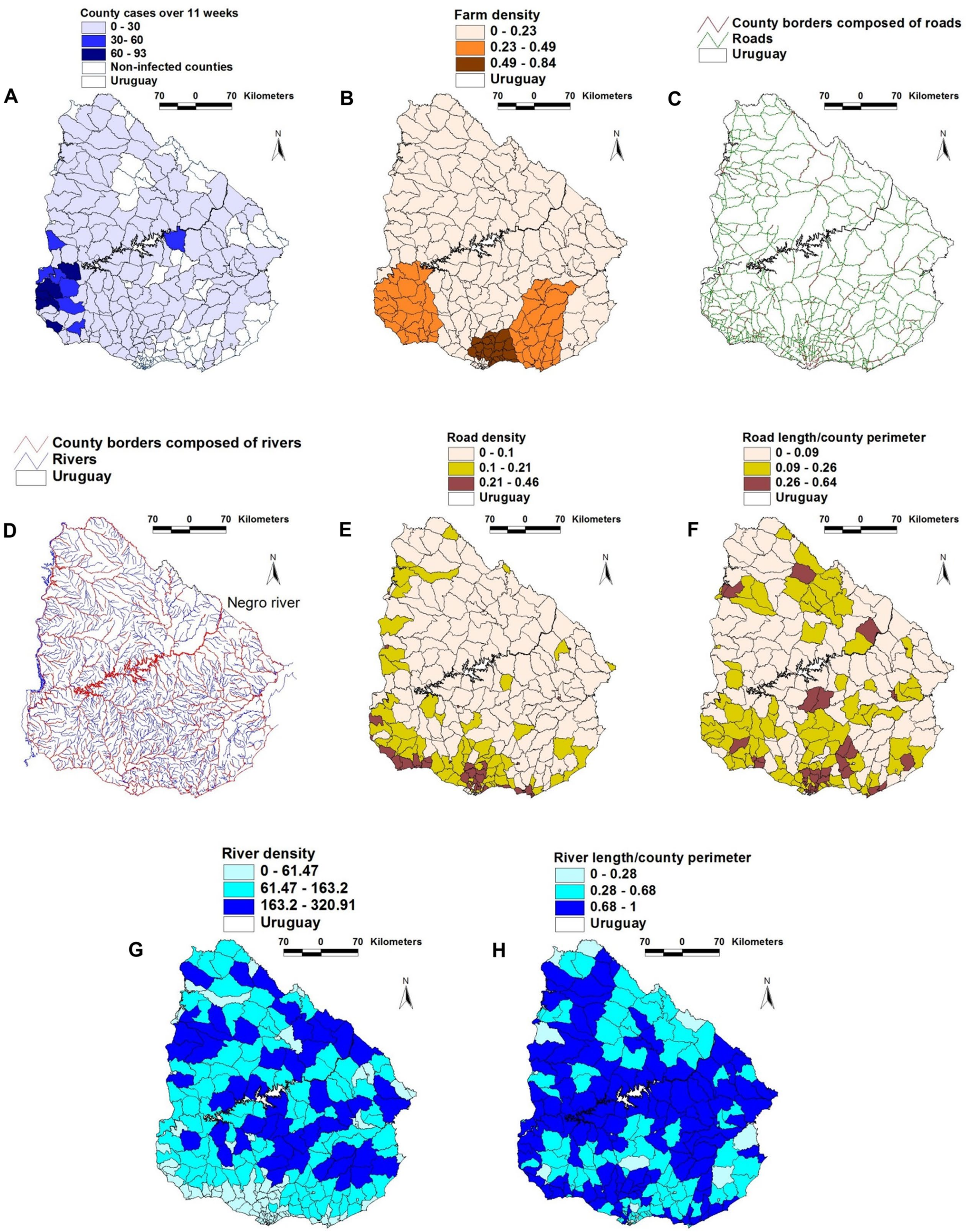

FIGURE 1
Geographical features of the 2001 FMD epizootic that took place in Uruguay. **(A)** county-wide FMD case density (cases/sq. km) reported in the first 11weeks of the epizootic. **(B)** county farm density (farms/sq. km). **(C)** road and county boundaries composed of roads **(D)** rivers and county boundaries composed of rivers. **(E)** county road density. **(F)** county road length/county perimeter composed of roads. **(G)** county river density. **(H)** county river length/county perimeter composed of rivers. County case density was higher in the south-western region **(A)**, south of the Negro river, which flows diagonally trough the country, from the north-eastern to the south-western border **(D)**. The south-western region, as well as much of the southern coast shows the highest road density **(F)**. Such geo-epidemic structure suggests that the Negro river acted as a *de facto* obstacle for the dissemination of the epidemic, which was first reported to the south of this river.

(infected farms/sq. km), farm density (farms/sq. km), road density (km of county road length/county area), river density (km of river length/county area), and the percent of county perimeter occupied by roads or rivers (road [river] length/county perimeter).

Method

An open-ended, combinatorial approach was used, which investigated georeferenced and/or epidemiologic variables until visually distinct data patterns were detected in the early epidemic phase (first 2 weeks). Such patterns were then used to classify counties into two categories: facilitator [F] or barrier [B] of epidemic dispersal. Subsequent data analyses focused on detecting properties associated with complex and dynamic systems, such as emergence, irreducibility, and/or unpredictability. The method operated as an open-ended series of multivariate, map-based analyses which, after comparing several data ranges of each variable, concluded when at least one distinct pattern of geographical units (counties) was identified. Such a pattern should include not more than one dissimilar unit into a cluster of units otherwise similar, e.g., a cluster of was characterized by counties with similar values of the same geographical feature or not more than one county exhibiting dissimilar values.

Using a commercial Geographical Information Systems (*ArcGIS 9.3*, ESRI, Redlands, CA, United States) package, a layer (shapefile) of county boundaries was created. The seven variables used in the analysis were generated from variable layers and the county boundaries layer utilizing buffer, intersection and other data manipulation tools. The resulting data for these seven variables were combined into a single table which was then joined (appended) to the county boundaries layer using the common county identifier. The resulting dataset was then used to conduct a *query* for counties that fell within specified intervals (e.g., "road density greater than … AND river length segments in perimeter less than …"), and a *new set* was created. Such procedure was conducted for the entire 11-week long epidemics and for each epidemic week. The corresponding tables were then exported to a commercial statistical package (*Minitab 22*, Minitab Inc., State College, PA, United States) for further analyses. Correlation analysis was used to explore simple relationships among variables. The Mann–Whitney test for comparison of medians was applied to compare groups of counties. The same package was utilized to generate three-dimensional plots. A proprietary algorithm was used to facilitate the open-ended cycle that included map-based and 3D plot-based assessments.

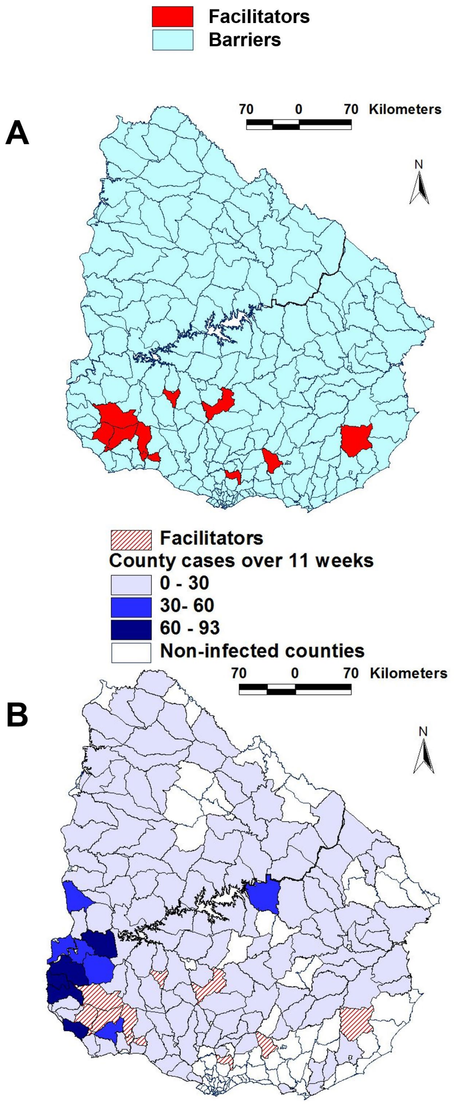

FIGURE 2
Geographic features of counties suspected to facilitate or block epidemic dispersal. Most 'facilitator' counties were clustered in the south-western region, where they occupied a continuous area **(A)**. To elucidate whether such pattern was due to chance or geo-biology, time-related epidemic data were investigated. Most 'facilitator' counties were contiguous to a group of counties where the highest case density was reported over the entire course of the epizootic **(B)**.

Results

The geographical location of FMD cases, farms, rivers, and roads is shown in Figures 1A–H. A physical barrier was observed: most FMD cases were located south of the Negro river (Figure 1D).

When geographical variables were analyzed (without considering temporal-epidemiologic data), *farm density* was positively associated with both *road density* and *road length* (both with $r \geq 0.34$, $p < 0.01$). In contrast, *road length* was negatively and statistically significantly associated with *river length* (Supplementary Table 2). When epidemic and temporal data were assessed, positive and significant correlations were found between *case density* and both *farm density* and *road density* in at least one of the first two epidemic weeks. In the early epidemic phase, *farm density* was positively and significantly correlated with *road length* –a variable associated with *road density* (Supplementary Table 3). The values of the geographic variables changed over time: while infections were only reported in 29 of the 275 counties in epidemic week I, 71 counties reported FMD cases 1 week later (Supplementary Table 4A).

While correlation analysis indicated relationships, it did not distinguish county categories. In contrast, map-based assessments of complex data combinations detected a group of 11 counties here labeled as 'facilitators of epidemic spread' (F). Most 'facilitator' (F) counties clustered in the south-western region of the country (Figure 2A). 'Facilitator' counties were characterized by: (i) *farm density* > 0.15; (ii) *road density* > 0.1; (iii) *river density* > 0.1 but <0.3; (iv) *road length / county*

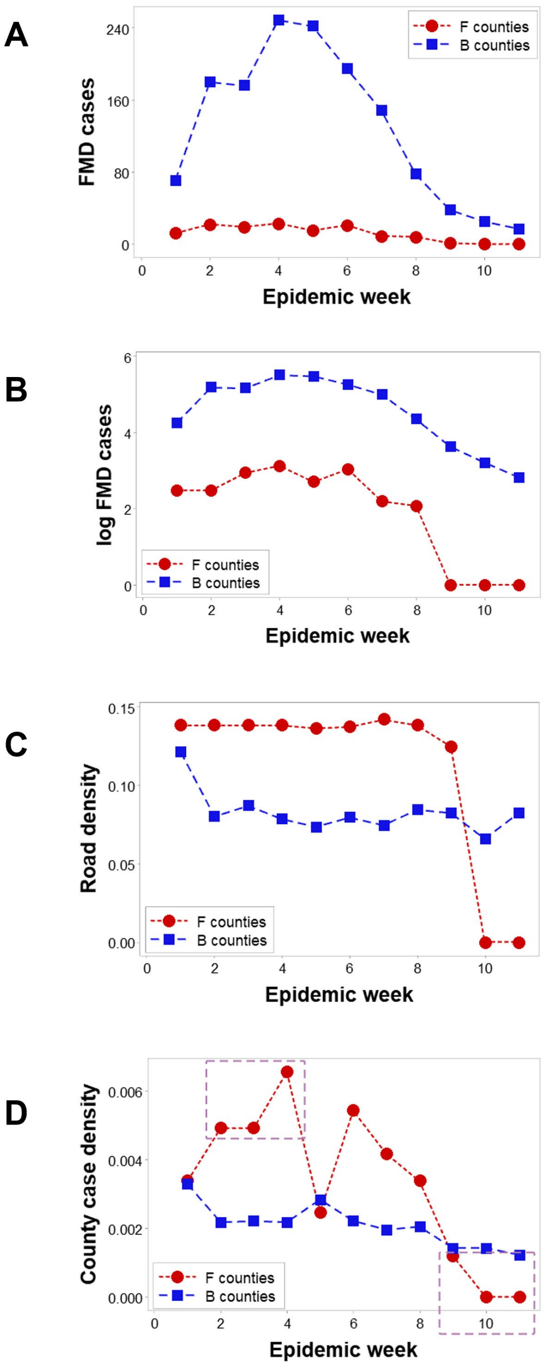

FIGURE 3
Dynamics of counties suspected to facilitate or block disease dispersal. In all 11 epizootic weeks, 'barrrier' (B) counties reported FMD cases; in contrast 'facilitator' (F) counties only presented cases in the first 9weeks **(A)**. Log-transformed FMD case data revealed that F and B counties revealed similar trends in the first 9weeks **(B)**. The median road density (km of road length/sq.km of county area) was significantly higher in F than B counties in the first 9weeks **(C)**. FMD case density was higher in F than B counties in three of the first 4weeks (oval, **D**), becoming zero after week 8 (box, **D**).

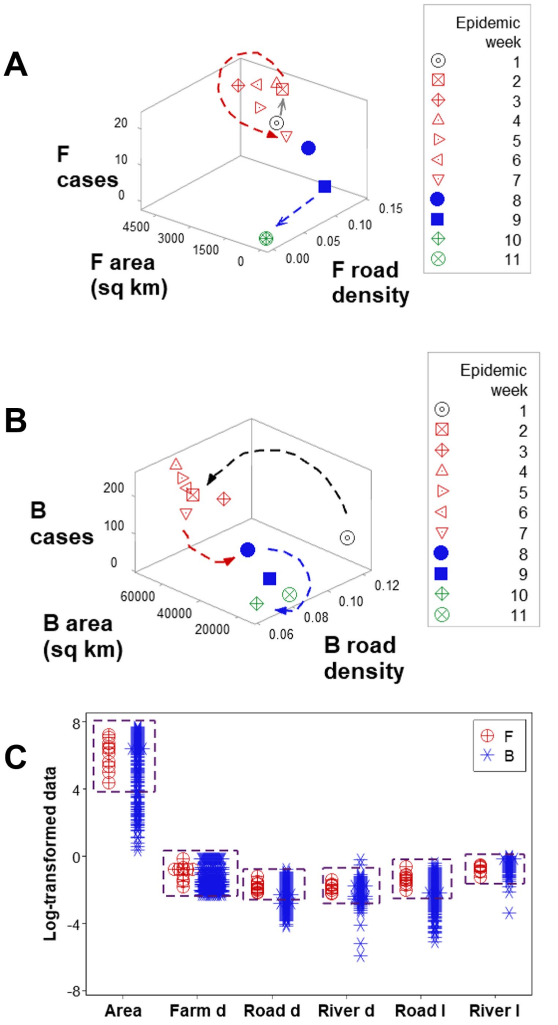

FIGURE 4
Complex and simple data assessments When F counties were considered, three epidemic phases were detected (arrows, **A**). When county classes were not considered (F and B classes were not differentiated), only two epidemic phases were differentiated (arrows, **B**). While three-dimensional, complex assessments distinguished F from B counties, no variable, alone, differentiated F from B counties: overlapping data distributions were observed (boxes, **C**).

perimeter > 0.1; and (v) *river length/county perimeter* > 0.38 but <0.6. The remaining 264 counties were classified as 'barriers' (B, Figure 2A).

To validate such a classification, FMD case data were assessed over time in F and B counties. Over 11 epidemic weeks, 130 and 1,420 cases were reported in F and B counties, respectively (Supplementary Table 4B). That is, the percentage of all cases associated with 'facilitator' counties (8.4% or 130/1550) was 2.1 times higher than the percent of cases within all counties (4.0% or 11/275). The F cluster was also geographically connected: it showed a continuous and contiguous structure within which, over 11 weeks, the highest FMD case density was observed (Figure 2B).

Spatial epidemic dynamics differed markedly between F and B counties. While FMD cases were reported in B counties in every week, no infection took place in F counties at weeks 10 and 11 (Figures 3A,B). The median *road density* associated with F counties was higher than that of B counties in the first 9 epidemic weeks ($p < 0.01$, Mann–Whitney test; Figure 3C). Epidemic dynamics also differed between county classes: the *case density* (cases/sq. km) was higher in F than B counties in epidemic weeks 2–4, but lower, later (rectangles; Figure 3D).

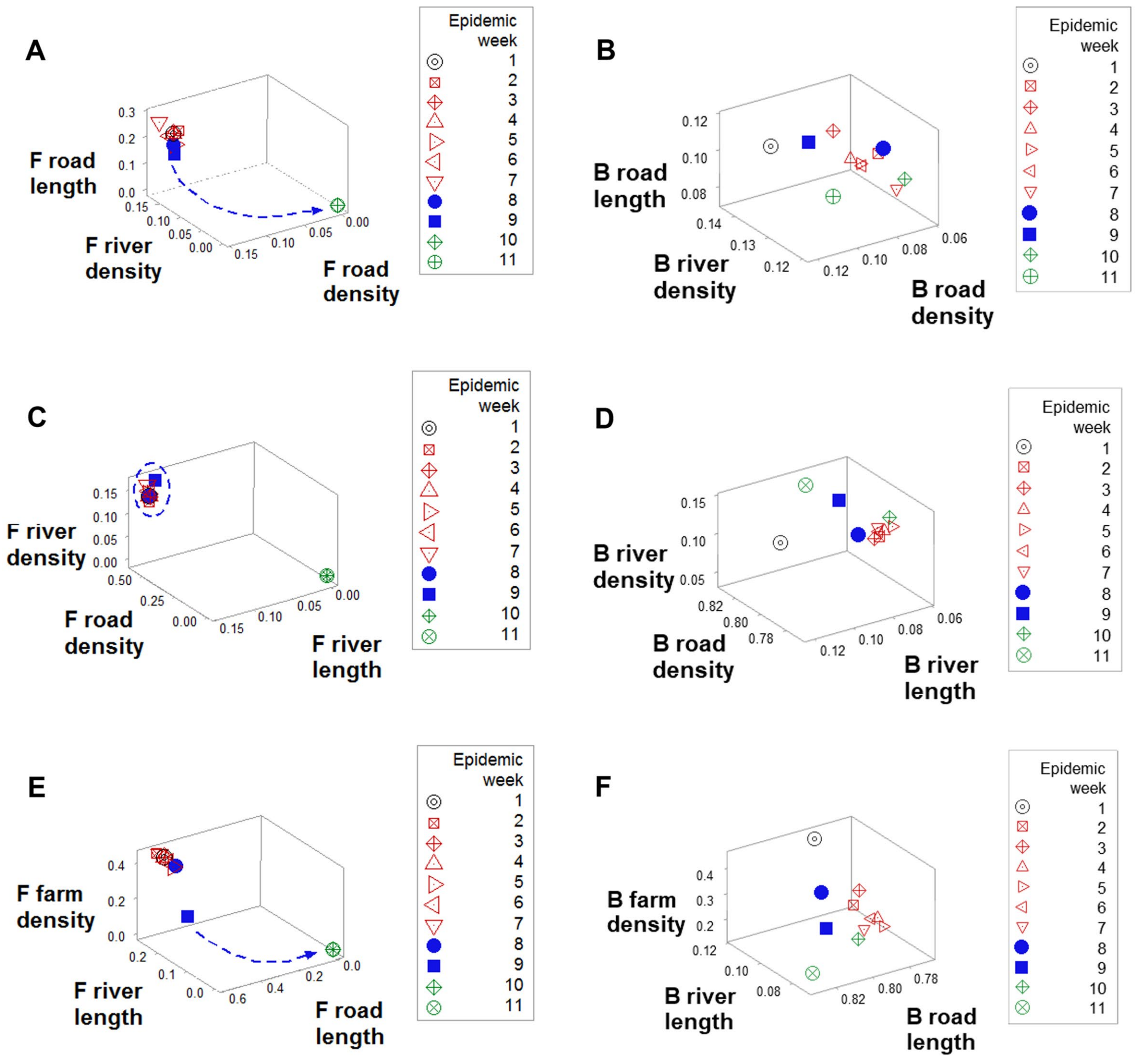

FIGURE 5
Geographical differences between F and B counties. Data clustering, as well as a perpendicular data inflection (shown by F counties after epidemic week 8), **(A)** were not observed in B counties **(B)**. While F counties displayed a high road density in the first 9 epidemics (circle, **C**), in B counties road density was high only in week 1 (arrow, **D**). F road length data revealed a sudden decrease data that predicted resolution (arrow, **E**), which was not shown the road length of B counties **(F)**.

Three-dimensional (3D) analyses revealed three temporal data inflections when the *road density* associated with F counties was measured together with the weekly *case count* and the *area* (sq km) of such counties (Figure 4A). Such indicators differentiated three epidemic phases, here described as early, intermediate, or late (or resolution; red, blue, and green symbols; Figure 4A). However, when such variables were assessed in B counties, only two data inflections were detected, and the last epidemic phase (resolution) was not observed (Figure 4B). Hence, a new (*emergent*) pattern (three, as opposed to two data patterns) was only displayed by F counties. In contrast, unstructured data, alone, did not distinguish F from B counties (Figure 4C). Because *emergence* was not predicted by or reduced to the properties of any one unstructured variable, the data demonstrated three typical properties of biological complexity: *emergence, irreducibility* and *unpredictability*.

Emergence was also documented when *road length*, *road density*, and *river density* were considered: a perpendicular data inflection, observed in F counties (arrow, Figure 5A), was not revealed by B counties (Figure 5B). While F counties exhibited a high *river density* only in the first epidemic week (oval, Figure 5C), B counties displayed a high river density throughout the first 9 epidemic weeks (Figure 5D). While, in F counties, a sudden decrease in *road length* values predicted resolution (blue arrow; Figure 5E), B counties did not express such a feature (Figure 5F).

Findings also revealed data *circularity* and *spatial–temporal biological relativity*. Circularity was shown in numerous expressions (Figures 4A,B and also in Figures 6A,B). Relativity was expressed both as data points that occupied a large portion of the space analyzed but involved a small period of time (blue lines; Figures 6A,B) and also as observations generated over a long period of time, which occupied a small plot space (red ovals; Figures 6A,B).

Ambiguity was also observed: observations similar in all variables and values could have different meanings (green boxes; Figures 6A,B). To further explore complexity, geographic data were also analyzed

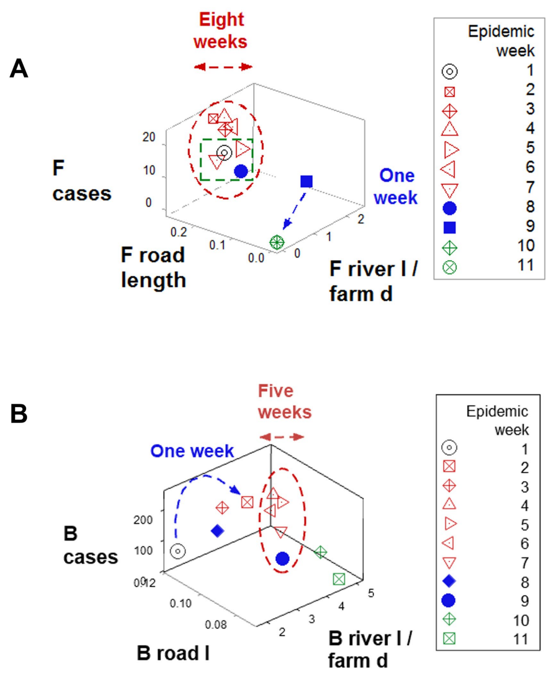

FIGURE 6
Demonstration of spatial–temporal biological relativity (ambiguity). **(A)** Oscillatory or circular data patterns were observed in numerous plots, including this one. One associated property is spatial–temporal biological relativity. Such a property is expressed as data points that describe long temporal periods but occupy a small space of the plot (red oval, **A,B**), as well as the opposite pattern: data points collected over a short time period, which occupy a large portion of space (blue lines, **A,B**). For instance, observations collected over 6weeks (red oval, **B**) occupied a smaller space than observations collected over 1week (blue line, **B**). This property results in ambiguity: some data points of similar values in all variables may possess different meaning, e.g., the open (red) symbols refer to the early epidemic stage, while the closed (blue) symbols reflect the resolution phase (green boxes, **A,B**).

without consideration of epidemic data. Using the identifiers that, in the temporal (11-week long) analysis characterized F counties (those that reported most of the infections), a county-centered analysis explored whether combinations of variables could reveal patterns that differentiated the same 11 counties from the remaining (B-like) 264 counties. Three levels of complexity were then evaluated: (i) complexity level I (bi-dimensional relationships between two variables; Figures 7A–C); (ii) complexity level II (bi-dimensional relationships between three variables; Figure 7D); and (iii) complexity level III [three-dimensional (3D) relationships among complex [more than three] variables; Figures 8A–C). Statistically significant differences were found between F and B-like counties when four indicators (complexity levels I and II) were assessed (all with $p \leq 0.01$, Mann–Whitney test; Figures 7A–D). Three-dimensional analyses detected an additional emergent pattern: most B-like counties were orthogonal to F counties. While the vertical data subset included all F and some B-like counties, the horizontal subset only included B-like counties (Figures 8A–C).

The 3D analysis of physical structures distinguished two subsets that were perpendicular to one another (Figures 8A–C). If epidemiologic data were considered, the 'vertical' subset would include all F counties (and a few B counties) while the 'horizontal' subset would be 100% free of infections (Figures 8A–C). Because the orthogonal patterns were detected regardless of epidemiologic status, if these patterns were repeatable, any county included in the 'horizontal' subset could be suspected to become a barrier if an epizootic took place.

Discussion

This study supported two novel inferences: (i) properties associated with complexity may be found when methods utilize geo-referenced and temporal data; and (ii) complex combinations of non-biological, geo-referenced data (such as the road and river networks) may reveal non-randomly distributed structures with potential influence on disease dispersal, which may be detected even in the absence of epidemiologic data. Methodological consequences associated with these inferences and some areas of possible applications are here discussed.

While unstructured data –observations on simple or isolated variables– were non-informative (Figure 4C), data structures that captured several levels of complexity described both a dynamic process (the epidemic) and a static (geographical) structure (Figures 4A,B, 8A–C). Findings support the view that epizootics reveal *emergence*, *irreducibility*, and *unpredictability* –properties typical of complex systems. The analysis of geo-referenced complexity may, at least partially, explain FMD outbreaks (21, 30, 31).

Two properties that may affect data analysis were also documented: data *circularity* and *spatial–temporal biological relativity* (32, 33). Such properties may prevent the use of models that analyze finite data intervals because circular data structures have no beginning and no end and, therefore, there are no explicit endpoints (34). As shown in Figures 6A,B, when relativity occurs, observations with similar numerical values may have different, if not opposite meaning (35). Yet, geo-referenced and dynamic analyses may distinguish such false similarities: the potential problems associated with 'biological relativity theory' and/or data circularity can be circumvented when complex properties are assessed with pattern recognition-oriented approaches. When an unambiguous pattern is determined, discrimination is possible (32, 35, 36).

Specifically, time-related arrows (data directionality) facilitated interpretation, even when circularity and relativity were observed. For example, two observations that showed similar numerical values were distinguished epidemiologically when temporal information (arrows that indicated where a data point was coming from/going to) were considered: one preceded the early epidemic phase, the other preceded the later phase (black open circle and blue closed square, respectively, Figure 4A).

Findings also indicated that, when data circularity is observed, no dichotomy is true (37). In contrast, non-binary models (those that consider there may be three or more epidemic stages) may prevent errors. As expected, complexity analysis extracted more or new information (20). Additional information was associated with data structures that captured many levels of complexity: level III indicators (which simultaneously captured 7 or more interactions, as shown in Figures 7A–D) yielded more information than simple (non-structured) variables (such as those reported in Figure 4C).

To discriminate, 'top-down' and 'bottom-up' aspects were considered (38). In particular, two challenges were addressed: (i) the

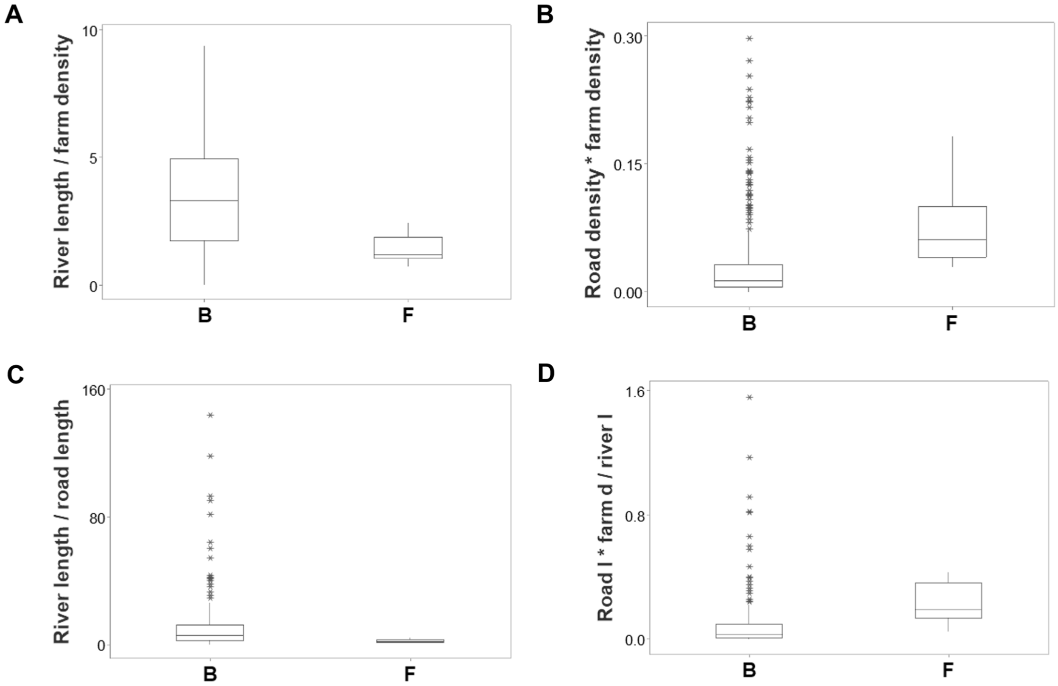

FIGURE 7
Bi-dimensional analysis of geo-referenced, complex physical relationships that may influence disease dispersal. Dimensionless indicators that included ratios and/or products showed statistically significantly different medians when F and B counties were compared (all at $p \leq 0.01$, Mann–Whitney test, **A–D**). **(A)** River length/farm density (the ratio resulting from dividing river length [percent of county perimeter] over farm density). **(B)** Road density * farm density (the product resulting from multiplying road density times farm density). **(C)** River length/road length (the ratio resulting from dividing river length [percent of county perimeter] over road length [percent of county perimeter]). **(D)** [Road density/river density] * farm density (the result from dividing road density over river density, multiplied by farm density). After two products were calculated (river density times river length, and road density times farm density), the first product was divided by the second product.

need to structure the data in a way such that a complex host-microbial-geo-temporal system could be evaluated even without knowing, *a priori*, which data components would inform (a 'top-down'-related problem); and (ii) the computational challenge associated with a very large number of data combinations to be analyzed (a potential problem associated with 'bottom-up' approaches). Both obstacles were overcome using an operation oriented to reveal distinct spatial patterns.

The adopted strategy prevented the 'combinatorial explosion' (39). This problem (also known as the 'curse of dimensionality' or 'combinatorial complexity') refers to analytical situations in which the number of possible combinations exceeds the number of variables and may approach infinity. For example, if 10 locations may experience 3 different events (to be disease-free, to be currently infected and within the exponential growth phase, or to be still infected but within the late or resolution phase), there are 3^{10} (~59,000) possible combinations. If the analysis of each of such combinations took 1 h, the whole analysis would require 6.7 years. While some numerical approaches have attempted to reduce the length of combinatorial analyses (40), other approaches have addressed the combinatorial explosion by focusing on spatial relationships (41). Because they tend to be more informative than one- or two-dimensional alternatives, this study followed the 3D approach.

Complex data structures demonstrated to be less variable than unstructured data (42, 43). Such features matter when validity is explored. This study investigated four dimensions of validity (44). *Construct validity* (detection of emergence, expressed as F and B counties) was shown at least eight times, as Figures 3 and 7 document. Because different data structures revealed emergence, *internal validity* was demonstrated. Because one physical geo-referenced pattern (a river acting as a barrier) has been reported in South African FMD epidemics (45), *external validity* was supported. Because statistical significance was documented at least four times (Figures 7A–D), *statistical validity* was demonstrated.

The notion that the *number* of 'facilitators' was not as relevant as their *geo-demographic location* and *structure* was supported: the observed geo-referenced network may be a part of a connecting network (28, 46). In several diseases, the geographical structure may determine whether disease dispersal occurs synchronically (47, 48). However, other factors (such as a 'network of networks') may also influence disease dissemination (49).

Findings may apply to human diseases, including human cholera and mosquito-borne diseases such as malaria and dengue (50). Climate-related factors –such as El Niño –induce ocean warming, which promotes long-distance dissemination of infectious agents (51–53). While the approach here explored is not necessarily applicable to epidemics of low morbidity, such as Ebola (54), it may apply in rapidly disseminating infectious diseases (46). These concepts also apply to wildlife surveillance and One Health approaches, where positive correlations have been reported between forest density and improved public health (55–59).

These considerations may improve interventions meant to stop epidemics. For example, practices that assume static situations and lack of interactions could be discontinued (60). They could

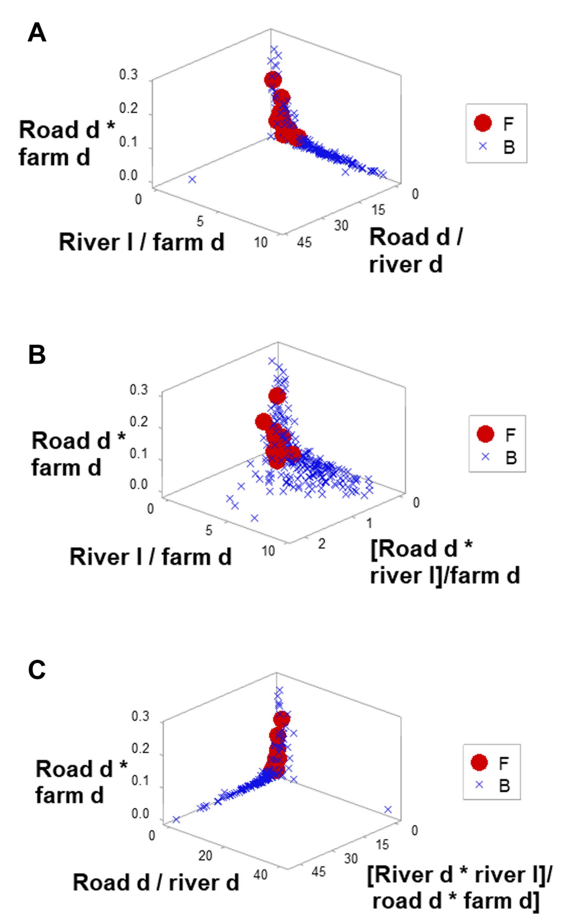

FIGURE 8
Three-dimensional analysis of geo-referenced, complex physical relationships that may influence disease dispersal. Three-dimensional analysis of complex indicators that only involved physical variables, such as the county-related length of rivers or county-related road density, displayed one or more perpendicular data inflections **(A–C)**. Such inflection differentiated two data subsets, of which one did not include counties affected by FMD (B counties). Because all variables interact in 3D space, it is assumed that the overall pattern captures, at least, seven interactions. Because spatial patterns can be assessed even in the absence of infections, if these distinct spatial patterns were shown to be repeatable, they could be considered in anticipatory testing.

be replaced with assumption-free, dynamic assessments of the local geography, which facilitate anticipatory allocation of resources and may lead to less costly and/or more effective control policies (46).

Because complexity is associated with hidden interactions (61) and physical geo-referenced structures are independent of and/or precede epidemics, research on multiple geographic variables suspected to facilitate (or prevent) disease dispersal can uncover patterns usually unobserved. Hence, the analysis of geographical complexity is suggested. To that end, additional validations conducted in different bio-geographies are recommended.

Data availability statement

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

Author contributions

AH, JF, and MK: writing. AR and FF: methodology. SS: software. All authors contributed to the article and approved the submitted version.

Conflict of interest

MK is employed by KB One Health LLC.

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

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1149460/full#supplementary-material

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Disentangling transport movement patterns of trucks either transporting pigs or while empty within a swine production system before and during the COVID-19 epidemic

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Transport of pigs between sites occurs frequently as part of genetic improvement and age segregation. However, a lack of transport biosecurity could have catastrophic implications if not managed properly as disease spread would be imminent. However, there is a lack of a comprehensive study of vehicle movement trends within swine systems in the Midwest. In this study, we aimed to describe and characterize vehicle movement patterns within one large Midwest swine system representative of modern pig production to understand movement trends and proxies for biosecurity compliance and identify potential risky behaviors that may result in a higher risk for infectious disease spread. Geolocation tracking devices recorded vehicle movements of a subset of trucks and trailers from a production system every 5 min and every time tracks entered a landmark between January 2019 and December 2020, before and during the COVID-19 pandemic. We described 6,213 transport records from 12 vehicles controlled by the company. In total, 114 predefined landmarks were included during the study period, representing 5 categories of farms and truck wash facilities. The results showed that trucks completed the majority (76.4%, 2,111/2,762) of the recorded movements. The seasonal distribution of incoming movements was similar across years ($P > 0.05$), while the 2019 winter and summer seasons showed higher incoming movements to sow farms than any other season, year, or production type ($P < 0.05$). More than half of the in-movements recorded occurred within the triad of sow farms, wean-to-market stage, and truck wash facilities. Overall, time spent at each landmark was 9.08% higher in 2020 than in 2019, without seasonal highlights, but with a notably higher time spent at truck wash facilities than any other type of landmark. Network analyses showed high connectivity among farms with identifiable clusters in the network. Furthermore, we observed a decrease in connectivity in 2020 compared with 2019, as indicated by the majority of network parameter values. Further network analysis will be needed to understand its impact on disease spread and control. However, the description and quantification of movement trends reported in this study provide findings that might be the basis for targeting infectious disease surveillance and control.

KEYWORDS

transport, pork industry, network analysis, disease surveillance, Midwest, USA

Introduction

Ground transportation of pigs between sites within a production system occurs frequently for age category and production phase segregation because it has been shown to increase production efficiency and animal health. Although trucks and trailers are designed to load batches of animals with protocols that safeguard animals' wellbeing, health, and safety, it is well-established that animal movements are a source of transmission of infectious disease within the swine industry (1–6), and vehicles which transported animals need to be correctly cleaned and disinfected before loading the next batch of pigs to prevent the risk of pathogen spread (7, 8).

With the intent to mitigate the risk of potential disease spread among pig farms, biosecurity practices such as isolation of incoming breeding stock, testing before commingling animals, shower-in/shower-out, disinfection, and drying rooms for supply entry are recommended among others (9). In addition, cleaning and disinfection of trucks and trailers transporting pigs between loads is an area of focus especially when vehicles transport gilts, boars, recently weaned pigs, and culled animals. Although all these biosecurity measures are useful, they are not completely effective as these depend on a combination of standardized processes and their consistent implementation (9). As an example, it has been described that washing and disinfecting vehicles without the appropriate drying has a similar risk for disease transmission as unwashed vehicles (7, 8). Furthermore, a recent study indicated that PRRSv transmission associated with movements of trucks used for feed and personnel transportation is not negligible (4), adding a layer of complexity when understanding disease spread and designing risk-mitigation (e.g., biosecurity) practices for the swine industry.

Social network analysis has proven to be a useful tool for understanding infectious disease dissemination among populations by analyzing pig movement patterns among animal production systems (10–14). Disease surveillance and control have been traditionally assessed by tracing animal movements as a source of disease spread since infected animals are the most frequent way to spread pathogens to susceptible populations. Furthermore, the potential impact of pathogen dissemination through animals in the US has been assessed *in silico* using retrospective shipment records (4) that can introduce bias in the analysis, when dealing with missing or incomplete data. Limited studies have focused on understanding the potential roles that the vehicles used for feed transport and the personnel involved may have on pathogen dissemination (4, 15, 16), but in fact, the majority of studies have overlooked the role that trucks may have on pathogen spread or assumed zero risk for disease transmission when vehicles visit a site after stopping in a cleaning station (i.e., truck wash station) (4, 6).

Here, as a first step to understanding the role of trucks transporting pigs or empty-unwashed trucks in the spread of diseases, we described and analyzed for the first time the vehicle movement patterns and network structure of one of the largest multi-site pig production systems in the Midwestern US using GPS trackers and including vehicles in the network, regardless of the load status or origin/destination of the movement. We assessed the utility of GPS trackers as a reliable source to record truck movements, characterized vehicle network before and during the COVID-19 pandemic, fidelity (defined as the consistency of vehicle movements over time), and time spent at each site, with the ultimate goal of understanding movement trends that will help to identify potential risky behaviors and spatio-temporal variabilities that can inform epidemic-preparedness and support decisions to improve biosecurity practices and compliance for reducing the spread of diseases compromising swine health.

Materials and methods

Population and data source

Data were collected from a pig-producing company participating in the University of Minnesota Morrison Swine Health Monitoring Project (MSHMP) (17) that voluntarily agreed to participate in the study. The selected company is a typical multi-site system representative of modern pig production systems in the US This company accounts for approximately 1% of the swine production in the country. The system agreed to have a selected subset of 12 out of 128 owned pig transportation vehicles monitored through geolocation-tracking devices installed on seven trucks transporting only recently weaned pigs and five trailers that transport either gilts or culled sows. On the trailers, the Verizon Networkfleet Asset Guard was attached through screws, whereas the Verizon Networkfleet 5000 Series Model 5500N4VL was connected to the onboard diagnostics (OBD) port on the tractor cab. These devices (Figure 1) are designed to endure long periods of outdoor or other rugged environments while maintaining communication and GPS signal during the journey and through vehicle washing.

Vehicle movement monitoring occurred between January 2019 and December 2020. A total of 6,213 records of vehicles entering 114 georeferenced pig sites were initially considered for this study over the study period (3,659 and 2,554 in

FIGURE 1
Geolocation tracking devices installed in the vehicles **(A, B)**. Full description of the devices is available online at: https://www.verizon.com/about/news/verizon-expands-asset-tracking-portfolio-introduction-networkfleet-asset-guard-0.

2019 and 2020, respectively). Connections among sites (i.e., vehicle movements) were recreated based on the time and date of entry to each of the geofences and the trajectory of the vehicles tracked.

Spatiotemporal characterization of the movements

We described the frequency of incoming movement patterns yearly, seasonally, and weekly over the study period by source farm (e.g., farm production type) and time spent by the vehicles at each site. Farm production type was categorized as either sow, nursery, finisher, wean-to-market, or gilt development unit. Differences in the frequency of movements were assessed with univariable and multivariable negative binomial regression which allowed adjusting for over-dispersed data (e.g., frequency of incoming movements mean = 18.71, variance = 274.47) (18) and adjusted for multiple comparisons. We described the proportion of movement performed between pairs of sites grouped by production category (e.g., sow to nursery, sow to sow) and vehicle fidelity over time. Incoming movement density was mapped by year to identify areas of major movement activities.

Network nomenclature and metrics definition

A yearly static directed non-weighted network was constructed for the complete period (2019–2020). Network nodes or vertices (i.e., elements or units of the network) (19) were polygons created within the extension of preexisting system site locations (i.e., landmarks), which included the abovementioned five different categories of farms and truck wash facilities. The movement of vehicles was captured when tracking devices located in the vehicles that entered or exited each predefined polygon, and thus, the time spent within the polygon was recorded for each vehicle and node.

Edges were created by linking nodes sequentially visited by each vehicle. We selected those nodes in which a stop was recorded for more than 5 min as these were considered an "operational stop", such as loading/unloading animals or washing the vehicle. When vehicles showed a recorded stop in a node for <5 min, this was considered a drive-by or an incorrect record (i.e., a result of rearranging vehicles for loading/unloading animals or inaccurately captured by the geofences surrounding the nodes-polygons when the vehicle was circulating close to the polygon boundaries). Furthermore, movements recorded by vehicles entering sequentially onto the same node-polygon for a period of <15 min within an hour and without visiting any other

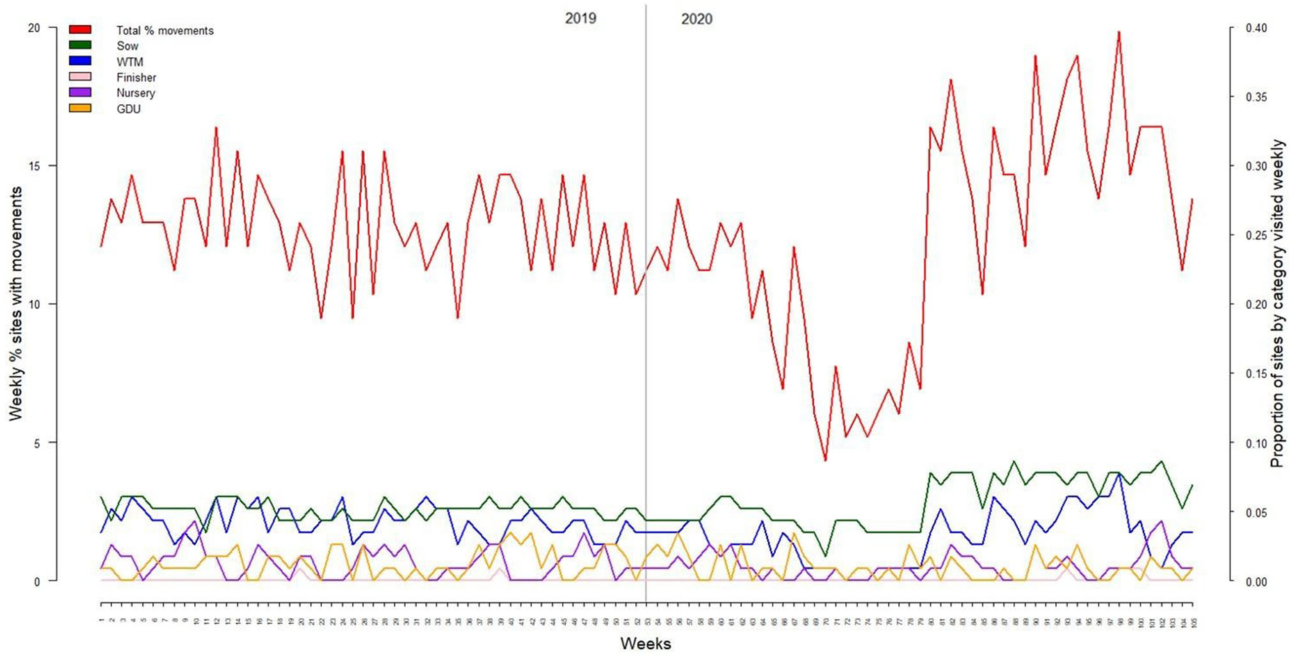

FIGURE 2
Weekly in-movement distribution by farm categories (proportion) and in total (%) for 2019 **(left)** and 2020 **(right)**.

node in between were merged and analyzed as one movement (i.e., loop to the same node). Additionally, those vehicles stopping for more than 24 h at the nodes were considered as "parked" and not performing an "operational activity" as they could be under maintenance or quarantine (e.g., downtime). The latter situation was included in the network analysis but excluded from our temporal analysis since they did not represent daily routine operation and the time spent at each node can be overestimated.

Network characteristics and features were described by the calculation of (a) network parameters, including the number of nodes and edges, average path length, diameter, edge density, and clustering coefficient, and (b) centrality metrics, such as degree (in and out), closeness (in and out), and betweenness for each node in the networks created. Definitions for each network parameter and metrics have been reviewed and described in a previous study by Martínez-López et al. (19). In brief, *average path length* is the average number of additional nodes contacted or visited in the trajectory to go from node$_i$ to node$_j$ in the network, *diameter* is the length of the shortest path within the two most distant nodes in the network (i.e., nodes that need the largest number of intermediate nodes to be able to connect each other), *edge density* indicates the proportion of contacts that occur in the network over the potential ones happening, and *clustering coefficient* (i.e., transitivity) is the frequency of loops connecting nodes reciprocally between each other (5). The *degree* is the number of contacts that each node has overall and, when directionality is applied, the number of connections originated (*out-degree*) or received (*in-degree*) by that node. *Closeness* is an estimate of how closely one node is connected to every other node in the network based on all incoming (*in-closeness*) or outgoing (*out-closeness*) connections, and *betweenness* represents the number of times certain node lies on the shortest paths when all the shortest paths are traced between nodes in the network.

To identify cohesive groups in the network, we recognized fully connected nodes in the network (i.e., *cliques*) and densely connected nodes (i.e., *communities*) across the networks constructed. Communities were detected using the algorithm base on propagating labels as an efficient and ease-of-implementation algorithm (20). In brief, unique labels are assigned initially to each node, and after each iteration of the algorithm, new labels are adopted by the nodes, based on the most frequent labels of the neighbors until they converge into one label which represents the community. In the case of ties among neighboring labels, one is randomly selected before the next algorithm iteration.

All statistical analyses were performed using R Studio v4.1.0 software (21). Network creation, description, parameter calculations, and visualization were performed via the igraph package v1.2.6 (22).

Results

Site and movement descriptive results

From the predefined 114 sites (i.e., nodes), 12 vehicles moved among them within 2 years of the study period, comprising 12 sow farms, 8 gilt development units (GDU), 17 nurseries, 71 wean-to-market (WTM), 4 finishers (i.e., sites/unique nodes), and 2 truck wash facilities. The nodes were located in Minnesota, South Dakota, Nebraska, and Iowa. After excluding 26% of the movements recorded as described earlier, 4,579 movements were analyzed, with 60% (2,749/4,579) of them occurring in 2019 and the remaining 40% (1,830/4,579) in 2020.

TABLE 1 Frequency of in-movements by farm-type (%). Graded scale colors represent a higher (red) and lower (white) percentage of movements across dyad connections in the 2 years of study (2019–2020).

Origin/ destination	GDU	NURSERY	SOW	TRUCK WASH	WTM	FINISHER
FINISHER	0.07	0.02	0.04	0.07	0.00	0.00
GDU	1.62	0.07	1.81	0.07	0.02	0.00
NURSERY	0.20	1.62	1.18	3.49	0.28	0.00
SOW	1.27	4.02	5.48	5.87	19.76	0.00
TRUCK WASH	0.50	0.63	20.92	2.42	2.07	0.00
WTM	0.02	0.44	7.29	14.48	1.05	0.00

GDU, Gilt development unit, WTM, Wean to Market.

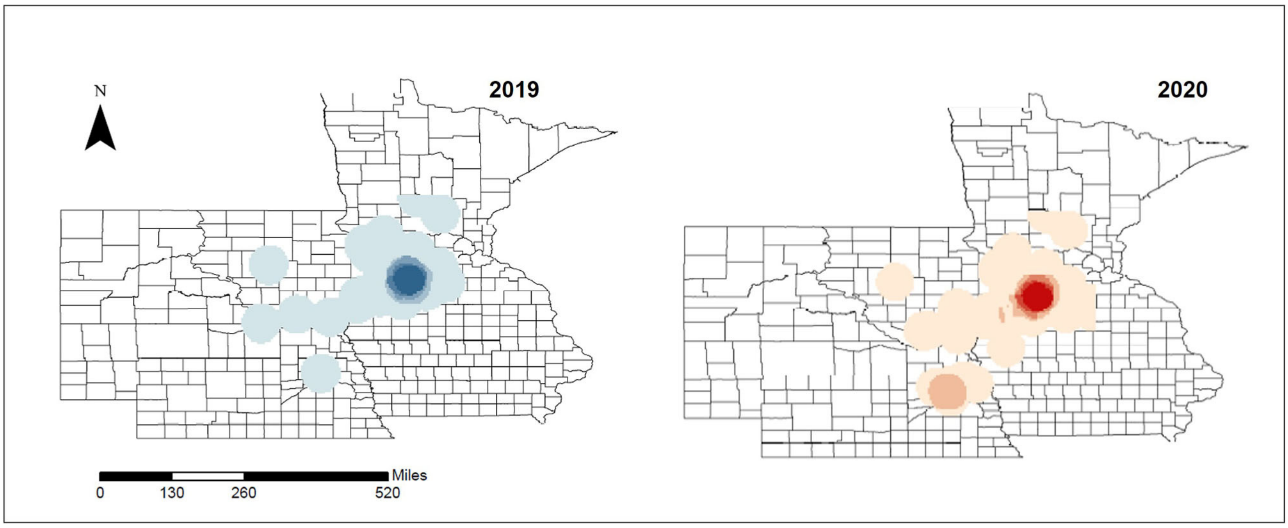

FIGURE 3
Density of incoming movements within the study area. Higher (dark) and lower (light) density areas (movements per county) are represented for 2019 and 2020, respectively.

Spatiotemporal results

The monthly and seasonal in-movement counts were similar across 2019 and 2020 (negative binomial regression $P > 0.05$), with 10–15% of the sites performing at least one movement weekly. The lowest frequency of movements was observed in weeks 70–78 of the study (i.e., May and June 2020, Figure 2). Movements toward sow farms in the winter and summer of 2019 were significantly higher ($P < 0.05$) than movements toward any other farm category or season-year with mean risk ratios ranging from 1.10 to 2257.34. Furthermore, there were fewer movements to finishers in spring 2020 ($P < 0.01$) than any other category or season-year, with risk ratios ranging from 0.0018 to 0.5 (Supplementary Table 1). The seven trucks performed the majority of the movements in both years studied, with 76.8% (2,111/2,749) in 2019 and 66.99% (1,226/1,830) in 2020, while the five trailers did the rest of the movements, 638 in 2019 and 604 in 2020.

In-movements classified by production type dyad indicated that 55.43% of the in-movements were performed by three dyads, sow farm to WTM sites (19.76%/4,579), WTM to truck wash (14.48%), and truck wash to sow farm (20.92%). The remaining in-movements were performed by multiple dyads (Table 1 and Supplementary Table 2). The spatial distribution of movements was consistently higher in the central area of the Midwest states analyzed for both years mapped (Figure 3).

Overall, time spent in the nodes was higher (RR: 1.1, $P < 0.03$) in 2020 than in 2019, with higher time spent at truck wash sites in 2020 but without any significant variation observed when analyzed by season (Table 2). However, time spent at truck wash facilities was significantly higher ($P < 0.001$) than any other site categories, with the occurrence of this category being 3.32, 5.83, 14.86, 16.00, and 21.19 times higher, on average, than sow farms, GDU, finishers, nursery, and WTM sites, respectively.

Network characterization

Descriptive statistics for network parameters and centrality metrics for the complete network and the network constructed for 2019 and 2020, respectively, are shown in Table 3. For the global network, on average, every site can be reached typically in three steps (*average path distance*), with less than a one-step reduction in 2019 and an increase in 2020, respectively. Although less than approximately 35% of the potential connections that

TABLE 2 Median (max-min) minutes spent in each node (i.e., sites) overall by production type and by season during the study period.

Production type	FINISHER		GDU		NURSERY		SOW		TRUCK WASH		WTM		Overall	
Stop time	Median (Min, Max)	*n*	Median (Min, Max)	*n*	Median (Min, Max)	*n*	Median (Min, Max)	*N*	Median (Min, Max)	*n*	Median (Min, Max)	*n*	Median (Min, Max)	*n*
Winter19[a]			44.0 (6.00, 602)	25	36.0 (14.0, 76.0)	66	28.0 (6.00, 1,220)	215	162 (11.0, 1,360)	173	26.0 (9.00, 75.0)	150	36.0 (6.00, 1,360)	629
Spring19	50.0 (50.0, 50.0)	1	64.0 (6.00, 478)	25	36.0 (9.00, 60.0)	32	26.0 (8.00, 1,290)	246	149 (9.00, 1,410)	195	22.0 (6.00, 146)	167	32.0 (6.00, 1,410)	666
Summer19	NA		60.0 (18.0, 160)	15	29.0 (10.0, 46.0)	62	26.0 (6.00, 1,280)	267	149 (8.00, 1,440)	210	20.0 (6.00, 101)	171	32.0 (6.00, 1,440)	725
Fall19	20.0 (20.0, 20.0)	1	44.0 (10.0, 148)	34	30.0 (8.00, 76.0)	43	27.5 (6.00, 1,260)	244	152 (6.00, 1,390)	187	20.0 (10.0, 64.0)	167	32.0 (6.00, 1,390)	676
2019	35.0 (20.0, 50.0)	2	54.0 (6.00, 602)	99	32.0 (8.00, 76.0)	210	27.0 (6.00, 1,290)	992	150^{+} (6.00, 1,440)	779	22.0 (6.00, 146)	668	34.0 (6.00, 1,440)	2,750
Winter20[b]			70.0 (8.00, 167)	25	26.0 (6.00, 60.0)	39	28.0 (6.00, 1,270)	173	870 (11.0, 1,420)	137	24.0 (10.0, 179)	116	34.0 (6.00, 1,420)	490
Spring20	NA		98.0 (26.0, 229)	13	22.0 (16.0, 128)	12	31.0 (6.00, 1,280)	112	127 (10.0, 1,350)	73	20.0 (10.0, 66.0)	43	42.0 (6.00, 1,350)	253
Summer20	NA		110 (38.0, 155)	15	31.0 (14.0, 88.0)	24	43.5 (6.00, 1,240)	218	733 (6.00, 1,430)	125	24.5 (6.00, 94.0)	120	45.0 (6.00, 1,430)	502
Fall20	32.0 (9.00, 58.0)	7	77.0 (44.0, 167)	16	31.0 (6.00, 77.0)	40	51.0 (7.00, 1,330)	233	459 (6.00, 1,440)	136	26.0 (8.00, 80.0)	144	47.0 (6.00, 1,440)	576
Winter21[c]			110 (110, 110)	1	25.0 (24.0, 26.0)	2	44.5 (18.0, 1,260)	30	185 (12.0, 1,350)	13	28.0 (14.0, 59.0)	17	44.0 (12.0, 1,350)	63
2020	32.0 (9.00, 58.0)	7	81.0 (8.00, 229)	70	26.0 (6.00, 128)	110	40.0 (6.00, 1,330)	746	345^{+} (6.00, 1,440)	470	24.0 (6.00, 179)	427	42.0 (6.00, 1,440)	1,830
Overall	32.0 (9.00, 58.0)	9	60.0 (6.00, 602)	169	30.0 (6.00, 128)	320	32.0 (6.00, 1,330)	1,738	176* (6.00, 1,440)	1,249	22.0 (6.00, 179)	1,095	36.0 (6.00, 1,440)	4,580

n, number of stops per season and production type. Multivariate negative binomial is significantly different ($P < 0.05$), (*) from other production types overall or between estimates from 2019 and 2020 (+). GDU, Gilt development unit, WTM, Wean to Market. a, Winter 19 includes January and February 2019; b, Winter 20 includes December 2019, January and February 2020; c, Winter 21 includes December 2020.

could occur really occurred (*edge density*) in the complete network when assessing it on a per-year basis of the study period, the proportion doubled in 2019 compared with 2020 (43.5 vs. 25.6%). Furthermore, *degree*, *in-degree*, and *out-degree* were higher for 2019 than 2020 with important higher outliers observed, corresponding to truck wash facilities and sow farms. A similar tendency was observed for centrality *closeness*. However, *betweenness* showed higher values for 2020 than 2019, and the nodes with the highest values were the two truck wash facilities (Figure 4B, red and orange circles).

Complete network cluster analysis recognized three communities, and 85.3% (2,808/3,292) of the cliques observed were fully connected in pairs (i.e., cliques' size of two). When yearly analyses were performed, larger cliques with up to seven nodes fully interconnected were identified each year (Table 4). Furthermore, two and four communities were observed in 2019 and 2020, respectively, after excluding those with two or fewer members.

Discussion

This study, to the best of our knowledge, was the first description of vehicle movements using GPS data of a typical pig production system in the Midwestern US. Moreover, we have provided the first comparison between networks from a typical year of production (2019), and 2020, a year in which the swine industry was strongly affected due to the temporary decline in slaughter rates as a result of the COVID-19 pandemic in the USA (23).

A total of 74% of the records were accurate and used in these analyses. Data generated by vehicle-tracking devices have shown to be a robust tool to understand connectivity among farms, which can give a more in-depth picture of the vehicle trajectory, sites visited, and time spent at each stop. This data can serve as a proxy for compliance regarding times dedicated to animal loading/unloading, vehicle flow and vehicle cleaning, and disinfection and downtime procedures (7). The majority of the movements were performed in 2019, with a 20% reduction during the following year and strong fluctuations in the proportion of farms with weekly movements in 2020 (5–20% of the sites performing weekly movements). As expected, trucks that performed the majority of movements were those used for transporting weaned piglets that, according to production management practices, were weaned from sow farms more than a week ago. The highest reduction of vehicle movements was during spring 2020, with a follow-up increase in mid-2020, mostly due to sow and WTM movements. One potential explanation could be the limitations associated with the high rates of COVID-19 illness among workers at slaughter plants, limiting the capacity for slaughter in abattoirs during the spring of 2020 (24) and, in other cases, the temporary shutdown of major slaughter plants in the region during April and May 2020 (23). Although, movements to slaughter were not included in this study, limitations on pig-slaughter is expected to affect the pig flow within the systems in the initial levels of production, which will recover later in that year. Another potential explanation could be an increase in sites covered by the trucks in response to the initial movement reduction, despite only 5 extra nodes being observed in 2020 compared to 2019 (85/80).

TABLE 3 Network parameters and centrality metrics at the node level calculated for the complete vehicle network and the 2019 and 2020 networks, respectively.

Global parameters	Complete network	2019	2020
Nodes	114	80	85
Edges	4,579	2,749	1,830
Edge density	0.3493	0.435	0.2563
Diameter	7	7	7
Average path length	3.0925	2.596	3.4837
Transitivity (global)	0.2218	0.205	0.2573
Node parameters	**Mean (min–Max)**	**Mean (min–max)**	**Mean (min–max)**
Degree	79.63 (2–2,199)	68.72 (2–1,546)	43.06 (2–653)
In	39.82 (1–1,095)	34.36 (1–770)	21.53(1–325)
Out	39.82 (1–1,104)	34.36 (1–776)	21.53 (1–325)
Closeness	0.0024 (7.63e-05–0.0034)	0.004 (1.6e-04–0.0058)	0.003 (1.4e-04–0.004)
In	0.0022 (7.628e-05–0.0033)	0.0036 (1.5e-04–0.0052)	0.0027 (1.4e-04–0.004)
Out	0.0022 (7.68e-05–0.0028)	0.0035 (1.5e-04–0.0048)	0.0027 (1.4e-04–0.0036)
Betweenness	234.40 (0–5694.07)	122.94 (0–3539.24)	203.72 (0–3017.81)

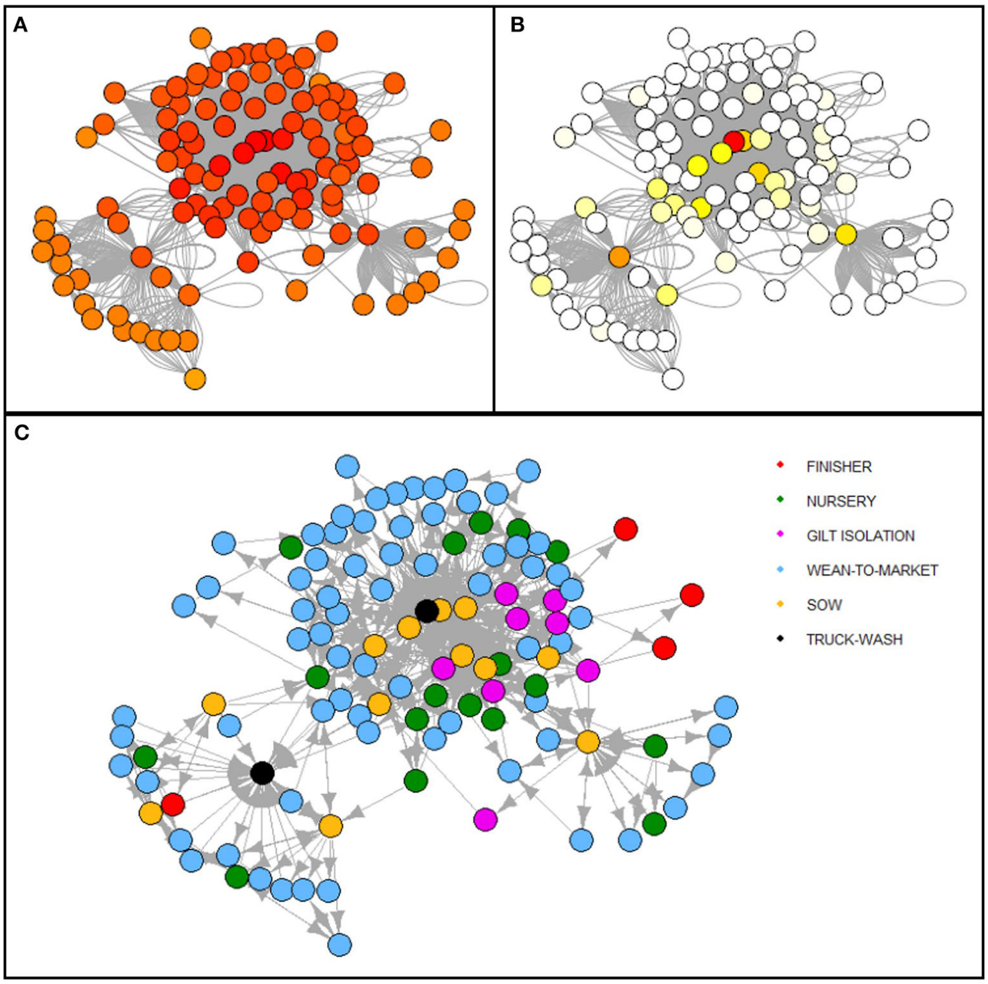

FIGURE 4
Network representation of the complete period analyzed (2019–2020). Heat scale colors represent **(A)** closeness and **(B)** betweenness relative values for each node, with the highest (red) and lowest (white) node values for each centrality metric, respectively. The five different categories of nodes and their connections are shown in different colors as reference **(C)**.

TABLE 4 Cliques and communities identified by the year of study (2019 and 2020).

2019				2020			
Cliques ($N = 805$)		Community	Nodes member ($N = 80$)	Cliques ($N = 594$)		Community	Nodes member ($N = 85$)
Size	*n*		*n* (n/N)	Size	*n*		*n* (n/N)
2	250	1	66 (0.825)	2	220	1	36 (0.424)
3	297	2	10 (0.125)	3	205	2	10 (0.118)
4	178			4	109	3	12 (0.141)
5	62			5	44	4	24 (0.282)
6	16			6	14		
7	2			7	2		

Communities with one or two members (i.e., nodes) are not included in this table.

As expected, a level of "fidelity" was observed within sites, with vehicles connecting sow, WTM, and truck wash facilities in the direction indicated by the production system. We observed that approximately 20% of the movements were performed from truck wash facilities to sow farms and from sow farms to WTM farms. However, approximately 14.5% of movements were connecting WTM to truck wash instead of the approximately 20% expected, which seemed to be balanced with movements from WTM to other sites, such as sow farms and WTM. Although WTM to WTM movements are common when pigs need to be finished in different sites, the movements of WTM to sow farms at the commercial level are rare as their terminal genetic makeup of the commercial market pig populations is not suitable if these animals were to be used as replacement breeding stock. Additionally, commercial growing pigs housed in WTM facilities are usually located in highly dense regions where the prevalence of endemic pathogens is high, which will represent a risk to the sow farm if these were to be introduced. WTM to sow farm movement exists but these are related to moving high-health gilts from the genetic multiplication herds that tend to have better health than commercial sow farms. Overall time spent in truck wash facilities (∼3 h) denotes a sufficient time for the washing and drying process recommended for animal transport within the industry (25), with a noticeable median increase among seasons in 2020, although maximum times remain constant. This can indicate changes in disinfection procedures due to COVID-19 awareness which resulted in longer times at the truck wash facilities. It is important to mention that trucks could spend more time at the truck wash after being cleaned as they would be parked until needed again. This could bias the parameter, overestimating the mean time spent at the truck wash facilities overall. This metric needs to be further studied and analyzed but is the first insight as a proxy for biosecurity compliance. Furthermore, there were more paths connecting truck washes in 2020, which together with the increase in the median time at truck wash observed can indicate that these changes in cleaning and disinfection procedures could potentially be a result of an increase in biosecurity concerns as a whole and affected the connectivity among the network that year. The observed larger number of communities in 2020 could be reflecting management practices applied to segregate the network and avoid disease transmission between sites. While we lack data to identify the reason for observing these nodes largely interconnected (i.e., clusters, cliques, and communities), it exposes the potential usefulness of these datasets when applying control or surveillance strategies targeting groups of sites at high/low risk of infection through vehicle movements (16, 26).

Network metrics displayed a high interconnection among sites through vehicles, in which regardless of the geographical distance among farms, they were only three steps away from each other (i.e., *mean distance*). Areas with a high density of vehicle movements were detected. This finding shows that movements are not randomly distributed among all sites integrating the system, highlighting potential target areas for disease active surveillance when resources are limited as described in other production systems globally (5, 27, 28). The validity of this finding in other states is yet to be evaluated. Comparison of these network metrics with others can be challenging since systems, data collection, movements, inclusion criteria, and time frame can vary across studies (5, 29, 30). However, the need to improve within-system movement records has been reported, as it represents an important factor in disease spread (6), and our study provides information on one of the denser swine production regions within the USA, and insights are representative of the Midwestern USA swine industry.

A limitation of this study is that the network characterization was performed in one production system representative of current pig production practices in the US and might not fully represent patterns of different states or regions of the USA. Another limitation is related to the number of trucks and trailers monitored, which perhaps underestimated connectivity trends. Although a low (9.3%) proportion of company vehicles were enrolled in the study, these vehicles were those used the most and continuously during 2019–2020, resulting in a robust representation of typical movement patterns before and during the COVID-19 pandemic. In addition, the network built in this study was composed of breeding farms, growing pig farms, and truck wash facilities which leave out the connectivity with slaughter plants and market-hog transport cleaning and disinfection facilities. Furthermore, the unusual aspects observed during the COVID-19 global pandemic highlight a unique situation within the swine industry. However, this analysis builds on the understanding of the complex network interactions of vehicles within the Midwest swine industry and gives insights into how unique events can affect the flow of the pork supply production, informing preparedness for future infectious disease epidemics and events.

Conclusion

In this study of vehicle movements within a pig production system, we observed a highly connected and structured organized network with high fidelity among the site triad of sow, WTM, and truck wash. We also described the impact on movements and network connectivity in 2020, when the peak of the COVID-19 epidemic was observed within the USA. The directionality of movements among sites and time spent at truck wash facilities represented a proxy for good biosecurity practices within the system. Monitoring and understanding spatiotemporal and network patterns of movements beforehand can prove useful in identifying high-risk areas and preparedness in the face of a new disease epidemic. The results also showed the grouping of farms within the system when considering the vehicles, indicating that many farms were connected by these indirect means. Knowledge obtained from this movement characterization in the Midwest may assist with targeting interventions, prevention, and control strategies for infectious diseases within the swine industry, with an emphasis on monitoring and reducing the prevalence in different communities connected by vehicles.

Data availability statement

The datasets presented in this article are not readily because available are confidential. Requests to access the datasets should be directed to corzo@umn.edu.

Author contributions

CP-R, CV, and CC: conceptualization. CV, JS, and CC: fieldwork and data collection. CC and MS: resources. CP-R: methodology and data analysis and writing the original draft. CP-R, CC, MK, CV, and JS: writing—reviewing and editing. CC: project administration. CC and CV: funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1201644/full#supplementary-material

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Contribution of cats and dogs to SARS-CoV-2 transmission in households

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Introduction: SARS-CoV-2 is known to jump across species. The occurrence of transmission in households between humans and companion animals has been shown, but the contribution of companion animals to the overall transmission within a household is unknown. The basic reproduction number (R_0) is an important indicator to quantify transmission. For a pathogen with multiple host species, such as SARS-CoV-2, the basic reproduction number needs to be calculated from the partial reproduction numbers for each combination of host species.

Method: In this study, the basic and partial reproduction numbers for SARS-CoV-2 were estimated by reanalyzing a survey of Dutch households with dogs and cats and minimally one SARS-CoV-2-infected human.

Results: For households with cats, a clear correlation between the number of cats and the basic reproduction number (Spearman's correlation: ρ 0.40, p-value: 1.4×10^{-5}) was identified, while for dogs, the correlation was smaller and not significant (Spearman's correlation: ρ 0.12, p-value: 0.21). Partial reproduction numbers from cats or dogs to humans were 0.3 (0.0–2.0) and 0.3 (0.0–3.5) and from humans to cats or dogs were 0.6 (0.4–0.8) and 0.6 (0.4–0.9).

Discussion: Thus, the estimations of within-household transmission indicated the likelihood of transmission from these companion animals to humans and vice versa, but the observational nature of this study limited the ability to establish conclusive evidence. This study's findings support the advice provided during the pandemic to COVID-19 patients to maintain distance from companion animals as a precautionary measure and given the possibility of transmission, although there is an overall relatively limited impact on the pandemic when compared to human-to-human transmission.

KEYWORDS

final size, zoonosis, multispecies, mathematical model, reproduction number, multilevel transmission, COVID-19, companion animal

Introduction

Since the beginning of the COVID-19 pandemic, SARS-CoV-2 infections have been reported in captive and domestic animals. Felines in the Bronx Zoo were found to be infected (1), and ~14.7% of cats (*Felix catus L.*) tested seropositive in early 2020 in the Wuhan region (2). It was found that domestic cats are susceptible to infection, and virus reproduction can occur (3). Furthermore, infection of cats by infected humans or other species (i.e., mink) has been reported (4). In later experimental studies, transmission between cats was confirmed

(5, 6). A Dutch seroprevalence study including companion animals with unknown SARS-CoV-2 exposure status showed 0.4% of cats and 0.2% of dogs (*Canis lupus familiaris* L.) to be positive for COVID-19 in both ELISA-assays and virus neutralization tests (7). Another survey in the Netherlands with dogs and cats from households with at least one human with a confirmed SARS-CoV-2 infection revealed 20.4% of cats and 17.3% of dogs to be seropositive and/or PCR positive (8). Experiments indicated that transmission between cats in confined spaces can be efficient (9). The epidemiological study on the cat population in Wuhan indicated the reproduction number to be low (1.09), implying the cat-to-cat transmission to be certainly possible but not highly efficient (6, 10). The presence of other endemic coronaviruses and reinfection with these viruses in domestic cats indicates that ongoing horizontal transmission of the coronavirus between domestic cats is not unprecedented (11). In 2021, Shia et al. were the first to report a cat-to-human transmission event of SARS-CoV-2 (12). This important finding indicates that cat-to-human transmission is possible, and given the human-to-cat transmission (2, 8) and the cat-to-cat transmission (5, 6, 9), these animals might contribute to the overall reproduction number in households.

Besides cats, SARS-CoV-2 research has also been conducted on dogs, albeit to a lesser extent. The study by Sit et al. showed that the SARS-CoV-2-positive dogs were most likely infected by humans (13). In one transmission experiment, none of the five contact dogs were infected, while only two out of the five inoculated dogs were seroconverted. No infectious virus was found in the swabs collected from inoculated dogs. The other tissues, including the lungs, were negative (3). To our knowledge, this is the only experiment with dogs. It is, however, not possible to draw an inference on the possibility of transmission between dogs due to the small number of animals in this experiment.

In the Netherlands, ∼23% of households own one or more cats, with an average of 1.7 cats per household. For dogs, these numbers are 18% and 1.2, respectively (14). Cats and dogs are likely to have intense contact with their owners, such as licking their faces or sleeping in a bed with them (15). This warrants an investigation of the potential role of cats and dogs in spreading SARS-CoV-2 in households. In this study, we quantified the potential role of cats and dogs in the transmission of SARS-CoV-2 within Dutch households by computing the basic and partial reproduction numbers.

Materials and methods

Datasets on humans, dogs, and cats in households

For this study, we made use of datasets obtained by a survey in households with at least one cat or dog and with at least one person who tested positive for SARS-CoV-2 by PCR. Samples were collected between July 2020 and April 2021, and during this period, the α-variant (PANGO lineage b.1.1.7) was dominant in the Netherlands (16). The survey was conducted (8) by recruiting households via the municipal health service. Persons could express their interest in participating by sending an e-mail, and they were then contacted for an appointment. The households were visited by a mobile veterinary clinic for the sampling of the animals, which was available twice per week. Animals that tested PCR-positive underwent a follow-up examination, which occurred 1–3 weeks after the initial visit.

For the modeling performed in this study, we assumed that the final size was reached within these households. This implies no occurrences of new infections due to within-household transmission after the moment of recording the number of cases. This assumption was viewed as realistic, given the generation time of around 5 days (17) for the dominant virus variant at the time in the Netherlands and the delay between health service testing and inclusion in this study.

The survey was conducted amongst 196 households, of which five households had missing data for the number of humans in the household, so these were excluded. Of the 191 remaining households, 95 households had one or more cats and 121 households had one or more dogs. Only households for which the test results of all adult household members and companion animals were available were included for data analysis in this study. The resulting dataset comprised a total of 150 cats, 153 dogs, and 593 humans. Following the assumption that an infection in a household started with one human index case, 123 secondary human cases were found in 87 households, 30 infected cats in 24 households, and 27 infected dogs in 24 households.

These data were analyzed for households with cats and dogs assuming equal transmission (which we will name for convenience "companion animals"), with cats only, and with dogs only. It was not feasible to estimate transmission between cats and dogs due to the low number of 25 households in this study with both cats and dogs.

Within household transmission

The final size of an outbreak is the number of individuals that have been infected during the entire duration of the outbreak. The number of infected individuals during a household outbreak (i.e., the final size) follows a probability distribution, given the probability of all integer numbers between 1 (index) and all individuals being infected. This so-called final size distribution can be determined based on a stochastic epidemiological SIR model for single and multiple types of individuals (18, 19). Thus, we considered the situation where the infection is introduced by one individual in the household.

Correlation between R_0 for humans and animals

First, we estimated the reproduction number of humans only but included the ratio of animals to humans in the analysis as a covariate. For this analysis, only households with at least two humans were used because, otherwise, transmission between humans within the household was not possible. The regression coefficient indicates the effect of increasing the ratio of animals to humans in the household on the basic reproduction number. Additionally, we estimated the basic reproduction number for each household separately and determined a correlation with the ratio of companion animals to humans with a Spearman rank correlation test.

Estimating partial reproduction numbers for within- and between-species transmission

Next, the partial reproduction numbers (see Figure 1) were calculated for both companion animals, cats only, or dogs only. Partial reproduction numbers R_{ij} are the number of new infectious individuals of type j (1 = human or 2 = animal) produced by an individual of type i (1 = human or 2 = animal). For example, R_{12} is the number of new infectious animals (type 2) caused by one infectious human (type 1) during its entire infectious period. The actual number of new infections also depends on the number of susceptible individuals of a certain type (S_1 or S_2) and the total household size determined by the total number of humans in the household H.

The final size distribution for two types (human and animal) after an introduction by one individual was determined by the number of individuals of each type in the household and these partial reproduction numbers R_{ij} (18–20). The partial reproduction numbers R_{ij} are the elements of the next-generation matrix (NGM) that determine the number of new cases of type j caused by type i. The overall within-household reproduction number R_0 is the largest eigenvalue of the next-generation matrix for a given household composition (i.e., number of humans and companion animals). The equations for the final size distribution and basic reproduction number are given in the Supplementary material and provided as an algorithm in a mathematical notebook.

We used the two-type SIR model to estimate the transmission parameters for a household with humans and companion animals, with humans and cats, or with humans and dogs. For the two-type SIR model, we considered that mixing between humans, companion animals, and human and companion animals is proportionate to the number of humans alone, i.e., the resulting NGM will only contain the ratio of companion animals to humans ($\frac{C}{H}$). See Supplementary material. The basic reproduction number for a household with a specific ratio of companion animals to humans was then calculated by the following Equation (20):

$$R_0 = \frac{1}{2}\left(R_{11} + R_{22}\frac{C}{H} + \sqrt{\left(R_{11} - \frac{C}{H}R_{22}\right)^2 + 4\,R_{12}\,R_{21}\frac{C}{H}}\right) \quad (1)$$

Parameter estimation for the within-household model was obtained by maximum likelihood estimation based on the final size distribution. The confidence intervals for the partial reproduction numbers R_{ij} (the elements of the NGM) were derived using profile likelihood methods. The confidence interval for the overall reproduction number R_0 were obtained using a bootstrapping procedure.

Scenarios

To calculate the effect of reducing contact with animals and between animals, we adapted our model to include a proportionate decrease in time spent by animals in contact with other household members, for example, because these are outdoors. To enable the calculation of differences in time spent in contact with infected families, we distinguished between the number of contacts made by an infectious animal (ω_I) and susceptible animals (ω_S). Although, in practice, this is often impossible, it allowed us to study the effect of reducing spread by preventing transmission to animals and by preventing transmission from animals. The transmission matrix within the household **R** is as follows:

$$\mathbf{R} = \begin{pmatrix} R_{11} & (1-\omega_I)\,R_{21} \\ (1-\omega_S)\,R_{12} & (1-\omega_I)(1-\omega_S)\,R_{22} \end{pmatrix} \quad (2)$$

With the heterogeneity within the household model, we calculated the mean final sizes for different household compositions when the infection is introduced by an infected cat or by an infected human. Four different scenarios are shown with cats either spending their time completely in contact with household members, having no contact with susceptible animals, having no contact with infected animals, or having no contact with animals at all.

Software

All data processing and calculations were conducted using Wolfram Mathematica version 12.0. The Mathematica notebook can be found in the Supplementary material.

Results

Estimation of within-household transmission

Human-to-human with cats and dogs, cats, or dogs as a covariate

First, we estimated the reproduction number of humans without considering animals as a different type in the transmission model but included them in the analysis as a covariate. For this analysis, only households with at least two humans were used because, otherwise, transmission within the household was not possible. The overall human-to-human reproduction number R_0 was 1.17 (0.92–1.47) in this dataset.

The individual household reproduction numbers were calculated, and these were positively correlated with the number of animals (Spearman ρ 0.28, p-value 1.8 x 10^{-5}) and cats (Spearman ρ 0.40, p-value 1.4 x 10^{-5}) but not with dogs (Spearman ρ 0.12, p-value 0.21). Including the human-to-animal ratio and human-to-cat ratio in the estimation of the basic reproduction number R_0 improved the fit of the model and again showed an increase in R_0 with cats per human. In contrast, the human-to-dog ratio did not improve the model fit (Table 1).

The household sizes of cat (mean 2.3, se 0.1) and dog owners (mean 2.4, se 0.1) did not differ (t-test −0.75, $p = 0.45$).

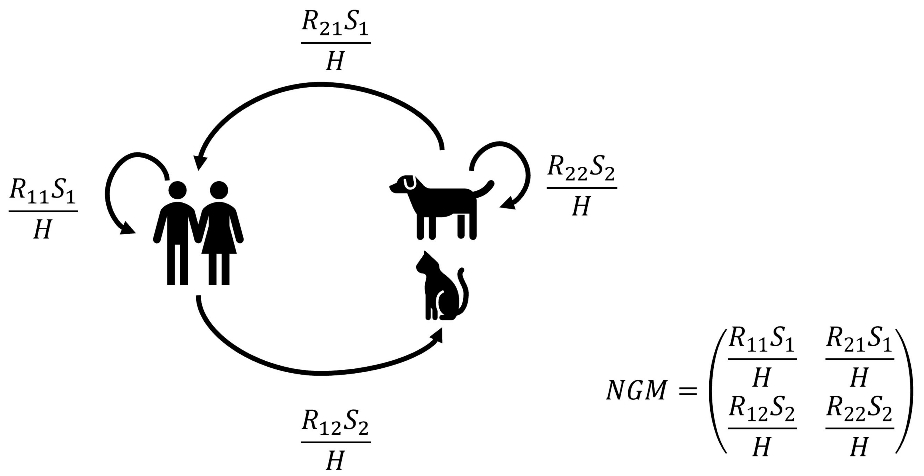

FIGURE 1
Graphical representation of the transmission model and the next-generation matrix (NGM). Partial reproduction numbers R_{ij} are the number of new infectious individuals of type j (1 = human or 2 = animal) by an individual of type I (1 = human or 2 = animal). For example, R_{12} is the number of new infectious animals (type 2) caused by one infectious human (type 1). The actual number of new infections does also depend on the number of susceptible individuals of a certain type (S_1 or S_2) and the total household size H, i.e., the number of humans in the house.

TABLE 1 Reproduction number for human-to-human transmission with the effect of animal-to-human ratio as a covariate.

Model	R_0	95%-CI	Effect of animal/human-ratio	95%-CI	AIC
Baseline	1.17	(0.93–1.47)			363
Companion animals	0.89	(0.57–1.34)	1.97	(0.18–4.79)	355
Cats	0.91	(0.57–1.39)	1.19	(-0.05–2.97)	358
Dogs	1.10	(0.69–1.66)	0.33	(-0.15–2.19)	364

The effect of animals should be interpreted as an increase in reproduction numbers in a household with an increase of one animal per human. R0, reproduction number, 95%-CI, 95%-confidence interval, and AIC, Aikaike's information criterion.

TABLE 2 Estimates for transmission between companion animals and humans. The partial reproduction numbers R_{ij} have subscripts indicating transmission of *i* to *j*; 1 = human and 2 = animal (companion animal, cat, or dog).

	Human and companion animal		Human and cat		Human and dog	
R_{11}	1.19	(0.90–1.44)	1.26	(0.92–1.49)	1.11	(0.88–1.44)
R_{21}	0.39	(0.00–2.15)	0.30	(0.00–2.02)	0.30	(0.00–3.48)
R_{12}	0.63	(0.42–0.77)	0.56	(0.36–0.78)	0.57	(0.41–0.86)
R_{22}	0.00	(0.00–0.27)	0.00	(0.00–0.48)	0.00	(0.00–1.26)

Human and companion animals as separate types in transmission modeling

In total, 191 households with complete records containing one or more companion animals, i.e., cats, dogs, or both, were included in the estimation procedure. In these households, 314 out of 593 humans and 56 out of 303 dogs and cats tested positive, respectively.

This gives a partial reproduction number for human-to-human transmission of 1.19 (0.90–1.44) and for human-to-companion animal transmission of 0.63 (0.42–0.77). The estimates on transmission from companion animals to humans and between companion animals have a higher level of uncertainty due to the low numbers of infected companion animals and the assumption that, for each household, a human introduced SARS-CoV-2. The companion animal-to-human transmission was 0.39, for which the lower limit remains undetermined, indicating that it was very close to zero, and the upper limit was 2.15, and companion animal-to-companion animal transmission was 0 with an upper limit of 0.27. The lower limit of an estimate equals the point estimate if the point estimate is zero. The results are summarized in Table 2.

The basic reproduction number in a household increases with the ratio of companion animals to humans, from 1.19 (0.90–1.44) in the absence of companion animals to 1.94 (1.59–2.23) with six companion animals to one human (Figure 2).

Humans and cats as separate types in transmission modeling

In total, 191 households with complete records, of which 91 contained one or more cats, were included in the estimation procedure. In these households, 314 out of 592 humans and 30 out of 150 cats tested positive. We did not consider dogs to be infectious in these analyses.

This resulted in a partial reproduction number for human-to-human transmission of 1.26 (0.92–1.49) and for human-to-cat of

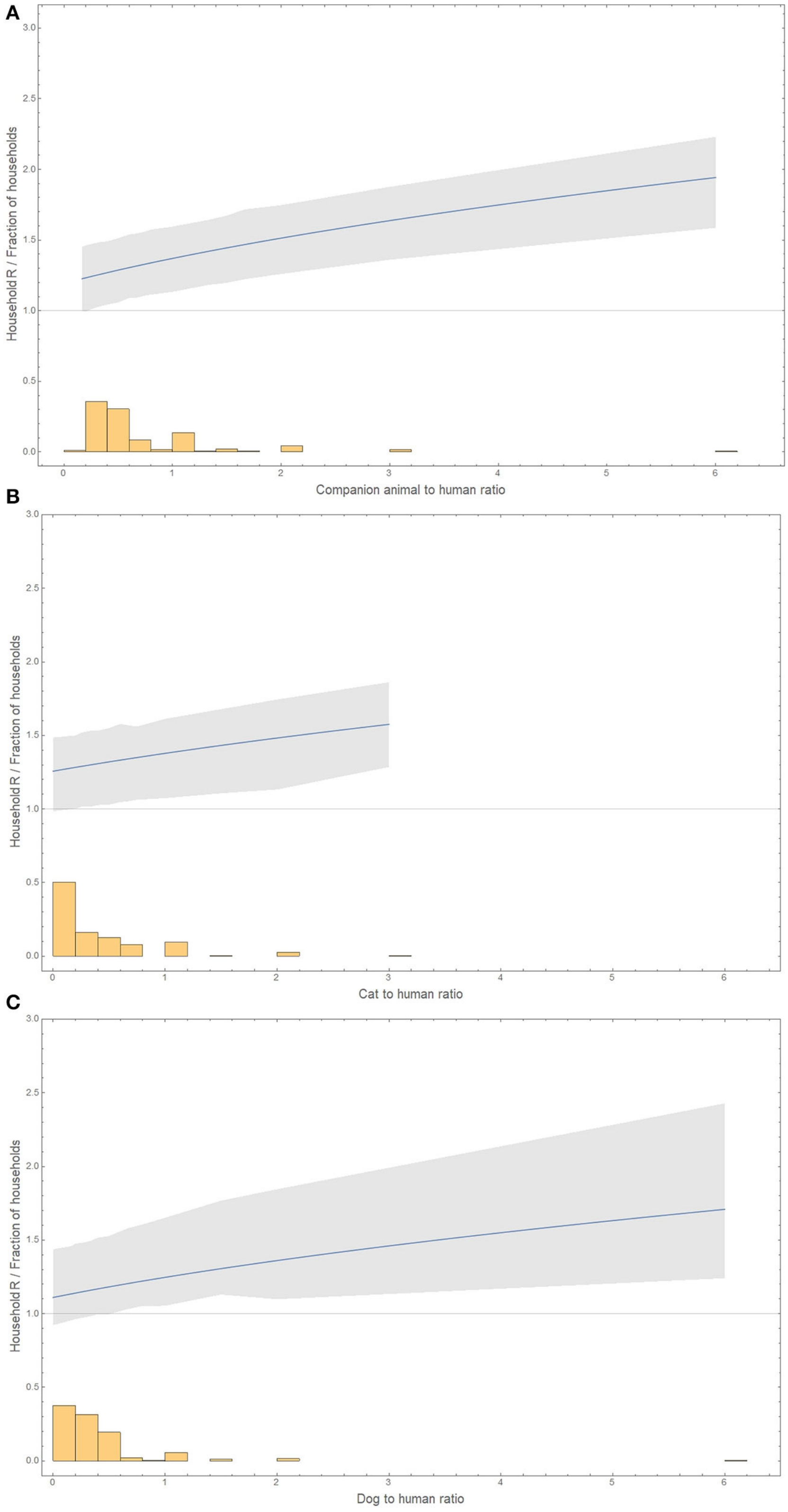

FIGURE 2
Animal-to-human ratio distribution (histogram) and the basic reproduction number for these household compositions (line and bootstrap 95% confidence interval as a gray area). **(A)** Companion animals. **(B)** Only cats. **(C)** Only dogs. The x-axis has for dogs or companion animals to humans a maximum of 6, and for cats to humans, the maximum was 3. The y-axis represents either the household's basic reproduction number or the fraction of households with a certain ratio of animals to humans. The basic reproduction number is shown only in the range of observed values of animal-to-human ratios.

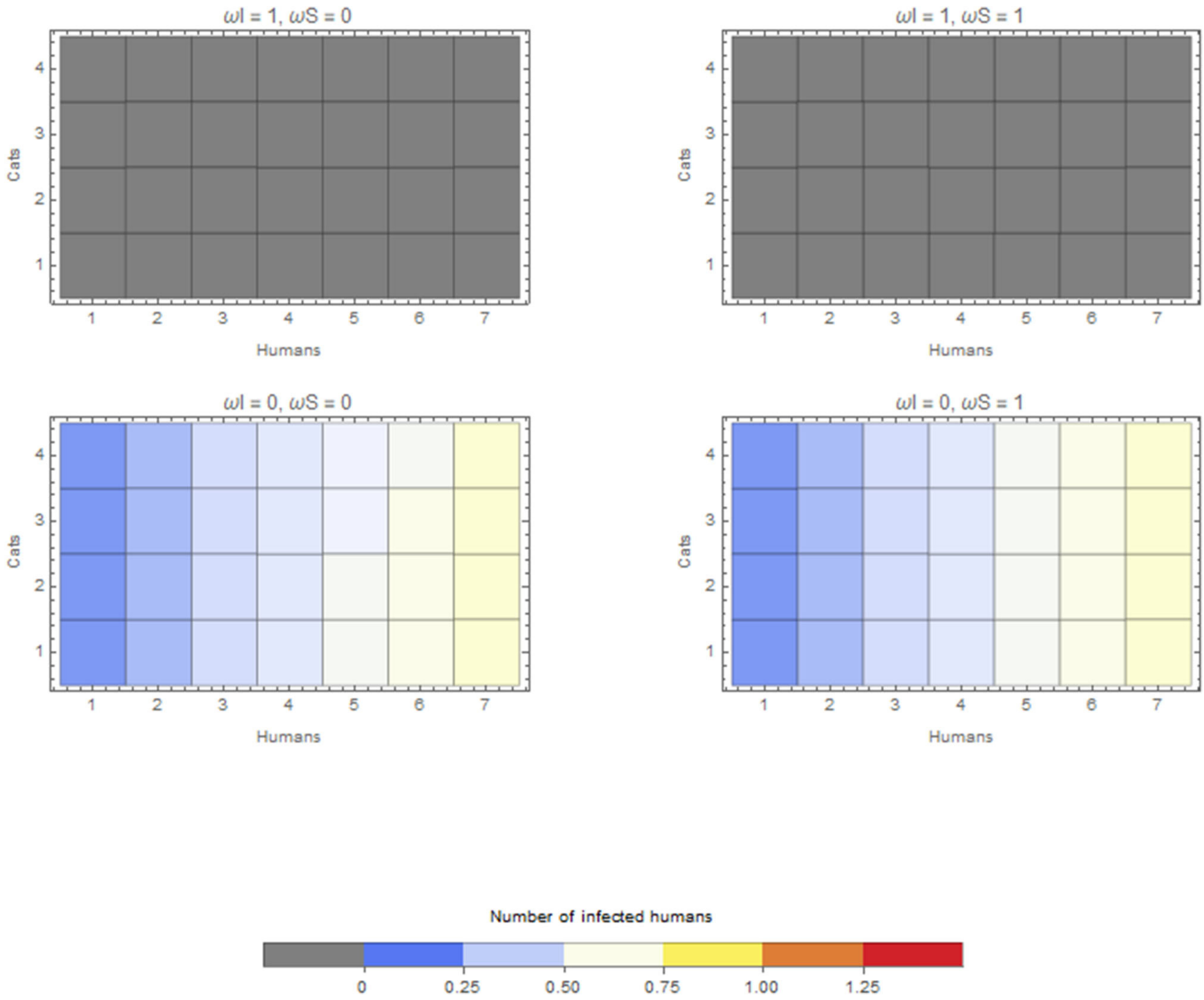

FIGURE 3
Average final size of infected humans when the infection is introduced by a cat for extreme values of ω_I and ω_S. Cats that do not go outside $\omega = 0$ or are always outside $\omega = 1$.

0.56 (0.36–0.78). The transmission estimates from cats to humans and between cats have a higher level of uncertainty due to the low numbers of infected cats and the assumption that, for each household, a human introduced SARS-CoV-2. The cat-to-human transmission was 0.30 (0.00–2.02), and the cat-to-cat transmission was zero, with an upper limit of 0.63. The lower limit of an estimate equals the point estimate if the point estimate is zero. The results are summarized in Table 2.

The basic reproduction number increases with the ratio of cats to humans, from 1.26 in the absence of cats to 1.58 (1.29–1.86) with three cats to one human (Figure 2).

Humans and dogs as separate types of transmission modeling

In total, 110 households with complete records containing one or more dogs were included in the estimation procedure. In these households, 186 out of 378 humans and 26 out of 134 dogs tested positive, respectively. We did not consider cats to be infectious in these analyses.

This resulted in a partial reproduction number for humans-to-humans of 1.1 (0.88–1.44) and the human-to-dog of 0.57 (0.41–0.86). The estimates on transmission from dogs to humans and between dogs have a higher level of uncertainty due to the low numbers of infected dogs and the assumption that, for each household, a human introduced SARS-CoV-2. The dog-to-human transmission was 0.33, for which the lower limit was 0.00, the upper limit was 3.38, and the dog-to-dog transmission was 0, with an upper limit of 1.26. The lower limit of an estimate equals the point estimate if the point estimate is zero. The results are summarized in Table 2.

The basic reproduction number increases with the ratio of dogs to humans from 1.11 in the absence of dogs to 1.71 (1.24–2.43) with six dogs to one human (Figure 2).

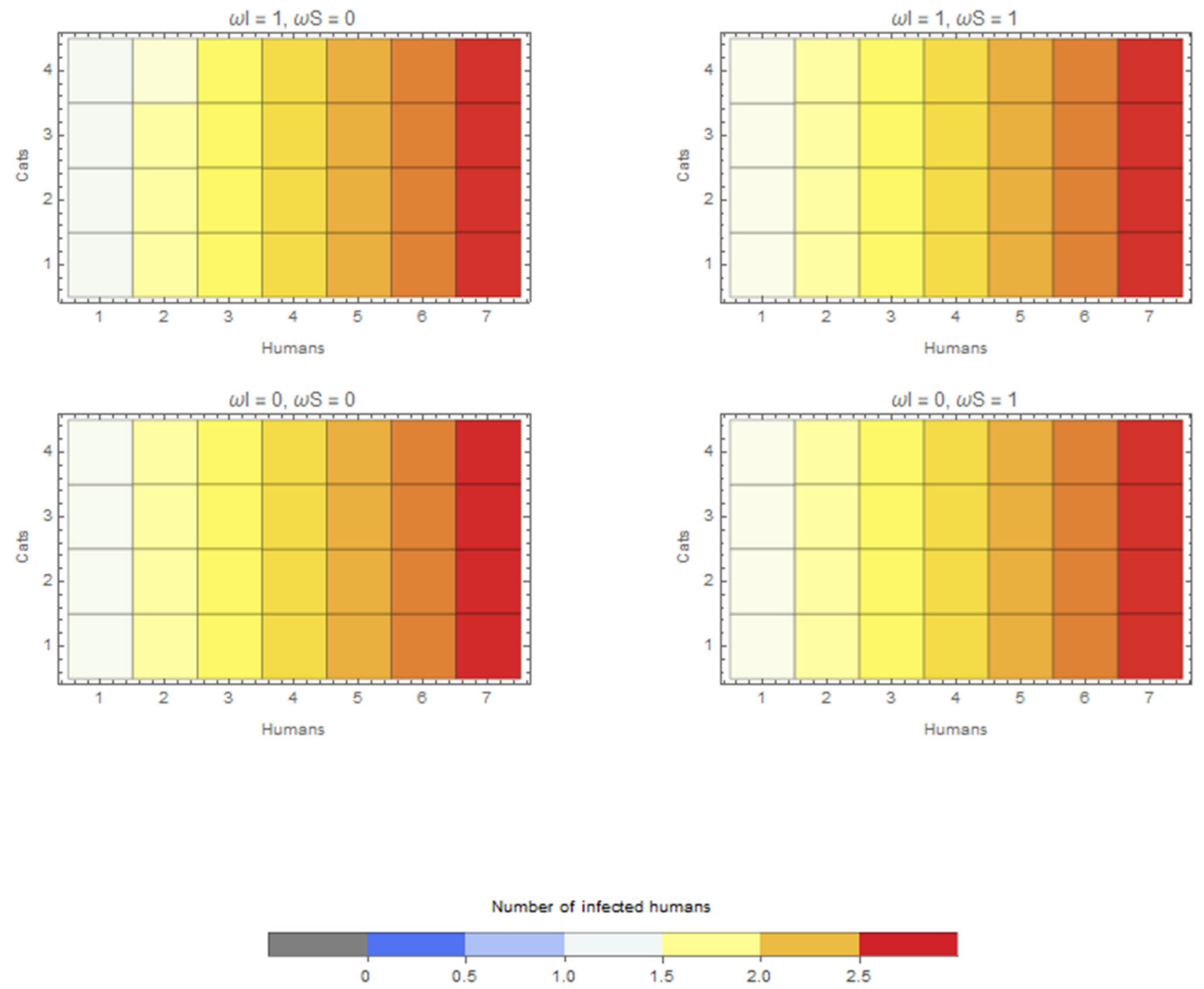

FIGURE 4
Average final size when the infection is introduced by a human for extreme values of ω_I and ω_S. Cats do not go outside $\omega = 0$, or cats are always outside $\omega = 1$.

Scenarios

Within-household

The within-household outbreak size in relation to the duration of time spent in the household by companion animals is presented here for cats only. Cats have the largest effect, given the estimates above. A similar pattern was found for companion animals and dogs. In the scenario considering a cat as the index case in a household, if this infectious cat does not spend any time within the household ($\omega_I = 1$), this cat will not cause an outbreak in the household (top two panels of Figure 3). If this index case cat is kept within the household all the time ($\omega_I = 0$), the final size depends on the number of cats in the household and depends on whether these susceptible cats are kept inside (bottom two panels of Figure 3). Keeping both susceptible and infectious cats inside (left bottom panel of Figure 3) causes the largest within-household outbreaks. However, these differences are subtle.

In the scenario with a human as the index case in a household, the median final size of the outbreak is 2.17, irrespective of whether cats are kept inside the house or outside. The maximum final size is, however, slightly different, that is, 2.78, when cats are kept inside the house and 2.75 when kept outside. The overall effect is thus limited compared to the role of humans regarding within-household transmission (Figure 4).

Discussion

The findings regarding within-household transmission showed a likely but not conclusive indication of SARS-CoV-2 transmission from companion animals to humans, especially for cats. The ratio of cats and companion animals was associated with an increase in the reproduction number in a household, which was not found for dogs alone. For both species, a non-zero estimated value was found for animal-to-human transmission, but this could not be distinguished statistically from zero.

Although our findings may indicate a potential role for companion animals and, in particular, cats in the within-household transmission of SARS-CoV-2, some caution in the interpretation is required. We did not consider other confounding factors like behavior, housing conditions, or the age of the owner, which could be related to increased transmission and ownership of cats or dogs, and the number of companion animals in a household. Still, given the evidence for spill-over between species of SARS-CoV-2 (2, 4, 7, 12), a role in transmission is conceivable, and these analyses provide additional evidence and quantification of the partial reproduction numbers.

Both the results of analyses of the correlation between R_0 when only considering humans as hosts and the ratio of companion animals and the estimates for the model with both transmissions to and from companion animals indicate stronger evidence for cats having a potential role in transmission than dogs. For both species, we found non-zero estimates for transmission from animal to humans, but the overall uncertainty for dogs was greater. Additionally, we found a positive correlation for cats in the overall R_0 when only considering humans as hosts, but not for dogs. R_0 does not depend on the household size; thus, an increase in R_0 with more animals per human suggests additional transmission by these animals, or, as stated above, it can be due to a confounding factor. Furthermore, the average number of cats per household is higher, which increases the potential transmission to and from these animals. The role of dogs can, however, not be disregarded (yet), given the current limited knowledge. It is worth noting the overall limited role of cats and dogs compared to human-to-human transmission in light of the course of the pandemic.

The reproduction rate of both cats and dogs in experimental settings is highly uncertain. Inferring R_0 the data of Bosco-Lauth et al. (9) gives an estimate of 0.37 to infinity. Experiments by Gehrards et al. (6) obtained a basic reproduction number of 2.50 (0.97–5.15). Our results on households are lower than those experiments and bounded by 0. For stray cats in Wuhan with no known contact with humans, the basic reproduction number was estimated to be $\sim$1.1 (10). In the sensitivity analysis, when the cat-to-cat reproduction number was fixed at a value of 1.1, no relevant change in parameters was observed. When both the cat-to-cat and human-to-human reproduction numbers were fixed at values of 1.1 and 1.4, respectively, the cat-to-human transmission was estimated to be zero. However, it is important to note that there was an upper limit of 1.63 in the confidence interval (see Supplementary material). This shows that our results cannot unequivocally prove cat-to-human transmission in these households nor disprove this route. Experimental studies provide evidence that cats reproduce the virus and can transmit it to other cats and, thus, most probably also to other mammals (6, 9). Animal-to-human transmission of SARS-CoV-2 has been observed in farmed mink and farm workers, showing the potential for transmission from animals to humans (21). Furthermore, the report of a cat-to-human transmission event in Thailand makes this route more probable (12).

An experimental study with dogs did not observe transmission between dogs. This could indicate that dogs might not be able to transmit the infection, but this study was too small in size to draw such conclusions (3). Only two out of five inoculated dogs did seroconvert, and solely two susceptible dogs were added as contacts. Consequently, owing to this lack of power, the confidence interval on the reproduction number was wide, with an upper limit of 44.

In the Netherlands, the number of people per household (the household size) is typically relatively small (on average 2.13). We had not considered other living conditions with intense contacts, such as a student housing with shared sanitary and kitchen facilities. Most domestic cats live in households alone or with one other cats (14), so there cannot be a sustained endemic within a household. It should be taken into consideration that we did not include feral cats or catteries. These larger groups of cats could become reservoirs from which reintroduction of the virus is possible. In Wuhan, China, a high prevalence of cats shows the potential for a reservoir among cats found on the streets (2). However, this has not been identified in the Netherlands to date based on the low seroprevalence detected in shelter cats (22). Quantification of the likelihood of a reservoir in feral cats requires another modeling approach, including a thorough quantification of the interaction between different colonies of feral cats, between catteries, and between animal shelters. Moreover, dogs tend to be kept alone in households. The population of feral or semi-feral dogs and, of course, shelters could also be potential populations for sustained transmission amongst dogs.

In contrast to feral cats (23), little is known about the interaction between domestic cats related to infectious diseases outside their own households. Interactions between cats from other households could occur during fights or by transmission via the environment in overlapping territories. Dog-to-dog transmission outside the household can occur when walking the dog or in shared airing areas. Transmission from companion animals to humans or human-to-companion animals could occur via the same pathway via the environment or by petting. Furthermore, feeding feral or neighboring cats might be a way in which cats are stimulated to visit more than one household. This can potentially contribute to between-household transmission (see Supplementary material), but to determine this with any certainty, data on between-household transmission are required.

Although vaccination coverage in many countries is reaching the required level (https://covid19.who.int/table), the virus can still spread among the vaccinated population due to waning immunity or new variants (24). It is, therefore, warranted to keep track of SARS-CoV-2 susceptible companion animals in the household when considering the epidemiology of SARS-CoV-2 in humans. Close contact with companion animals by SARS-CoV-2-positive humans increases the probability of this animal becoming infected, but if animals are kept inside for a sufficiently long period, this will mitigate the risk of animals acting as vectors between households (see Supplementary material). In the Netherlands, people with a SARS-CoV-2 diagnosis/suspicion are advised to keep their distance from companion animals, which is also supported by this study, although it should be taken into consideration that the overall contribution of companion animals is limited. Moreover, avoiding contact with companion animals from other households, even when visiting these households, can be viewed as a general method to reduce the impact of companion animals acting as potential vectors for the transmission of infectious diseases between households.

Data availability statement

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

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Ethical review and approval was not required for the animal study because this study was conducted on previously obtained data. Sampling of the animals was approved by the Animal Care and Ethics Committee of Utrecht University, in accordance with the Dutch law on experimental animals (approval number AVD1080020209666). Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

EF and MCMD had an equal contribution to the development and application the mathematical models. MCMD and JS initiated the study. EB, MMTD, and HK provided the data and discussed the interpretation of the outcomes of mathematical models. All authors contributed in writing the manuscript.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1151772/full#supplementary-material

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Building subnational capacities in animal health to deliver frontline cross-sectoral health services in Kenya

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Introduction: Operationalizing effective subnational veterinary services as major contributor to disease surveillance, reporting, diagnoses and One Health requires resources and mindset change. Here we describe workforce capacity building in animal health in Kenya and an approach that can be used to skill-up this workforce to respond beyond animal health challenges to emergent One Health realities and public health emergencies. Furthermore, triggering a paradigm shift has been identified for impactful delivery of health services, thus mindset change are important for learning new skills, but they also affect the way that we think about everything, for instance training in field epidemiology. Emphasis was therefore placed on skills, beliefs, and mindset shift.

Methods: Contextualized within the Kenyan environment, this description identifies problems likely to be found elsewhere: They are (a) The limited programs that offer structured and routine on-the-job training for animal health workers; (b) Unequal distribution and inadequate quantity and quality of highly skilled workforce with appropriate technical training and scientific skills to combat public (and animal) health challenges at the frontline; (c) Health challenges occasioned by climate change and drought, including feed, and water scarcity; and (d) Inadequate contingency, preparedness, and response planning for effective deployment of ready-to-trigger workforce capacity. In-Service Applied Veterinary Epidemiology Training (ISAVET) is a four-month long training program targeted at capacity building of frontline animal health professionals. The training, which is currently implemented in 17 African countries, is innovative and a customized field epidemiology program, which responds to specific needs in animal health and contribute to approaches utilizing One Health.

Results: Several trainees have marked mindset change as shown in the outputs and outcomes. Positive attitudes towards improving animal health surveillance were noted during the evaluation process.

Discussion and Conclusion: Most existing workforce capacities in the animal and public health systems were built for specific fields, and hardly respond optimally for cross-sectoral purposes. We proposed customised in-service applied veterinary epidemiology training that bypasses narrow-scoped workforce development but meets multifunctional, multidisciplinary and multisectoral needs before and during emergencies.

KEYWORDS

cross-sectoral engagement, capacity building, ISAVET, localization, Kenya

1. Introduction

The International Health Regulation 2005 (IHR 2005) guidelines recognizes the need for World Health Organization (WHO) member states to meet their core capacity requirements for surveillance, reporting and response activities (1). Using the whole of society approach to mobilize multiple sectors and disciplines to work together in fostering well-being and tackling threats to health and ecosystems is important. This approach must address critical societal needs, act on climate change mitigation, and contribute to sustainable development and health systems at national and subnational levels (2).

The JEE is a voluntary, collaborative, multisectoral process to assess country capacities to prevent, detect and rapidly respond to public health risks. Kenya participated in the Joint External Evaluation (JEE) in February to March 2017 (3). In the follow-up report, the lack of a structured and routine on-job training for animal health workers was identified as a major hindrance to attaining workforce with appropriate technical training and scientific skills to combat public (and animal) health challenges at the frontline (3). Furthermore, Kenyan battles persistent challenges that need innovative solutions including: (a) Limited Programs that offer structured and routine on-the-job training for animal health workers (4, 5); (b) Unequal distribution and inadequate quantity and quality of highly skilled workforce with appropriate technical training and scientific skills to combat public (and animal) health challenges at the frontline (4); (c) Health challenges occasioned by climate change and drought, feed, and water scarcity (6, 7); and (d) Inadequate contingency, preparedness, and response planning for effective deployment of ready-to-trigger workforce capacity (8, 9).

The animal health services in Kenya are decentralized into the national and county (subnational) services, and these are led by veterinary doctors, and supported by animal health scientists, technicians and other veterinary paraprofessionals. The dearth of veterinarians (quantitatively) are observed in the government ministries, para-veterinarians constituted the majority animal health workforce in Kenya, both at the national, counties, and the private companies. The capacities of this workforce to respond, prevent and detect disease specific zoonoses and One Health concerns range from 1.17 to 3.25; 1.19 to 3.44, and 1.14–3.67 respectively, on a scale of 5.00 (10). This is linked to the uneven level and intensity of training among the different categories that form the animal health workforce, as well as available resources. Hence, these challenges warrant that innovative methods are used to address them, and with the aim of capacity building of frontline animal health workers with the requisite skills to enhance early detection and response in mitigating the impact of potentially transboundary and zoonotic pathogen threats at their source was determined as issue of high priority (11, 12).

To respond to these challenges, the Food and Agriculture Organization of the United Nations (FAO), the Institute for Infectious Animal Diseases (IIAD) of Texas A&M University, through funding from the United States Agency for International Development, developed a comprehensive curriculum (13), for Frontline In-Service Applied Veterinary Epidemiology Training (ISAVET). The curriculum that was launched in a pilot training in October 2018 to April (13) in Kampala Uganda has since been rolled out in 17 countries of West, Central, East and Southern Africa. This training program primarily focuses on building the capacity of field animal health workers particularly veterinarians and paraveterinarians who are critical in addressing endemic, emerging infectious and transboundary animal diseases on the frontline. It also deliver additional benefit by providing workforce that meets the cross-sectoral collaboration strengthening by focusing on One Health training in field epidemiology to enable common understanding between public and animal health workforce, because structurally, it complement the Field Epidemiology and Laboratory Training Program (FELTP). In addition, because it is necessary to have mindset change to deliver impactful animal health services at the frontline, the learning of new skills and thinking around health service delivery were included using the concept that focuses on paradigm shift in service delivery. In countries, FAO works with the relevant ministries responsible for agriculture, health, wildlife and environment to implement the training Program.

In Kenya, the first Cohort of ISAVET training was launched on 7th June 2021, in Nakuru County and to date, three cohorts have been trained. Specifically, the ISAVET in Kenya is targeted at building capacities in 8 domains, 14 core competencies and 47 skills developed using a consultative approach among stakeholders [Supplementary Table S1; (13)]. The training consisted of 4 weeks of classroom training (3 weeks of didactic and 1 week of field experience). This was followed by 12 weeks of implementation of field activities relevant to the areas where trainees originated from.

1.1. Description of the in-country ISAVET program

1.1.1. Preparation for ISAVET training

1.1.1.1. Building national consensus and establishment of national ISAVET coordination structures

To facilitate the adoption of the training program in-country, FAO together with the relevant national partners organized a pre-inception stakeholders' consultative meeting to introduce the ISAVET program and get buy-in from the national and subnational governments. A comprehensive stakeholder mapping and analysis was also conducted in consultation with the State Department of Livestock's Directorate of Veterinary Services (DVS) and the Zoonotic Disease Unit (ZDU). This process led to the identification of relevant stakeholders by institutional domain (major government stakeholders, minor government stakeholders, regulators, research institutions, partners/funders) and their area of interest (influence) (Supplementary Table S1).

Important stakeholders were mandated to nominate representatives to the *National ISAVET Program Steering Committee* (NIPSC), a body that is chaired by the Director of Veterinary Services. Other members of the NIPSC were from the Ministry of Agriculture, Livestock, Fisheries and Cooperatives (MoALFC), Ministry of Health, Kenya Wildlife Services, Council of Governors, Academia, Development Partners, Regulatory and Professional Bodies. The NIPSC operates through a terms of reference that include the provision of strategic guidance and support, and the monitoring and evaluation of progress and impact of the ISAVET program at country level.

Furthermore, a team of professionals were carefully selected to form the ISAVET Technical Working Group (ITWG), including staff from the MoALFC, ZDU, County Veterinary Services, Academia, Research and Development Partners. The terms of references for the ITWG was to provide advice and support in planning, preparation and implementation of the frontline ISAVET course and the provision of oversight of trainer and mentor competencies and skills. In preparation for the 1st cohort of ISAVET training, the global curriculum was first contextualized to Kenya, endorsed and validated by national stakeholders in November 2020. In the context of ISAVET, The trainers were persons who are technically sound in matters of epidemio-surveillance, and who were involved in the one-month didactic training of ISAVET trainees. The mentors were either academic or institutional mentors. The academic mentor provided advice to trainees on technical matters in epidemiology for the three-month field activities while the institutional mentor assisted in the design of a realistic and achievable study and played oversight during the three-month field study period.

1.1.1.2. Planning and organization of the in-country ISAVET training

To implement the holistic training and enable mentoring approach of the ISAVET program, three training components were conducted. These included the 4-month in-service training program for frontline veterinarians and paraveterinarians, and a separate two-week long training of trainers and training of mentors workshops. The *National ISAVET Coordinator*, a former a graduate of the Field Epidemiology and Laboratory Training (FELTP), and a staff from the Directorate of Veterinary Services, coordinated the ISAVET program on behalf of the government of Kenya. The National ISAVET Coordinator worked closely with the *FAO-ECTAD National Epidemiologist*, who coordinated and facilitated the ISAVET program from FAO Kenya (Supplementary Figure S1).

1.1.1.3. Training of mentors and trainers

In preparation for the rolling out of frontline ISAVET Program in Kenya, 10 mentors and trainers were trained in addition to the 5 trainers and 5mentors initially trained in the regional training workshop held in Nairobi, Kenya in October 2018. Following an open call, a competitive process was utilized to select qualified individuals from the national and sub national veterinary services, the Meat Training Institute and the Kenya Wildlife Services. Briefly, the potential trainers and mentors were taken through a 5-day training on specific roles, responsibilities and required competencies expected to support the trainees during the 4 months applied veterinary epidemiology training. Content of the training included exercises on the technical requirements to ensure successful trainee program until completion, the curriculum and case study development tools, the monitoring and evaluation tools, core dimensions of the trainer-trainee and trainer- mentor relationship and trainer responsibilities and expectations. This specific training was facilitated by mentors and trainers who had an initial training in the regional pilot workshop for trainers and mentors held in Nairobi in October (13). The national training was based on the approved curriculum adopted by the NIPSC.

1.1.1.4. Scoping mission

A key pre-course activity in planning for the frontline ISAVET training workshops was the scoping mission exercise. The goal of the mission was to assess the suitability of the selected County (subnational facility) to host ISAVET training of frontline health workers with the specific objectives of determining the prevalent disease(s) present at the County that can be used for field activities; identifying the specific location of field activities, and preparing and locating available resources suitable for the field activities. Statutorily, the scoping mission team was led by the government ISAVET focal person, but was inclusive of officers from FAO, and DVS, MoALFC. During the mission, consultations were made with the various stakeholders using a guided scoping mission tool that comprises of the stakeholder consultation checklist, field planning and laboratory planning tools. The scoping mission reports was shared with the stakeholders (FAO country team, the DVS and the respective counties that should host the month-long didactic training). The reports informed the comprehensive follow up administration and logistic actions, communication and other preparations.

1.1.1.5. Trainees selection and preparation for the training

The calls for application of potential veterinary and paraveterinary trainees from national and sub national veterinary services and institutions were sent out twice *per annum* (2 months before the commencement of the training) to all animal health and public health networks. Applications to the ISAVET training were done through the standardized ISAVET application form, and each application must be endorsed by respective County Director of Veterinary Services (CDVS) and the immediate work station supervisor. The CDVS was the designated as the Institutional Mentor during the field-based training. Using a pre-defined set of criteria, all applications were vetted by the 10-member selection committee representative of the DVS (Veterinary Epidemiology and Economics Unit and Training division), the ZDU and Council of Governors (CoG). From a set of applications, only 25 successful applicants were selected to constitute a cohort of ISAVET trainees. Each applicant was then pre-notified of the success of his or her application and informed of the venue of training, reporting dates, content and duration of the training, required proposed field case study concept note, and the necessary items to bring along to the training.

Approximately 4 weeks before the training, proposed weekly lessons were assigned to trainers considering each trainer's competency, experience, and availability during the didactic duration of the training. Trainees were also matched to mentors based on geographical location of the trainees and mentors. Virtual meetings were held between the course coordinators at DVS and FAO with the trainers to go through the developed lessons, exercises and

TABLE 1 Clustering of participants including the areas of routine practices and activities.

Criteria	Cohort 1	Cohort 2	Cohort 3*
Focused region of Kenya	Random nationwide	Random nationwide	North Rift Economic Bloc (NOREB)
Number of counties involved	24 (inclusive of 6 border counties and a Meat Training Institute)	22 (inclusive of one Regional Veterinary Investigation Laboratory, a point of entry and one national food processing and export facility)	9 (inclusive of the Kenya Wildlife Services (KWS) and Regional Veterinary Investigation Laboratory).
Description of areas covered	The high animal production and Arid and Semi-Arid regions of Kenya.	The high animal production and Arid and Semi-Arid regions of Kenya.	Arid and Semi-Arid regions in the North Rift Economic Bloc (NOREB)
Numbers in the Cohort	25	25	25
Period of implementation (Classroom didactic)	7 June, 2021 to 2 July 2021	7 March 2022 to 1 April 2022	6 June 2022 to 1 July 2022
Period of implementation (3-month Field work)	5 July, 2021 to 24 September 2021	4 April 2022 to 24 June 2022	4 July 2022 to 23 September 2022
Male: Female ratio	16 males: 9 females	17 males, 8 females	17 males: 8 females
Professional diversity of trainees	10 veterinarians, 15 paraveterinarians	8 veterinarians, 17 paraveterinarians	11 Veterinarians, 14 Paraveterinarians
Areas of work	24 County disease surveillance officers, (8 serving in border counties), 1 officer from the Meat Training Institute who is now posted at border inspection point.	22 County disease surveillance officers, (4 serving in border counties), 1 border control officer, 1 veterinarian at the Regional Veterinary Investigation Laboratory and (1 paraveterinarian at the national food processing and export facility)	24 County disease surveillance officers, (3 serving in border counties), I veterinarian at Kenya wildlife service s and 1 veterinarian at the Regional Veterinary Investigation Laboratory

Cohort 3: classwork is completed and field component is ongoing. The list of specific topics covered to date are available in the Supplementary Table S2.

contextualize case studies as required. The mentors and trainees also have virtual and phone call engagements to refine the proposed field case study concept notes.

1.1.2. Implementation and delivering of frontline ISAVET training

The trainees were introduced first to the concept of importance of mindset change and its output in reference to field epidemiology following training. On weekly basis, trainees were taken through courses in epidemiological surveillance, field investigations, preparedness, disease prevention and response. Other topics covered included communication, ethics, and professionalism during disease investigation. Furthermore, trainees were taken through training on collaborative engagement and shared resources and modalities for implementing field project using the One Health perspectives. The didactic portion of the training were delivered over 4-week period structured as outlined in the Supplementary Table S2a and Supplementary material S2b.

2. Materials and methods

Using biodata provided by trainees and the geo-coordinates of the place of primary assignment of each trainee, the spatial–temporal maps of the ISAVET field implementation for the three cohorts were conducted. All serial maps were created using QGIS v3.3[1].

Additional data used to evaluate the ISAVET program and trainees were collected using mixed method (quantitative and qualitative). Specifically, three surveys (plus four supplementary questionnaires) were designed and pretested among three technical staff (see Supplementary Appendices S1–S7). Based on the feedback, the questionnaire was modified to ensure consistency, clarity and validity, and coded on Google forms. The final version was self-administered online, or the links for the online surveys were shared *via* email and social media (WhatsApp). Responses on (1) Self-reported comparison of pre and post-training survey among ISAVET trainees; (2) Scores for all areas of competencies and veterinary skills assessed before and after the 4 weeks didactic training; and (3) Scores for areas of intended or ongoing applications of veterinary epidemiology skills, and perceptions on the most useful veterinary epidemiology skills post-training were captured on a Likert scale (i.e., 1 = strongly disagree, 2 = disagree, 3 = neutral/no change, 4 = agree and 5 = strongly agree). Other responses were either dichotomous in nature or non-structured (qualitative). Whereas the pre-training and post-training practices surveys were carried out among all the 76 ISAVET trainees, the 6-month post training practices survey was only carried out to target only the 25 ISAVET Cohort 1 trainees.

3. Results

In total, 75 trainees have undergone the in country ISAVET program in Kenya to date with approximately 67% male and 33% female (Table 1), in addition to the three originally trained through the pilot Program in Uganda, 2018. Over 93% of the trained workforce are direct frontline officers in the subnational system. A total of 40/47

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[1] https://www.qgis.org/en/site/

(85.1%) of the counties have been represented in the training, although the number trained per counties varied (Table 1 and Figure 1). The high animal production (HAP), the Arid and Semi-Arid lands (ASALs) and the North Rift Economic Bloc (NOREB) regions of Kenya have been covered to date. Participants have been drawn from county veterinary services (including from 6 border counties), the Meat Training Institute, a Regional Veterinary Investigation Laboratory, a border post (point of entry), a national food processing and export facility, and the Kenya Wildlife Services (KWS) (Table 1).

The trainees included 33 veterinarians (44%) and 42 paraveterinarians (56%). On the specific areas of operations, 70 of the trainees operate as County disease surveillance officers (93.3%), including 14, who work in the border counties, and one representative each as meat training officer, border control officer, laboratory veterinarian, national food processor at the export facility and a wildlife veterinarian (Table 1). The trainees have had marked mindset changes as shown in their positive attitudes towards improving animal health surveillance in their localities and based on the outcomes of the evaluation process. For instance, positive attitudinal shift and commitments were seen in the mean gain of the veterinary epidemiologic skills of the ISAVET graduates [increase of at least 44.9%: ((4.46/3.08 *100) − (3.08/3.08 *100))] (Figure 2), and the confidence in field implementation, field applications of epidemiologic skills and One Health approach all improved (Table 2).

On a scale of 1–5, the mean value of the veterinary epidemiologic skills of the trainees at the beginning of the training was 3.08/5.00 (61.6%), which increased to 4.46/5.00 (89.2%) by the end of the training (Figure 2). At the pre-training level, the three skills with the highest scores were: (a) identifying important stakeholders in the locality (district) (3.96/5.00; 79.2%); (b) adopting roles assigned during field activities as a team member (3.76/5.00; 75.2%); and (c) calculating the measures of central tendency (mean, median and mode) and preparing a written report and oral presentation to technical and non-technical audiences (3.56/5.00; 71.2% each) (Figure 2). The weakest skills at the pre-training stage were the application of the seven-step approach to ethical decision-making (2.32/5.00; 46.4%) and the application of methods used for interpreting data to improve situational awareness (2.48/5.00; 49.6%) (Figure 2). To highlight a few, at post-training level, the three strongest scores remained identifying important stakeholders in the locality (district) and adopting roles assigned during field activities as a team member (4.76/5.00; 95.2%), and calculating the measures of central tendency (mean, median and mode) (4.72/5.00; 94.4%) (Figure 2).

On the likely immediate post-training application of epidemiologic knowledge and skills gained during the didactic training, 50% indicated willingness to apply their knowledge to improve surveillance and reporting, while 14.7 and 11.8% wanted to improve outbreak investigation and feedback mechanisms, respectively. Only 2.9% indicated the utilization of the new knowledge to improve disease prevention (Figure 3). Approximately 8.8% believed that the new knowledge could improve their professionalism and 5.9% each believed that it should improve advocacy and applied One Health (Figure 3).

Six-month post training, 86.6% of the trainees were adopting roles assigned to them during field activities as team members, 78.4% were identifying important stakeholders in the locality (district) and 70.8% each were still producing summary reports with recommendations for action, and preparing and following up plans to capture and describe data by animal, place and time (Figure 4A). Six-month post training, trainees perceived that the most useful veterinary epidemiology skills gained were: preparing and following up plans to capture and describe data by animal-place [16/25 (64%)], and producing summary reports with recommendations for action [15/25 (60%)]. None of the trainees perceived that creating a standard outbreak histogram and interpreting the results were as useful as other skills (Figure 4B).

Furthermore, in terms of attitude, confidence, and commitments, 12 areas were evaluated, and the trainees gained marginal increases in some areas ($p > 0.05$) and only in the area of

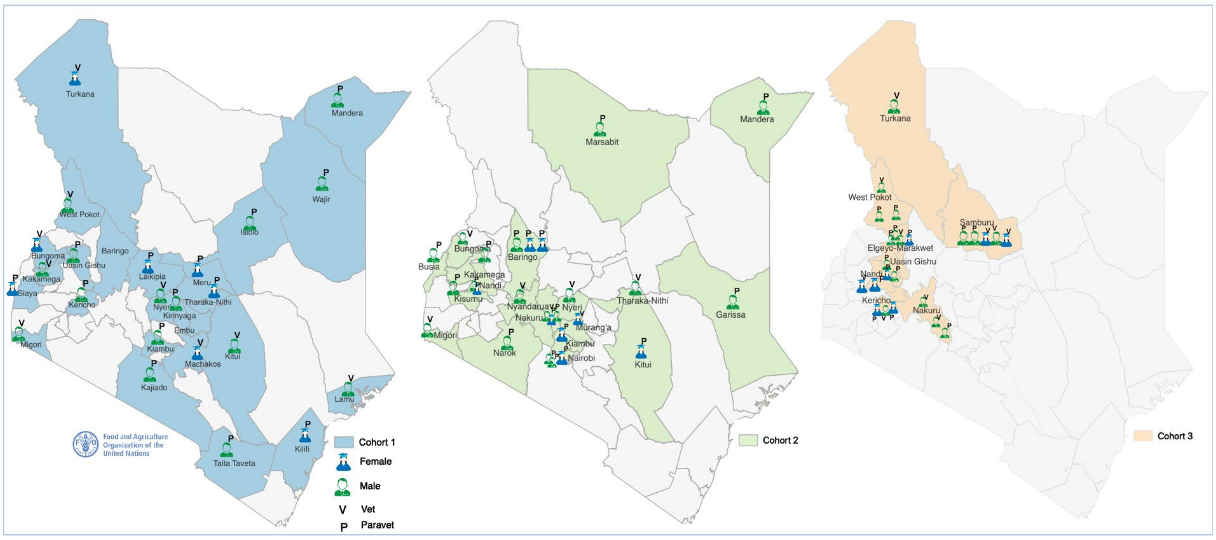
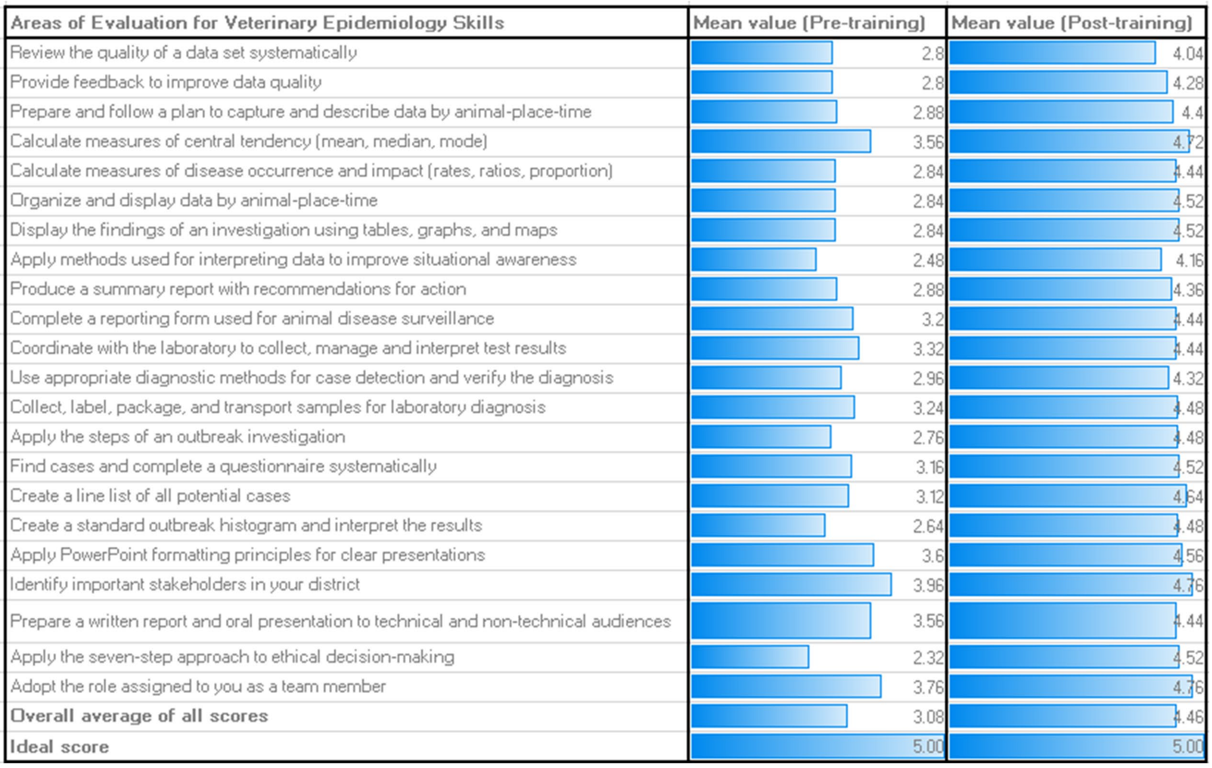


FIGURE 1
Spatial spread of trained cohorts of in-service applied veterinary epidemiology training (ISAVET). Note that Cohort 1 is represented in (blue), Cohort 2 (green), and Cohort 3 (light brown).



Areas of Evaluation for Veterinary Epidemiology Skills	Mean value (Pre-training)	Mean value (Post-training)
Review the quality of a data set systematically	2.8	4.04
Provide feedback to improve data quality	2.8	4.28
Prepare and follow a plan to capture and describe data by animal-place-time	2.88	4.4
Calculate measures of central tendency (mean, median, mode)	3.56	4.72
Calculate measures of disease occurrence and impact (rates, ratios, proportion)	2.84	4.44
Organize and display data by animal-place-time	2.84	4.52
Display the findings of an investigation using tables, graphs, and maps	2.84	4.52
Apply methods used for interpreting data to improve situational awareness	2.48	4.16
Produce a summary report with recommendations for action	2.88	4.36
Complete a reporting form used for animal disease surveillance	3.2	4.44
Coordinate with the laboratory to collect, manage and interpret test results	3.32	4.44
Use appropriate diagnostic methods for case detection and verify the diagnosis	2.96	4.32
Collect, label, package, and transport samples for laboratory diagnosis	3.24	4.48
Apply the steps of an outbreak investigation	2.76	4.48
Find cases and complete a questionnaire systematically	3.16	4.52
Create a line list of all potential cases	3.12	4.64
Create a standard outbreak histogram and interpret the results	2.64	4.48
Apply PowerPoint formatting principles for clear presentations	3.6	4.56
Identify important stakeholders in your district	3.96	4.76
Prepare a written report and oral presentation to technical and non-technical audiences	3.56	4.44
Apply the seven-step approach to ethical decision-making	2.32	4.52
Adopt the role assigned to you as a team member	3.76	4.76
Overall average of all scores	3.08	4.46
Ideal score	5.00	5.00

FIGURE 2
Mean scores for all areas of competencies and veterinary skills assessed before and after the 4 weeks didactic training. Only 65% of the trainees understood the key One Health concepts at the beginning of the didactic training but this increased to approximately 88% during the post-training practices survey.

contribution to post-incident assessments by validating and providing field level data were significant gain observed ($p = 0.01$) (Table 2).

4. Discussion

In this work, the implementation of ISAVET was explored in Kenya and showed how international development organization and national partners can collaborate and co-create to build the national workforce with resultant positive effects on country's epidemio-surveillance systems, with reflections on the One Health approach. The need for such trained workforce cannot be underestimated (13). Specifically, in the delivery of ISAVET Program in Kenya, only 78 trainees have completed the training in three cohorts, but the Program intends to generate additional 50 participants *per annum*, unless the scaling up is facilitated by other partners. It is acknowledged that the current numbers being trained *per annum* is inadequate in a country like Kenya, where skilled workforce demand is huge, and animal resources are enormous. While it is understood that this work is almost completely externally funded, it is acknowledged that there is a need to ramp up local and national resources to rapidly expand the pool of trainees and place them where they are most needed in order to meet the epidemio-surveillance needs of Kenya. Over-dependency on externally sourced funds have been identified as a limitation to scale up the implementation of best initiative in countries of Africa (14–17).

The spatial spread of trainees from counties and where they are working appeared balanced; this covers 85% of the geographical landscape; however, the number of trained personnel per county range from 1 to 5, figures that need to be up-scaled. Perhaps a training of trainer approach can be utilized to fast-track the trained personnel per county, through the extended training of officers at the county level by the ISAVET graduates. Future training can thereafter be prioritized through upskilling of these locally trained individuals. From the third cohorts, the focus of selecting trainees has been re-organized from having a national spread to prioritizing a region in each cohort. The change was occasioned by the different challenges and needs that exist in the different zones. For instance, the ASALs faces challenges of drought, with resultant loss of livestock resources due to lack of water and feed scarcity. Such special needs are therefore considered in packaging regional-level training and discussed alongside the training of the cohorts. Localizing animal and public health solutions have been considered beneficial than a generalized approach (18). This observation is important because it brings the veterinary services in direct contact with the people who need the services, and reduce the critical response time to public health and animal health events. The benefits of reducing critical response time have been emphasized previously (19). This also have implications on One Health at local level as animal health issues often result human health in the frontlines, e.g., anthrax, rabies, brucellosis and trypanosomiasis among others.

TABLE 2 Self-reported comparison of pre and post-training survey among ISAVET trainees.

Criterion to measure attitude, confidence and commitment to epidemio-surveillance capacities	Pre-training average (n = 25)	Post-training average (n = 25)	Value of p	X^2
Map value chains with stakeholders during peace time	3.48	4.56	0.06	3.6
Contribute to post-incident assessments by validating and providing field level data	2.76	4.4	0.01	6.5
Based on the ISAVET curriculum I have been introduced to, ISAVET will help me gain skills in field epidemiology	4.76	4.92	0.52	0.4
Participating in ISAVET will be important for my professional development	4.92	5.00	0.53	0.4
I will use what I will learn from ISAVET in my job	4.96	4.88	0.66	0.2
I am only going to participate in ISAVET because my supervisor asked me to	1.44	1.12	0.61	0.3
I know what is expected of me after I complete the course	4.44	4.84	0.28	1.2
I believe that ISAVET will help me develop professionally	4.92	4.96	0.80	0.1
I am eager to apply the knowledge I will gain in ISAVET in my job	4.96	5	0.66	0.2
ISAVET will improve my field epidemiology skills	4.96	4.96	1.00	0.0
My expectation from the ISAVET training is to able to improve quality of data and reports	4.92	4.96	0.80	0.1
I am confident my supervisor/management will provide me with the necessary support to apply the skills I learnt through ISAVET in my daily work.	4.64	4.64	1.00	0.0

The maximum score obtainable in the pre and post-training evaluation is 5.0.

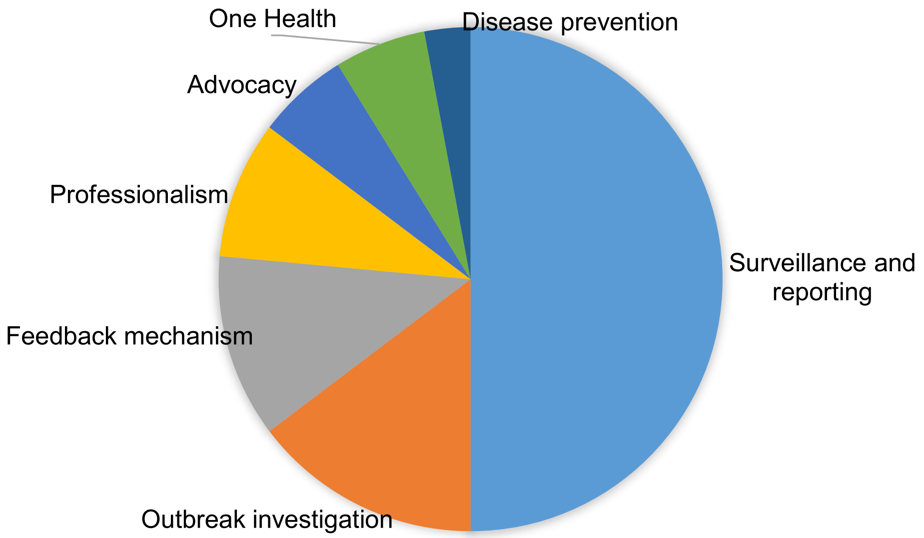


FIGURE 3
Plans on how ISAVET knowledge and skills will be utilized post-training.

Important observations were the attitudinal shift especially in the mean veterinary epidemiologic skills, which increased by 44.9% and the observed boost in confidence in field implementation, field applications of epidemiologic skills and One Health approach. These is important for professionalism and effectiveness of the frontline officers. The training highlighted the weakest areas of skills of the

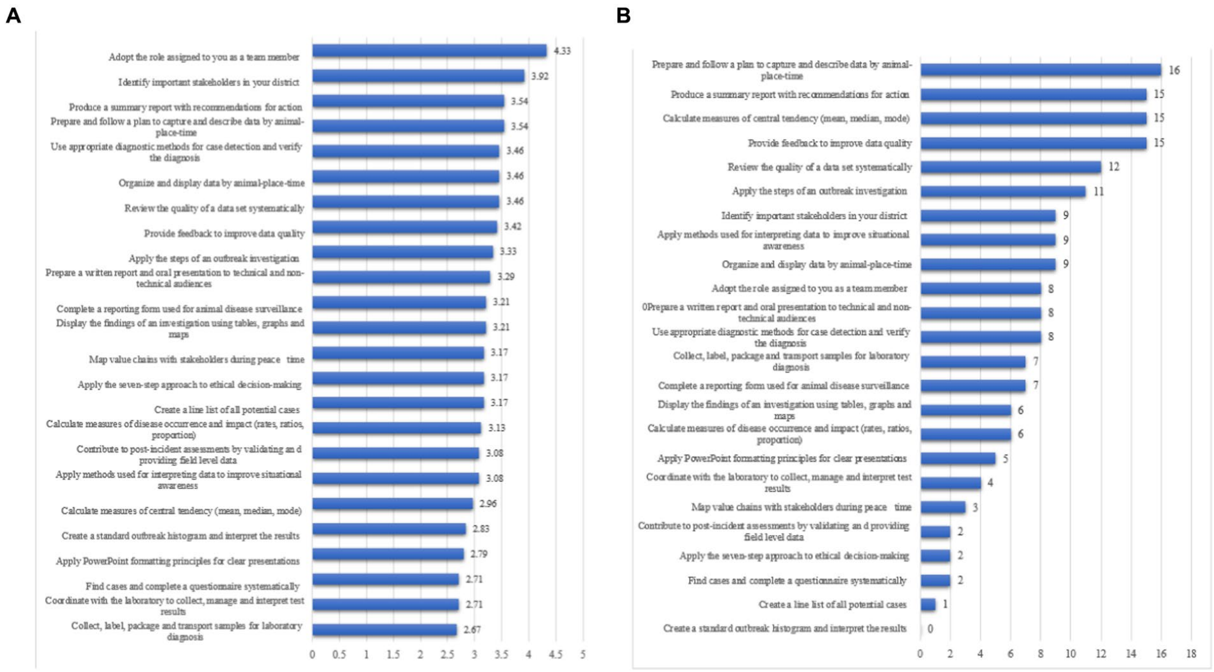


FIGURE 4
(A) Mean scores for areas of intended or ongoing applications of veterinary epidemiology skills, and **(B)** perception of trainees on the most useful veterinary epidemiology skills gained, 6 months post-training.

frontline officer and provided feedback to the training institutions (universities and higher colleges). The follow-up monitoring and evaluation was particularly useful because it tracked each training in real time and served as additional evaluative tool in service delivery and career progress of animal health workforce. Although the training exposed the trainees to a two-way (epidemiology-laboratory) and four-way (epidemiology-laboratory in public health and animal health) linkages, and the direct supervisors of the graduates understood the skills and knowledge gained by trainees, the CDVSs were somewhat disconnected from the program thus necessitating a need to introduce executive session of the program.

It is confirmed that the current ISAVET graduates have reached beyond the limited scope of epidemio-surveillance in animal health alone, but some have contributed to or have been utilized for support services where public health officials are in short supply, for instance, during the surge in COVID-19 epidemics, and during county-level unusual public health events or incidences needing One health approach. Data-driven approach in animal health and broad training in animal health for the benefit of public health have been emphasized (20, 21). It should be understood that most African states have inadequate capacity (number and quality) of animal and public health workforce to meet the immediate animal and public health challenges. In the recent COVID-19 challenges, countries like Ghana, Liberia, Senegal, Sierra Leone, Tanzania, and Nigeria utilized the veterinary laboratories and its workforce to increase diagnostic capacities and contribute to response to COVID-19 outbreak when the public health laboratories were over whelmed (22–24).

Basically, the ISAVET program has addressed some of the originally identified challenges mentioned in the introduction above: (1) It is offering a structured and routine on-the-job training for animal health workers; (2) Although, it cannot even out the unequal distribution and inadequate in quantity of highly skilled workforce, it has shown capacity to improve the quality and effectiveness of this workforce through appropriate technical training and scientific skills to combat both animal health and public health challenges at the frontline; (3) The training also capacitated innovative thinking to resolve associated health challenges of climate change and drought; and (4) It has also contributed significantly to improving contingency, preparedness, and response planning for effective deployment of frontline rapid response team members.

Finally, the field interventions and follow-up reports have presented both the national and subnational governments with opportunities to prioritize locally significant disease situations, level of awareness, knowledge and practices, and skill levels with regards to zoonotic, emerging, re-emerging and transboundary diseases, as well as other issues including antimicrobial resistance and One Health.

In conclusion, valuable capacities already exist in the animal and public health systems in Africa. These capacities were built for specific fields in isolation, and hardly are made available for other purposes. This lack of integration is costly, though these costs are under-appreciated most times. By implementing some minor adjustments and additional surge training that programs like FELTP and ISAVET are doing, such originally trained workforce can be upskilled to meet multifunctional needs including addressing emergencies, when they arise. Because, emergency does not allow much room for preparation when it occurs, these surge capacities must be prepared ahead, and can be rapidly adjusted to meet need when necessary.

Data availability statement

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

Author contributions

RS, KC, SO, CL, ON, and FF coordinated the research implementation. RS, MN, SO, HO, MA, JO, and TO conducted the training. CL, ON, CB, and FF played oversight roles. RS and FF did the original manuscript draft. ET, RS, and KC conducted the monitoring and evaluation. All authors contributed to the review and finalization of the draft.

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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.2023.1150557/full#supplementary-material

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A study on the impact of double external shocks on Chinese wholesale pork prices

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Introduction: Fluctuation in pork prices has always been a focus of academic attention. This paper examines the impact of double external shocks on pork prices, to provide reference for the impact of future outbreaks on the pork market.

Methods: This paper constructs a natural experiment based on the time and regional differences in the occurrence of the epidemics. Double difference models and triple difference models are used to identify the impacts of African swine fever and COVID-19 on Chinese pork prices.

Results: The results found that both African swine fever and COVID-19 positively affected pork prices, but African swine fever had a greater degree of impact; before the COVID-19 epidemic, African swine fever caused a more significant increase in pork prices; the impact of a single African swine fever shock was greater than the double shocks.

Discussion: The COVID-19 epidemic may have curbed the further increase in pork prices, due to the decreased market consumption demand caused by the epidemic.

KEYWORDS

African swine fever, COVID-19, pork prices, wholesale prices, natural experiment

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1. Introduction

African swine fever (ASF, the same as below) first emerged in various regions of China in August 2018. Measures, including infection culling, disease-related mortality, and trans-provincial embargoes, severely disrupted the pork market, creating a significant imbalance in supply and demand, and profoundly affected the swine industry (1). The continuous repercussions of ASF were not yet mitigated when COVID-19 (the same as below) emerged in December 2019, exacerbating the impact on pig production, consumption, and pricing (2). As an indicator of economic activity, prices best encapsulate the real-time status of market supply and demand. Agricultural product prices are among the first to be impacted when an epidemic strikes (3). The swine industry and the pork market find themselves in a challenging position, dealing with a complex and overlapping set of external factors. These “double external shocks” have caused instability in the Chinese hog market and further fluctuated pork prices.

Many previous studies have investigated how some epidemic outbreaks can affect the market. Some scholars point out that disease outbreaks can affect hog production (4, 5). Also, epidemics outbreaks can affect the pork trade (6) and lead to bias against the origin (7), while prolonged trade bans can reduce the pork supply (8). After the outbreak of ASF, some scholars have studied that ASF has led to an increase in pork prices and a decrease in demand (9). But currently, research on ASF mainly focuses on the impact of exports, and export losses are the main reason for the increase in the total cost of this epidemic (10). After the COVID-19 outbreak, some scholars pointed out that the COVID-19 epidemic has to some extent disrupted the pork supply chain (5).

Thus it can be seen, the dual external impacts of ASF and COVID-19 are bound to bring a series of serious impacts on the pork market, which will lead to the structural adjustment of the meat market, and the industrial development is full of uncertainty. China is the world's largest producer of live pigs and consumer of pork (11). The fluctuation of pork prices has always been highly concerned by all sectors of society, ensuring the stability of pork prices, which is related to the well-being of people and the stability of the market. In view of this, the Central Committee of the Communist Party of China and the State Council made an essential deployment in the No.1 central document of the Central Committee in 2020 to ensure the price and stable supply of agricultural products such as pigs, to promote industrial stability and market stability.

Based on previous literature and research findings, many scholars contend that factors influencing pork prices encompass policy regulations, production costs such as feed, transportation expenses, and exogenous shocks (12–15). According to the equilibrium price theory, the key factors affecting pork prices lie in supply and demand. From a supply perspective, production costs and circulation costs can be summarized as internal factors that affect supply, which determine the scale of pig farming. The impact of the epidemic (including disasters) can be summarized as external factors that affect supply, causing great uncertainty to the pig industry and the pork market. There is a wealth of literature on this aspect. For example, Wang Mingli and Xiao Hongbo believe that the impact of the epidemic on the pig market is bidirectional. On the one hand, the epidemic will directly trigger public panic, impact consumer demand, and cause drastic fluctuations in pork prices; On the other hand, the epidemic has led to a decrease in the stock of live pigs, resulting in a decrease in output and impacting market supply (16). Liang Xingqun and Xia Qingli analyzed the chain policy response caused by the epidemic and proposed that the ASF led to market segmentation caused by the government's implementation of the embargo policy, which restricted the transportation of products between production and sales areas, and subsequently led to drastic fluctuations in pork prices (17).

From the perspective of demand, with the development of the social economy and the increase in people's income, the demand for pork in the market has undergone profound changes. Drawing on the discussions of Li Binglong and He Qiuhong, from an economic perspective, pork is a normal good with a positive income elasticity of demand. As residents' incomes increase, so does their demand for pork consumption, leading to higher prices in society. But if, with the development of the economy, residents' income reaches a higher level, consumers' consumption habits and concepts are also changing, and the demand for a diversified diet and balanced nutrition increases. Therefore, pork becomes an inferior product, and the effect of income changes on pork prices is exactly opposite to the direction when pork is a normal product. In recent years, the growth in urban residents' consumption of pork in China has been sluggish, and the high-income group has exhibited a downward trend (18). In other words, an increase in public income is likely to result in a reduction in pork consumption.

In general, there are many studies on the supply and demand situation of the pork market and the impact of the epidemic, but there is still some research space. Previous literature on the impact of external shocks on pork prices has mostly focused on a single external shock perspective, with few discussions on the complex impact of multiple shocks on pork prices. In addition, ASF and COVID-19 in reality occur in a wide range and last for a long time, which provides a place for building Natural experiments, thus providing conditions for analyzing the impact of multiple external shocks on pork prices, which makes the paper supplement and contribute to the previous literature and research theories.

Based on the practical situation described above and the limitations of existing research, we propose the following scientific issues: Since the impact of dual external shocks on the pork market is complex and staggered, to what extent will ASF and COVID-19, respectively, affect the pork market? Is there any difference in ASF impact before and after COVID-19? Will COVID-19 aggravate the impact on the pork market, or will it not cause a new impact? To answer these questions in depth is conducive to sorting out the development and changes of China's pork market under the dual external impact, and can better explain the phenomenon, reveal the law, and put forward targeted suggestions. This is of great practical significance to ensure the smooth development of the pork market. Considering that price is an important indicator to reflect the stable development of the market, it is feasible and representative to analyze the impact of dual external shocks on the pork market from the perspective of the change in pork wholesale price, and analyzing from this perspective not only has certain practical theoretical significance but also serves as a supplement to relevant literature and theory.

Therefore, under the dual impact of ASF and COVID-19, in the reality of the rapid rise of pork prices, using the data of China's agricultural wholesale market (sourced from the wholesale market monitoring database of the Ministry of Agriculture and Rural Affairs of the People's Republic of China), according to the different time differences and regional differences of the two shocks in different regions, Chongqing, Sichuan Province, Zhejiang Province, and Hubei Province were selected to form natural experiments respectively, the difference-in-difference model is constructed to identify the impact of ASF and COVID-19 on the prices of pork wholesale market. On this basis, the triple difference model, replacement sample period, and placebo test are further used for the robustness test. The results showed that ASF and COVID-19 significantly increased the wholesale price of pork; Compared with COVID-19, ASF has a greater impact on the wholesale prices of pork; In addition, the impact of ASF on the wholesale prices of pork is different before and after COVID-19. COVID-19 leads to a decline in market consumption demand, which may inhibit the further increase of pork prices.

Possible contributions of this paper: under the realistic conditions of ASF and COVID-19 double shocks, analyze the changes in pork prices, and analyze the impact of external shocks on pork prices according to the special realistic conditions, which to a certain extent broadens the realistic boundary of factors affecting pork prices; In addition, it is a supplement to the existing literature to study the impact of dual external shocks on pork prices and analyze the impact intensity of different impacts and try to sort out the trend of pork prices changes in complex situations.

The writing structure is as follows: The second part is theoretical analysis; The third part is sample selection, data analysis, and scheme design; The fourth part is the empirical test; The fifth part is conclusions and suggestions.

2. Conceptual framework

According to previous studies, the key to the impact mechanism of external shocks on product prices lies in supply and demand. The same is true for ASF and COVID-19. Generally, when a catastrophic or public hazard event occurs, the consumer market is bound to suffer from short-term depression, which will gradually spread to the production end, and then the entire market will experience short-term huge fluctuations. Especially when the external impact directly affects the production end, the whole market will be subject to greater intervention, and the market price change is particularly unstable. To analyze the impact mechanism of ASF and COVID-19 on pork prices, the difficulty lies not only in sorting out the impact of the two on pork prices but also in the complex impact mechanism during the staggered period of the two (see Figure 1).

First of all, the impact mechanism of ASF on pork prices before the occurrence of COVID-19 was analyzed. From the perspective of supply, the sudden outbreak of swine fever has resulted in a significant number of live pigs being infected and many farm households being impacted or even withdrawing from the market, potentially leading to a decline in production (19, 20). At the same time, considering the safety and stability, the government has taken measures such as killing, burning, and burying to further reduce the number of live pigs in the plague area. In addition, pig transportation and other processes are subject to stronger supervision, which increases the difficulty of product supply. The supply of pork in the whole market showed a sharp decline. According to the equilibrium price theory, when supply falls, the supply curve shifts to the left. On the premise of constant demand, prices are bound to rise.

From the perspective of demand, ASF, as an animal disease, greatly affects food safety. Consumers are not aware of swine fever and have consumer panic psychology, which will urge them to change their consumption behavior and seek alternative consumption. In the short term, consumers will increase their demand for alternative products such as poultry and beef, and mutton, thereby driving up the overall price of livestock products (1, 21–23). Although governments at all levels actively publicized ASF-related knowledge, the panic mentality was difficult to eradicate in the short term, the consumer market fell in the short term, and the demand curve also moved to the left. Considering the actual situation, the impact of pig hunting, policy adjustment, and other factors on the pork market is much higher than consumer panic psychological factors, and the degree of left shift of the supply curve may be much greater than the demand curve, so the pork price shows a relatively large upward trend.

Next, the impact of ASF on pork prices during the occurrence of COVID-19 was analyzed. First of all, after the outbreak of COVID-19 (including partial COVID-19 in the post-epidemic era), the local government took closed control measures, blocked transportation roads, and strengthened the supervision of trans-provincial transportation, resulting in traffic disruption. In this case, the transportation of live pigs is blocked, the farms are passively closed, and even some slaughterhouses are closed, affecting the short-term supply of pork. It is worth emphasizing that the supply of pigs is always in a tight state due to the extension of the pig feeding period, and the traffic jam may also further expand the impact of the supply shortage. Due to a series of closed control and transportation blocking measures, at this time, the infection of pig epidemics caused by ASF is reduced, and the government killing is reduced. In addition, considering the poor performance of the international external environment COVID-19, imported pork products are under strict control, further strengthening the uncertainty of pork market supply. That is, from the perspective of supply, under the closed control policy during COVID-19, the impact of ASF was significantly reduced.

In addition, under COVID-19, people are isolated at home, the canteen and restaurants are closed, the people's food consumption is transferred to their homes, and the catering industry is rapidly declining. Considering the widespread waste of food consumption, the food consumption shift is likely to cause a decline in the market demand for agricultural products. At the same time, the shrinking of the catering industry is bound to have an impact on the agricultural product consumption market. As a major consumer product in the catering industry in China, the demand for pork has declined significantly in the short term. That is, from the perspective of demand, the change in people's eating habits during COVID-19 has no impact on the pork consumption panic caused by ASF. Therefore, the impact of ASF on the pork demand side has not changed before and after COVID-19.

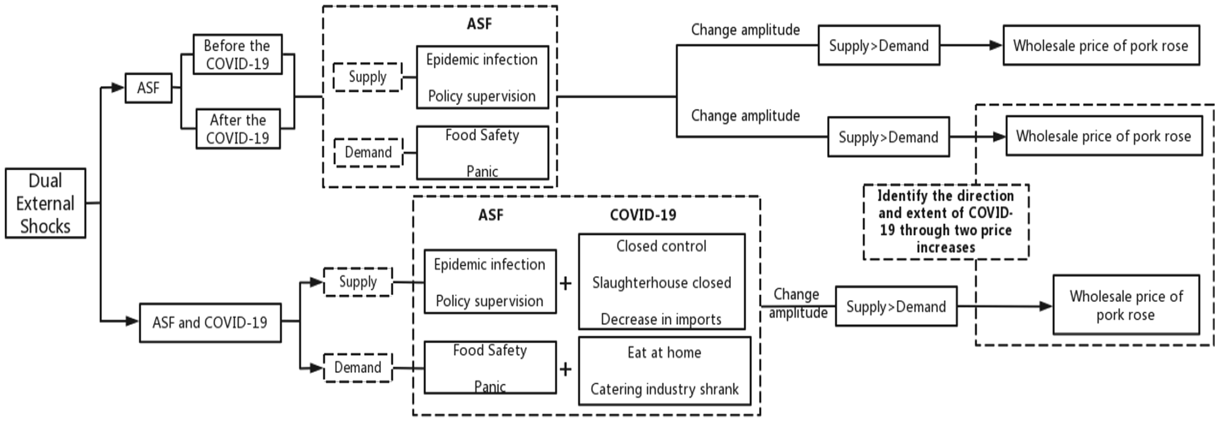

FIGURE 1
Conceptual framework of the impact of ASF and COVID-19 on the wholesale price of pork.

Finally, analyze the dual impact of ASF and COVID-19 on pork prices. In the COVID-19 state, the market on both sides of supply and demand has shrunk to varying degrees, and the degree of shrinkage is difficult to determine. At this time, there are ASF cases, and measures such as catching and killing are staged again, which will inevitably lead to a stronger impact on the supply side. Therefore, under the double impact of ASF + COVID-19, the degree of impact on pork prices is bound to be higher than that under the single impact of COVID-19. Although Mingli et al. (16) have demonstrated that the impact of epidemic diseases on pork price fluctuations is more severe than that of natural disasters, the severity of COVID-19's impact has yet to be verified. So, there is uncertainty in the direction of COVID-19 affecting pork prices. The impact of ASF and ASF + COVID-19 on pork prices can be assessed and compared post-COVID-19 to determine the extent and direction of COVID-19's influence on pork prices. Considering the actual situation, ASF and COVID-19 have a stronger impact on the wholesale price of pork on the supply side. Therefore, the change in supply volume is greater than the demand volume, which ultimately affects the wholesale price of pork and causes price changes. Figure 1 shows the theoretical framework of the impact of ASF and COVID-19 on pork prices.

Next, discuss the relationship between the two from an economic perspective. Draw a diagram of the impact of external shocks on pork prices on supply and demand (see Figure 2). First, analyze the impact of ASF on the pork market (as shown in Figure 2A). Both supply and demand declined. At the supply level, the pork supply decreased sharply, and the supply curve moved to the left, from S to S′. At the demand level, the pork demand decreased due to the substitution transfer of consumption structure, and the demand curve moved from D to D′. Considering that supply is more affected than demand, the supply curve moves farther to the left. Therefore, according to the equilibrium price theory, the equilibrium point E_0 will move to E_1, and the equilibrium price will rise significantly from P_0 to P_1.

Secondly, analyze the impact of ASF on pork prices during COVID-19 (as shown in Figure 2B). From the supply side, closed control, blocked roads, blocked pig transportation, the passive barrier of farms and the international external environment caused the tight supply of pork market. At this time, the impact of ASF under the background of COVID-19 was weakened by a series of COVID-19 policy measures. At this time, the left shift of the supply curve caused by ASF was smaller than (a); From the demand side, closed control, people are isolated at home, restaurants and canteens are closed, the catering industry shrinks, and the demand for pork market decreases. At this time, the changes in the demand side caused by COVID-19 can not affect people's panic about the impact of ASF. Therefore, the left shift of the demand curve caused by ASF is the same as that caused by (a). The result shows that in (b), the price of pork rose from P_0 to P_2, less than that in (a) ($P_2 < P_1$).

Finally, analyze the role of ASF + COVID-19 dual effects (as shown in Figure 2C). Under the complex influence of COVID-19, curve S is bound to move a large distance to the left by superposing the influence of ASF. At this time, P_3 is above P_2 ($P_2 < P_3$), while the influence of COVID-19 on pork price is uncertain, resulting in the single impact of ASF being stronger than the combined effect of the above two types of external impacts ($P_3 < P_1$).

To sum up, in the context of COVID-19, a series of closed prevention and control measures will weaken the impact of ASF from the supply side, so the impact of ASF on the wholesale price of pork before COVID-19 is the strongest, followed by the dual impact of ASF + COVID-19, and finally the impact of ASF on the price of pork during the occurrence of COVID-19, namely $P_2 < P_3 < P_1$.

3. Data and scheme design

3.1. Data sources

The data comes from the wholesale market monitoring database of the Ministry of Agriculture and Rural Affairs of the People's Republic of China, which covers the daily transaction wholesale prices and volume of various agricultural and sideline products. According to the research needs, the daily wholesale prices of pork in all wholesale markets in Chongqing, Zhejiang, Sichuan, and Hubei were selected as the research objects. The data type is daily data, and the period is from March 1, 2017, to March 23, 2020.

In addition, 0–1 dummy variables are used to reflect ASF and COVID-19. When ASF or COVID-19 occurs in the sample period, the indicator is equal to 1, otherwise, it is equal to 0. The deadline for the impact of ASF is based on the release of ASF in the region on the official website of the Ministry of Agriculture; The deadline for

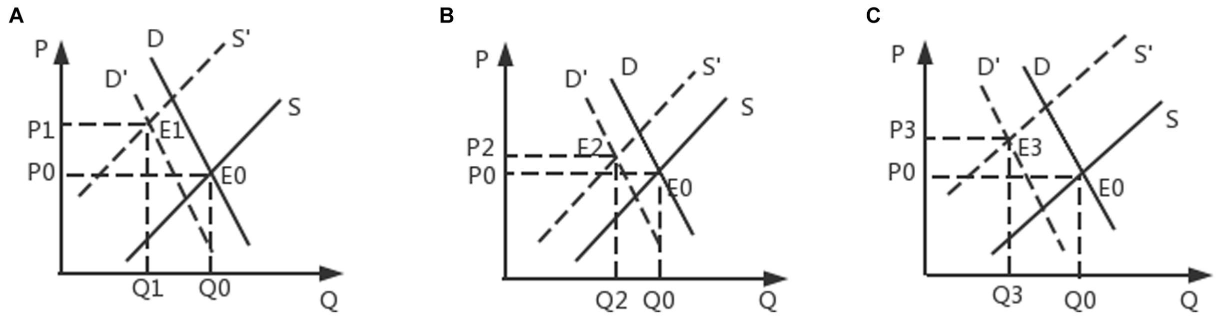

FIGURE 2
Supply and demand diagram of the impact of external shocks on the wholesale price of pork. **(A)** Effect of ASF before COVID-19 on wholesale price of pork. **(B)** Effect of ASF wholesale price of pork during COVID-19. **(C)** The dual impact of ASF+COVID-19 on the wholesale price of pork.

COVID-19's impact is based on the reduction of the risk response level of the notification issued by the region to level 3 or below.

3.2. Experimental design

3.2.1. Experimental background

ASF and COVID-19 come one after another, affecting most of China. This realistic background provides a natural experimental ground for identifying the impact of dual external shocks on pork prices. First, there are samples of provinces that are impacted and samples of provinces that are not impacted in the same period. The experimental group and the control group can be set up to identify the processing effect of the impact by using the difference-in-difference model; Second, there are differences in the impact time. On the premise that the economic and social development rate of the sample in a single province is consistent year by year, using the method of Hanming (24) for reference, compare the external impact period of a single sample with that of the same period in previous years, and establish an interpreted experimental group and a control group to identify the impact treatment effect (24). Based on this, after sorting out the occurrence of ASF and COVID-19 in each province, three practical situations in line with the research purpose were summarized. See Figure 3 for details.

The first reality is to study the impact of ASF on pork prices before COVID-19. The observation sample area is Chongqing. Since Sichuan Province is a major pork production province (25), Chongqing is located next to Sichuan, which is a typical pork main selling area and has good representativeness. ASF occurred in Chongqing on November 4, 2018, and ended on January 31, 2019. Based on this situation, a single-province inter-temporal difference-in-difference model is constructed.

The second reality is used to study the impact of ASF on pork prices after the COVID-19 epidemic. The observation sample areas are Sichuan Province and Zhejiang Province. COVID-19 of the same level broke out in both places from January 24, 2020, to March 23, 2020. On this premise, the ASF epidemic occurred in Sichuan Province on March 12 of the same year (ended on March 25), but not in Zhejiang Province. Therefore, the daily data were used to construct a control experiment according to the differences between the two places.

The third reality is used to study the dual impact of COVID-19 and ASF on pork prices. The observation sample area is Hubei Province. The province had COVID-19 on December 29, 2019 (unsealed on April 8, 2020) and ASF on March 3, 2020 (ended on April 11), which means that during the period from March 3, 2020, to April 8, 2020, the two impacts caused overlapping impacts. From this, we can also build a single-province inter-temporal difference-in-difference model.

3.2.2. Experimental construction

Based on the above social reality, three groups of control experiments were constructed to identify the impact of external shocks on pork prices in different contexts (see Table 1 for details). The first group of control experiments took Chongqing as the target sample. Referring to the method of Hanming (24), a natural experiment was constructed with the pork price in Chongqing from March 1, 2018, to January 31, 2019, as the experimental group and the pork price in Chongqing from March 1, 2017, to January 31, 2018, as the control group. Among them, during the action period of the experimental group, ASF broke out in Chongqing on November 4, 2018, and ended on January 31, 2019, which can be regarded as the impact action period of ASF, and before the impact action period from March 1 to November 3, 2018. Under the assumption that the social and economic development rate of Chongqing is consistent year by year and the price cyclical change is constrained, the difference between the average price from November 4, 2018, to January 31, 2019, minus the average price from November 4, 2017, to January 31, 2018, and the difference between the average price from March 1 to November 3, 2018, minus the average price from March 1 to November 3, 2017, can

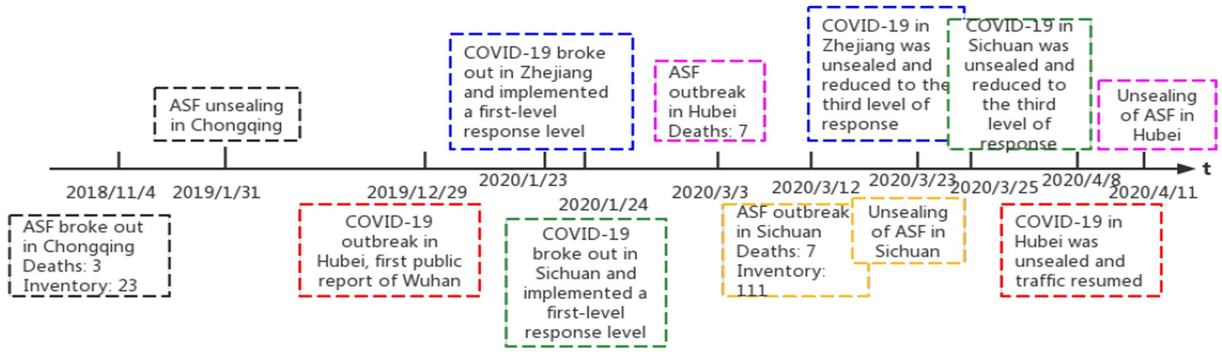

FIGURE 3
Impact period of ASF and COVID-19 in the sample area. The severity of COVID-19 is divided into four response levels (According to the Emergency Response Law of the People's Republic of China and the National Emergency Response Plan for Public Emergencies, emergencies are divided into four categories: natural disasters, accident disasters, public health events and social security events. According to the nature, severity, controllability and scope of influence of the event, the emergency level is divided into Level 1 (especially serious) and Level 2 (Major), Level 3 (Major), and Level 4 (General). This COVID-19 is a public health event). The first and second level response refers to the very serious epidemic and the highest risk level; The risk level of Level III and Level IV response is relatively low, and the traffic is restored. Therefore, lowering the response level to Level III and below is COVID-19 unsealing.

TABLE 1 Three groups of control experiments.

Group	Research object	Time
The first group: identified the ASF treatment effect before COVID-19		
Experimental group	Chongqing	March 1, 2018 – November 3, 2018 (before impact)
		November 4, 2018 – January 31, 2019(after impact)
Control group	Chongqing	March 1, 2017 – November 3, 2017 (before impact)
		November 4, 2017 – January 31, 2018 (after impact)
The second group: identified the ASF treatment effect when COVID-19 occurred		
Experimental group	Sichuan	January 24, 2020 – March 11, 2020 (before impact)
		March 12, 2020 – March 23, 2020 (after impact)
Control group	Zhejiang	January 24, 2020 – March 11, 2020 (before impact)
		March 12, 2020 – March 23, 2020 (after impact)
The third group: identified the dual processing effects of COVID-19 and ASF		
Experimental group	Hubei	November 22, 2019 – December 29, 2019 (before impact)
		March 3, 2020 – April 8, 2020 (after impact)
Control group	Hubei	November 22, 2018 – December 29, 2018 (before impact)
		March 3, 2019 – April 8, 2019 (after impact)

be used as a subtraction method to preliminarily identify the effect of ASF on pork price treatment.

The second group of control experiments took Zhejiang Province and Sichuan Province as the target samples. A natural experiment was constructed with the pork price in Sichuan Province from January 24 to March 23, 2020, as the experimental group and the pork price in Zhejiang Province from January 24 to March 23, 2020, as the control group. The above period is in the background of the COVID-19 outbreak (the response levels of the two places are the same). Among them, ASF broke out again in Sichuan Province from March 12 to March 23, 2020, which can be regarded as the impact period of ASF under the background of COVID-19. January 24–March 11, 2020 can be regarded as before the impact period. Assuming that there is a fixed difference in the economy and society between the two places, that is, the difference does not change with time in the short term, and the price fluctuation difference in the short term is constrained, the difference between the average price of pork in Sichuan Province from March 12 to March 23, 2020, and the average price of pork in Zhejiang Province from March 12 to March 23, 2020, is deducted, Subtract the difference between the average price of pork in Sichuan Province from January 24 to March 11, 2020, minus the average price of pork in Zhejiang Province from January 24 to March 11, 2020, to identify the effect of ASF on pork price in the context of COVID-19.

The third group of control experiments took Hubei Province as the target sample. COVID-19 broke out in Hubei Province from January 23 to April 8, 2020, and the ASF epidemic occurred from March 3 to April 8 of the same year. To identify the dual treatment effect of two external shocks, the period of dual impact effect is selected from March 3 to April 8, 2020. Due to the particularity and typicality of the selected samples and the practical constraints, it is difficult to match other sample areas as the control group. We still use the method of Hanming (24) for reference to build a difference-in-difference model based on the sample time difference of a single province. Considering the cleanliness before the policy action period, the pre-action period of the experimental group was from November 22 to December 29, 2019. Similarly, the pork prices of Hubei Province from November 22, 2018, to December 29, 2019, and March 3 to April 8, 2019, were taken as the control group. Under the assumption that the social and economic development rate of Hubei Province is consistent year by year, and the price cyclical change is constrained, the difference between the average price from March 3 to April 8, 2020, minus the average price from March 3 to April 8, 2019, and the difference between the average price from November 22 to December 29, 2019, minus the average price from November 22 to December 29, 2018, can be subtracted to preliminarily identify the treatment effect of ASF and COVID-19 double shocks on pork prices.

4. Empirical analysis

4.1. Identify treatment effect

According to the above three groups of control experimental design, the difference-in-difference model can be used for estimation. The general model is set as follows.

$$P_{it} = \alpha_0 + \alpha_1 time * treat + \theta_t + \mu_i + \varepsilon_{it} \tag{1}$$

Where, P_{it} refers to the price of pork on the date t of region i. To weaken the possible impact of the pig cycle, the price is logarithmic. *Time* reflects the dummy variable of external impact time, with values of 1 and 0 (1 represents the time of external impact, and 0 represents the time of no external impact). *treat* reflects the dummy variables of the external impact sample, and the values are 1 and 0 (1 represents the area affected by external impact, and 0 represents the area not affected by the external impact). θ_t represents fixed date effect, and we control for temporal factors influencing pork prices, which include macroeconomic shocks, monetary policy, cyclical changes, and prices of substitutes. μ_i represents regional fixed effect, and we control for static regional characteristics such as the level of regional economic

TABLE 2 Difference-in-difference estimation results.

	OLS			FE		
	①	②	③	④	⑤	⑥
	ASF (Before COVID-19)	ASF (After COVID-19)	ASF and COVID-19	ASF (Before COVID-19)	ASF (After COVID-19)	ASF and COVID-19
Treatment effect	0.2463***	0.0478***	0.1316***	0.2463***	0.0300**	0.1316**
(*Treat*Time*)	(0.0117)	(0.0109)	(0.0339)	(0.0279)	(0.0132)	(0.0539)
Cons	2.9282***	3.8394***	2.8681***	2.9282***	3.8799***	2.8681***
	(0.0741)	(0.0079)	(0.0114)	(0.1980)	(0.0074)	(0.0140)
θ_t	NO	NO	NO	YES	YES	YES
μ_t	NO	YES	YES	NO	YES	NO
Obs	674	120	150	674	120	150
R^2	0.6941	0.7627	0.9873	0.6941	0.6292	0.9873

Robust standard error in brackets.
***$p < 0.01$.
**$p < 0.05$.
*$p < 0.1$.

development, management expertise, and climatic conditions. ε_{it} is the random error term. The model focuses on the coefficient α_1 in front of $time * treat$, which reflects the effect of external shocks on pork prices.

In addition, the General Office of the Ministry of Ecology and Environment and the General Office of the Ministry of Agriculture and Rural Affairs jointly issued the Notice on Further Standardizing the Delineation and Management of Livestock and Poultry Farming Prohibited Areas to Promote the Development of Pig Production on September 3, 2019, which put forward requirements for pig environmental protection and breeding. This document has brought great intervention in environmental protection, the division into districts, supervision, and other aspects of pig production, and to a certain extent affected the level of pig supply. Therefore, to analyze the relationship between the dual external impact and the wholesale price of pork, we must eliminate the additional intervention brought by the environmental protection policy. In the three groups of control experiments designed, the first group of sample period occurred before the environmental protection policy and was not affected; the second sample period occurs after the policy. Assuming that the control group and the experimental group are affected by the environmental protection policy, the treatment effect in the estimation model (1) is consistent, and the error is negligible; the more complex is the third group of control experiments. The sample period of the experimental group is after the environmental protection policy, while the sample period of the control group is before the policy, which means that the calculated treatment effect may be the result of the triple effects of ASF, COVID-19, and environmental protection policy. Therefore, the difference between the experimental group and the control group in the sample period is controlled, and the impact of the policy is eliminated as far as possible, The effect of introducing the triple difference of annual difference is relatively better.

For the three groups of control experiments, using model (1), the *OLS* estimation without controlling the fixed effect of time and the *FE* estimation with controlling the fixed effect of time were successively used for regression processing. The results are shown in Table 2. Among them, ① and ④ columns reflect the regression results of the first group of control experiments, ② and ⑤ columns reflect the regression results of the second group of control experiments, and ③ and ⑥ columns reflect the regression results of the third group of control experiments. It can be seen from Table 2 that for the first group of control experiments (Chongqing), the effect of ASF on pork price treatment is significantly positive, regardless of *OLS* estimation or *FE* estimation, and through the 1% significance level, it preliminarily shows that the single external impact of ASF significantly promotes the increase of pork price. This conclusion is very close to reality. In particular, as a typical population gathering area, Chongqing has the largest price increase (columns ① and ④ in Table 2 α_1 values are significantly higher than other columns).

The second group of control experiments (Sichuan Province and Zhejiang Province) showed the same status as the first group of control experiments, indicating that ASF in the context of COVID-19 still had a significant positive impact on pork prices. Different from the first group, the columns ② and ⑤ α_1 values are far less than that of columns ① and ④. There are two reasons for this situation: first, the regression objects are Sichuan Province and Zhejiang Province (Sichuan Province can be regarded as the main pork-producing province, and Zhejiang Province can be regarded as the main pork-selling province[1]). According to the law of price transmission, ASF leads to a lower rise in the price of pork in the main producing areas than in the main selling areas. Sichuan Province, as an experimental group, was hit by ASF. The impact on its price is relatively small. Second, under the

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[1] Sichuan Province, Henan Province, Hunan Province, Shandong Province and Hubei Province are the five provinces with the largest pig production in China. Among them, Sichuan Province has become the province with the largest number of pigs in China with an annual output of 65,791,000 pigs. See link for details https://baijiahao.baidu.com/s?id=1626175637776462025&wfr=spider&for=pc; Tianjin, Jiangsu, Zhejiang, Guangdong and Fujian are the main pork sales areas in the southeast coastal areas of China. See the link for details https://baike.baidu.com/item/ Three-year action plan for accelerating the recovery and development of pig production.

outbreak of COVID-19, social demand fell in the short term, people's expected income fell, and people tended to hoard durable agricultural products such as grain and edible oil. The closure of restaurants and canteens also led to a decline in demand for meat products. The decline in market consumption demand caused by COVID-19 may be one of the factors that inhibit the further rise of pork prices.

In the third control experiment (Hubei Province), the double impact of COVID-19 and ASF significantly promoted the increase of pork prices. ③ and ⑥ columns α_1 values are higher than that of columns ② and ⑤, which shows that COVID-19 is likely to cause the rise in pork prices without considering regional and time differences; However, because of the particularity of Hubei Province in China's COVID-19 and the fact that its annual pork output is lower than that of Sichuan Province and far higher than the actual structure of the pork market in Zhejiang Province, the strength and direction of the effect of COVID-19 on pork prices cannot be accurately determined. Combined with columns ③ and ⑥ α_1 values are lower than the result in columns ① and ④. It can be speculated that after the occurrence of COVID-19, the impact of ASF on pork prices will decrease; However, COVID-19 has an uncertain impact on pork prices, which leads to the result that the single impact of ASF is stronger than the combined impact of the above two types of external shocks.

In general, ASF notably elevates pork prices and exhibits the most substantial effect. However, the impact of COVID-19 on pork prices remains undetermined. This conclusion is consistent with the view put forward by *Xiaohua Yu* in 2020 (26). After the outbreak of COVID-19, the impact of ASF on the wholesale price of pork weakened, and COVID-19 led to a decline in market consumption demand, which may inhibit the further rise of pork prices.

4.2. Dynamic effect analysis

The precondition for the coherence of the double-difference estimation results is the fulfillment of the parallel trend hypothesis by both the experimental and control groups (27). This postulates that, in the absence of ASF and COVID-19 impacts, the progression of the outcome variables would have been consistent across both groups. For this reason, this paper empirically tests the dynamic effects of ASF and COVID-19 regarding the *Event Study Approach* proposed by Jacobson et al. (28), and uses the dynamic effect model to estimate. The general model is set as follows:

$$P_{it} = \alpha_0 + \sum_{1}^{m} \alpha_t treat * \theta_t + \mu_i + \varepsilon_{it} \tag{2}$$

Where, m represents the number of days/months in the sample period of the three groups of experimental designs (based on the long sample period of individual natural experiments, it is difficult to present the results in a centralized manner, so the number of months reflects the change). In the first group of control experiments, m was 11 in some months of the sample period; In the second group of control experiments, the number of days in the sample period is 30; In the third group of control experiments, the number of days in the sample period is 75. t represents time, and the value is between 1 and m. In the control experiment of different groups, there are different series of specific estimates, and the definition of other variables is the same as that of the regression model (1). Figure 4 shows the estimated results in three control experiments under a 95% confidence interval.

Figure 4A shows that the change is not obvious in the first 18 months (March 1, 2017, to January 31, 2018), which preliminarily shows that there is no significant difference between the experimental group and the control group before the occurrence of ASF. In addition, the estimated coefficient began to be significant and gradually increased after the occurrence of ASF. Figure 4B shows that before the outbreak of ASF in Sichuan Province on March 12, 2020, there was no significant difference in the wholesale price fluctuation of pork between the two places, and the estimated coefficient was basically below 0, indicating that the pork price in Sichuan Province was lower than that in Zhejiang Province for a long time; After the African pork epidemic, the price difference between the two provinces has changed significantly, with an estimated coefficient of more than 0, and gradually showing a trend that is significantly different from 0. The price of pork in Sichuan Province is significantly higher than that in Zhejiang Province. Figure 4C shows that under the impact of the double shocks, the price of pork rose significantly and remained at a high level for about 22 days, after which the price slowly fell back to the level before the shock.

To summarize, the three control experiments comply with the parallel trend test. Notably, the ASF outbreak preceding COVID-19 had the most significant impact on wholesale pork prices, resulting in short-term continuous increases. The combined impact of post-COVID-19 ASF and the two external shocks induced a relatively moderate effect on wholesale pork prices, which aligns with previous findings.

4.3. Triple difference

For the sake of preciseness, considering the possible interference of other unobservable factors (especially the year difference) that affect the wholesale price of pork overtime on the treatment effect, a cross-year triple difference model is constructed to eliminate the above effects. The specific method is to find another pair of "experimental group" and "control group" that is not affected by external shocks in the three control experiments. In the first group of control experiments, another pair of experimental groups and control groups were constructed by pushing forward 1 year. The difference only came from the year and other factors. The difference between the original experimental group and the control group (including the difference of ASF and year and other factors) was subtracted from the difference between the new experimental group and the control group, and the net effect of ASF on pork price was obtained. Similarly, the second group of control experiments (Sichuan Province and Zhejiang Province) and the third group of control experiments (Hubei Province) were both pushed forward by 1 year to construct new experimental and control groups, forming a triple difference, respectively, to obtain the net treatment effect of ASF after COVID-19 and the double impact on the wholesale price of pork. Therefore, the following model is constructed:

$$\begin{aligned} P_{it} = {} & \alpha_0 + \alpha_1 time * treat * group_1 + \alpha_2 time * treat \\ & + \alpha_3 time * group_1 + \alpha_4 treat * group_1 + \theta_t + \mu_i + \varepsilon_{it} \end{aligned} \tag{3}$$

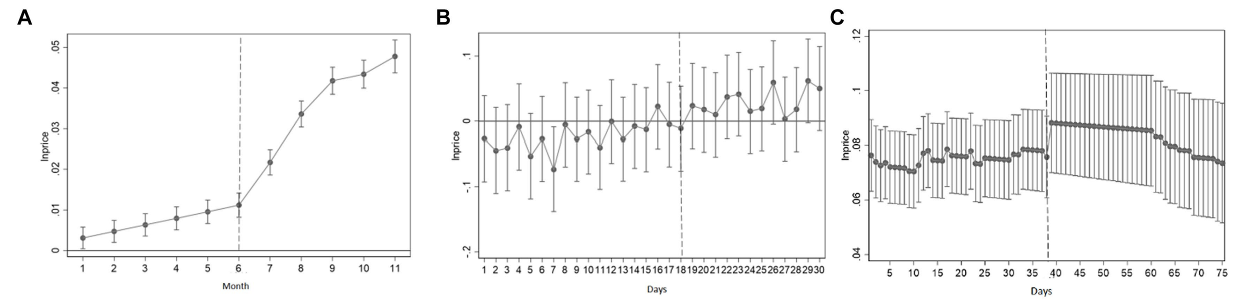

FIGURE 4
Dynamic effect of double difference. **(A)** ASF (before the COVID-19) dynamic effect. **(B)** ASF (after the COVID-19) dynamic effect. **(C)** ASF and the COVID-19 dynamic effect.

TABLE 3 Triple difference estimation results.

		OLS		FE		
	①	②	③	④	⑤	⑥
	ASF (Before COVID-19)	ASF (After COVID-19)	ASF and COVID-19	ASF (Before COVID-19)	ASF(After COVID-19)	ASF and COVID-19
Treatment effect	0.2199***	0.0705***	0.0950***	0.2771***	0.0705***	0.0950***
($Treat*Time*Group_1$)	(0.0113)	(0.0000)	(0.0077)	(0.0638)	(0.0000)	(0.0118)
Cons	3.1364***	2.8994***	2.9782***	3.1937***	3.0118***	2.9817***
	(0.0044)	(0.0152)	(0.0038)	(0.0641)	(0.0215)	(0.0060)
θ_t	NO	NO	NO	YES	YES	YES
μ_t	NO	YES	NO	NO	YES	NO
obs	1,011	238	225	1,011	238	225
R^2	0.6925	0.9408	0.4623	0.7410	0.9174	0.4649

Robust standard error in brackets.
***$p < 0.01$.
**$p < 0.05$.
*$p < 0.1$.

Where, $group_1$ is the newly generated grouping variable. When the newly generated sample data is in the sample period of the original experimental group and the control group, $group_1$ is 1. When the sample data is in 1 year forward, $group_1$ is 0 (the first group of control experimental sample data is from March 1, 2017, to January 31, 2019, $group_1$ is 1, and the sample data is from March 1, 2016, to January 31, 2017, $group_1$ is 0; The sample data of the second group of the control experiment is 1 in 2020 and 0 in 2019; The data of the third group of control experiment samples is from November 22, 2018, to April 8, 2018, $group_1$ is 1, and the sample data is from November 22, 2017, to April 8, 2018, $group_1$ is 0). $time * treat * group_1$ is 1 indicates the period when the pork price in the region is subject to external shocks. The estimated coefficient α_1 is a triple difference estimator, representing the average treatment effect of external shocks on the regional pork price. The definition of other variables is the same as that of the regression model (1).

For the three groups of control experiments, using model (3), the OLS estimation without controlling the fixed effect of time and the FE estimation with controlling the fixed effect of time were successively used for regression processing, and the triple difference estimation results were obtained (see Table 3). For the regression results of the first group of control experiments (①④), the coefficient of OLS estimation without controlling the fixed effect of time is 0.2199, which has a significant positive impact. Compared with the coefficient of difference-in-difference estimation, the coefficient of OLS estimation is slightly lower, which is about 2 percentage points lower. However, in the FE estimation with fixed effect of control time, the coefficient of triple difference estimation is slightly higher than the coefficient of difference-in-difference estimation by 0.03; The regression results of the second group of control experiments (②⑤) showed that, like the difference-in-difference results, the treatment effect of external shocks on pork prices was significantly positive, and the degree of treatment effect was lower than that of the first group of control experiments, but the OLS and FE estimation coefficients of the triple difference were slightly higher than the difference-in-difference by 2–3 percentage points; The regression results of the third group of control experiments (③⑥), that is, the treatment effect of double external shocks on pork prices is significantly positive, and the estimated coefficient of the triple difference is about 3% lower than the estimated coefficient of the difference-in-difference, which is slightly larger than the estimated coefficient of the second group of control experiments, and smaller than the estimated coefficient of the first group of control experiments.

To sum up, the three groups of control experiments, whether OLS estimation or FE estimation, are consistent with the difference-in-difference results in the direction of action. The effect of external shocks on pork price treatment is significantly positive and passes the 1% significance level. In terms of activity intensity, when excluding the intervention caused by the year difference, the action intensity of ASF and double impact before COVID-19 is lower than that of the difference-in-difference result, the action intensity of ASF after COVID-19 is higher than that of the difference-in-difference result, and the action intensity of ASF after COVID-19 is slightly lower than that of the double impact effect.

The insights derived from this result are: on the one hand, before COVID-19, ASF had the strongest impact on the wholesale price of pork; After COVID-19, the impact of ASF on the wholesale price of pork was significantly reduced (consistent with the difference-in-difference result), and COVID-19 may hinder the further increase of pork price. On the other hand, the impact of dual external shocks on the wholesale price of pork is slightly stronger than that of ASF after the epidemic, indicating that COVID-19 also promotes the increase of the wholesale price of pork, but the impact intensity is slightly smaller. This revelation provides a possible complement to the view of Xiaohua Yu et al. that "COVID-19 has an unknown impact on pork price" (26), and this conclusion is more robust than the double difference result.

4.4. Robustness check

4.4.1. Replace sample period

To further prove the reliability and robustness of the results, the method of replacing the sample period is used to test the robustness of the above results. The method is consistent with the construction of the triple difference model. The replacement sample period is to push forward the period of the control group of the three groups of control experiments by 2 years to form a replacement sample, which is tested by the triple difference model. The specific model is consistent with the model (3). The results are shown in Table 4.

For the first group of control experiments (Chongqing), the OLS estimation coefficient without controlling the time-fixed effect is slightly higher than the triple difference by 0.01 percentage points, and the FE estimation coefficient of controlling the time-fixed effect is slightly higher than the triple difference by 0.01, and the results after replacing the sample period are not significantly different; In the second group of control experiments (Zhejiang Province and Sichuan Province), the estimated results after replacing the samples are the same as the above triple difference results, the estimated coefficients are 0.0705, and the estimated results are completely consistent; In the third group of control experiment (Hubei Province), the OLS estimation result is slightly lower than the triple difference by 0.02, and the FE estimation result is only 1 percentage point higher than the triple difference, and the results are almost the same. There is almost no difference between the treatment effect coefficient of the three groups of control experiments and the triple-difference estimation results. The reason is that using the triple-difference model to replace the sample period to eliminate the interference results of uncertain factors such as year can enhance the reliability and explanatory power of the model, but the cross-year, environmental protection policies and the reality of regional development differences in the sample period still affect the results.

The enlightenment is that after the replacement sample period, as with the triple difference result, the action intensity of ASF and double shocks before COVID-19 is lower than that of the difference-in-difference result, and the action intensity of ASF after COVID-19 is higher than that of the difference-in-difference result. After the replacement sample period, the action intensity of a single ASF is still the highest, and COVID-19 has a weak role in promoting the rise of pork prices. The robustness of the above conclusions is confirmed again.

4.4.2. Placebo test

To further test whether the above results are driven by unobservable factors at the year level, a placebo test was conducted by randomly allocating the period affected by external impact factors (29). Specifically, three groups of control experiments were randomly selected as the experimental group in the sample period, and the remaining sample period was used as the control group to construct

TABLE 4 Robustness test.

		OLS		FE		
	①	②	③	④	⑤	⑥
	ASF (Before COVID-19)	ASF (After COVID-19)	ASF and COVID-19	ASF (Before COVID-19)	ASF (After COVID-19)	ASF and COVID-19
Treatment effect	0.2200***	0.0705***	0.0679***	0.2882***	0.0705***	0.1054***
$(Treat*Time*Group_1)$	(0.0151)	(0.0349)	(0.0119)	(0.0166)	(0.0346)	(0.0022)
Cons	3.0107***	3.0463***	3.0451***	3.3286***	3.0978***	3.1205***
	(0.0047)	(0.0162)	(0.0042)	(0.0071)	(0.0139)	(0.0044)
θ_t	NO	NO	NO	YES	YES	YES
μ_t	NO	YES	NO	NO	YES	NO
obs	1,011	238	225	1,011	238	225
R^2	0.2358	0.9708	0.9812	0.8009	0.9713	0.9904

Robust standard error in brackets.

***$p < 0.01$.

**$p < 0.05$.

*$p < 0.1$.

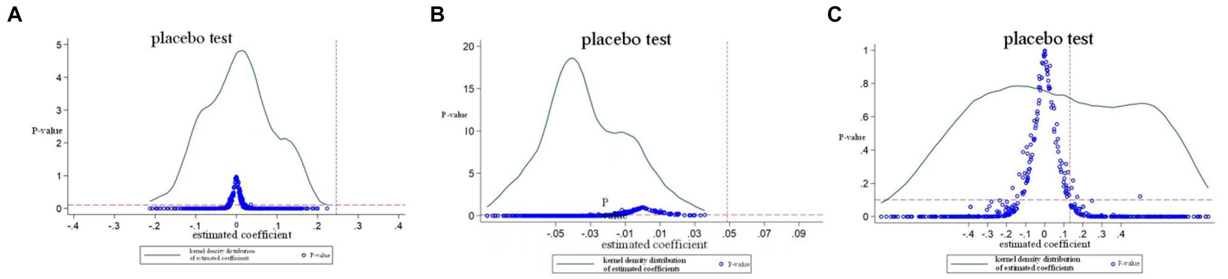

FIGURE 5
Placebo test. **(A)** ASF (before the COVID-19) estimated coefficients and p-value distribution. **(B)** ASF (after the COVID-19) estimated coefficients and p-value distribution. **(C)** ASF and the COVID-19: estimated coefficients and p-value distribution. The X-axis represents the estimated coefficient of time * treat from 500 randomly assigned. The curve is the estimated kernel density distribution, and the point is the relevant p value. The vertical line is the true estimate of Table 3.

a double difference model. Random sampling ensures that the constructed independent variable *time*treat* has no impact on the wholesale price of pork. In other words, any significant findings will indicate that the above regression results are biased. The three groups of control experiments were randomly selected for the length of the sample period with the external impact period (88 days for the first group of control experiment, 12 days for the second group of control experiment, and 36 days for the third group of control experiment), The duration of external impact was randomly selected from three groups of control experimental study sample periods (674 days in total for the first group, 60 days for the second group, and 150 days for the third group) as the experimental group, and the remaining period was the control group. Benchmark regression was performed, the above sampling was repeated 500 times, and the following coefficient distribution and relevant *p* values were drawn (see Figure 5).

The results show that the mean value of the estimated coefficients of all *time*treat* is almost zero. The distribution of 500 estimated coefficients and their related *p* value distribution are all concentrated near zero, and the p value of most estimated values is greater than 0.1 (not significant at the level of 10%). In addition, the true estimate (from Table 3) is an obvious outlier in the placebo test, which further proves the robustness of the results.

5. Conclusions and suggestions

Leveraging data from the wholesale market monitoring database of the Ministry of Agriculture and Rural Affairs of the People's Republic of China, this paper explores the impact of ASF and COVID-19 on pork prices. By utilizing difference-in-difference and triple difference models, alongside a series of robustness tests, we conduct an empirical analysis of the effects of standalone ASF, and the compound external shocks from ASF and COVID-19 on the wholesale price of pork, leading us to the following conclusions.

First of all, from the direction of action, no matter the single external impact or the ASF and COVID-19 double external impact have a significant positive effect on the pork price treatment, and through the 1% significance level, the external impact promotes the pork price significantly. Secondly, in terms of the impact intensity, before COVID-19, ASF had the highest impact on the wholesale price of pork; After COVID-19, the impact of ASF on the wholesale price of pork decreased significantly, and COVID-19 may hinder the further increase of pork price. Finally, the impact of dual external shocks on the wholesale price of pork is slightly stronger than that of ASF after the epidemic, indicating that COVID-19 also promotes the increase of the wholesale price of pork, but the impact intensity is slightly smaller. The economic implication behind it is that when a catastrophic or public hazard event occurs, the pork consumption market will inevitably suffer from short-term depression, the demand curve will shift to the left, the market will gradually spread to the production end, the supply shortage, the supply curve will shift to the left, and the entire market will experience short-term huge fluctuations. Especially when the external impact directly affects the production end, the whole pork market will be subject to greater intervention, and the market price fluctuation will be greater.

The above conclusions can prove that the external impact is an important factor in the fluctuation of pork wholesale prices, and ASF and COVID-19 have obvious sudden external impacts, resulting in the drastic fluctuation of pork wholesale prices. Therefore, to stabilize the wholesale price of pork and maintain the green and orderly development of the pork market, the following suggestions are put forward based on the current situation.

First, strengthen the early warning mechanism and establish a joint prevention and control system. Improve the prevention and control mechanism of ASF and COVID-19 to prevent the impact of sudden external shocks on the pork market. Improve the emergency response system for major epidemic situations, optimize the internal reporting system of the epidemic situation monitoring and warning mechanism, and ensure that the epidemic situation is grasped at the first time; Improve the joint prevention and control system of the regional epidemic situation and establish the epidemic information sharing mechanism; Build a training system for epidemic prevention and control. Second, we should properly publicize and report, avoid panic, and cultivate market confidence. Strengthen scientific guidance for ASF. Propagandize and guide the ways and means of transmission of ASF, let the public have a scientific understanding, not believe rumors, rationally consume pork and its products, and guide the public to buy pork through formal channels. Third, regulate market prices. External shocks such as ASF and COVID-19 are important influencing factors in pork price fluctuations. Therefore, the government should strengthen the supervision and inspection of the wholesale price of pork and regulate the market price.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: the price data in this dataset is daily data with a short span. Requests to access these datasets should be directed to XY, yangxiaoli@syau.edu.cn.

Author contributions

RY and XY: conceptualization, software, and data curation. RY: methodology and writing – original draft preparation. XY: validation. XY and EM: resources and funding acquisition. RY, XY, and EM: writing – review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

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Geo-temporal patterns to design cost-effective interventions for zoonotic diseases -the case of brucellosis in the country of Georgia

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Introduction: Control of zoonosis can benefit from geo-referenced procedures. Focusing on brucellosis, here the ability of two methods to distinguish disease dissemination patterns and promote cost-effective interventions was compared.

Method: Geographical data on bovine, ovine and human brucellosis reported in the country of Georgia between 2014 and 2019 were investigated with (i) the Hot Spot (HS) analysis and (ii) a bio-geographical (BG) alternative.

Results: More than one fourth of all sites reported cases affecting two or more species. While ruminant cases displayed different patterns over time, most human cases described similar geo-temporal features, which were associated with the route used by migrant shepherds. Other human cases showed heterogeneous patterns. The BG approach identified small areas with a case density twice as high as the HS method. The BG method also identified, in 2018, a 2.6–2.99 higher case density in zoonotic (human and non-human) sites than in non-zoonotic sites (which only reported cases affecting a single species) –a finding that, if corroborated, could support cost-effective policy-making.

Discussion: Three dissemination hypotheses were supported by the data: (i) human cases induced by sheep-related contacts; (ii) human cases probably mediated by contaminated milk or meat; and (iii) cattle and sheep that infected one another. This proof-of-concept provided a preliminary validation for a method that may support cost-effective interventions oriented to control zoonoses. To expand these findings, additional studies on zoonosis-related decision-making are recommended.

KEYWORDS

brucellosis, zoonosis, geo-temporal analysis, Georgia (country), cost-benefit analysis

1 Introduction

The COVID-19 pandemic brought many lessons and questions. One of them refers to whether epidemics should be countered with reactive or anticipatory approaches (1). To demonstrate why anticipatory approaches are necessary, cost–benefit oriented studies are needed.

How, when and *where* can implemented interventions lead to cost–benefit based results? To answer this composite question, the type of data analyzed is critical. The analysis of geo-referenced and temporal infectious disease-related data may determine whether intervening at specific geographical sites induce cost-effective policies (2).

Because numerous (if not infinite) geo-temporal patterns may be found in disseminating infectious diseases, geo-referenced and temporal data may also inform on covariates, such as soil, elevation, meteorology, seasonality, and sociology (3). Because they can –visually– reveal interactions, geo-referenced data can inform more than tabular data (4). Because the geographical context surrounding diseases may be unique and it may influence (promoting or preventing) their dissemination, geo-temporal analysis of diseases can capture relationships that reductionist approaches may omit or not anticipate (5, 6).

One specific question that decision-makers need to answer is where, exactly, interventions may lead to less costly, earlier and/or more beneficial results (7). To develop geo-referenced, decision-making oriented analyses, inter/transdisciplinary approaches have been recommended (8). Such approaches may consider bio-geographical and dynamical data that may feed models meant to interrupt disease transmission and/or be cost-effective (9).

While policy-making based on geo-referenced data has been promoted (10) and several studies have explored brucellosis (11–13), the overall as well as the specific (construct, internal, external and/or statistical) validity of such procedures have not yet been emphasized (14, 15). For example, external validity (which refers to multiple variables, metrics, locations, populations, and/or outcomes) has been reported to be infrequently evaluated with empirical data (16).

Geographically explicit, high-resolution, grid-based maps offer an actionable alternative to explore many sources of validity. Such maps have been used to investigate (non-infectious) interactions involving human and non-human species (17). These maps also circumvent the limitations of maps based on aggregate data, which miss local interactions among geo-referenced variables (18). In contrast, grid-based maps can display a high level of granularity (19). Furthermore, bio-geographical methods do not assume space homogeneity –an assumption associated with classic spatial statistics, which considers that neighbors are epidemiologically similar (20, 21).

Brucellosis-related dissemination patterns can be explored in Georgia, the country located in the South Caucasus. With brucellosis being a substantial endemic problem (22), Georgia has a large geo-temporal dataset on cases affecting cattle, sheep, and humans.

Such a context is also adequate to explore *One Health* processes, in which the environment interacts with potential hosts and non-human and human species may infect one another (23, 24). While numerous educational programs now focus on *One Health*, inter–/trans-disciplinary educational gaps have been reported in this field (25).

To evaluate cost–benefit oriented approaches, new concepts may be investigated. For example, the detection of small geographical sites where infections induced by the same bacterium affect two or more species may be desirable.

Accordingly, this study pursued two objectives: (i) to distinguish geo-temporal patterns of zoonotic disease dissemination, and (ii) to identify sites where interventions may induce cost-effective results.

2 Materials and methods

2.1 Data

Data on (ruminant) brucellosis were collected by the National Food Agency of the Ministry of Environmental Protection and Agriculture of Georgia between 2014 and 2019. Human data on brucellosis cases reported between 2015 and 2020 were provided by the Center for Disease Control and Public Health of Georgia. Because the purpose of this study was to generate a preliminary evaluation of a geo-referenced tool based on historical data, no inferences are here made on laboratory-related issues (such as the bacteriological tests used) or epidemiological-related issues (such as estimates of disease prevalence).

The data were filtered to extract geographically referenced and time-stamped records (records with latitude, longitude, and date) for cattle, sheep, and humans. This filtering resulted in the identification of 7,643 records for the 2014–2019 period (2,999 cattle and 4,644 sheep). Nine hundred and ninety-three human records were identified between 2015 and 2018. Due to space limitations of Brief Reports, human data from 2014 and 2019 are not reported but are available upon request.

2.2 Geo-referenced method

These tabular data were brought into a geographic information system (*ArcGIS Pro 3.1.3*, ESRI, Redlands, CA, USA) and a geodatabase point feature was generated. Starting with this initial point feature information, yearly species-specific point features were generated for mapping purposes.

To identify areas of case concentrations, a 5 km-by-5 km, country-wide grid (7,068 cells) was created, and scripts were developed to associate case data with the enclosing grid cell. Such size was selected as a compromise between larger areas (more likely to capture more cases but less precise in terms of specific case geo-location) and smaller areas (more precise in terms of specific case geo-location but more likely to exclude nearby cases).

Each yearly species-specific grid cell polygon feature was added to an *ArcGIS Pro* map and summarized by grid cell number. Each summarized table was exported to a text file for tabulation and analysis. Using the summarized data, a quantile (maximum of three) display of the grid cell's total cases was generated in *ArcGIS Pro*, and a map layout was created.

2.3 Construct, internal, external and statistical validity

The degree to which the concept of interest was actually investigated by the operation implemented (construct validity) was estimated by comparing the bio-geographical (BG) model with an alternative –the Getis-Ord Gi* or Hot Spot (HS) analysis, a method performed by *ArcGIS Pro* that assumes neighbors are bio-geographically similar. The method that yielded the highest case density (cases/square kilometer) was viewed as the most cost-effective.

Internal validity (lack of confounding) was assessed by testing several variables. Threats to internal validity were ruled out when two or more variables yielded similar results.

Statistical analysis was performed using *Minitab 22* (Minitab Inc., State College, PA, USA). Regression analysis explored relationships between the number of cases reported at sites where ruminants, humans, or both human and non-human species reported infections. By investigating two or more host species over two or more years, the external validity of the BG method was also explored.

3 Results

To investigate whether the bio-geographical tool could be used in different populations and/or different timeframes, cattle and sheep cases were plotted, side by side, annually (Figures 1–3). It was observed that cattle cases (Figures 1A, 2A, 3A) matched sheep cases (Figures 1B, 2B, 3C). However, time did not appear to be related with case location. For instance, earlier cases (Figures 1A,B) did not match later cases, even when a short temporal period (a year) was considered (Figures 2A,B).

While cattle and sheep cases displayed noticeable geo-referenced changes over time, most human cases did not. Over 4 years, most human cases were reported in the same area –the eastern region of Georgia (Figures 4A–D). Yet, a second pattern associated with human cases was also seen, which was heterogeneous and took place in the central municipalities of Georgia (Figures 4A–D).

Sites that reported cases affecting two species (cattle and sheep or ruminant sites) seemed to differ from the remaining sites. When the counts or percentages of cases and sites were considered, ruminant sites exhibited twice as many cases as uni-species sites (Supplementary Table 1).

Findings supported the differentiation of brucellosis cases into three geo-temporal patterns: (i) one only observed in the eastern region, which included human cases; (ii) one also affecting humans, which took place outside the eastern region; and (iii) non-human cases, reported outside the eastern region. Within the last variety, two presentations were distinguished: (a) sites where only one ruminant species was infected, and (b) sites where both cattle and sheep cases were reported (ruminant sites).

Findings also suggested an additional pattern of cattle and sheep cases, which was observed in the south-western region of the country, in 2015. Upon further investigation, it was found that the geographical location did not characterize cattle/sheep farms and/or summer pastures but the location of specialized laboratories (located close to ports) where animals exported to other countries are tested before they are embarked. This unexpected finding illustrates the usefulness of geo-referenced data analysis, which may shed light on procedures recorded but not always considered in routine epidemiological analyses.

While the historical nature of the data prevented inferences and predictions, possible educational applications included data-driven hypotheses on peak temporal patterns reaching in 2017 (Supplementary Figure 1). The number of cases observed in either ruminant species predicted the total number of cases found in multi-species (ruminant) sites ($p < 0.01$, Supplementary Figure 2). In one scenario under study, the bio-geographical procedure captured more cases than the HS analysis (583 vs. 521, respectively) in an area 28.4% smaller than the area occupied by the HS solution (Supplementary Figures 3A,B). Because each unit of area occupied 25 square kilometers, expressed as case density/square kilometer, the BG method captured 0.167 cases/sq km while the HS analysis detected 0.107 cases/sq km. Consequently, the case density of the BG approach was 56.2% higher than that of the HS. This difference in potential cost-effectiveness was explained by two factors: (i) the HS missed large areas that included numerous cases (Supplementary Figure 3C) and, (ii) in particular, the HS analysis did not detect nine mini-areas with very high case density (Supplementary Figure 3D). If cost effectiveness of interventions was measured as the ratio of benefits over costs (cases captured/area unit), then the ratio of the BG method would be 2.18 (156.2/71.6), i.e., the BG method exhibited a benefit/cost ratio twice as large as the one shown by the HS analysis.

To explore possible applications in decision-making, the BG method was further investigated according to the (zoonotic vs. non-zoonotic) content of the infected sites. Using the data reported in 2018, the case density of sites reporting zoonotic (human, cattle and/or sheep) cases was at least 2.6 and up to three times higher than the number of sites where only one species was infected (Supplementary Figure 4). Accordingly, if a policy meant to be cost-effective was designed to be applied in this scenario, the first priority of interventions would focus on zoonotic sites (locations that reported a high case density where humans and non-humans were infected), non-zoonotic sites (where only one –human or non-human– species was infected) would be the second priority and, as the last priority, non-zoonotic sites reporting the lowest case density would be intervened.

4 Discussion

4.1 Caveats

Findings are prone to bias due to many factors, which include but are not limited to the varying frequency and magnitude of testing conducted over time and the threats to validity associated with the analysis of historical data (26). Accordingly, this study should not be construed to represent the current status of brucellosis-related conditions existing in Georgia but, instead, a realistic learning scenario that can support research and education in geo-epidemiology.

While zoonotic infections disseminate by only three (direct, indirect, or both direct and indirect) types of transmission, the expression of such transmissions may vary according to the local geography. Consequently, cost-effective interventions may be designed and selected according to specific geo-temporal expressions.

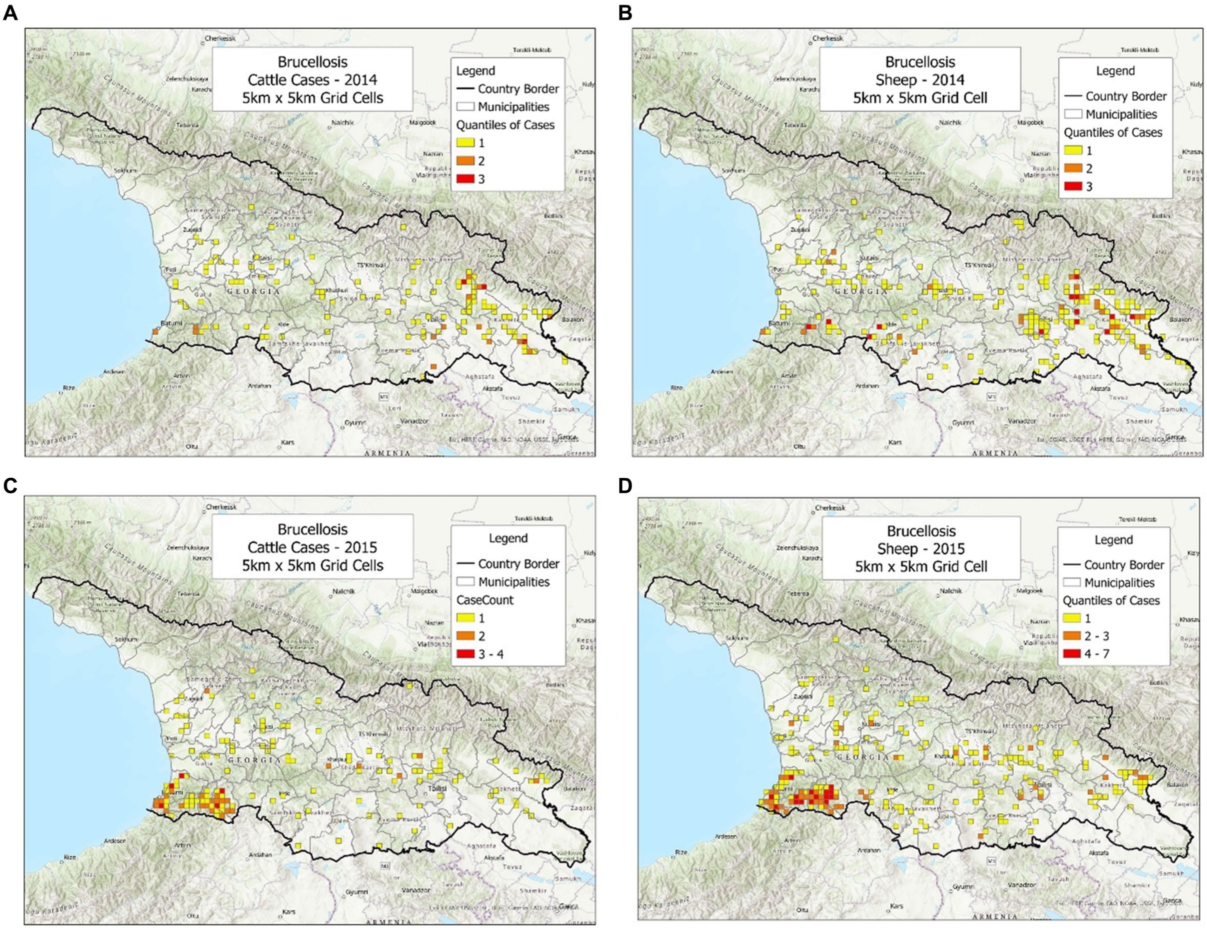

FIGURE 1
Brucellosis cases reported in cattle and sheep, in 2014–2015. **(A)** Cattle cases, 2014. **(B)** Sheep cases, 2014. **(C)** Cattle cases, 2015. **(D)** Sheep cases, 2015.

4.2 Geo-geographical patterns of zoonotic disease transmission

The hypothesis of direct contacts between non-human infected species and susceptible humans was supported by the pattern observed in the eastern region of Georgia (27). Disease transmission, in this modality, is thought to be facilitated when migrant shepherds move their sheep, twice a year, between the southern and the northern borders of the country, along a path flanked by mountains –a geographical feature that determines a rather constant geographical pattern, detected regardless of time (Figures 4A–D). In contrast, other human infections (reported outside the eastern region) are possibly facilitated by the consumption of contaminated milk and meat (26). The hypothesis of cattle-sheep contacts (apparent in several regions of Georgia) is facilitated by a common agricultural practice –also observed in many countries–, in which cattle and sheep share summer pastures (28).

This study emphasized high-resolution, geo-referenced and cost-effective epidemic control measures. To avoid loss of resolution, this study was not centered on municipalities (which differ in area, population, connectivity and many other aspects) but on small areas of equal size (cells of 25 sq km). Such operation facilitated the detection of specific geographical sites where infections affecting two or more host species were found. While approaches that aggregate data and assume homogeneous data distributions over large spatial areas tend to result in large areas to be intervened (e.g., higher costs), the geo-biological method identified small areas with high case density which, if intervened, are likely to induce more beneficial interventions (more cases to be covered) at lower costs (in smaller areas to be intervened), and –due to their smaller areas– such interventions may be completed earlier. Furthermore, small areas with high case density can be mapped over other layers and, consequently, inform on connectivity and many other variables, as described before (29, 30).

Such a geographical approach can be complemented with a biological emphasis.

When the data are divided into classes (e.g., zoonotic or human and non-human, only ruminants, and only humans), the number of cases may be much higher in zoonotic sites and, consequently, interventions that prioritize such sites may be less costly and/or more beneficial (Supplementary Figure 4).

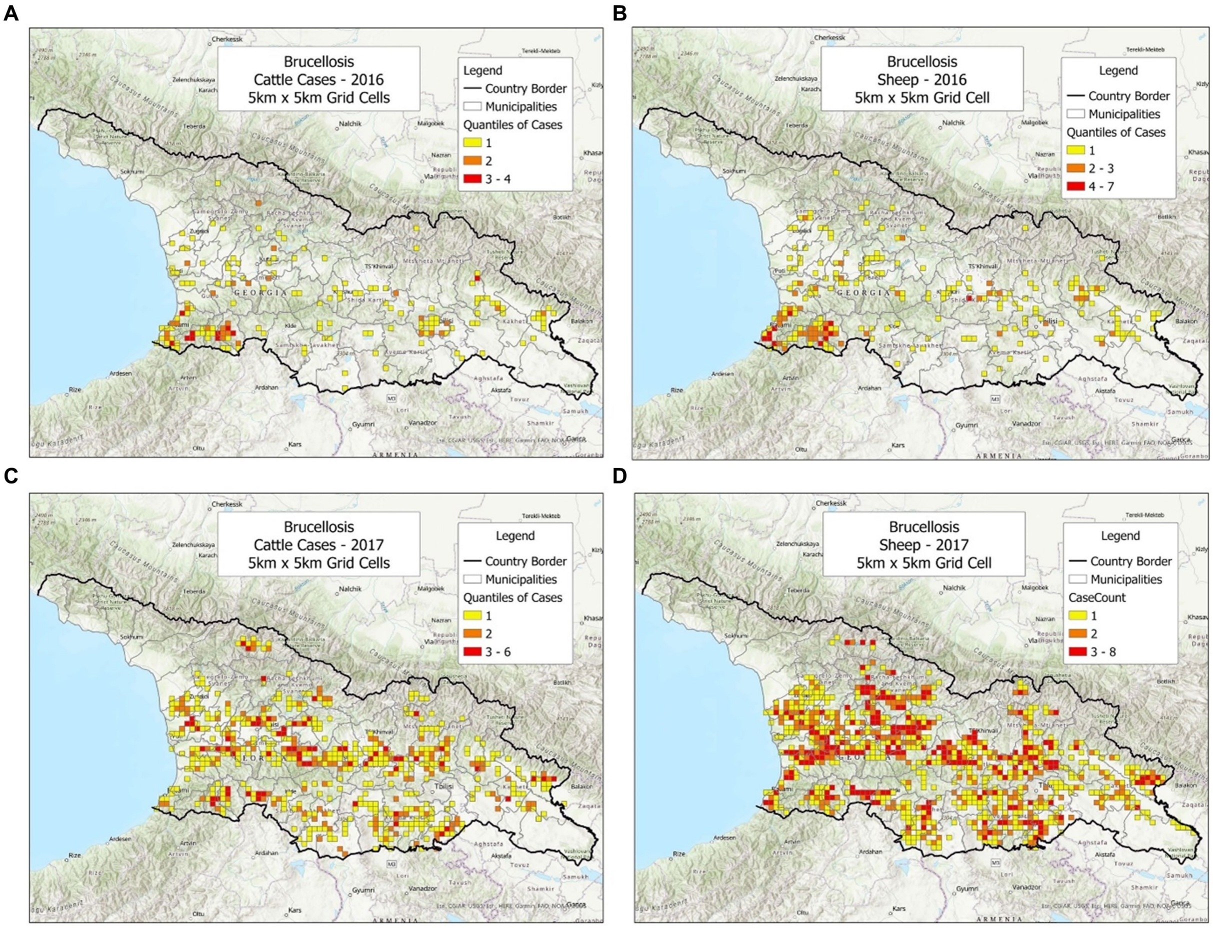

FIGURE 2
Brucellosis cases reported in cattle and sheep, in 2016–2017. **(A)** Cattle cases, 2016. **(B)** Sheep cases, 2016. **(C)** Cattle cases, 2017. **(D)** Sheep cases, 2017.

4.3 Validation

Several estimates of construct, internal, external and/or statistical validity were facilitated by the method under study. Although the historical nature of the data is prone to several threats to validity, the fact that three host species were investigated (and tested for several years) demonstrates that this tool may possess external validity. Because the analysis of five variables yielded similar patterns (and, therefore, confounding was ruled out) and revealed statistically significant associations, internal and statistical validities were supported (Supplementary Figure 1). Because the bio-geographical approach captured more cases per sq. km than the alternative method –a finding also observed when geographical locations reporting infections were classified according to their ability to generate or disseminate zoonotic cases–, construct validity was not ruled out (Supplementary Figures 3, 4).

However, the previous comparison did not consider all possible scenarios (which may include additional bio-geo-temporal variables). To better estimate cost-effective interventions, the benefits associated with interrupting disease transmission (especially, long-term interactions) need to be considered (9).

4.4 Potential applications and future studies

These geo-referenced findings facilitated the development of, at least, five new studies or projects. One refers to prioritizing interventions in locations that report human and non-human cases ('zoonotic disease' sites).

Because such sites can induce secondary infections along ruminant and non-ruminant hosts, they may be prioritized when cost–benefit oriented interventions are planned. Support for ranks that prioritize where interventions should be implemented may be facilitated by a cost–benefit oriented analysis. For example, when there is evidence that zoonotic mini-sites capture more cases than sites presenting infections in a single species (as shown in Supplementary Table 1; Supplementary Figure 4), zoonotic sites could be prioritized, followed by sites that do not show zoonotic cases, and, finally, sites that display the lowest case density and are not zoonotic. Such priority ranks could later be modified or expanded when additional information becomes available. For example, earlier cases could receive the first priority to be intervened when they also display the shortest inter-case distance along actual road networks (30).

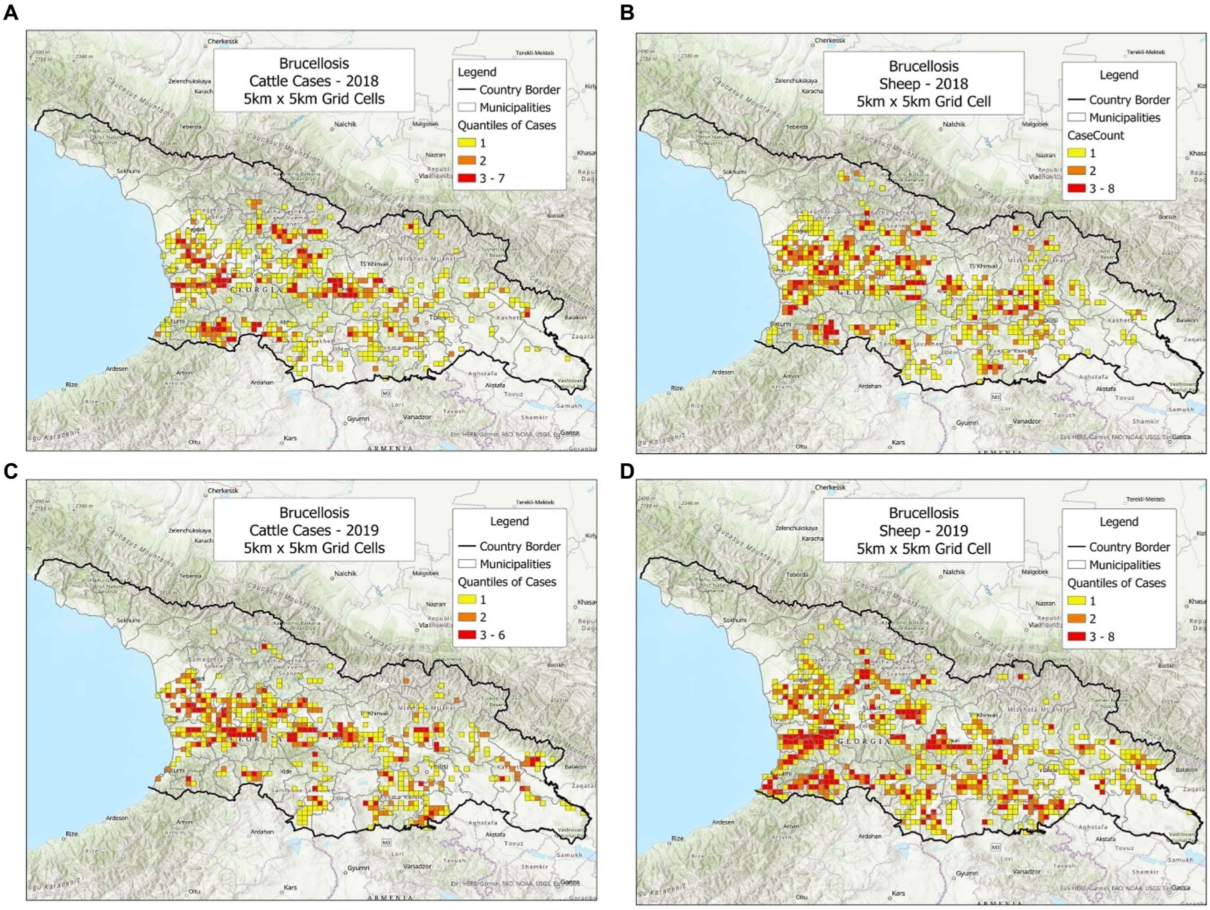

FIGURE 3
Brucellosis cases reported in cattle and sheep, in 2018–2019. **(A)** Cattle cases, 2018. **(B)** Sheep cases, 2018. **(C)** Cattle cases, 2019. **(D)** Sheep cases, 2019.

The second potential intervention refers to migrant shepherds. Because their cases are consistently reported, every year, at the same places, it appears that some behaviors are repeated over time, which occur at specific places where interventions are likely to be beneficial. Because available information suggests there may be inadequate brucellosis-related, educational campaigns in the Tusheti and Kakheti regions (22), future investigations may focus on sociological-educational variables at specific geographical areas, such as associations between educational packages received on the role of uncooked meat and/or unpasteurized milk consumption and disease occurrence (31).

Third, findings may support new studies that seek to identify covariates associated with zoonotic sites. To that end, inverse-problem methods could be considered. Such approaches start with a known solution and then attempt to identify the predictors (32). Inverse-problem approaches have been proposed for epidemiological studies (33). In this case, the precise geo-referenced location of 'zoonotic sites' (a site that may be associated with a rather unique combination of factors) could be the starting point for such inquiries.

Furthermore, future studies based on these or similar geo-referenced procedures could explore alternative methods that may estimate the costs induced by bacterial zoonoses and the potential benefits of preventive campaigns. While similar studies have been conducted for viral zoonoses (1), no cost–benefit estimates on bacterial zoonoses-related decision-making have been emphasized. One exception in this area refers to Mongolia, where a 3.2 benefit/cost ratio has been reported (34). Future studies on cost effectiveness may also consider pastoralist practices (35).

Last, but not least, the development of new educational programs is recommended. It is suggested that, to generate and validate new tools that integrate three fields (epidemiology, geographical data analysis and decision-making), the academic creation of such an interdisciplinary field is required.

5 Conclusion

It is suggested that the geo-temporal analysis of brucellosis may be instrumental to investigate and teach how zoonoses emerge and disseminate. Methods similar to the one reported may be considered in the design of geographical site-specific, time-sensitive, cost-effective interventions.

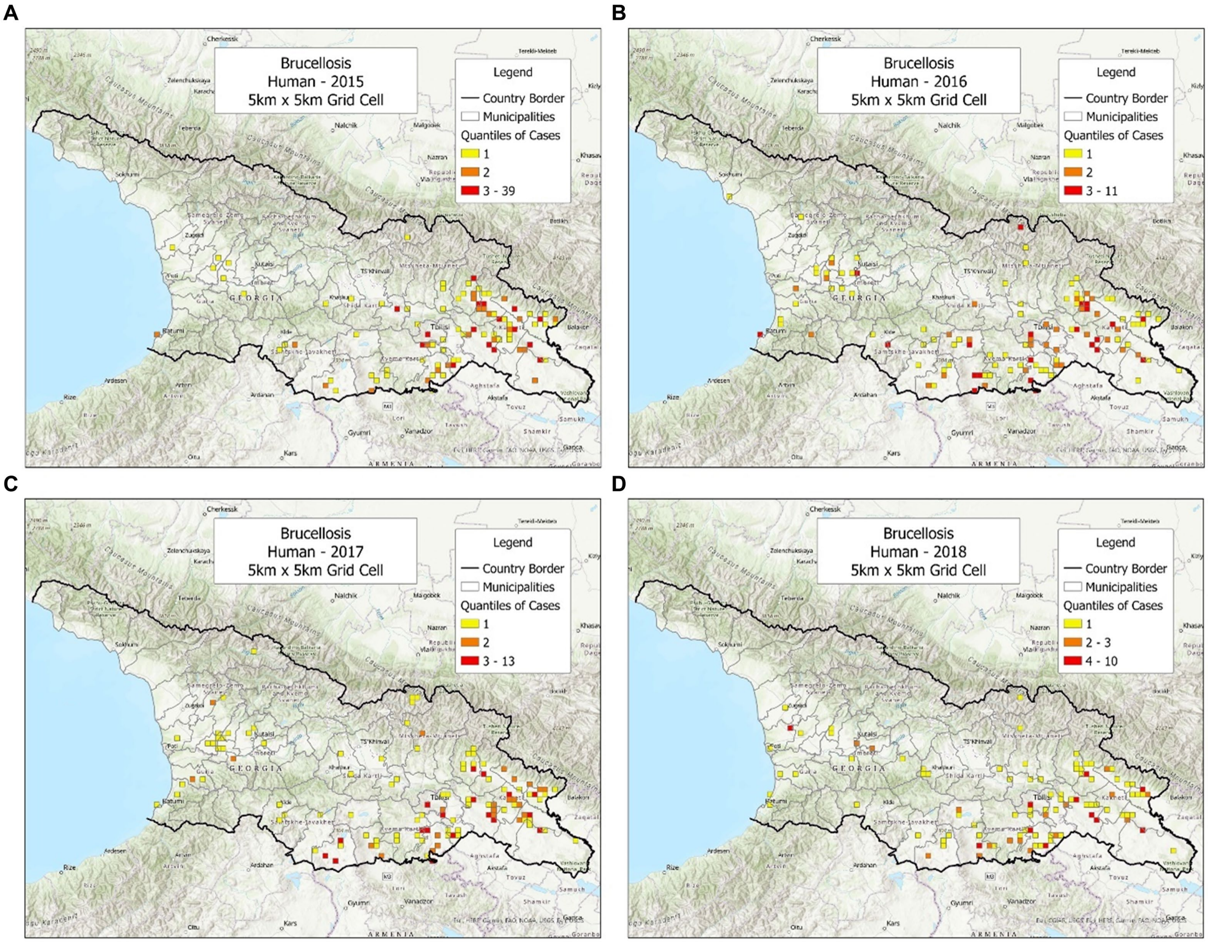

FIGURE 4
Brucellosis cases reported in humans, in 2015–2018. **(A)** Cases reported in 2015. **(B)** Cases reported in 2016. **(C)** Cases reported in 2017. **(D)** Cases reported in 2018.

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

AR: Conceptualization, Writing – original draft. SS: Formal analysis, Visualization, Writing – review & editing. VB: Data curation, Supervision, Writing – review & editing. TCha: Data curation, Writing – review & editing. LM: Data curation, Project administration, Writing – review & editing. IB: Data curation, Writing – review & editing. TChi: Data curation, Writing – review & editing. NS: Data curation, Writing – review & editing. NB: Data curation, Supervision, Writing – review & editing. AH: Investigation, Writing – review & editing. KG: Data curation, Writing – review & editing. JB: Data curation, Writing – original draft. JF: Writing – review & editing. CW: Project administration, Writing – review & editing. PI: Supervision, Writing – review & editing. MK: Conceptualization, Funding acquisition, Writing – original draft.

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Conflict of interest

MK was employed by KB One Health LLC.

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

The reviewers SR and AS declared a shared affiliation with the authors KG, JB, CW to the handling editor at the time of review.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1270505/full#supplementary-material

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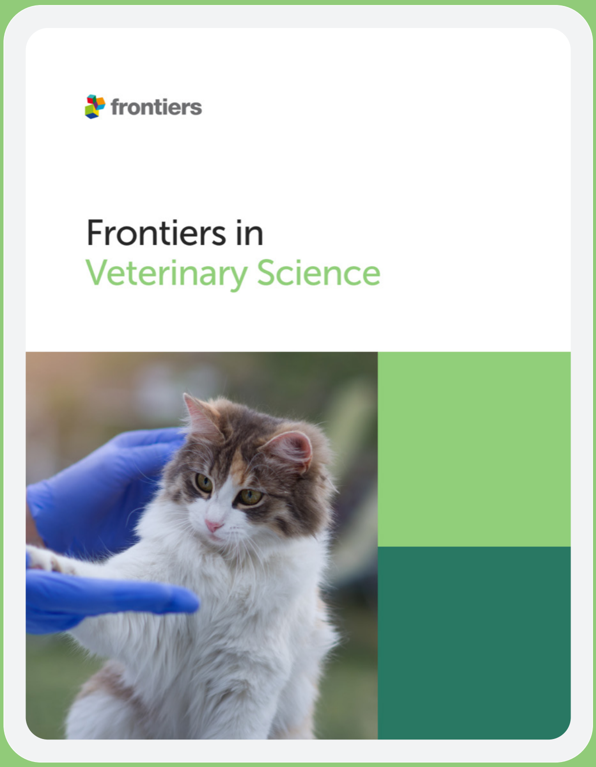

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