SOCIETY FOR VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

Proceedings of a meeting held in

Berlin, Germany

26th – 28th March 2025

Edited by F. Dórea & D. Brodbelt

and the SVEPM Executive Committee

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Cite as: Dórea, F. and Brodbelt, D. (eds). Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine annual meeting held in Berlin, Germany, SVEPM

(Views expressed in these proceedings are not necessarily those of the Editor or the Executive Committee of the Society)

ISBN 978-0-948073-80-9

ACKNOWLEDGEMENTS

SVEPM 2025 was supported by:

Friedrich-Loeffler-Institut (FLI)

The following bodies provided financial support for the conference:

Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)

Zoetis

Elsevier

CIRAD

MSc Epidemiology Postgraduate Online (University Medical Center Utrecht)

Scotland's Rural College (SRUC)

Royal Veterinary College (RVC)

WOAH Collaborating Centre for Risk Analysis & Modelling (APHA + RVC)

EpiMundi (formerly known as Ausvet)

Frontiers in Veterinary Science, A journal by Frontiers

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RISK MODELING

QUANTIFYING THE RISK OF HIGH PATHOGENICITY AVIAN INFLUENZA VIRUS SPILLOVER FROM WILD BIRDS TO POULTRY FARMS IN DENMARK

Y. LIU*, L.J. KJÆR, A. E. BOKLUND, M. WARD, Y. LIANG AND C.T. KIRKEBY

SUMMARY

A spatiotemporal mechanistic model was developed to assess the spillover risk of high pathogenicity avian influenza (HPAI) from wild birds to poultry farms in Denmark, with a focus on the 2020/2021 epidemic season. The model firstly estimates environmental contamination over time, from five wild bird species selected for their ability to spread HPAI. Secondly, the model quantifies the risk of transmission to poultry farms from the environment. For six domestic flock types (chicken, duck, turkey, mallard, other single-gamebird, and mixed species) transmission parameters regarding susceptibility were derived from literature, and dose-response relationships were used to quantify the potential of spillover into poultry farms. Cluster analysis identified consistent high-risk areas in South Zealand throughout the year, corresponding to a hot spot identified in the reported outbreak data. A good model precision (78%) was found, whereas the specificity ranged from 50% to 77%, depending on the time of the year.

INTRODUCTION

Since late 2020, Denmark experienced increasing detections of HPAI in wild birds and poultry farms. By the end of September 2024, the dominant viral type was the HPAI H5 clade 2.3.4.4b, which caused 49 poultry outbreaks, and 845 wild birds were lab-confirmed with HPAI virus (HPAIV) (FVST, 2024). Infected wild birds were suspected to be the most relevant source of farm outbreaks via direct and indirect contact. During the 2020/2021 epidemic, 189 HPAIV-infected birds belonged to the order Anseriformes, accounting for 56% of wild bird cases reported via passive surveillance. At the time, among the Anseriformes species, 60 whooper swans (*Cygnus cygnus*), 59 barnacle geese (*Branta leucopsis*), 36 mute swans (*Cygnus olor*), and 14 greylag geese (*Anser anser*) were the most reported positive species. Since the autumn 2023, Charadriiformes species, such as terns and gulls, were increasingly found hit by HPAIV in Denmark (EFSA et al., 2023). Massive die-offs were observed along the coastline. Fortunately, since 2024, the epidemic has slowed down with fewer outbreaks, as indicated by the overall low number of detections in Denmark and other European countries (EFSA et al., 2024a, EFSA et al., 2024b). Investigation of poultry outbreaks suggested a close genetic association with wild bird cases in Denmark, which was also reported in earlier epidemics before 2020 (Liang et al., 2021), indicating that there is a need to assess the spillover risk of HPAIV from wild birds to poultry.

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Wild birds are considered the natural reservoir of HPAIV and are suspected to move the virus over long distances with seasonal migrations. The first HPAI H5 mass mortality was documented in 2005 in western China at a breeding location for migratory birds, which overwintered in Southeast Asia, Tibet, and India (Li et al., 2024). Thereafter, the virus showed a trend of moving westward (Li et al., 2024). To investigate the HPAI risk to poultry farms posed by wild birds, much effort has been made in field and lab studies. For example, camera trapping in the vicinity of poultry premises in Northern Italy reported magpies, pheasants, and doves being the most frequent visitors (Graziosi et al., 2024); models built for disease occurrence risk dynamics in Sweden quantified spatiotemporal risk levels depending on wild bird presence (Stiles et al., 2024), and incubation experiments in pheasants and the successful onward transmission to chickens revealed efficient infectivity from game birds to poultry (Liang et al., 2022). Denmark is located on a busy migration route and is home to various birds of the Northwest and Central European flyway population: most waterbirds (e.g., ducks, geese and swans) migrate to Denmark for wintering and are distributed widely in the country, particularly around water-rich areas (Nielsen et al., 2023). Voluntary bird watchers and the national bird census program contribute to gaining knowledge of bird abundance across Denmark, where bird data are collected in time and space.

In this study, a spatiotemporal model was used to assess the HPAIV spillover risk from wild birds to poultry farms in Denmark, using knowledge of wild bird populations and poultry farms, passive HPAIV-infected wild bird detections in 2020/2021, and poultry outbreaks from 2020/2021 to 2023/2024. The aim of the study is to construct a tool for assisting decision-makers to evaluate the risk of HPAIV outbreaks in poultry farms in Denmark.

MATERIALS AND METHODS

Denmark was rasterised into 10 by 10 km grid cells and a 1 km buffer zone was added around the coastal line to account for the habitat use of waterbirds. Based on HPAIV-positive wild bird detections reported in the 2020/2021 season, Several water bird species were selected — barnacle goose, whooper swan, mute swan, and greylag goose — to represent the total HPAI-risk species. Mallard (*Anas platyrhynchos*) was also included because of its widespread distribution and the role of mallards in transmission, being able to sustain the silent spread of HPAIV as shown in experimental studies (Pantin-Jackwood et al., 2016; Ahrens et al., 2022).

To assess HPAIV spillover risk to poultry, a previously developed a model was used, namely the DanHPAIwild model, which predicts the weekly environmental contamination derived from bird migration and environmental transmission (unpublished data).

Brief description of the DanHPAIwild model

The DanHPAIwild simulates weekly bird movement using the number of birds and disease states of birds through a susceptible-infectious-recovered/removed (SIR) model with indirect transmission via the environment. The model was calibrated to reflect outbreaks in the period October 2020 to September 2021 with regards to seasonality and assuming a 2% detection rate of infected wild birds. Corresponding to the HPAI incidence pattern and bird migration behaviour, an epidemiological season begins in week 39 of the first year and ends in week 38 of the next year. Furthermore, a season was equally divided into four quarters, with the first quarter starting in October.

Spatiotemporal bird abundance data of the selected species were obtained from BirdLife Denmark and the NOVANA database (GBIF, 2022; Nielsen et al., 2023), which provided weekly ratios and the overwinter (week 2) bird population, respectively. To predict the environmental contamination load, the DanHPAIwild model was initialised using spatiotemporal bird abundance data and HPAIV-detections in wild birds in 2020/2021, and simulated two consecutive seasons: the first season was a burn-in season in which HPAIV-infected wild birds were introduced, and the second season was the season of interest in this study, without any cases introduced, only environmental circulation of virus shed from the wild bird population. To account for variability in the bird population between years and the HPAIV entry locations, the model was run stochastically for each iteration by 1) random sampling of bird abundance from data collected in five previous seasons, i.e. 2016/2017 to 2020/2021 and 2) random cell allocation of the site of infectious bird entries during migration. In this way, a model-based weekly median viral load ($V_{w,c}$, with medians taken from 400 iterations) of HPAIV was obtained in each grid cell.

Spillover risk

The estimated risk of spillover to poultry farms used the information of on-farm species and farm locations (Fig.1). To quantify the spillover risk from environmental contamination in each cell, the literature-derived or assumed median infectious dose ($ID_{50,f}$) was used for six farm types categorised by the on-farm species: chicken, duck, turkey, mallard, other mono-species game, and mixed species (Table 1). This dose reflected the relative risk of ingesting virus from the environment, so a magnitude parameter, $r_f = \ln(2)/ID_{50,f}$, was generated. By assuming a homogenous spread of the environmental contamination within each cell, an exponential function that was based on infection probabilities was used to calculate the relative risk, $RR(V_{w,c}) = 1 - e^{-r_f V_{w,c}/N_{Env,c}}$, with $V_{w,c}$ being the weekly environmental viral load in the cell c, and $N_{Env,c}$ being the cell-specific dilution effect. The baseline value of the dilution effect of viral particles into water bodies in grid cells was 2×10^{14}, which was obtained by assuming an average depth of 2 meters for lakes in Denmark and a cell-sized (i.e. 100 km^2) area (MST, 2000). Then, the cell-specific dilution effect was scaled to reflect the actual proportion of water areas in a cell. This procedure ensured that the dilution of virus particles in each cell corresponded to the relative proportion of standing water in the cells.

Figure 1. Location of Denmark and distribution of poultry farms with the farm types categorised according to the on-farm species in Denmark at the end of 2021. Data were obtained from Danish Central Husbandry Register (CHR). Only commercial farms with more than 100 birds were considered in the study.

Table 1. The median infection dose (ID_{50}) used in the model for each farm type with additional information provided, including the challenge strains in the experiment, magnitude parameters r_f, and source.

Farm type (categorised by on-farm species)	Literature-based challenge HPAIV strain	ID_{50} (EID_{50}/ml)	r_f	Source
Chicken	Clade 2.2 H5N1 A/turkey/Turkey/1/05	$5\times10^{3.4}$	0.00005519	(Aldous et al., 2010)
Duck	Clade 2.2 H5N1 A/turkey/Turkey/1/05	5×10	0.01386294	(Aldous et al., 2009)
Turkey	Clade 2.2 H5N1 A/turkey/Turkey/1/05	5×10	0.01386294	(Aldous et al., 2009)
Mallard	-	5×10	0.01386294	Assumed the same as in ducks
Mono-species game (partridge, pheasant, or quail)	-	5×10^{2}	0.001386294	Assumed
Mixed species	-	5×10^{2}	0.001386294	Assumed

Cluster analysis and model performance

Four snapshots representing the RR in the first week of each quarter were mapped for individual poultry farms, illustrating the changes over a year. Then, for each week,

dichotomised farms into at-risk and not-at-risk groups using the median of weekly RRs as the cut-off value. Spatial clusters of farm-specific relative risks were identified using SatScan version 10.1.2 and the rsatscan package in R Version 4.2.1 (R Core Team, 2022; Kleinman et al., 2023). The Bernoulli-based spatial scan statistics with an elliptical scanning window was employed and then the mean observed to expected ratios (ODE) was checked (Kleinman et al., 2023). The value of the ODE assigns either a hot spot (ODE > 1) or a cold spot (ODE < 1). Statistically significant clusters were reported based on the Gini coefficient, which provides a quick and intuitive way to evaluate the degree of heterogeneity of the collection of clusters (Han et al., 2016). To compare the results of the estimated spillover risk to farms with actual farm outbreaks, a similar cluster analysis was conducted for reported poultry outbreaks aggregated from 2020 to early 2024, together with poultry farms without outbreaks retrieved from the CHR database at the end of 2021. Visual inspection was carried out to compare the identified significant clusters of model estimates and actual reported outbreaks.

The performance accuracy of model estimates was assessed using the precision-specificity method by comparing the estimated risk of presence/absence in poultry farms with reported farm outbreaks from 2020 to 2024. Model precision was reported for the whole simulated period representing the proportion of reported outbreaks being categorised as at-risk farms during the same week of the outbreak occurrence out of the total outbreaks. Model specificity was reported at a weekly level, representing the proportion of farms without outbreaks being categorised as not-at-risk. Weekly sensitivity of the model was also calculated, but it was less informative in indicating model performance due to the limited number of outbreaks.

RESULTS

The model-estimated farm-specific HPAIV spillover risk from wild birds showed that the overall risk in week 39 (early October) was in general lower than week 52 (late December/early January) and the risk continued increasing slightly to week 13 (early April) followed by a decreased overall pattern in week 26 (early July) (Fig.2). The development of RR suggested the overall spillover risk was generally higher during winter/spring and lower in summer.

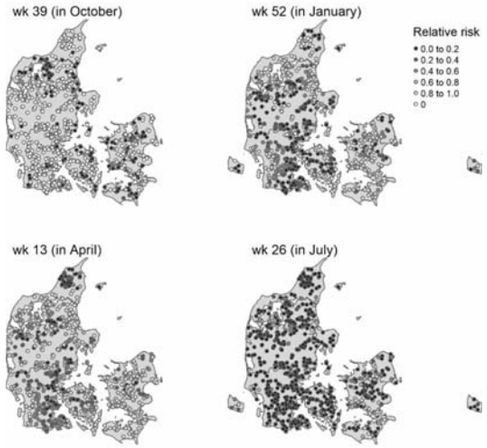

Figure 2. Model-estimated relative risks of each poultry farm in the first weeks of each quarter. Each dots represent a poultry farm in Denmark.

By dichotomising farms into at-risk and not-at-risk, based on the weekly medians, the result showed an increase in the proportion of at-risk farms in early January compared to early October by 26.2%, followed by a relatively stable risk presence in Danish poultry farms in early April and early July (49.7% and 50.0%, respectively) (Fig.3). The SatScan cluster analysis detected significant clusters (both hot- and cold spots) for the HPAIV spillover risk for poultry farms. For all quarters of the year, a hot spot was consistently found in the southern area of Zealand (Fig.3), coinciding with the hot spot found for poultry outbreaks, indicating a good model performance (Fig.3). Comparing the estimated at-risk farms with reported outbreak farms, it was found that 38 of 49 reported farm outbreaks were identified as at-risk farms during the week of outbreak occurrence, indicating a good overall (78%) model precision. Specificity varied for each week, from 50% to 77%.

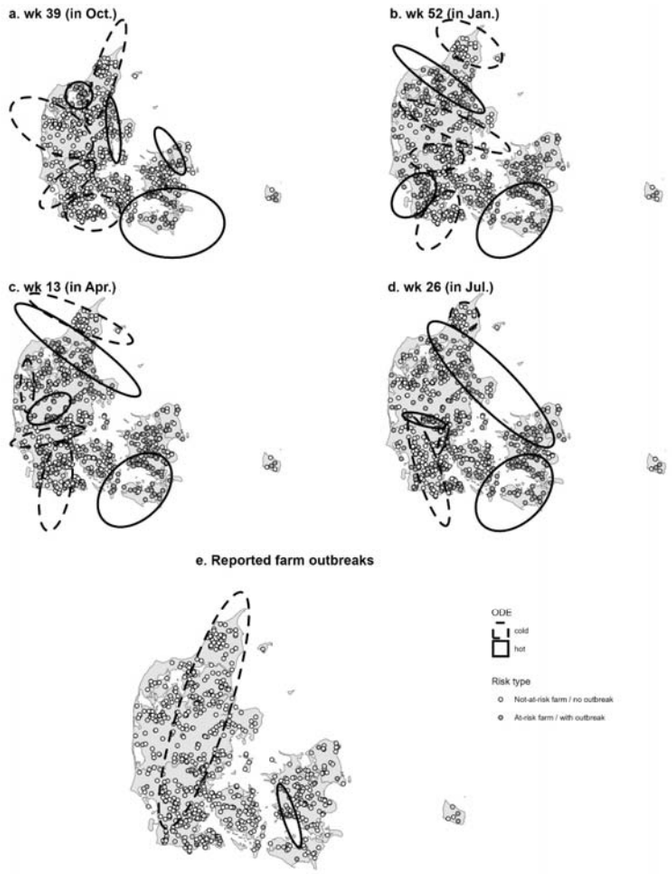

Figure 3. The dichotomised risk of high pathogenicity avian influenza virus spillover from wild birds to poultry farms in Denmark, actual reported farm outbreaks from 2020/2021 to 2023/2024 in Denmark, and the identified significant spatial clusters during the first weeks of each quarter. Orange and green points indicate farms at risk and not at risk or with and without outbreak respectively. Solid and dashed ellipses indicate hot and cold spots, respectively.

DISCUSSION

The spillover risk estimation, based on the environmental contamination load driven by wild birds and the distribution of poultry farms with different on-farm species, showed seasonal differences and spatial heterogeneity. Compared to actual reported outbreaks, model-estimated risks well reflected the reality regarding HPAI seasonality and high-risk areas, despite the limited number of reported outbreaks. The study did not only quantify absolute farm-specific RR values that changed week by week, but also provide a user-intuitive way to present risks into hot/cold spots, which could help decision-makers evaluate spatiotemporal distribution of the HPAIV spillover risk and layout monitoring strategies with limited resources. It is worth considering both absolute farm-specific RR values (Fig.2) and hot/cold spots (Fig.3), because whereas RR values show a better indication of the overall weekly change in the spillover risk across poultry farms, the identified hot/cold spots clustering areas give more intuitive management information. Among the simulated four weeks, the overall RR values were highest during early winter, and South Zealand was identified as having a hot spot in all weeks, which was supported by previously estimated clusters for both active and passive wild bird AI virus (AIV) surveillance data prior to 2020 (Kjær et al., 2021).

Epidemiological features of HPAI keep evolving from year to year, showing remarkable variations in affected species, seasonality, and magnitude (EFSA et al., 2023, EFSA et al., 2024a, EFSA et al., 2024b). In this study, previously collected surveillance data of wild birds from 2020/2021 was used to assess the farm risk for a general year. Although the model performed well indicated by the prediction, the DanHPAIwild model and the spillover model need updating with new epidemiological information. In 2022/2023 the most noticeable changes were the great HPAIV mortality in Charadriiformes (i.e. seabirds) and the high incidence rate in wild birds during the summer. However, the epidemic seems to have slowed down in 2024 with the virus circulating at a low level, maybe as a result of immunity build-up in certain wild bird species (Knief et al., 2024). As a consequence of not including the information from the 2022/23 season, the present model might underestimate the RRs around the coastline due to the lack of seabird populations in the DanHPAIwild model, and overestimate the overall RRs due to not accounting for increased immunity.

The reported outbreaks showed that in the southern area of Zealand several commercial farms and hobby farms located in close proximity to each other experienced HPAIV outbreaks in both the 2020/21 and 2021/22 seasons; a farm-to-farm transmission route was suspected for a few outbreaks in South Zealand, where they were closely located or belonged to the same cooperation. However, the identified hot spot suggested that a possible introduction pathway by wild birds cannot be ruled out. However, it could also indicate other factors coinciding with the increase of wild bird density and higher risk of secondary outbreaks, e.g. weather conditions (de Vos and Elbers, 2024). The alignment of the identified hot spots in the model and the data highlights the utility of the DanHPAIwild model in assisting in planning risk-based surveillance strategies and in indicating warning levels on farms of different locations. The validity of the model was further supported by its good precision throughout the simulation period. While the accuracy in excluding non-outbreak farms showed greater variability depending on the time of year, the model demonstrated a 78% success rate in correctly identifying outbreak farms. This performance highlights the model's utility in guiding targeted preventive measures for farms assessed as being at risk.

ACKNOWLEDGEMENTS

This study was funded by the ENIGMA project, a veterinary contingency project funded by the Danish Veterinary and Food Administration. The authors appreciate volunteers and bird watchers for reporting diseased birds and counting bird numbers in Denmark, and the data available from Birdlife Denmark.

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WILD BOAR SUITABILITY DROVE ASF DYNAMICS IN KOREAN WILD BOAR POPULATIONS

J.-S. LIM*, B. HAYES, E. KIM, T. VERGNE AND M. ANDRAUD

SUMMARY

African swine fever (ASF) is a highly contagious disease both in domestic pig and wild boar, threatening the global pig industry. During the ongoing ASF epidemic in the Korean wild boar populations the association between wild boar density and ASF transmission emerged as a key question. Here, a spatially-explicit mechanistic model was developed to simulate ASF transmission across a grid of 25 km² hexagons. The model included a constant transmission rate, imperfect detectability of affected hexagons, and wild boar habitat suitability influencing on infectivity and susceptibility of hexagons. The model showed that a wild boar-favourable infectious hexagon would infect a wild boar-favourable susceptible hexagon every 2.38 weeks, with only a quarter of infectious hexagons being eventually detected. It was estimated that wild boar-favourable hexagons were five times more susceptible and infectious than wild boar-unfavourable hexagons. This model provides quantitative evidence to support decision-makers in placing fences, considering the true disease dynamics.

INTRODUCTION

African swine fever (ASF) is caused by ASF virus in susceptible swine species, including wild boar (*Sus scrofa*) and domestic pigs (*Sus scrofa domesticus*), with a high case-fatality rate close to 100%. No internationally commercialised vaccine or treatment against ASF has been developed yet. The ongoing ASF epidemic, which began in 2007 in Georgia (Rowlands et al., 2008), has affected numerous European and Asian countries (European Food Safety Authority et al., 2024; Ge et al., 2018), threatening the global pig industries and animal-derived protein economy (Mason-D'Croz et al., 2020). In September 2019, South Korea reported its first ASF case on domestic pig farm near the border with North Korea (Kim et al., 2020), followed by cases in wild boar in the nearby regions in October of the same year (Jo and Gortazar, 2020). Korean authorities have responded by implementing three control measures to limit the ASF spreading within the northern region near the border, including a fencing strategy, active carcass searching and depopulation (Jo and Gortazar, 2021). Despite intensive responses, ASF in wild boar breached the implemented measures and have invaded southern regions of the country. As of August 2024, over 4,100 wild boar cases have been reported (Ministry of Environment of South Korea, 2024).

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Two major challenges emerged during this five-year ongoing epidemic in South Korea. First, a better understanding of ASF dynamics related to wild boar density is needed, as 70% of South Korea is covered by largely unfragmented forested mountains, providing the favourable habitats for wild boar populations (Lim et al., 2023). Second, authorities need to address the partially-observed ASF dynamics caused by limited surveillance sensitivity, as it has implications for disease dynamics itself. ASF-positive wild boar carcasses that go undetected by surveillance activities cannot be removed or sanitised and therefore remain infectious until the virus loses its viability through decomposition. From a decision-making perspective, the partially-observed ASF dynamics is highly likely to bias the understanding of the current disease dynamics, leading to less effective decisions on control measures (Lim et al., 2023). For example, a recent study in South Korea suggested that the misplacement of the fences with regards to the true wavefront location could explain the limited impact of the fences on ASF progression (Lim et al., 2024).

South Korea offers an ideal setting for addressing the challenges outlined above, due to its unique epidemiological context characterized by a very high number of ASF cases detected in wild boar compared to other countries of the Asian continent (Vergne et al., 2020). This context presents a critical opportunity to apply flexible models that can accurately capture the hidden disease spread. To that purpose, a mechanistic model was developed explicitly structured to account for undetected states, in order to reproduce spatiotemporal dynamics of the ASF epidemics among wild boar populations in South Korea. This model uncovers the underlying disease dynamics, provides evidence to support disease management policies, and improves the understanding of ASF dynamics.

MATERIALS AND METHODS

Surveillance data and study region

Cases reported in domestic pigs were assumed to not significantly affect the ASF dynamics among wild boar populations during the study period from September 2019 to September 2022. Indeed, by September 2022, only 24 sporadic outbreaks have been reported in domestic pig farms, with no evidence of transmission to wild boar from these farms (Ministry of Agriculture Food and Rural Affairs, 2022). Therefore, the analysis only used ASF data from the wild boar compartment.

In total, 2,661 wild boar cases were recorded in the nationwide surveillance system operated by the Korean Ministry of Environment from the first reported ASF case during study period (Ministry of Environments, 2022). The dataset includes the date of wild boar detection, the date of diagnosis, the estimated date of death (only for carcass sample), the sample type (e.g. carcass, hunted and trapped), and the geographical coordinates of the wild boar tested (longitude and latitude).

The study region was defined to encompass areas that reported ASF cases, with 60 km spatial buffer around each geographical points of wild boar cases. This area was then confined within South Korea territory excluding maritime areas (Fig.1). This area covering 52,969 km² was discretised into grid of 2,280 hexagons with a resolution of 25 km². This was based on the home range size of wild boar (Guberti et al., 2022). These hexagons served as the epidemiological unit in the mechanistic model developed in this study, as will be further described in subsequent sections.

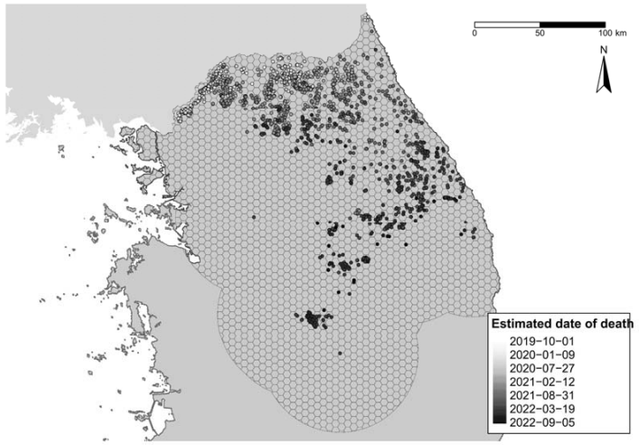

Figure 1. Spatial distribution of the reported ASF cases from October 2019 to September 2022. The study region is layered using 2,280 25-km² hexagons, covering the areas where ASF cases were reported.

Landscape topography is crucial as it shapes the wild boar abundance and thereby ASF disease dynamics (Lim et al., 2021a). Due to varying human accessibility, the topography also affects surveillance intensity (Lim et al., 2021b). In this study, two different landscape topography metrics were used to reflect the wild boar abundance and the sensitivity of surveillance for each hexagon: forest coverage and elevation. Forest coverage was used as a proxy for wild boar abundance (Fonseca, 2007; Lyang and Lee, 2010; Kim et al., 2019; Podgorski et al., 2020) given the lack of high-resolution information to estimate the wild boar abundance for each unit. The forest coverage within each hexagon was determined by calculating the overlapping area of each hexagon with forested land cover. Then, the calculated forest coverage was categorised in each hexagon by matching it with the habitat suitability of wild boar (Kim and Pak, 2020) to classify the hexagons as wild boar-favourable or -unfavourable. Those with forest coverage greater than or equal to 80% were defined as wild boar-favourable, while those with less than 80% forest coverage were defined as wild boar-unfavourable. Among total of 2,280 units, 1,463 (64%) units were categorized as wild boar-favourable units. Elevation was used as proxy for the sensitivity of surveillance, as it was identified as one of key factors associated with sensitivity of surveillance from previous study (Lim et al., 2021b). The average elevation of each hexagon was calculated by taking the mean value of the overlapping raster cells of elevation with each hexagon.

Reconstructing the observed ASF epidemics at the hexagon level

As the ASF model simulates the spread of ASF between 25-km² hexagons, the observed wild boar-level dynamics were aggregated and reprojected into hexagon-level dynamics. First, each individual wild boar case was assigned to its corresponding hexagon. Then, the estimated date of death of each case was used to back-calculate the date of infection by assuming a fixed

three-week period between infection and death (Guberti et al., 2022). To account for viral persistence in the environment, an individual wild boar was considered infectious from infection until three weeks after its carcass was detected (and removed). For each hexagon that contained reported wild boar cases, the hexagon-level date of infection was defined as the earliest wild boar-level date of infection and considered that the hexagon was infectious from that date. The date of detection of the hexagon was defined as the date the first infected wild boar was reported. It was considered that the hexagon remained infectious as long as infectious periods of individual wild boar (i.e. from infection to three weeks after detection) overlapped one another. If the periods did not overlap, the hexagon was considered re-infected. From the reconstructed ASF dynamics, 452 out of 2,280 hexagons reported ASF.

ASF model description

A spatially-explicit stochastic grid-based compartment model was developed. The structure of the model was based on the ASF model developed for the Romanian context, which incorporated transmissions both among and between wild boars and domestic pig farms (Hayes et al., 2024). To fit the research questions and the Korean context, this model was adapted by focusing only on the wild boar populations, and by incorporating processes to reflect wild boar abundance, the imperfect sensitivity of surveillance and delays in reporting (Fig.2).

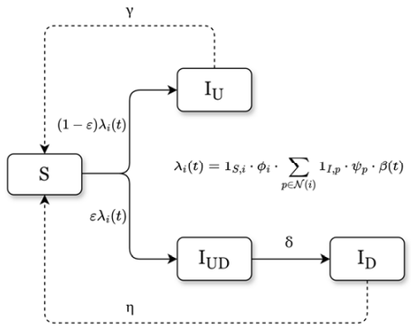

Figure 2. The scheme of the model S indicates the susceptible state; IUD indicates "infectious to be detected" state; ID indicates "infectious-Detected" state; IU indicates "infectious-undetected" state. ε is the detectability probability; $\lambda_i(t)$ is the force of infection experienced by unit i; $1_{S,i}$ is the indicator function for whether unit i is susceptible; ϕ_i is relative susceptibility of unit i; $N(i)$ is the ensemble of units neighbouring i; $1_{S,i}$ is the indicator function for whether unit p is infectious or not; ψ_p is relative infectivity of unit p, and $\beta(t)$ is transmission rate at time t.

The model was composed of four different states defined at the hexagon level (Fig.2): Susceptible (S, all wild boar in the hexagon are not infected) – Infectious-undetected (I_U, at least one wild boar is infectious in a hexagon, but none of these cases are not detected by surveillance activities) – Infectious-to-be-detected (I_{UD}, at least one wild boar is infectious in a hexagon, and at least one of these cases will be detected by surveillance activities) – Infectious-

detected (I_D, at least one infected wild boar is detected) (Fig.2). Upon infection, each hexagon could follow two distinct transition pathways, depending on whether the infectious hexagon would eventually be detected or not. When the hexagon becomes infected, it turns into I_U or I_{UD} states based on a Bernoulli process with the probability of detection (ε) reflecting sensitivity of wild boar surveillance. When in I_{UD} state, a hexagon transitions to the I_D state based on the detection rate (δ) reflecting detection delays. Then, infectious and detected hexagon will be sanitized due to the intervention and transitions back to S at a rate determined by the sanitation rate (η). For I_U, this unit naturally recovers (e.g. transitions to S) without any interventions at a rate determined by the recovery rate (γ) and can then be re-infected.

The model assumed that hexagons in any of the three infectious states (I_U, I_{UD}, I_D) can transmit the infection to any susceptible first-order neighbouring hexagon. It was further assumed that each unit has a different level of susceptibility and infectivity depending on wild boar favourability of a hexagon. Under the density-dependent assumption, at time *t*, the force of infection (FOI) experienced by the susceptible unit *i* ($\lambda_i(t)$) was defined as the sum of forces of infection exerted by the infectious neighbouring units *p*, and given by Eq.(1).

$$\lambda_i(t) = 1_{S,i} \cdot \phi_i \cdot \sum_{p \in N(i)} 1_{I,p} \cdot \phi_p \cdot \beta(t) \quad (1)$$

where $1_{S,i}$ is the indicator function for whether unit i is susceptible, ϕ_i is the relative susceptibility and infectivity of unit i depending on its wild boar favourability, $N(i)$ is the ensemble of units neighbouring i, $1_{I,p}$ is the indicator function for whether unit p is infectious or not, and $\beta(t)$ is transmission rate at time t between two hexagons presenting the highest density of wild boar in the study region.

The relative susceptibility and infectivity ϕ_i was used to reflect the different disease dynamics depending on wild boar favourability of a hexagon. It adjusts the baseline susceptibility and infectivity of a susceptible hexagon *i*. This scaling factor indicates how much less susceptible or infectious a hexagon is compared to the wild boar-favourable hexagon in the study region. The susceptibility and infectivity of each hexagon were formulated using a step function associated with wild boar favourability of that hexagon. This formulation was under the assumption that the wild boar-favourable units had a higher susceptibility and infectability (Eq.(2))

$$\phi_i = \begin{cases} 1 & \text{if i is a wild boar} - \text{favorable hexagon} \\ \phi_{min} & \text{if i is a wild boar} - \text{unfavorable hexagon} \end{cases} \quad (2)$$

where ϕ_{min} stands for the relative susceptibility and infectivity parameter, and was constrained between 0 and 1.

The probability of ASF infection for susceptible unit *i* at time *t* was calculated as shown in Eq.(3).

$$Infection_i(t) = 1 - e^{-\lambda_i(t)\Delta t} \quad (3)$$

Probability of detection was formulated as constant fixed value. Constant probability of detection indicates a uniform detection probability across the entire study region and period.

Probabilities of other transitions between states such as I_{UD} to I_D, I_D to S, and I_U to S in a time interval Δt were calculated as descrbied in Eq.(4), Eq.(5) and Eq.(6).

$$\text{Prob}_i(I_{UD} \rightarrow I_D) = 1 - e^{-1_{I_{UD,i}} \cdot \delta \Delta t} \quad (4)$$

$$\text{Prob}_i(I_D \rightarrow I_S) = 1 - e^{-1_{I_{D,i}} \cdot \eta \Delta t} \quad (5)$$

$$\text{Prob}_i(I_U \rightarrow I_S) = 1 - e^{-1_{I_{U,i}} \cdot \gamma \Delta t} \quad (6)$$

where $1_{I_{UD,i}}$ is indicator for whether unit i is in I_{UD} state, δ is the detection rate, $1_{I_{D,i}}$ is indicator for whether unit i is in I_D state, η is the sanitation rate, $1_{I_{U,i}}$ is indicator for whether unit i is in I_U state, γ is the recovery rate.

Three rate parameters were fixed for these transitions above, including detection rate δ, sanitation rate η, and recovery rate γ. From the reconstructed ASF dynamics, the detection rate δ at the hexagon level was calculated as the inverse of detection delay, defined as the duration between the dates of hexagon infection and its first detection. The sanitation rate η was calculated as the inverse of sanitation period, defined as the duration between the dates of hexagon detection and the last detection ending the infectious period. The calculated mean of the detection and sanitation periods from surveillance data were assumed to be 4.66 and 5.88 weeks, respectively. Thus, detection rate and sanitation rate were defined as $1/4.66$, and $1/5.88$, respectively. To define the recovery rate γ for undetected (but infected) hexagons γ, undetected hexagons were assumed more likely to have very few infected carcasses. Consequently, a scenario where an infectious hexagon had only one individual wild boar infected with ASF virus was considered, and this case does not occur any secondary infections, resulting in no detection in a hexagon by limited surveillance activities. This ASF-positive wild boar carcass (and therefore the infected but undetected hexagon in which it is contained) was assumed to remain infectious from the time of infection until it is skeletonized. According to the previous experimental study (Cho et al., 2021), which examined wild boar decomposition in a Korean environmental context, a wild boar carcass was skeletonized in 75 days (≈10.7 weeks) from the time of death on average. To account for the entire recovery period from infection to skeletonization, this period was assumed to be an additional 3 weeks. Therefore, the recovery period was calculated as 13.7 weeks. Thus, recovery rate γ was defined as $1/13.7 \approx 0.073$.

The epidemic simulation was initialized using nine hexagons corresponding to each distinct index cases that established independent wild boar-mediated spatiotemporal clusters suspectedly due to human-mediated viral translocation events, or potential continuous introductions from the North Korean border, as identified in a previous study (Lim et al., 2024).

Model fitting

An Approximate Bayesian Computation (ABC) was used, coupled with adaptive population Monte Carlo algorithm (APMC) to explore the posterior distribution of the parameters in the model (Toni et al., 2009; Lenormand et al., 2013).

To make summary statistics reflecting the spatiotemporal ASF dynamic as accurately as possible, the study region was partitioned into sixteen areas. Summary statistics included 1) the overall number of newly infected hexagons per quarter, 2) the number of newly infected

hexagons per quarter per area, 3) the overall number of newly infected hexagons per week, 4) the average distances between detected hexagons within areas and 5) the average of the forest coverage and of elevation amongst detected units per quarter for the entire study region. In total, 299 summary statistics were used to estimate model parameters.

Since all parameters to be estimated were assumed to be bounded by 0 and 1, non-informative prior distributions of the parameters for each formulation were set with uniform distribution ranging from 0 to 1. For APMC-ABC, the *EasyABC* packge was used in R software (Jabot et al., 2013).

Model selection and goodness-of-fit

From fitted model, the goodness-of-fit of the model was inspected by simulating the ASF epidemics using 500 parameter sets derived from the estimated posterior distributions. Temporal trajectories and spatial risks of detected and infected units were generated. Spatial risks were calculated as the number of simulations each unit was detected or infected at least once across the study period. To assess performance of the fitted model to reproduce the detected units, the area under the receiver operating characteristic (ROC) curve was calculated using the spatial risks for the detected units.

RESULTS

The posterior distributions for the estimated parameters are summarised in Table 1. The transmission rate between units of high wild boar abundance was estimated as 0.07 hexagon^{-1}·week^{-1} (95% Credible Interval: 0.05 – 0.10). This means that, on average, an infected wild boar-favourable hexagon transmits the virus to one neighbouring wild boar-favourable susceptible hexagon, out of total of six hexagons, every 2.38 week. Probability of detection was estimated as 0.27 (95% CrI: 0.05 – 0.52) suggesting that only one quarter of the infected units were detected and sanitized. It was found that susceptibility and infectivity of wild boar-unfavourable hexagons was only 0.19 (95% CrI: 0.01 – 0.54) that of wild boar-favourable hexagons. The simulated temporal trajectories and estimated spatial risk were illustrated in Fig. 3 and Fig. 4. The area under the ROC curve for the final model was 0.88.

Table 1. Estimates of model parameters for the best-fitting model.

Parameter	Median	95% credible interval
Transmission rate	0.07	0.05 – 0.10
Detectability	0.27	0.05 – 0.52
Relative Susceptibility/Infectability	0.19	0.01 – 0.54

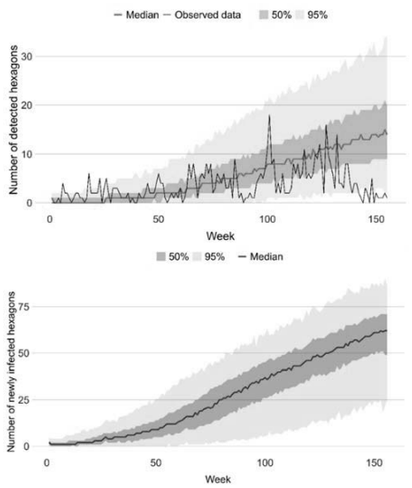

Figure 3. Simulated temporal trajectories of the infected (top) and detected hexagons (bottom).

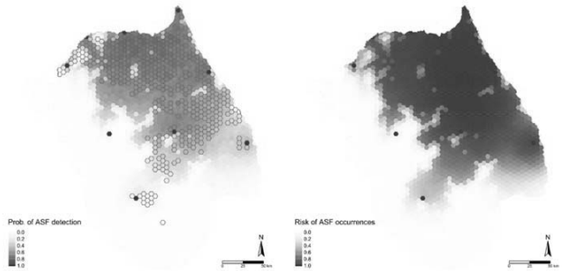

Figure 4. Spatial distribution of the probability of detecting ASF-infected hexagons (left) and of infection (right). The risk was calculated as the number of simulations that a unit is detected or infected at least once across the study period among 500 simulations.

DISCUSSION

Various models have been developed to study ASF transmission in wild boar and domestic pigs (Hayes et al., 2021). However, the unique geographical and epidemiological context of South Korea necessitated the integration of specific features to better understand the ongoing epidemic since 2019, as discussed in previous studies (Lim et al., 2021a; Lim et al., 2021b; Lim et al., 2023). Based on the model by Hayes et al. (2024), a spatiotemporal stochastic grid-based compartment model was built to explore the roles of wild boar habitat while reflecting

the limited sensitivity of surveillance by fitting nationwide surveillance data reported from October 2019 to September 2022. The epidemic data suggest that an infected wild boar-favourable hexagon transmits the virus to one neighbouring wild boar-favourable susceptible hexagon every 2.38 week. Only one quarter of the infectious units have been detected and that elevation did not impact detectability. However, it was also showed that wild boar density, as approximated by forest coverage, had a substantial impact on ASF dynamics in South Korea, as wild boar-unfavourable hexagons (i.e. with less than 80% forest coverage) were associated with a five-fold reduction of their susceptibility and infectivity as compared to wild boar-favourable hexagons.

The transmission rate was estimated as 0.07 hexagon^{-1}·week^{-1}, indicating that an infected wild boar-favourable hexagon will transmit the virus to one neighbouring wild boar-favourable hexagon every 2.38 weeks on average. In contrast, in the Romanian context from which this model was stemmed, estimated the transmission rate as 0.25 week^{-1} (Hayes et al., 2024). This can be interpreted an infected wild boar-favourable hexagon transmit the virus to one neighbouring wild boar-favourable hexagon in about 4 weeks. The higher transmission rate observed in this study could be attributed to the fact that the wild boar-favourable hexagons were defined using a forest coverage threshold of 80%, which likely indicates a higher wild boar abundance compared to the wild boar-favourable hexagons in the Romanian study, which were defined with a threshold of 15%. This higher abundance could make these areas more susceptible and infectious. However, the difference in transmission rates is more likely a result of the distinct environmental contexts in each country. In the Romanian study, the study region focused on the early phase of the epidemic, where ASF did not significantly spread to the Carpathian Mountains (Andraud et al., 2021), which are expected to have a high distribution of wild boar (Hayes et al., 2024). Additionally, the wild boar-favourable hexagons classified under their threshold made up only 14% of the total hexagons (197 out of 1,376) (Hayes et al., 2024). In contrast, this study's region had 64% classified as wild boar-favourable hexagons, despite using a higher threshold of 80%. This indicates that more than half of the study area is highly suitable for wild boar, even with stricter threshold. A previous study for spatial distribution of the Korean wild boar showed that wild boar can be distributed across entire country, particularly along the mountain ranges, except for some urbanized regions (Kim and Pak, 2020). Thus, these environmental contexts provide higher quality wild boar habitat and support higher density of wild boar compared to the Romania and the other European contexts, as suggested by the predicted wild boar spatial density in a previous study (Lewis et al., 2017).

Additionally, the high elevation and resulting low human accessibility in these areas limit the implementation of control measures, which create an environment more conducive to the spread of the disease.

It was estimated that the Korean nationwide surveillance system detected only a quarter of the infected units. Given that a statistical model using 3 km^2 hexagons as epidemiological unit estimated the sensitivity of surveillance at 0.49 (95% CrI: 0.41 – 0.57) from October 2019 to January 2020, and at 0.73 (95% CrI: 0.66 – 0.81) from January 2020 to February 2020 (Lim et al., 2021b), it was expected that the detectability in the presented mechanistic model would be higher than 0.50. This might be because of the temporal variations of the sensitivity of the surveillance. During the study period analysed in Lim et al. (2021b), ASF in wild boar was predominantly concentrated in the northern region, characterized by lower elevations compared to the mountainous ranges. Additionally, it was the early phase of the epidemic, during which control measures were likely more rigorously implemented. However, the surveillance data used in this study covered three years of the epidemic, from October 2019 to

September 2022, a period during which ASF increasingly spread into mountainous regions with limited human accessibility. This challenging landscape may have hindered surveillance activities and consequently decreased the sensitivity of surveillance.

This study found that wild boar-favourable units were five times more susceptible and infectious than the wild boar-unfavourable units were. Wild boar has been known to have preferences among several types of forests (Kim et al., 2019). Moreover, other factors, such as elevation, slope, and micro-topology, also affect the feeding ground and abundance. Thus, the model incorporating these factors can elaborate and shed light on the different relative susceptibility and infectivity parameters.

Since the first wild boar case in October 2019 around the border with North Korea, over 4,100 cases have been reported as of August 2024, with the wavefront reaching the southern region of South Korea. Although the Korean authorities have implemented control measures to prevent ASF from spreading, this wavefront is still moving southward. The developed model, which reflects the sensitivity of surveillance and wild boar suitability, has helped to unravel the true dynamics of ASF as distinct from the apparent dynamics, showing that wild boar suitability drove the ASF dynamics in South Korea. The study findings are expected to contribute to the design efficient control measures, and the model can be further developed to enhance these measures and finally to eradicate the ASF in South Korea.

ACKNOWLEDGEMENTS

We extend our gratitude to the INRAE MIGALE bioinformatics facility (MIGALE, INRAE, 2020, doi: 10.15454/1.5572390655343293E12) for generously providing the necessary computing resources.

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PREVALENCE AND FARM FACTORS ASSOCIATED WITH THE OCCURRENCE OF *CAMPYLOBACTER* AND FLUOROQUINOLONE RESISTANT *CAMPYLOBACTER* ON UK BROILER FARMS

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SUMMARY

This study estimated the prevalence of fluoroquinolone resistant *Campylobacter* (FQRC) in UK broiler flocks and identified biosecurity and hygiene factors associated with an increased risk of *Campylobacter* and FQRC presence. Commercial broiler farms from four major broiler companies were sampled, and laboratory results analysed alongside questionnaire data on farm characteristics. *Campylobacter* was detected on 34/121 (28%) farms, and FQRC on 25/121 (21%) farms. Rodent presence in empty buildings and mechanical ventilation with roof chimney extraction were found to be risk factors for *Campylobacter* and FQRC. The study findings indicate best practices for controlling *Campylobacter* and FQRC and can be utilised to support optimisation of biosecurity across the broiler industry.

INTRODUCTION

Campylobacter is the most frequently reported cause of foodborne illness in the EU (ECDC, 2022). In 2022, there were 96.3 laboratory reports of *Campylobacter* in humans per 100,000 population in the United Kingdom (UK Health Security Agency, 2024).

Most *Campylobacter* spp. are intrinsically resistant to several antibiotics, including first- and second-generation penicillins, as well as to antibiotics with specific activity against Gram-positive bacteria such as vancomycin (Portes et al., 2023). Antimicrobial resistance (AMR) in *Campylobacter* can arise from spontaneous mutations, the acquisition of genes by horizontal gene transfer, or both (El Dessouky et al., 2022). Macrolides and fluoroquinolones are the main antibiotics used to treat *Campylobacter* infections, with the World Health Organisation (WHO) classifying fluoroquinolone (FQ) antibiotics as critically important (WHO, 2019). Food-producing sectors have been identified as one of the predominant environments where AMR proliferates, with high antimicrobial usage a contributing factor (EFSA Panel on Biological Hazards (BIOHAZ) et al., 2021). In the UK, concerted efforts to reduce antimicrobial use in food-producing animals have been in place over the past decade (Veterinary Medicines Directorate, 2023). In 2021, the total quantity of antibiotics sold for animal treatment in the UK was the lowest recorded to date, and the reduction in usage of Highest Priority Critically Important Antibiotics (HP-CIAs) such as fluoroquinolones was even greater, representing only

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0.4% of total antibiotic sales for animals (Veterinary Medicines Directorate, 2022). Despite this, fluroquinolone resistant (FQR) bacteria have been increasing in broiler flocks across Europe, and beyond, in recent years (European Medicines Agency, 2023). Given that availability of effective antimicrobials is essential for the treatment and prevention of infections in humans and animals, a rise in AMR in *Campylobacter* poses a threat to antibiotic efficacy. Thus, further study of AMR spread is required to elucidate strategies to tackle this issue.

In January 2019, the UK Government published its vision to "contain and control" AMR by 2040, supported by a five-year National Action Plan (NAP) (HM Government UK, 2019). The NAP was designed to begin fulfilling the Government's vision with a One Health approach between 2019 and 2024 (HM Government UK, 2019). In line with the NAP, this study aimed to evaluate the prevalence and risk factors for both *Campylobacter* and FQR *Campylobacter* on poultry farms and was developed to better understand control methods for FQR *Campylobacter* in broiler flocks.

MATERIALS AND METHODS

Farm selection

Farms were selected to be representative of the broiler farming industry in the UK. The population of interest included all flocks of *Gallus gallus* reared for the purpose of producing meat for human consumption as recorded within the GB Poultry register. Only conventional commercial holdings (>1,000 poultry) were considered, selected from four major broiler companies. The number of poultry farms invited to participate in the study was based on a population of 49,642 farms in GB (Joanna Lawes, personal communication) using the following assumptions: the assumed true prevalence of *Campylobacter* spp. was 56% (UK-VARSS, 2021), assuming 90% test sensitivity and 99.9% test specificity, with 95% confidence and 10% acceptable level of error. The sample size to estimate herd prevalence of *Campylobacter* was determined to be 121 farms, and this population was used to determine the proportion with fluoroquinolone resistance.

Data collection

Data on farm characteristics, management procedures, animal health, and antibiotic usage was collected from participating farms using a standardised questionnaire that was distributed to farms at the same time as sample submission. Questionnaire responses were submitted via post or online to the Qualtrics™ platform.

Sampling

The sampling procedure for bootswab sample collection was based on the UK National Control Programme (NCP) for *Salmonella* in chickens (Department of Food, Environment and Rural Affairs, 2008). Sampling was conducted between February and May 2023. Farms were sampled when birds were around 28 days of age and before thinning. Two houses from each farm were sampled: House A was the closest house to the farm entrance and House B the furthest. Two samples were collected by the farmer from each house, one inside the house in the bird area and one outside in front of the house (with a total of four samples obtained per farm). Samples were shipped in temperature-controlled (2-8°C) boxes for analysis.

Isolation and identification of *Campylobacter*

For each pair of bootswabs, a combined suspension was prepared using 6 ml of each bootswab suspension. For direct culture, 10µl of the combined suspension was streaked onto an MCCDA plate and an MCCDA plate with ciprofloxacin 4 µg/ml (MCCDA-CIP). For enrichment culture, 10 ml of combined suspension was transferred to 90 ml of Exeter broth. *C. jejuni* s16/0416 (ciprofloxacin susceptible), *C. jejuni* s16/0021 (ciprofloxacin resistant) and *C. coli* NCTC 12143 were used as growth controls. Agar plates and culture broths were incubated at 41.5 ± 1 °C in a microaerobic atmosphere for 44 ± 4 hours. Agar plates were then read, and 10 µl of enrichment broths were plated on MCCDA and MCCDA-CIP. These plates were also read after 44 ± 4 hours incubation.

Two suspected *Campylobacter* colonies from each MCCDA and MCCDA-CIP plate from direct culture and after enrichment were selected for sub-culture, if available. These colonies were sub-cultured on SBA 7% supplemented with actidione 0.1% and incubated at 41.5 ± 1 °C in a microaerobic atmosphere for 18-48 hours. At least one colony per sample was stored on glycerol broth 10% at -80 °C, giving priority to suspected ciprofloxacin resistant colonies isolated from plates containing ciprofloxacin.

Identification of presumptive *Campylobacter* isolates was performed using the Extended Direct Transfer (EDT) method as described in Randall et al. (2015). A broth microdilution method using the Sensititre system (Trek Diagnostics, Thermofisher) and based on ISO 20776-1:2006, determined the MIC for all six antimicrobials that are specified in 2013/652/EU (2013/652EC, 2013), using the EUCAMP3 Sensititre plate (Trek). This contained doubling dilutions of the antimicrobials; chloramphenicol, ciprofloxacin, ertapenem, erythromycin, gentamicin, and tetracycline. As specified in 2013/652/EU decision, EUCAST ECOFFs were used to interpret the MIC for each antimicrobial.

Statistical analysis

Questionnaire responses from participating farms were entered into a spreadsheet and data checked to remove typos, shorten variable names, and to correct formatting. Coding of explanatory and outcome variables was conducted using Stata version 15. For continuous variables, new variables were generated and data binned into categories, with the median used for any missing values. Analyses were conducted at farm-level, with the detection of any positive samples indicating a positive farm. For each exposure variable, the number and percentage of farms positive for the outcome variables *Campylobacter* and fluroquinolone resistant *Campylobacter* (FQRC) was tabulated. Categorical data were compared using a chi-squared test, and continuous data with normal distribution were analysed using a t-test.

To identify risk factors, a univariable screening step was first used to evaluate associations between outcome and exposure variables of interest. Variables included in this step were those indicating a potential association ($p<0.25$) with the outcome variables when tabulated. Variables were excluded from univariable analyses if 100% of observations were either positive or negative when tabulated.

Multivariable analysis using a forwards-stepwise logistic command model was conducted to further evaluate associations between outcome and exposures whilst accounting for the presence of other variables in the model. All variables with $p>0.25$ in univariable analyses were excluded before the multivariable stage. In subsequent steps, the significant variable ($p<0.05$) that most improved the model fit (lowest Akaike Information Criterion, AIC) was included

until a step was reached where no further variables were significant or could improve the model fit. Collinearity within the final model was assessed using a correlation matrix of model parameter estimates (estat vce). If a variable was found to have collinearity with another variable of 0.75 or greater, only the variable with the lowest p-value which had the largest effect on model fit was retained in the final model.

RESULTS

Study population

In total, 121 farms were sampled during the study. Participating farms were associated with four major broiler companies: Company 1 (n=28, 23.0% of study total), Company 2 (n=7, 5.8%), Company 3 (n=53, 43.8%), and Company 4 (n=33, 27.3%).

Detection of *Campylobacter* on participating farms

Campylobacter was detected on 34 out of 121 farms (28.1%). Most participating farms associated with Company 2 (71.4%) and 36.4% of farms associated with Company 4 tested positive for *Campylobacter* (Table 1). *Campylobacter* was detected at 17.9% and 22.6% of participating farms associated with Company 1 and Company 3, respectively and there was a significant difference in the proportion of positive farms between all companies (p=0.02).

Table 1. Number and percentage of *Campylobacter*-positive farms (n=34), broken down by company (p=0.02).

Company	Number and percentage of Campylobacter-positive farms (out of total farms)
Company 1	5/28 (17.9%)
Company 2	5/7 (71.4%)
Company 3	12/53 (22.6%)
Company 4	12/33 (36.4%)

Univariable logistic regression analysis of *Campylobacter*-positive farms

All exposure variables were tabulated against the binary *Campylobacter* risk status at the farm-level. A total of 94 variables indicated a potential association (p<0.25) with *Campylobacter*. Seven variables were excluded from further analyses as all of the farms for the answer of interest were either all positive or negative and so this could not be statistically analysed. Ten variables were dropped from further analyses as all responders have the same answer, resulting in 77 variables being taken forward for univariable logistic regression. These variables included those related to housing structure, presence of animals close to the site, thinning and depopulation practices, water treatment, rodent control, and waste disposal.

Multivariable model for *Campylobacter* detection on participating farms

The final multivariable logistic regression model is provided in Table 2. None of the variables in the final model indicated covariance hence there were no collinearity concerns.

The results showed that obtaining chicks from fewer hatcheries per crop (between 1-3 hatcheries) had a protective effect, with these farms 81% less likely to test positive for *Campylobacter* (p=0.007). In contrast, farms that obtained chicks from five or more hatcheries per crop were five times more likely to test positive for *Campylobacter*, although this was not found to be statistically significant (p=0.183). Of the 10 farms that reported using beetle spray as an insecticide, six tested positive for *Campylobacter*. Farms that used beetle spray were found to be 12.9 times more likely to test positive for *Campylobacter*, compared to farms that did not use beetle spray, and this was a statistically significant result (p=0.002). Most farms that reported presence of rodents inside empty houses tested positive for *Campylobacter* (7 out of 8 farms, 87.5%). There was a strong association between *Campylobacter* detection and presence of rodents inside empty houses (p=0.002), with these farms 37.2 times more likely to test positive for *Campylobacter*. Farms that reported having a combination of mechanical ventilation and roof chimney extract in houses were shown to be nearly five times more likely to test positive for *Campylobacter*, and this was found to be statistically significant (p=0.023).

Table 2. Multivariable logistic regression model for *Campylobacter*-positive farms (n=34).

Variable	Number of *Campylobacter*-positive farms (of total)	Percentage (%) of farms positive for *Campylobacter* (of total)	OR (95% CI)	p-value
Hatcheries per crop				
1 – 3	12 / 76	15.8%	0.19 (0.06 – 0.64)	0.007
3 - 5	16 / 38	42.1%	1.00 (ref)	-
5+	6 / 7	85.7%	5.14 (0.46 – 57.1)	0.183
Any beetle spray				
No	28 / 111	25.2%	1.00 (ref)	0.002
Yes	6 / 10	60.0%	12.9 (2.47 – 67.4)	
Rodents inside empty houses				
No	27 / 86	23.9%	1.00 (ref)	0.002
Yes	7 / 8	87.5%	37.2 (3.81 – 363.8)	
Mechanical and roof chimney[a]				
Other	21 / 102	20.6%	1.00 (ref)	0.023
Mechanical & roof chimney	13 / 19	68.4%	4.67 (1.24 – 17.6)	

[a] Farms with both mechanical ventilation and roof chimney extract are recorded as “Mechanical & roof chimney”. Farms with any other ventilation type including no ventilation type are recorded as “Other”.

Detection of fluoroquinolone resistant *Campylobacter* (FQRC) on participating farms

FQRC was detected on 25 farms, comprising 20.7% of all participating farms. In total, 73.5% of all *Campylobacter*-positive samples were FQR. Variation in detection of FQR *Campylobacter* between all companies was statistically significant (p=0.03) (Table 3).

Table 3. Number and percentage of fluoroquinolone-resistant *Campylobacter*-positive (FQRC) farms (n=25), broken down by company (p=0.03).

Company	Number of FQRC-positive farms (% of total farms owned by company)	Number of FQRC-positive farms (% of total *Campylobacter*-positive farms owned by company)
Company 1	2/28 (7.1%)	2/5 (40.0%)
Company 2	4/7 (57.1%)	4/5 (80.0%)
Company 3	12/53 (22.6%)	12/12 (100.0%)
Company 4	7/33 (21.2%)	7/12 (58.3%)

Univariable logistic regression analysis of FQR *Campylobacter*-positive farms

All exposure variables were tabulated against the binary FQRC risk status at the farm-level. A total of 63 variables indicated a potential association ($p<0.25$) with FQRC. Nine variables were excluded from further analyses as all the farms for the answer of interest were either all positive or negative and so this could not be statistically analysed. Three variables were dropped from further analyses as all responders had the same answer, resulting in 51 variables being taken forward for univariable logistic regression analyses. These variables included those related to housing structure, presence of animals close to the site, thinning and depopulation practices, to water treatment, rodent control, and waste disposal.

Multivariable logistic regression model for FQR *Campylobacter* on participating farms

The final multivariable logistic regression model is provided in Table 4. None of the variables in the final model indicated covariance, hence there were no collinearity concerns. The absence of rodents inside the anteroom and empty houses was shown to be protective (Table 4). Farms without rodents in anteroom were 73% less likely to test positive for FQRC, and farms without rodents in empty houses had 23.4 times decreased odds of FQRC. This was found to be statistically significant in both instances ($p=0.039$ for anteroom, $p=0.004$ for empty houses). Farms with mechanical ventilation in addition to a roof chimney extract had 15.6 times increased odds of FQRC compared to farms with any other ventilation type ($p<0.001$). Farms with between 1 and 3 poor chick quality deliveries per year were found to be 86% less likely to test positive for FQRC, compared to farms with zero poor chick quality deliveries ($p=0.012$). However, the likelihood of FQRC reduced as the number of poor chick quality deliveries per year increased, with farms with 5+ poor chick quality deliveries 58% less likely to test positive for FQRC. It should be noted that this finding was not statistically significant ($p=0.492$).

Table 4. Multivariable logistic regression model for fluoroquinolone resistant *Campylobacter* (FQRC) (n=25).

Variable	Number of FQRC-positive farms	Percentage (%) of FQRC-positive farms	OR (95% CI)	p-value
Presence of rodents inside anteroom				
No	10	11.5%	0.27 (0.08 – 0.93)	0.039
Yes	15	44.1%	1.00 (ref)	
Mechanical and roof chimney extract[a]				
Other	15	14.7%	1.00 (ref)	<0.001
Yes	10	52.6%	15.60 (3.99 – 61.3)	
Presence of rodents inside empty houses				
No	19	16.8%	1.00 (ref)	0.004
Yes	6	75.0%	23.40 (2.81 – 195.0)	
Number of chick deliveries subject to poor chick quality per year[b]				
0	13	35.1%	1.00 (ref)	
1-3	6	14.6%	0.14 (0.03 – 0.65)	0.012
3-5	5	14.3%	0.36 (0.08 – 1.55)	0.171
5+	1	12.5%	0.42 (0.03 – 5.03)	0.492

[a] Farms with both mechanical ventilation and roof chimney extract are recorded as "Yes". Farms with any other ventilation type including no ventilation type are recorded as "Other".

[b] Examples of poor chick quality included: chicks that were not clean, that did not stand firmly or walk well, that were not alert or active, or that presented mortality rates above 0.7% during the first 7 days of life.

DISCUSSION

The study determined that the prevalence of *Campylobacter* on participating farms was 28.1%, with *Campylobacter* detected on 34 out of 121 farms sampled during the study. The prevalence of fluoroquinolone resistant *Campylobacter* (FQRC) was determined as 21%, with FQRC detected on 25 out of 121 farms sampled. This is a lower reported prevalence of *Campylobacter* compared to a previous UK study, whereby *Campylobacter* spp. were isolated at least once from 67.9% of 137 farms (Ellis-Iversen et al*.,* 2009). However, in the Ellis-Iversen et al. (2009) study, monthly results varied between 13% and 43% which is more in line with the findings from this study. It should be noted that Ellis-Iversen et al. (2009) repeatedly sampled batches from the farms, making any direct comparison of results difficult.

This study has identified several biosecurity and hygiene risk factors associated with *Campylobacter* and FQRC on UK broiler farms. The presence of rodents inside empty houses was shown to be a significant risk factor for both *Campylobacter* and FQRC. The absence of rodents inside the anteroom was also shown to be protective against FQRC. This could be indicative of larger biosecurity issues on implicated farms or may suggest that rodents are important in transmitting *Campylobacter*. Presence of rodents in poultry houses has previously been identified as a risk factor for *Campylobacter* on broiler farms in Spain and Northern Ireland (Torralbo et al., 2014, McDowell et al., 2008).

It was identified that obtaining chicks from fewer hatcheries per crop (between 1 and 3 hatcheries) had a protective association. One possible explanation could be that obtaining

chicks from fewer sources could mean less chance of importing *Campylobacter* onto farms when birds enter, although this requires further exploration.

The study found that a combination of mechanical ventilation and roof chimney extract in houses was also a risk factor for both *Campylobacter* and FQRC. It is possible that this combination is more difficult to clean resulting in contamination and spillover of infection from old to new batches of birds.

An association between farms that reported poor chick quality and fluoroquinolone resistant *Campylobacter* detection, was identified during the study. In this context, poor chick quality was defined by the companies as chicks that were not clean, those that did not stand firmly or walk well, that were not alert or active, or that presented mortality rates above 0.7% during the first 7 days of life. These results could have been impacted by the fact that each company measured poor chick quality slightly differently. It is also possible that the recorded responses were biased, with companies with a higher prevalence of chick quality issues potentially more likely to have noted this.

The application of beetle spray as an insecticide was noted as a potential risk factor for *Campylobacter* in the study. Of the 10 farms that reported using beetle spray as an insecticide, six tested positive for *Campylobacter*. This could indicate that affected farms have a larger issue with insects within poultry houses, although this requires further exploration.

It should be acknowledged that the study population was not entirely representative of the GB broiler industry, as it only included farms associated with four major broiler-producing companies. The study was further limited by potential recall bias given that farmers or company staff were required to complete a detailed questionnaire.

In conclusion, the study has identified biosecurity issues and potential control methods for reducing the spread of *Campylobacter* and FQR *Campylobacter* in broiler flocks. The study has highlighted that further action is required to improve the resilience of the poultry sector to the spread of AMR pathogens.

ACKNOWLEDGEMENTS

This work was undertaken at Animal and Plant Health Agency as part of a wider GB Government-funded research project (OZ0514) supported by Defra, Welsh and Scottish Governments. We wish to gratefully acknowledge the British Poultry Council, poultry industry partners, vet practices, and participating farms for their support and involvement in the study.

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ANTIMICROBIAL USAGE AND RESISTANCE (AMU & AMR)

REACHING THE LIMIT: MOVING BEYOND ANTIMICROBIAL USE INDICATORS

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SUMMARY

Trends in antimicrobial use for conventional broiler chicken production in France were analysed based on a dataset of 193,526 sales for 2,120 farms and 33,831 flocks for 2015-2023, including 21,218 antibiotic prescriptions. The percentage of flocks prescribed antibiotics dropped from 65% in 2013 to 20% in 2023, plateauing in 2020-2023 where it oscillated between 13% and 23%, and observed a reduction in the use of critical antibiotics. A multiple correspondence analysis and hierarchical clustering on principal components of 1940 antibiotic prescriptions and associated clinical data for 2021-22 produced five clusters of antibiotic prescribing patterns, each characterised by a specific clinical context. These findings indicate that antimicrobial prescribing patterns can be characterised by a combination of clinical indicators in addition to traditional AMU metrics. This calls for a shift to a more sustainable approach to monitoring antimicrobial stewardship, using integrated indicators which consider both AMU and the clinical context.

INTRODUCTION

The global challenge of antimicrobial resistance (AMR) is been increasingly recognised with AMR positioned as a threat to global health and development. Over the last 20 years, the international and domestic policy responses to this challenge have predominantly focused on reducing antimicrobial use (AMU) in both humans and animals. In France, significant progress has been made in reducing AMU in livestock species within the framework of the Ecoantibio plans (the French national plans for the reduction of risks of AMR in veterinary medicine) run successively over the periods 2012-2016, and 2017-2022 (MAAF, 2016a; Anses, 2023). Over the period 2011-2022 there was an overall reduction of 52% in the exposure to antibiotics among livestock species, with the most marked reductions seen in the pig and poultry sectors (67 and 72% respectively) (Anses, 2023).

However, while AMU surveillance and associated interventions can be considered a success in terms of having reduced AMU, these trends raise two key questions – firstly, whether the reduction in AMU comes at the cost of compromised animal health and welfare, and secondly, whether further AMU reductions may be feasible or sustainable (Ducrot et al., 2022). Both these challenges lend themselves to being addressed by a different, more holistic approach – looking beyond quantitative reductions in AMU to instead place AMU in the context of the clinical settings where decisions on AMU are made. This broader approach ties in with recent

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efforts to improve antimicrobial stewardship, a concept that encompasses the idea that "appropriate" or "prudent" AMU will depend on the context (Dyar et al., 2017).

One approach to incorporating context into AMU surveillance is to interpret AMU alongside other indicators – either as composite indicators or as additional information to inform AMU analysis (Schrag et al., 2022). Other research has demonstrated the potential of multivariate factorial analyses to investigate the influence of contextual factors on AMU decision making by farmers, showing that farm health and management characteristics can be associated with different AMU practices (Best et al., 2024). Such contextualised information necessarily implies the use of farm-level prescription or sales data, which can provide added value compared to national level sales data (Sanders et al., 2020). Many studies using these approaches have taken farmers as their starting point for analysis. However, it is also important to study AMU from the perspective of veterinarians, as veterinarians play an important role in the management of antibiotics and as guardians of public health in relation to AMR (Fortané, 2019). It has been shown that veterinarians' prescribing practices are influenced by contextual factors such as unwritten rules and empirical clinical experience (Moya et al., 2024), indicating the importance of studying prescription-level data in relation to veterinarians alongside clinical information.

Our objective was to use farm-level prescription data to confirm at a local level the general trend of AMU reduction in France, and subsequently characterise antimicrobial prescribing patterns in their clinical context during a time period where AMU was already at a low level.

MATERIALS AND METHODS

The dataset consisted of veterinary sales for conventional broiler chicken production from a large network of veterinary practices in France for the period 2013-2023, from the four main producer organisations. A deliberate choice was made to analyse data from conventional broilers so as to have a population with homogenous production standards in a country where there is otherwise considerable diversity in broiler production. For the subsequent multidimensional analyses, data on antimicrobial prescriptions were then extracted for the years 2021-2022, identified as the time period where AMU had plateaued. These data were combined with clinical data for the same population and time period consisting of results from bacteriologies, necropsies, and antimicrobial sensitivity testing (AST).

For the descriptive of overall trends, two types of information were analysed– the number of prescriptions per year, and the percentage of flocks in the dataset that were prescribed antibiotics per year, over the period 2013-2023.

Multidimensional analyses were then used to explore antibiotic prescriptions in the context of clinical information (necropsies, bacteriology, and AST) using the FactoMineR (Husson et al., 2024) and factoextra packages (Kassambara and Mundt, 2020). A multiple correspondence analysis (MCA) was performed followed by a hierarchical clustering on principal components (HCPC). Multiple correspondence analysis is a form of exploratory dimensional multivariate analysis that can be used to explore the relationship between categorical variables. It can be used to identify similarities between individuals on the basis of selected variables – in this study, the individuals (the epidemiological unit of interest) were antibiotic prescriptions. The full list of variables used for the MCA is presented in Table 1.

Table 1. List of variables used in the multiple correspondence analysis.

Variable type	Variable name	Variable description
Active	Age at prescription	A categorical variable with seven age groups (<0, 0-1, 2-7, 8-14, 15-21, 22-28, and >28 days of age).
Active	Active ingredient	The active ingredient of the prescribed antibiotic (from the summary of product characteristics.) Where two active ingredients were present, a composite name was generated.
Active	Necropsy/ bacteriology	Whether necropsy or bacteriology was performed (yes/no).
Active	AST [a]	Whether AST was performed (yes/no).
Supplementary	Syndrome [b]	The principal syndrome recorded in the necropsy dataset.
Supplementary	Lesion [b]	The principal lesion recorded in the necropsy dataset.
Supplementary	Diagnosis [b]	The principal diagnosis recorded in the necropsy dataset.
Supplementary	Pathogen [b]	The main pathogen isolated with bacteriology or for AST (separate variables were created for each).
Supplementary	Farmer	The anonymised identification code for a given farm.
Supplementary	Flock	The anonymised identification code for a given flock.

[a] AST = antimicrobial sensitivity testing
[b] For these variables, "NA" was used to indicate that testing was not performed for a given prescription (rather than missing information).

The patterns of prescribing identified in the MCA were confirmed and elaborated using the HCPC. An HCPC is a means of constructing a hierarchical tree to visualise the variability and similarities between individuals (between antibiotic prescriptions). Hierarchical clustering was performed using Ward's criterion and consolidated using the k-means method. The dendrogram (hierarchical tree) was cut based on the decrease of inertia and the identification of clusters which could be described in a coherent way. The clusters were characterised with regards to three values – the test statistic (v-test), Cla/Mod, and Mod/Cla. The test statistic (v-test) is a way of converting the p-value into a quantile of a normal distribution – if the absolute value of the v-test is greater than 1.96, then the category is considered to either over-represent (if positive) or under-represent (if negative) the cluster. Cla/Mod and Mod/Cla are ways of describing the frequency of a category in a cluster – across the whole dataset and across the cluster respectively. For example, if the variable was "active ingredient", and the category "amoxicillin", Cla/Mod would indicate the percentage of all amoxicillin prescriptions in the given cluster, while Mod/Cla would indicate the percentage of prescriptions within a given cluster which are for amoxicillin.

RESULTS

Descriptive analysis of trends in antibiotic prescriptions from 2013-2023

The dataset consisted of 193,526 sales of antibiotics, antiparasitics, disinfectants, feed supplements, farm visits, veterinary acts, and laboratory tests for 2015-2023, for 2,120 farms and 33,831 flocks with a median farm size of 21,230 chickens. It included 21,218 antibiotic prescriptions. The absolute number of antibiotic prescriptions decreased during the period 2013-2023, from a maximum of 3250 antibiotic prescriptions in 2015 to 460 antibiotic prescriptions in 2022, before rising again to 758 antibiotic prescriptions in 2023 (Fig. 1).

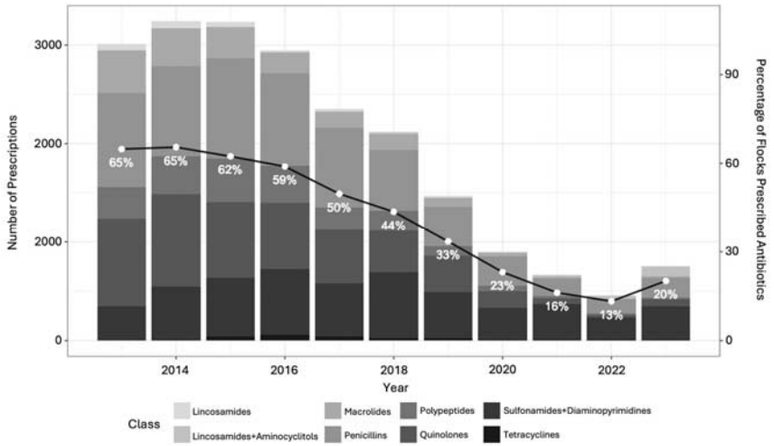

Figure 1. Number of antibiotic prescriptions per antibiotic class per year (top eight antibiotic classes corresponding to 85% of prescriptions) and evolution of the proportion of flocks prescribed antibiotics from 2013-2023.

In particular, the greatest decreases were in the classes quinolones, polymyxins, and macrolides (corresponding to the active ingredients enrofloxacin, colistin, and tylosin respectively). The two classes accounting for the highest proportion of prescriptions since 2018 are penicillins (amoxicillin) and trimethoprim-sulfonamide combinations. The main exception to the overall reduction in antibiotic prescriptions is the rise in the use of lincomycin and spectinomycin combinations (the class lincosamides and aminocyclitols). The decrease in the absolute number of prescriptions was also reflected in a decrease in the proportion of flocks that received antibiotic prescriptions, which dropped from 65% in 2013 to 20% in 2023, plateauing in 2020-2023 where the proportion of flocks prescribed antibiotics oscillated between 13% and 23%. The period 2021-22 was identified as the target for the subsequent multidimensional analysis.

Characterisation of antibiotic prescriptions in the context of clinical information from 2021-2022

Multidimensional analyses were then used to explore antibiotic prescriptions in relation to contextual clinical information (necropsies, bacteriologies, and AST) for 1940 prescriptions for 325 farms and 956 flocks in 2021-2022. The first dimension of the MCA was mainly influenced by the variables related to diagnostic testing ("necropsy/bacteriology" and "AST", both with 0.78 contribution), while the second dimension was mainly influenced by the variables "age at prescription" and "active ingredient" (both with 0.89 contribution). In the subsequent HCPC, the dendrogram (hierarchical tree) was cut to establish five clusters (Fig. 2). These five clusters of antibiotic prescriptions were analysed in relation to the first two dimensions of the MCA (Fig. 3).

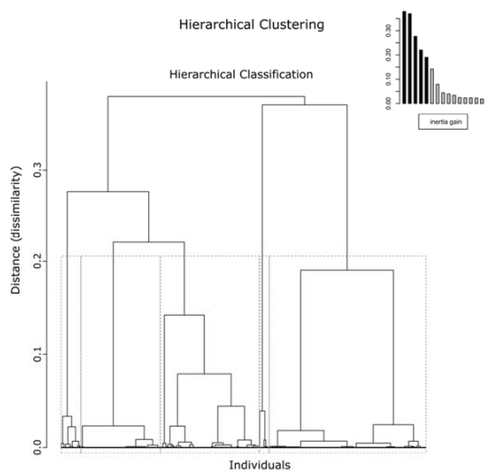

Figure 2. Dendrogram from the hierarchical clustering analysis with indication of the cut-point used to establish five clusters.

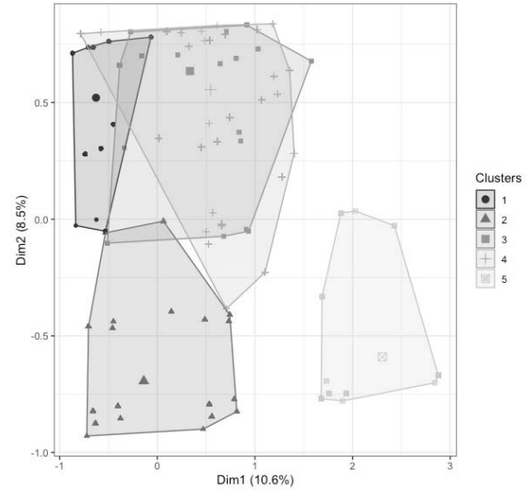

Figure 3. Visualisation of the five clusters identified using hierarchical clustering on principal components (HCPC) on the two first dimensions of the multiple correspondence analysis (MCA).

The number and proportion of prescriptions in each cluster are presented in Table 2, along with the number of farms and flocks associated with these prescriptions.

Table 2. Summary of clusters from the hierarchical clustering on principal components (HCPC), on the two first dimensions of the multiple correspondence analysis (MCA).

Cluster	Characterisation	Antibiotic prescriptions	Farms	Flocks
1	Mid-production locomotor problems	490 (25.26%)	70 (21.54%)	97 (10.15%)
2	Early mortality	808 (41.65%)	222 (68.31%)	465 (48.64%)
3	Late-age disease	105 (5.41%)	42 (12.92%)	60 (6.28%)
4	No diagnostics	485 (25%)	169 (52%)	375 (39.23%)
5	Treatment before or at chick arrival	52 (2.68%)	23 (7.08%)	50 (5.23%)

The main characteristics of these clusters are described below, alongside a summary of the categories with the highest v-test values for each cluster, and the associated values for Cla/Mod and Mod/Cla in Tables 3, 4, 5, 6, and 7.

Cluster 1 "mid-production locomotor problems", 490 (25.26%) of antibiotic prescriptions (Table 3)**:** This cluster was characterised by prescriptions at 15-21 days of age, locomotor syndromes, and arthritic lesions (approximately 60-80% of prescriptions in these categories were found in this cluster), as well as identification of *Enterococcus cecorum* on bacteriology and for AST. All prescriptions in this cluster (100%) had necropsy or bacteriology performed, and almost all had AST results (96.53%). This related to almost 50% of all antibiotic prescriptions for 2021-22 where necropsy, bacteriology, or AST was performed. The majority of prescriptions in this cluster were for amoxicillin (57.96%).

Table 3. The top 10 over-represented variable categories for cluster 1, ranked by v-test.

Variable	Category	Cla/Mod[a]	Mod/Cla[b]	v.test
Necropsy/bacteriology	Yes (necropsy or bacteriology performed)	44.34	100.00	25.84
AST	Yes (AST performed)	46.69	96.53	24.91
Age prescription	15-21 days	65.63	73.27	24.87
Pathogen (bacteriology)	*Enterococcus cecorum*	80.13	51.02	22.76
Pathogen (AST)	*Enterococcus cecorum*	84.05	39.80	20.38
Syndrome (necropsy)	Locomotor	78.89	43.47	20.31
Lesion (necropsy)	Arthritis	85.87	32.24	18.39
Active ingredient	Amoxicillin	50.62	57.96	15.86
Lesion (necropsy)	Airsacculitis/pericarditis	62.69	34.29	14.13
Syndrome (necropsy)	Respiratory	61.24	32.24	13.29

[a] Cla/Mod = The percentage of prescriptions in the dataset with the category of interest in the cluster.
[b] Mod/Cla = The percentage of prescriptions in the cluster with the category of interest.

Cluster 2 "early mortality", 808 (41.65%) of antibiotic prescriptions (Table 4): This cluster was highly characterised by antibiotic prescriptions at 2-7 days of age, early mortality, colibacillosis, and hepato/splenomegaly. The pathogens identified most commonly in this cluster were *Escherichia coli* (52.1%) and to a lesser extent *Enterococcus faecalis* (7.43%, result not shown). The majority of the prescriptions in this cluster (53.22%) were for sulfadiazine + trimethoprim, followed by a lower number of prescriptions for enrofloxacin (15.1%) – almost all of the prescriptions of these antibiotics across the 2021-22 dataset belonged in this cluster (97.95% and 93.85% respectively).

Table 4. The top 10 over-represented variable categories for cluster 2, ranked by v-test

Variable	Category	Cla/Mod[a]	Mod/Cla[b]	v.test
Age prescription	02-07 days	96.63	85.15	Inf
Active ingredient	Sulfadiazine + trimethoprim	97.95	53.22	29.14
Syndrome (necropsy)	Early mortality	96.88	46.16	26.11
Lesion (necropsy)	Hepato/splenomegaly	99.43	21.41	17.65
Diagnosis (necropsy)	Colibacillosis	73.57	33.42	13.77
Active ingredient	Enrofloxacin	93.85	15.10	13.07
Lesion (necropsy)	Omphalitis	95.58	13.37	12.64
Pathogen (AST)	*Escherichia coli*	58.31	52.10	11.45
Diagnosis (necropsy)	Omphalitis	94.05	9.78	10.40
Lesion (necropsy)	Abnormal vitellus	100.00	7.30	10.04

[a] Cla/Mod = The percentage of prescriptions in the dataset with the category of interest in the cluster.
[b] Mod/Cla = The percentage of prescriptions in the cluster with the category of interest.

Cluster 3 "late-age disease", 105 (5.41%) of antibiotic prescriptions (Table 5): This cluster is most strongly characterised by prescriptions for late production (>28 days) broilers for cellulitis with colistin, with almost 100% of all prescriptions relating to each of these categories present in the cluster. Some prescriptions in this cluster were also associated with *Enterococcus* spp. infections. The presence of several individual farmers suggests that this is perhaps representative of a few individual farmers prescribing in this way.

Table 5. The top 10 over-represented variable categories for cluster 3, ranked by v-test

Variable	Category	Cla/Mod[a]	Mod/Cla[b]	v.test
Active ingredient	Colistin	94.81	69.52	21.38
Age prescription	>28 days	98.15	50.48	18.05
Diagnosis (necropsy)	Cellulitis	100.00	13.33	8.86
Flock	Flock ID number	32.43	22.86	7.50
Flock	Flock ID number	100.00	9.52	7.39
Farmer	Farmer ID number	30.77	22.86	7.32
Farmer	Farmer ID number	90.91	9.52	7.07
Farmer	Farmer ID number	55.00	10.48	6.14
Farmer	Farmer ID number	83.33	4.76	4.71
Diagnosis (necropsy)	Infection with Enterococcus	20.00	15.24	4.66

[a] Cla/Mod = The percentage of prescriptions in the dataset with the category of interest in the cluster.
[b] Mod/Cla = The percentage of prescriptions in the cluster with the category of interest.

Cluster 5 "treatment at or before chick arrival", 52 (2.68%) of antibiotic prescriptions (Table 6): The cluster was highly characterised by prescriptions at less than 0 days of age (i.e. on or

before chick arrival on the farm) in the absence of diagnostic testing (indicated by the NA results). All prescriptions for lincomycin and spectinomycin combinations and for florfenicol in the 2021-22 dataset belonged to this cluster, with the vast majority (71.15%) of prescriptions in this cluster being the former.

Table 6. The top 10 over-represented variable categories for cluster 5 ranked by v-test

Variable	Category	Cla/Mod[a]	Mod/Cla[b]	v.test
Active ingredient	Lincomycin + spectinomycin	100.00	71.15	17.22
Age prescription	<0 days	100.00	69.23	16.94
Pathogen (bacteriology)	NA	6.27	100.00	9.24
Necropsy/bacteriology	No (necropsy or bacteriology not performed)	6.23	100.00	9.20
Syndrome (necropsy)	NA	6.22	100.00	9.19
Lesion (necropsy)	NA	5.85	100.00	8.83
Pathogen (AST)	NA	5.61	100.00	8.58
AST	No (AST not performed)	5.61	100.00	8.58
Diagnosis (necropsy)	NA	5.01	100.00	7.85
Farmer	Farmer ID number	38.10	15.38	5.58

[a] Cla/Mod = The percentage of prescriptions in the dataset with the category of interest in the cluster.
[b] Mod/Cla = The percentage of prescriptions in the cluster with the category of interest.

Cluster 4 "no diagnostics", 485 (25%) of antibiotic prescriptions (**Fehler! Verweisquelle konnte nicht gefunden werden.**): Three age groups of 8-14, 15-21, and 22-28 days were overrepresented in this cluster, although 22-28 days was the most overrepresented, suggesting that this cluster regroups prescriptions across these different age groups that were not associated with any testing.

Table 7. The top 10 over-represented variable categories for cluster 4, ranked by v-test

Variable	Category	Cla/Mod[a]	Mod/Cla[b]	v.test
Pathogen (AST)	NA	49.30	94.23	25.27
AST	No (AST not performed)	49.30	94.23	25.27
Necropsy/bacteriology	No (necropsy or bacteriology not performed)	48.74	83.92	21.44
Syndrome (necropsy)	NA	48.68	83.92	21.41
Pathogen (bacteriology)	NA	48.37	82.68	20.90
Lesion (necropsy)	NA	46.57	85.36	20.80
Diagnosis (necropsy)	NA	40.85	87.42	18.17
Age prescription	22-28 days	66.77	43.92	17.57
Active ingredient	Amoxicillin	44.92	51.96	12.52
Active ingredient	Ampicillin	100.00	6.80	9.39

[a] Cla/Mod = The percentage of prescriptions in the dataset with the category of interest in the cluster.
[b] Mod/Cla = The percentage of prescriptions in the cluster with the category of interest.

This cluster was overwhelmingly characterised by an absence of diagnostic testing, with 83.92% of prescriptions in this cluster not being associated with either necropsy or bacteriology, and 94.23% not associated with AST. Most of the prescriptions in this cluster were for amoxicillin (51.96%), although the cluster was also characterised by lower numbers of prescriptions of ampicillin, oxytetracycline, tylosin, and lincomycin.

DISCUSSION

Our findings demonstrate that over the last 10 years, in our study population of conventional broilers in the West of France, AMU has decreased overall, both in terms of absolute number of antibiotic prescriptions and in the proportion of flocks prescribed antibiotics. This is consistent with the findings of national-level reports of AMU across France in recent years (Anses, 2023).

These trends can be contextualised in relation to the landscape of AMU initiatives in France and in Europe more broadly. In France, the results can be understood in the context of the Ecoantibio plans in 2012-2016 and in 2017-2022 which consist of regulation, research actions, and veterinary-led initiatives (MAAF, 2016a). The decrease in quinolone (enrofloxacin) prescriptions are consistent with a decree listing fluoroquinolones and 3rd and 4th generation cephalosporins as critically important antibiotic agents, banning their use for preventive purposes, and requiring an AST before their prescription (MAAF, 2016a, MAFF, 2016b). There was also a decrease in colistin despite this antibiotic not being subject to specific regulation in France, suggesting that both regulatory and bottom-up approaches to reducing AMU can be effective. In relation to the European context, when considering the European Medicine Agency (EMA)'s categorisation of antibiotics (EMA, 2019), it can be observed that across the 2013-2023 period the classes for which there was the most demonstrable decrease were the category B ("restrict") classes of quinolones and polymyxins (i.e. enrofloxacin and colistin), and the category C ("caution") class macrolides (i.e. tylosin). The two antibiotics accounting for the highest proportion of prescriptions since 2018 are penicillins (amoxicillin) and trimethoprim-sulfonamide combinations, which are category D ("prudence" i.e. first-line antibiotics).

The exploratory multidimensional approach allowed us to structure the data into five clusters representing different patterns of antibiotic prescriptions in relation to different clinical situations. This approach allowed us to describe antibiotic prescribing in terms of clinical syndromes associated with different age groups. The clusters included three clinical situations where further reductions in AMU might be challenging, as antibiotics were prescribed in association with diagnosed disease:

- Cluster 1 related to use of amoxicillin to treat mid-production (15-21 days of age) locomotor problems associated with *Enterococcus* infections (mostly *Enterococcus cecorum*). This cluster, and the overall increase in amoxicillin use seen over the 2013-2023 period, should be viewed in the context of the increasing prevalence of *Enterococcus cecorum* infections in conventional broiler production in France over the last 15 years (Souillard et al., 2022). It would be useful to identify if the farms associated with this cluster have recurrent *Enterococcus cecorum* infections.
- Cluster 2 concerned treatment of early mortality at 2-7 days of age associated with *Escherichia coli* and *Enterococcus faecalis* infections. Treatment was mostly with sulfadiazine and trimethoprim in combination, or more rarely with enrofloxacin. It would be worthwhile to further investigate this in relation to resistance data (to see if the use of enrofloxacin correlates with sensitivity on AST) and to identify if there are particular patterns (such as seasonality) to the early mortality.
- Cluster 3 related to treatment of cellulitis or *Enterococcus* infection at a late age (>28 days) with colistin. The use of colistin in this age group can likely be attributed to the fact that it is one of few antibiotic agents available with a short (one day) withholding

period, bearing in mind that most birds will be slaughtered for consumption at 35 days of age (Anses, 2024).

There are two clusters which indicate situations that could be targets for further AMU interventions, because these were associated with an absence of diagnostic testing

- Cluster 5 concerned prescriptions of lincomycin and spectinomycin combinations at less than zero days of age i.e. before or at the moment of chick arrival on farm. This could be interpreted as prophylactic or metaphylactic treatment. It may also be associated with the rise in *E. cecorum* infections as this combination has been reported to be used as preventive treatment for *E. cecorum* in other European countries (Schreier et al., 2022). It would be useful to confirm whether this concerns farms previously affected by *E. cecorum* infection.
- Cluster 4 related principally to prescriptions in the absence of clinical testing. This suggests that these prescriptions are made within the remit of the annual farm health plan (which allows prescriptions of antibiotics without clinical testing if certain conditions are met) (Briand et al., 2015). It was overrepresented by three different age groups across the overall range of 8-28 days, suggesting that this is perhaps collecting together a range of different prescriptions where clinical testing is not performed. For this cluster, it would be useful to identify whether the farms concerned face specific barriers to performing diagnostic tests such as poor access to veterinary clinics.

This study had a number of limitations, notably that context was only considered with regards to clinical test results. As a result, some of the clusters are challenging to interpret in the absence of further information. There are also potentially broader contextual factors that influenced AMU over the study period that were not accounted for. For example, the recent epidemics of highly pathogenic avian influenza in France which led to significant culling of the poultry population (Lambert et al., 2022), and could have led to a decrease in the number of antibiotic prescriptions due to the reduced population. However, as the decrease in prescriptions was mirrored by a decrease in the proportion of flocks treated, this does seem to be indicative of a real shift in antibiotic prescribing practices over time.

These findings highlight the challenges with trying to interpret AMU data – or indeed any quantitative data – without information on context. They demonstrate that antimicrobial prescribing patterns can be characterized by a combination of indicators in addition to traditional AMU metrics, related to age at treatment, clinical signs, lesions at necropsy, and isolated bacteria. They also demonstrate that significant and sustained reductions in AMU have been made in recent years, to a point where further reductions may not be sustainable – evidenced by both the stagnation in reductions in overall AMU trends from 2013-2023, and the finding that the majority of antibiotic prescriptions are associated with a diagnosis of bacterial disease. This work is hoped to contribute to a shift in the way we think about AMU such that not just how much, but the why and how of AMU become of greater consideration.

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IDENTIFYING BARRIERS AND STRATEGIES TO IMPROVE ANTIMICROBIAL STEWARDSHIP IN PIG PRODUCTION USING A BOTTOM-UP APPROACH AMONG DANISH PIG VETERINARIANS

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SUMMARY

A survey was distributed to Danish pig veterinarians (n = 73), achieving a 58% response rate. Questions covered antimicrobial use (AMU) practices, changes over time, and drivers and barriers to reducing AMU. Based on the answers and a systems thinking approach, a diagram was created to depict interrelationships between elements of AMU practices. Results show that veterinarians adjusted their AMU practices over time, mainly due to legislative restrictions. While veterinarians and pig producers are willing to adopt AMU reduction strategies, practical implementation depends mostly on financial constraints. Key barriers include the lack of effective alternatives to antimicrobials and costs associated with improved vaccination schemes, management, housing and feeding strategies. The recent implementation of EU legislation to reduce AMU unintentionally increased it. This study highlights the need for feasible and effective AMU reduction strategies backed by robust scientific evidence while considering farming realities and the interconnectedness of drivers and barriers.

INTRODUCTION

The rise of antimicrobial resistance (AMR) presents a critical threat to global health, causing a substantial burden on healthcare systems worldwide. Consequently, a strong global movement towards reducing antimicrobial use (AMU) has emerged and gained strength over time (Aarestrup et al., 2008; Andersen et al., 2023). Antimicrobials (AMs) are essential in treating bacterial infections in humans and animals, and AMR genes can spread among the interconnected systems of the environment, animals and humans. To mitigate this risk, a coordinated One Health approach is needed, and responsible AMU across sectors is critical to ensure the availability of effective AM treatments for both humans and animals, now and in the future (Aenishaenslin et al., 2021).

Effective and feasible strategies to reduce AMU in food-producing animals should follow robust scientific evidence and aim to prevent and combat infections while minimising

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production losses. Essentially, AM treatments should be available for sick animals to ensure animal health and welfare (Carmo et al., 2018). During the last decade, Europe has seen a general decline in AM sales, with several countries reporting significant reductions (European Medicines Agency, 2023). We argue that understanding the drivers of AMU and barriers to its reduction - such as infection prevalence, clinical needs, livestock management practices, national policies, the financial system, and the underlying structure of livestock sectors - is critical for developing targeted and effective strategies to achieve further reductions without jeopardising animal health and welfare.

In Denmark, the pig industry continuously adopts comprehensive AM stewardship practices (Danish Agriculture & Food Council, 2019). The sector has implemented a voluntary ban on 3rd and 4th generation cephalosporin usage since 2010 (FAO & Denmark Ministry of Environment and Food – Danish Veterinary and Food Administration, 2019), which was immediately complied with, leading to no use of these AM substances in Danish pig production (DANMAP, 2024). This ban is voluntary only by definition because most pig producers are part of the Danish Product Standard and are obliged to comply with industry-set bans (Alban et al., 2024). At the national level, a legislative measure called the Yellow Card Initiative was first implemented in 2010. This initiative establishes national maximum permissible limits for AMU by pigs' age groups (i.e., sows including piglets, weaners, and finishers) applicable to all pig farms, to prevent excessive use (DANMAP, 2024). When calculating the limits, different weighting factors are assigned to reflect the negative impact of an AM class on AMR development and/or their importance in treating human bacterial diseases (DANMAP, 2024). Currently, a weighing factor of 10 is ascribed to 3rd and 4th generation cephalosporins, colistin and fluoroquinolones. Subsequently, these AMs are not used, as they highly affect a farm's calculated AMU. In addition, a weighing factor of 1.5 is ascribed to tetracyclines, while other AM classes have a weight factor of 1. In practice, AMU in animals is registered in the Danish veterinary database for medicinal products (VetStat*) and monitored at the farm level, allowing the Danish authorities to take legal action against pig producers with an unacceptably high AMU in any age group. In line with this, the Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP) follows AMR trends in livestock, food and humans.

Recent changes in the European Union (EU) legislation have influenced prescription practices among Danish pig veterinarians (DANMAP, 2024). Notably, the ban on veterinary medicinal products (VMPs) containing zinc oxide for food-producing animals, implemented in June 2022, has been a major change (Standing Committee on Veterinary Medicinal Products, 2017). Additionally, two EU regulations on veterinary medicines and medicated feed, EU Regulation 2019/6 and EU Regulation 2019/4, respectively, were implemented in January 2022. These regulations include provisions limiting the prescribed quantities of VMPs to the amount required for specific treatments or therapies and restricting the prescription of AMs for metaphylaxis or prophylaxis. Furthermore, they require that VMPs be used according to their marketing authorisations, as specified in the Summary of Product Characteristics (SPC). This implies that the recommended dosage and duration specified in the SPC must be followed when treating with a VMP.

Veterinarians are key actors in achieving feasible AMU reductions due to their education, authority to prescribe AMs, and their role in advising farmers on animal health, welfare and management. This role might be even stronger in Denmark than elsewhere because a pig

* https://vetstat.fvst.dk/vetstat/

producer must have a herd health agreement with a veterinarian involving regular visits to the herd, in practice resulting in four, six or 12 visits per year depending on the production type (FAO & Denmark Ministry of Environment and Food – Danish Veterinary and Food Administration, 2019). As a result, Danish pig veterinarians have firsthand contact with well-functioning to suboptimal pig herds. In this way, pig veterinarians transfer knowledge and experience between pig producers, making them invaluable for informing policy decisions to optimise AMU. Despite extensive work, there are still significant knowledge gaps regarding the specific factors influencing veterinarians' prescribing behaviours both in Denmark and elsewhere (Carmo et al., 2018).

AMR is often presented as a wicked problem implying that it is difficult to solve because of incomplete, contradictory, and changing requirements that are often difficult to recognise (Rickard et al., 2023). To address a wicked problem, systems thinking might offer a solution as it provides a holistic approach to unravelling the existing complexities by mapping out the interconnections between various elements within systems, in this case, the Danish pig production system. Systems thinking uses feedback loops and system maps to depict the relationships between components (Monat & Gannon, 2015), such as farm management practices, preventative interventions and legislative requirements. Moreover, systems thinking moves from looking at individual events to the patterns, underlying structure and mental models behind them (Monat & Gannon, 2015).

Our study aims to investigate the current stewardship strategies regarding AMU in Danish pig farming, focusing on the role of veterinarians in this process. Specifically, we were interested in hearing the views of Danish pig veterinarians regarding the effectiveness of AM stewardship strategies to understand the interrelatedness of different drivers and barriers. Understanding this is essential to refine future AMU policies and hereby enhancing AM stewardship practices, ultimately contributing to animal health and welfare as well as the global effort against AMR.

MATERIALS AND METHODS

Survey design and data collection

The survey was designed to capture AM prescription practices, barriers and preventive strategies influencing successful AMU reductions over time among Danish pig veterinarians. It consisted of five sections, each with a subset of questions: 1) respondent characteristics, 2) practices in AM prescription, 3) changes over time and associated reasons/barriers, 4) successful preventive practices allowing AMU reductions, and 5) the feasibility and barriers of further reducing AMU. The survey included binary, multiple choice, ranking, slider rating scales (0 - 100%), and open-ended questions. The responses were collected anonymously through EUSurvey version v1.5.3.1 (DG DIGIT, 2024). All private pig veterinary practices in Denmark (n = 6) were identified. Veterinarians' email and telephone contact information were publicly available, except for one practice, where only email contacts were available. Initial contact was made with each practice manager to explain the purpose and importance of the survey. After, an email was sent to all veterinarians with a brief explanation and a link to access the online survey. Shortly after, a follow-up telephone call was made to provide help if needed. The survey was launched on 11 December 2023 and was open to responses until 15 May 2024.

Data analysis

Quantitative data were analysed using descriptive statistics to summarise the distribution of responses. Data from binary, multiple-choice, ranking, and slider questions were analysed to identify possible relationships with working experience and educational level. First, each respondent was classified based on their years of work experience: those with less than or equal to the median years of work experience of all respondents were categorised as having worked for a short period while those with years of work experience greater than the median were categorised as having worked for a long period. Additionally, respondents were classified according to their educational level as either veterinarians or veterinarians with a specialisation. Fisher's exact test was used for binary and multiple-choice questions, accounting for presence of one or more expected cell counts below 5. The Mann-Whitney test was applied to questions where slider rating scales were used. The alpha level for significance was 0.05. To allow easy comparison among the different options in the ranking questions, we assigned weights to each rank using a reverse scoring method, where the most common (highest rank) was assigned the highest score and the least common (lowest rank) the lowest. For each option, we multiplied each weighted rank by the proportion of respondents and summed these values to get the total score for each option.

Qualitative data from open-ended questions were analysed using Grounded Theory methodology. This method involves a systematic process of gathering and analysing data to condense and group themes and concepts based on the collected data (Glasser & Strauss, 1967), thus facilitating the examination of complex behaviours and practices associated with AMU without predefined categories. The analysis began with open coding, where each response was examined, and initial codes were assigned to meaningful text segments. These codes were then grouped into categories based on similarities and patterns identified across the responses.

All data, comprising responses from the pig veterinarians, were utilized to construct a diagram using a systems thinking approach to illustrate the interrelationships between different internal and external elements related to AMU practices among Danish pig veterinarians. Internal elements were defined as on-farm practices (e.g., feeding, management, housing), whereas external elements included legislative requirements, economic factors, and availability of resources. The basis for this analysis was an individual pig, which could shift through different stages: 1) pig is born, 2) pig is growing, 3) pig falls ill, 4) pig is treated, and 5) pig recovers or dies. The AM stewardship system map was generated iteratively. First, the mapping of the system was conducted manually (i.e., in paper) using the different answers provided by the pig veterinarians. Here, the interconnectedness of the various elements became clear. This included causal loops divided into those resulting in positive or negative feedback. Next, the manually crafted system map was transferred to an online tool (BioRender*).

RESULTS

A total of 75 Danish pig veterinarians were contacted and invited to complete our questionnaire, of which 73 were eligible. We received 42 responses resulting in a 57.5% response rate. The median years of working experience in a pig veterinary practice was 15 (range: 1 – 38 years). Regarding specific education on porcine health management, 35.7% had

* https://www.biorender.com/

undertaken a specific continued education (n = 15) and 61.9% indicated they had not obtained such education (n = 26).

Practices in antimicrobial prescription

Thirty-five veterinarians reported altering their AM prescription behaviours throughout their careers (87.3%, n = 35). Of these, 85.7% indicated these changes were primarily driven by legislative requirements. Figure 1 shows the distribution of producers willing to implement voluntary strategies to reduce AMU (75.0%, range: 10% - 100%), the perceived usefulness of VetStat as a tool to explain compliance with the Yellow Card rules (90.0%, range: 0% - 100%), and the proportion of pig producers using VetStat as a benchmarking tool to compare their AMU with other pig producers (27.5%, range: 0% - 100%). We found no statistically significant differences (P > 0.05) based on veterinarians' work experience or education level.

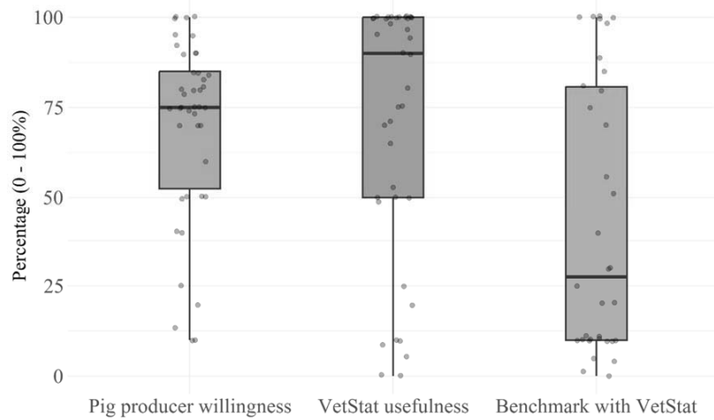

Figure 1. Distribution of Danish pig veterinarians' views regarding pig producers' willingness to implement changes to reduce antimicrobial usage (AMU; n = 42), the usefulness of VetStat as a tool to advise producers on compliance with the Yellow Card requirements (n = 39) and the use of VetStat as a benchmarking tool for AMU (n = 39), 2024.

Practices in antimicrobial prescription

Most pig veterinarians (57.1%, n = 24) reported no changes in prescription patterns to treat sows throughout their careers. Additionally, 64.3% (n = 27) indicated that an AMU reduction over time was primarily attributed to a decrease in disease prevalence (81.5%, n = 22).

In response to the ban on the therapeutic use of zinc oxide, 95.2% (n = 40) of the respondents reported that this new regulation resulted in an AMU increase during weaning. Most identified aminoglycosides as the AM class associated with this increase (n = 31). In addition to an AMU increase, a few veterinarians (n = 7) mentioned other strategies that helped cope with this ban. Namely, feeding strategies using biochar (n = 3), feed acidification (n = 3), seaweed meal (n =

2), high fibre (n = 1), reduced calcium (n = 1), rolled oats (n = 1), corn starch (n = 1), probiotics (n = 1); as well as water acidification and vaccination with the veterinary medicinal Coliprotect^{TM} F4/F18 (Elanco GmbH; depending on the *E. coli* strain present). The two veterinarians who did not observe an AMU increase after the ban on zinc oxide reported implementing other successful strategies such as switching to higher-quality feed and competitive flora.

Most pig veterinarians (81.0%, n = 34) changed prescription patterns over time to treat gastro-intestinal disorders in finishers, with most stating they managed to switch administration route from peroral to parenteral in some cases (61.9%, n = 26). Veterinarians ranked *Actinobacillus pleuropneumoniae* (APP) as the respiratory pathogen leading to the highest AMU in Danish pig production. Throughout their careers, most veterinarians were able to reduce AMU to treat respiratory diseases (73.8%, n = 31), of which the majority attributed this reduction to a decrease in disease prevalence (n = 28). Moreover, most respondents changed the administration route from peroral to parenteral (85.7%, n = 36). Those unable to make this change (n = 6) indicated that the main barriers were time constraints, ineffective treatment outcomes, and producers' concerns regarding productivity.

Following the implementation of the weighting factor of 1.5 assigned to tetracyclines in the Yellow Card scheme introduced by the Danish authorities in 2017, 85.7% of the respondents stated they were unable to reduce the use of tetracyclines without an increase in macrolides or other AMs. Most veterinarians indicated that their inability to reduce tetracycline use without increasing the use of other AMs was due to the following reasons 1) diarrhoea in pigs requires AM treatment (38.1%, n = 16%), 2) the substitution of tetracyclines with other AMs is not fully effective (23.8%, n = 10%), 3) changes involving vaccines, better feed, or more indoor heating are expensive (4.8%, n = 2%), and 4) that the only reason to reduce the use of tetracyclines is to avoid getting a yellow card (4.8%, n = 2 %). Additionally, most pig veterinarians indicated that, after the attribution of a weighing factor of 10 to colistin, introduced by the Danish authorities in 2017, colistin was replaced by neomycin (n = 29) and other aminoglycosides (n = 30). A total of 81.0% of respondents (n = 34) indicated that EU Regulation EU 2019/6 and EU Regulation EU 2019/4 (SPC requirements), which entered into force in January 2022, led to an increase in AMU, while 19.1% stated that their AM prescription practices remained unchanged (n = 8). Almost all (97.6%, n = 41) respondents believed these regulations did not contribute to reducing AMU in the Danish pig sector.

Practices allowing the successful implementation of AMU reductions

To identify the most important strategies to reduce AMU, pig veterinarians ranked different options related to external and internal biosecurity, feeding and water quality practices (Fig.2) and management and housing conditions. Respondents ranked strict gilt acclimatisation protocols as the most important external biosecurity practice, while all-in/all-out practices in grower and finisher pigs ranked highest for internal biosecurity. New feeding strategies involving higher quality feed and ventilation systems providing optimal temperature and air quality were the highest-ranking practices concerning feed and water quality, and management and housing conditions, respectively.

Other strategies were also considered important, with most respondents reporting changes in their diagnostic practices over time (88.1%, n = 37) and many indicating improved Porcine Reproductive and Respiratory Syndrome virus (PRRSv) diagnostics (n = 27), increased antimicrobial sensitivity testing with definitive diagnostics (n = 26), and necropsies performed

by specialised laboratories (n = 21). The optimisation of the management of sick pigs and hospital pens was also practised by most veterinarians (78.6%, n = 33), as well as the implementation of clear guidelines for the euthanasia of runt piglets (81.0%, n = 34). Increased weight and the minimisation of mixing at weaning were also strategies used by the majority of pig veterinarians (81.0%, n = 34 and 92.9%, n = 39, respectively). Regarding feeding strategies, most veterinarians indicated that they commonly included feed acidification (n = 32), enzymes to enhance digestion (n = 27) and probiotics (n = 26). Lastly, 92.9% of the 42 Danish pig veterinarians reported changes in their vaccination schemes to achieve reductions in AMU (n = 39), with only three veterinarians replying negatively. Relevant changes referred by most veterinarians were vaccination against *Lawsonia intracellularis* (n = 30), *E. coli* (n = 20), Porcine Circovirus (PCV2, n =17) and APP (n = 9).

Feasibility for further reducing AMU in the Danish pig sector

When asked to provide three measures to reduce AMU that could be both feasible and cost-effective, we received 35 responses. Pig veterinarians mentioned the increased use of vaccines (n = 13), highlighting the necessity for better and more affordable choices. Others mentioned improved housing, management and all-in/all-out practices as key strategies (n = 12); the need for higher feed quality and generally improved nutrition (n = 10); and improved diagnostics, treatment and eradication protocols (n = 9). A few veterinarians mentioned the removal of the SPC requirement (n = 6), the reversal of the ban on therapeutic use of zinc oxide (n = 3), and the reduction of Yellow Card limits (n = 3), respectively. Finally, 31.7% of the respondents identified financial constraints as the highest barrier to improving AM stewardship, followed closely by the lack of effective alternatives to AMs (29.3%) and limitations of the pig housing system.

Systems thinking

By aggregating all data generated by the responses of 42 Danish pig veterinarians, we mapped the primary drivers leading to AMU, which we classified as internal elements of the system (Fig.3).

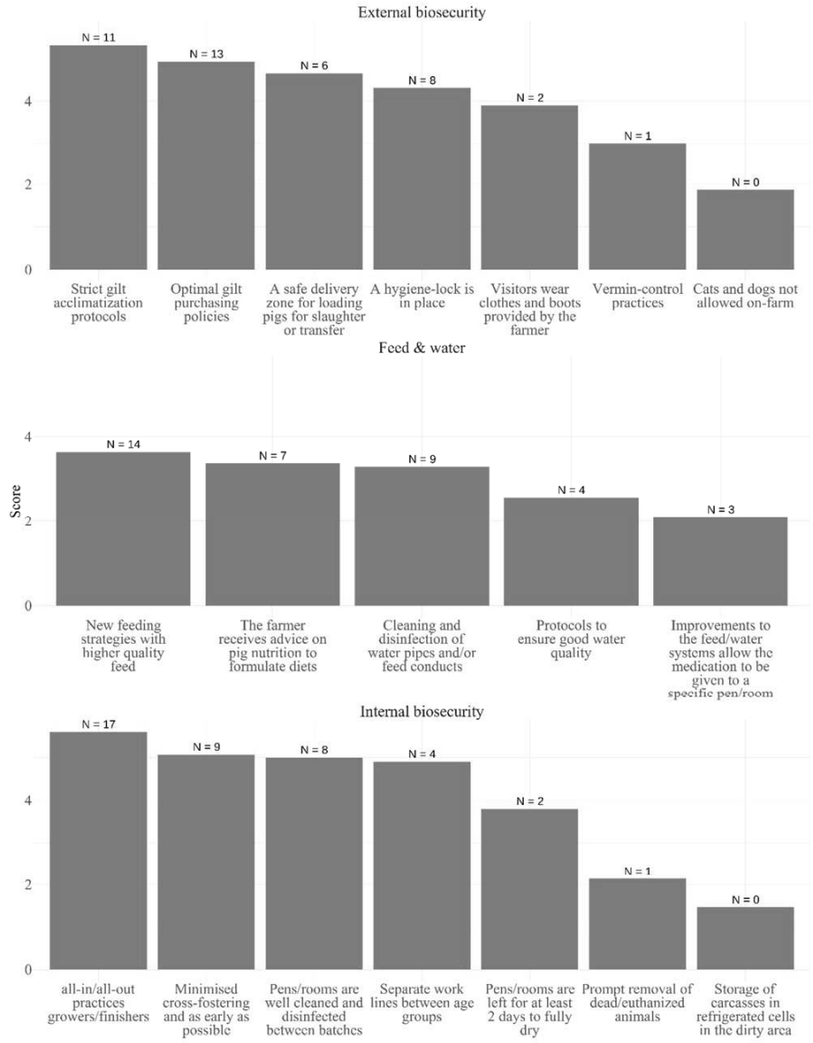

Figure 2. The rank of strategies regarding practices to reduce AMU according to Danish pig veterinarians, 2024. N: the number of veterinarians ranking each option in first place.

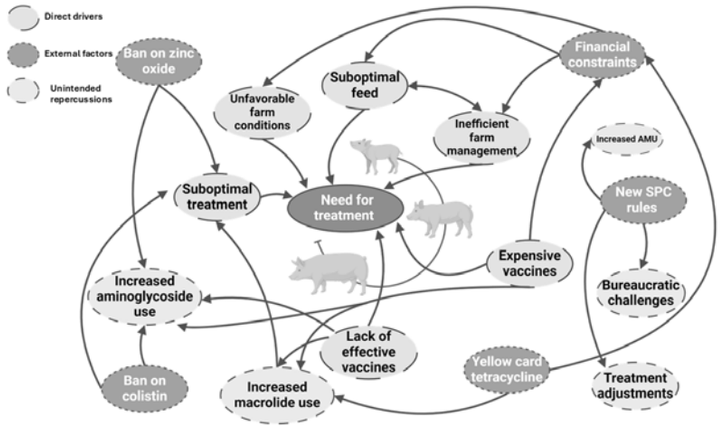

Figure 3. A systems thinking approach to illustrate views of Danish pig veterinarians regarding the direct drivers (internal factors) of antimicrobial use (AMU). External elements were identified as the most important barriers contributing to high AMU and resulting in unintended repercussions.

Furthermore, we identified several external components influencing the system. These included various legislative requirements and financial constraints, which Danish pig veterinarians indicated as the most important barriers impeding AMU reductions and negatively impacting the internal objects. These external components lead to several unintended repercussions (negative feedback loops).

DISCUSSION

We provide critical insights into the complexities of AMU within the Danish pig sector, emphasising the interplay between legislative requirements, on-farm practices, and the broader socio-economic context. Our findings underscore the importance of a systems thinking approach to understand and address the drivers and barriers to optimal antimicrobial stewardship, as well as the added value of receiving feedback from those working directly and daily on pig farms.

It is well established that AMU influences the level of AMR within a pig herd (Andersen et al., 2023). However, AMU in Danish pig production is also affected by internal and external factors capable of increasing or reducing disease prevalence in pigs (Fig.3). According to Danish veterinarians, the most influential factor in reducing AMU was the implementation of all-in/all-out practices in grower and finisher pigs. A change to an all-in/all-out production is only feasible if the movement of pigs within and between herds is minimised. Both external and internal biosecurity are essential to prevent disease spread (Filippitzi et al., 2018). At all stages, pigs are faced with constraints that impair their growth, however, due to the necessity to send uniform batches for slaughter, pig producers will mix pigs by size/weight to improve

profitability and avoid space and labour-intensive alternatives needed to avoid this practice (i.e., having buffer areas). These are barriers to implementing all-in/all-out.

Changes in feeding strategy and improved feed quality were also ranked high by Danish veterinarians. Several feeding strategies are known to effectively reduce diarrhoea in weaners and Eriksen et al. (2022) provide recommendations for herd health management of post-weaning diarrhoea, showing similar evidence as that mentioned by the Danish veterinarians in our study.

Our findings indicate that legislative requirements can substantially impact AMU practices at herd level. The Yellow Card initiative is recognised as a crucial strategy for regulating AMU in Denmark (DANMAP, 2024). However, the introduction of weighing factors for specific AMs, such as colistin and tetracyclines, has resulted in unintended consequences, including increased use of other AM classes such as aminoglycosides and macrolides (DANMAP, 2019, 2024). According to DANMAP (2024), cross-resistance between macrolides and tetracyclines and heightened gentamicin resistance stemming from increased apramycin use has been observed. This underscores that routine AMU in pig production can lead to a potentially problematic rise in AMR. The ban on the therapeutic use of zinc oxide has also increased AMU during weaning, with aminoglycosides being the primary substitute. This trend aligns with data reported in DANMAP (DANMAP, 2023, 2024). To ensure equitable conditions for all Danish pig producers, an easy way of lowering AMU at the national level could be to reduce the permit limits applied in the Yellow Card scheme – a suggestion mentioned by a few pig veterinarians. However, this might provide other unintended outcomes. Veterinarians highlight the financial constraints to improve vaccination schemes, management and housing conditions, with several veterinarians suggesting that government subsidies and different taxation schemes could positively impact the implementation of continuous and improved vaccination schemes.

The intention of the EU regulations on the SPC requirements was to reduce AMU. However, most Danish pig veterinarians indicated that these new regulations increased their AM prescriptions (DANMAP, 2024). When looking at AMU in 2023 this increase is not clear (DANMAP, 2024) however, Danish veterinarians highlight that outdated product summaries, failing access to important VMPs, and requirements for higher dosages and/or longer treatment periods are limiting their ability to adequately treat individual animals and herds (Melsen & Rindam in DANMAP, 2024). Alternatively interpreting the SPC information as a fixed set of rules, limits for dosage and treatment duration could be considered maximum values. Interestingly, a similar trend might not be seen in other countries, likely due to stewardship practices run in Denmark for more than 25 years (Moura et al., 2023).

Applying a systems thinking approach has allowed us to map out the interconnections between various elements within the Danish pig production system. This holistic perspective is essential for identifying leverage points where interventions can most effectively change AMU practices. By understanding the complex interplay of legislative, economic, and practical on-farm factors, more effective strategies to reduce AMU can be developed to combat AMR. The findings underscore the critical role of veterinarians and the need for a coordinated, systems-based approach to address this global “wicked” health challenge.

ACKNOWLEDGEMENTS

The pig veterinarians who participated in the interviews are acknowledged for their contributions. Frede Keller from DANVET is acknowledged for comments to the questionnaire during its development and for ensuring communication with the different pig veterinary practices in Denmark increasing the number of responses

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A FRAMEWORK TO UNDERSTAND RISK FACTORS OF ANTIMICROBIAL RESISTANCE IN AQUACULTURE SYSTEMS: A FOCUS ON VIETNAM

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SUMMARY

This study proposes a framework with four dimensions and variables influencing AMR emergence and spread in aquaculture: the micro-level, focusing on farm practices and species biology, the meso-level, examining environmental and value chain factors, the macro-level, addressing sector-wide production systems and trends, and the policy and governance landscape, including mitigation measures and enforcement. In Vietnam, priority systems such as pangasius, white-leg shrimp, black tiger shrimp, and tilapia production showed distinct profiles in intensification, water, waste, and input management, disease incidence, technology use, ecosystem impacts, value chains, and sector characteristics. Variables related to intensification and application of technology appeared to play an important role, and access to and uptake of AMR mitigation measures vary significantly between export-oriented and domestic market systems. These differences highlight the need for tailored interventions that consider each system's specific challenges and features to effectively address AMR.

INTRODUCTION

The vast development of aquaculture production systems worldwide, in particular in Asian countries, has been through rapid intensification not matched by appropriate disease prevention measures, leading to the occurrence of infectious diseases with significant economic and environmental impacts (Rico et al., 2012; Naylor et al., 2021). In response, there has been an indiscriminate use of veterinary medicinal products.

Aquaculture systems are considered an environmental “gateway” in the emergence and transmission of antimicrobial resistance (AMR). In these systems, the effect of interventions has been reported to have had limited effect, due to multiple barriers and unintended consequences. The heterogeneity of aquaculture production systems and their interconnectedness with the environment exacerbates the complexity of designing effective policies (Desbois et al., 2025). Aquaculture activities are also diverse, encompassing subsistence and commercial purposes, which in turn can be intended for domestic or export markets, with numerous value chains and stakeholders in the system (FAO, 2022). Consequently, factors influencing antimicrobial use (AMU) and resistance, and their potential

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impacts and consequences can be widely variable. As a result, multiple pathways, and hotspots for human exposure to antibiotic residues have been described (Brunton et al., 2019)

This study focused on Vietnam as the fourth largest global aquaculture producer and third exporter, and one of the top antimicrobial users in aquaculture globally (Lulijwa et al., 2020). The government of Vietnam, as main governing authority, reacted to the problem of AMR and residues due to the economic consequences from the impact on trade and the concerns to public health. Authorities engaged in the Global Action Plan on AMR (WHO, 2015), and led the elaboration and implementation of the "*National Action Plan (NAP) for management of antibiotic use and control of antimicrobial resistance in livestock production and aquaculture*" in 2017 (MARD, 2017a). This engagement triggered an investment in activities to understand the AMR problem in the country in multiple systems, including aquaculture, as well as for the implementation of policies and interventions. Most of the evidence was generated from point prevalence studies and the role of interventions is not understood. Vietnam has a diverse and complex aquaculture sector, characterised by a wide variety of species farmed, culturing methods and ecosystems (freshwater, brackish water, and marine water), as well as of heterogeneous value chains. All of which, contributes to the problem of AMR in the aquaculture systems of the country.

The objectives of this study were to i) identify potential factors influencing the emergence, spread and exposure to AMR in aquaculture systems, ii) elaborate a framework to develop relative risk profiles of aquaculture systems based on the factors, iii) apply it to the priority aquaculture systems in Vietnam.

MATERIALS AND METHODS

Conceptual approach

In this study, the problem of AMR in aquaculture systems is framed as a wicked problem that necessitates a systems thinking approach and embedding in socio-ecological systems as promoted by One Health (Häsler et al., 2023). They are complex adaptive systems, characterised by complex relationships between stakeholders and resulting processes. Further, these types of systems exhibit resilience, diversity, redundancy, and modularity that result in trade-offs when managing the system (Levin et al., 2013). The adoption of a systems thinking approach allows us to acknowledge and conceptualise the interdependence of the social and natural components of the ecosystem.

Selection of species – production systems - ecosystems

First, a boundary was defined in terms of species, production systems and ecosystems. An initial list was generated based on a group meeting with the collaborators and considering the Vietnamese authorities' nine aquaculture species development priorities, laid out in the Master Plan for the Fisheries Sector to 2020 with a Vision to 2030 (Government of Vietnam, 2013). From the list outlined in the Master Plan, the species included were pangasius species, white-leg shrimp, black tiger shrimp, and tilapia, while marine fish, lobster, molluscs, and seaweed were excluded. This decision was made based on the perceived relevance in production, role in export and domestic markets, available evidence of the usage and resistance to antimicrobials, and alignment with the work of the collaborators at the Research Institute for Aquaculture No 1. In Vietnam, *Pangasius* species (or striped catfish) and tilapia species, are

cultured in freshwater, predominantly in ponds, but also in cages in rivers. In contrast, the shrimp species considered are commonly produced in coastal or brackish ecosystems, using diverse culturing methods. The production of these species is common in other Asian countries and rapidly growing in African countries, and they are highly traded.

Evidence review, screening and data extraction:

A systematic literature search of scientific evidence was conducted to understand the problem of AMR in Vietnamese selected aquaculture systems, in particular to identify factors in the system that might influence the emergence, spread and exposure to AMR in aquaculture. The principles described by Tricco et al. (2017) were followed, as an adaptation of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009) for a sole reviewer. The articles were classified and read in detail and information extracted as described in Table 1.

Table 1. Overview of the scientific literature review process to provide evidence on antimicrobial use (AMU) and antimicrobial resistance (AMR) in different production systems in Vietnam. AB=Antibiotic.

Steps	Description
1.Databases	SCOPUS and CAB Direct
2.Search terms and Period for search	Search terms – in abstract and title ▪ (aquaculture OR fish OR pangas* OR catfish OR shrimp OR prawn OR tilapia), AND ▪ (antimicrobial* OR antibiotic* OR AMU OR ABU OR AMR OR ABR OR resistan*) AND ▪ Vietnam - Period for search: From 2000 to 2022
3.Exclusion criteria	Only records that referred to the following species were included: pangasius, white-leg shrimp, black tiger shrimp, tilapia and freshwater fish, and contained information about AMU and/or AMR or its drivers, policies and interventions
4.Analysis Step 1: Preliminary classification of documents	Documents were grouped under the following classifications, based on themes: (1) biological evidence, detection of resistant genes or bacteria, residues, biological hazards causing disease, (2) antimicrobial use practices, knowledge, attitudes and/or practices associated to antimicrobial use or resistance, as well as disease and health management. (3) Environment: evidence of antimicrobial residues, resistance genes, resistant bacteria, water quality in water sediments and soils of surrounding areas. (4) Experimental studies on AMR or disease (i.e., an intervention is introduced)
5.Analysis Step 2: Variables to extract and characterise information	The following information was extracted and analysed: ▪ Country, region, specie(s) ▪ Part of the system: farm, post-harvest, water, etc. ▪ Production system (intensification, culture method, ecosystem) ▪ Hazard ▪ AMR, AMU ▪ Method for detection or investigation ▪ Impact reported ▪ Intervention

Next, grey literature information, other scientific studies and expert consultation were used to obtain information about relevant characteristics of production systems (e.g., output, stocking density, export or domestic), sector level information (e.g., value chain connectivity, growth trends), and the existence of interventions, uptake, and barriers, which could play a role in the problem of AMR and were not documented in the systematic literature review.

Framework development and application to Vietnam

The framework was developed using information extracted from (1) systematic literature review of the AMR problem in the selected aquaculture systems (2) a grey literature search on aquaculture production systems parameters for Vietnam and scientific studies adopting a systems approach to address AMR in aquaculture from different perspectives which were not specific to Vietnam, and (3) consultation with expert collaborators.

Dimensions and variables

The variables characterising selected aquaculture production systems identified through the evidence review and from the expert consultation, were grouped into four dimensions. Variables represented features that might influence the occurrence of disease, pollution, AMR, or exposure to the problem. The first dimension encompasses biological and farm management (micro-level). The second dimension refers to the environment (meso-level), which refers to the dynamics with the connected ecosystems. The third dimension includes the macro-level factors, such as socio-economic information, trends, and sector governance and value chain. Finally, the fourth dimension refers to the potential availability of mitigation measures and interventions for the different production systems, and barriers. The selection of variables and understanding of dimensions was based on the results of the systematic literature search, reports and publications on characteristics of aquaculture production systems in Vietnam, and complemented with other relevant studies approaching the problem of AMR in aquaculture from a holistic perspective (Henriksson et al., 2018; Brunton et al., 2019; Desbois et al., 2021; Garza et al., 2022), and discussions with collaborators.

Application: relative risk profiles

The information collated was used to develop risk profiles for the production systems. Each variable represents a factor that can have an influence on increasing (+) or decreasing (-) the risk of AMR. To compare between different type of systems, **Fehler! Verweisquelle konnte nicht gefunden werden.**a relative, qualitative ranking was conducted for each production system and variable using the information collated, based on the level of risk, with high (red), medium (yellow) and low (green). For the forth dimension, that referred to interventions, the relative qualitative ranking referred to degree of application and effectiveness (green to yellow to red, from better, medium and worse)

RESULTS

Overview of evidence review

Figure 1 shows the result of the systematic literature search and screening process. Scientific results studies obtained were grouped based on the research aim and focus, including studies focusing on (1) the biological evidence (55/156), which covered the detection and identification of resistant pathogens and residues at the farm and value chain (national and international markets and processing sites), (2) studies characterising the farm practices and usage (25/156), (3) studies investigating pollution, resistance or residues in the environment (11/156), (4) studies assessing or reporting impact or policies (6/156), and (5) studies focused on technological solutions and alternatives to antimicrobials (59/156).

The group of technological solutions and alternatives to antimicrobials, was not considered to develop the framework. Grey literature search using Google provided reports on sector characteristics from industry from international institutions like the World Bank, and national sources from the Vietnamese government and industry bodies. Additional targeted search of scientific studies provided information to characterise aquaculture production systems, understand pathways to disease, pollution, and socio-economic characteristics. Three collaborators in the project provided sector statistics and provided expert opinion. The full results from the systematic literature review, as well as the results from the grey literature search and expert consultation reporting characteristics of the aquaculture production systems are reported elsewhere (Garza, 2023).

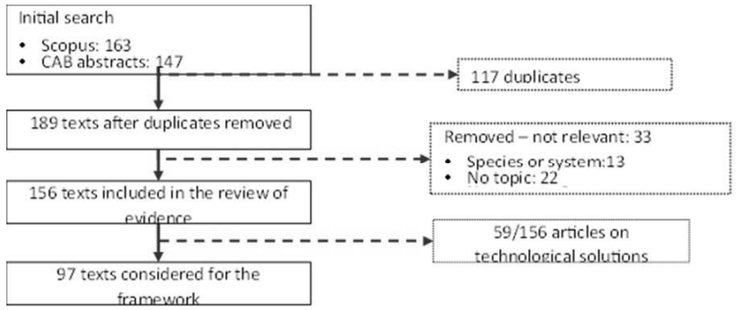

Figure 1. Summary of results from systematic literature search.

Framework

Tables 2-4 illustrate the main factors identified as relevant to understand emergence, spread and exposure to AMR in aquaculture production systems, and main variables to understand the problem. Factors in the first dimension (micro-level) outlined in Table 2, refer to species produced and farm characteristics that can influence the risk of disease, of increased use of antimicrobials and occurrence of AMR. Here, factors related to species characteristics were considered in terms of vulnerability and the potential genetic selection for disease resistance, as well as farm practices. This includes factors related to intensification, such as the type of stocking and density, use of inputs, quality of feed and chemicals. Parameters to assess the intensification can be the stocking density, farm area, use of inputs and use of water and production of solids (Anh et al., 2010a, 2010b). This dimension also includes any information about identification of chemical residues and AMR, that has been obtained in the relevant systems as well as the access to diagnostics.

Factors in the second dimension (meso-level) (Table 3), refer to the ecosystem of aquaculture systems and its connectivity, including elements about the impact on ecosystems and its linkages. It considers the ecosystem and public health pathways, as management of pollutants (treatment and disposal) and biosecurity practices will have an impact on neighbouring ecosystems and food production sites. Improper disposal of wastewater and solid disposal into agriculture and public canals will contribute to eutrophication (risk factor for

disease of animal population and ecosystem) and increases exposure to food safety hazards. In addition, aquaculture systems can be exposed through linked agricultural systems and to effluents from industrial areas and urban areas, including hospitals, through waterways. This will influence the exposure to pathogens, to chemicals and to resistant microorganisms and other stressors that will influence the health and welfare of the populations.

Table 2. Summary of micro-level characteristics (variables) of the system that can be influencing factors, their potential role in influencing the problem of AMR, and its application to main aquaculture systems in Vietnam.

Dimensions and Variables	Role	Production systems			
		Pangasius	WL shrimp	BT shrimp	Tilapia
Micro-level					
Species	Vulnerability to infectious disease (+) and effect breeding programs (-), polyculture (-) or monoculture (+)	M	H	H	M
Stocking density	More stocking density involves increased production of pollutants and risk of disease (+)	H	H	M	M
Disease impact	Impact of disease can lead to more use of antimicrobials (+)	M	H	M	M
Inputs	High use of inputs, in particular feed, leads to more solid production and risk of disease (+)	H	H	M	M
Usage of antimicrobials	A high use of antimicrobials can lead to increasing risk of emergence of AMR (+)	H	H	M	M
Management and technology	The application of technology, such as aeration of ponds, can lower the risk of disease (-)	M	M	H	M	H	H
Diagnostics and surveillance	Access and use of diagnostics can lower the risk (-)	M	M	H	M	H	H
Access to advice	It can prevent disease, and reduce the risk of misuse of antimicrobials and chemicals (-)	L	M	M	M

WL shrimp = white leg shrimp, BT shrimp = Black tiger shrimp; H=high; M=medium; L=low

The variables in the third dimension refer to macro-level factors (Table 4), to capture socio-economic characteristics, trends, and sector governance. This dimension considers the exposure to the product at a macro level to understand how widely the risks could apply, and the wider challenges and opportunities and their potential impact on the population and the economy. It considers the quantity of the commodity, type of market: domestic or export, involved livelihoods, consumption, regions. For, example, a higher production involves a higher biomass and potential use of antimicrobials, exposure to residues and AMR genes in the system: environment, related organisms, and consumers, in the absence of measures to prevent and mitigate the use of antimicrobials. The value chain characteristics, such as the dominance of smallholders or big farms, horizontal or vertical relationships, and the diversity of services and stakeholders, which can influence the prevention and control. A higher number of producers and processors, heterogeneous value chains can pose more difficulties to implement interventions across, monitor usage, AMR, and residues.

Table 3. Summary of meso-level characteristics (variables) of the system, their potential role influencing the problem of AMR, and its application to main aquaculture systems in Vietnam.

Dimensions and Variables	Role	Production systems			
		Pangasius	WL shrimp	BT shrimp	Tilapia
Meso-level					
Pollution: Wastewater and solid production	A high production of wastewater and solids can lead to the accumulation of sediments, residues, microbial overgrowth, and will affect water quality. (+)	H	H	M	M
Water use	Intensive systems require more use of water (+)	H	H	M	M
Management	For the disposal of wastewater and solids (-)	M	M	H	H
Impact on habitat	The infrastructure and size of farms can impact the surrounding (+)	H	H	M	M
Impact of linked systems	Production in lower course of rivers, near industrial areas or urban settings can involve a higher exposure to pollutants, pathogens and resistant genes (+)	H	M	H	M	H	M	H

WL shrimp = white leg shrimp, BT shrimp = Black tiger shrimp; H=high; M=medium; L=low

Table 4. Summary of macro-level characteristics (variables) their potential, role in influencing the problem of AMR, and its application to main aquaculture systems in Vietnam.

Dimensions and Variables	Role	Production systems			
		Pangasius	WL shrimp	BT shrimp	Tilapia
Macro-level					
Total Area	The bigger the area the more exposure to the environment (+)	H	M	M	M
Total production	The more production the more risk to use antimicrobials and of AMR (+)	H	H	M	M
Average annual growth	A higher annual growth will involve a potential higher risk for intensification (+)	L	H	L	H
Export quantity	Increased risk of spread of residues and AMR to other markets (+)	H	H	M	L
Producers	A higher number of producers involves heterogeneity and more needs to effective education and advice, management more difficult (+)	L	H	H	H
Domestic consumption	Exposure to local consumers (+)	L	M	M	H

WL shrimp = white leg shrimp, BT shrimp = Black tiger shrimp; H=high; M=medium; L=low

Finally, the fourth dimension (Table 5) refers to the potential availability of policies and interventions that can have a mitigating and preventive effect, and considers barriers, such as the resources, implementation and enforcement, as well as the compliance and adherence to the measures. For this, a typology of interventions for antimicrobial use and AMR in aquaculture systems (Garza et al., 2022) was used, which was built with information from

Vietnam and other key aquaculture countries, and includes regulations and legislative frameworks, National Action Plans for AMR, certification and standards, technology on farm, alternatives to antimicrobials, good management practices, training and awareness campaigns and activities with co-benefitis. All these groups can have a mitigating effect on AMR, from a direct, indirect or distant way, and are subjected to multiple barriers and factors.

Table 5. Summary of the main types of interventions to main aquaculture systems in Vietnam and its application: green (better), yellow (medium), red (worse).

Interventions and policy landscape	Main role for mitigation	Production systems			
		Pangasius	WL shrimp	BT shrimp	Tilapia
1. *Legislation and regulation*	Provide a framework for responsible use; restrict				
2. *Certification, labelling (market-driven)*	Promote compliance with good practices				
3. *NAP and other plans*	Promote interventions				
4. *Commercial technology and product alternatives*	Promote alternatives				
5. *On-farm management*	Prevent disease				
6. *Training and awareness*	Promote better practices				
7. *Activities with co-benefits (e.g. surveillance)*	Design targeted interventions				

WL shrimp = white leg shrimp, BT shrimp = Black tiger shrimp;

Application of the framework to selected production systems in Vietnam

The application of the framework to the Vietnamese context, shows a high heterogeneity of aquaculture systems, and their risk profiles (Tables 2-5). At the micro-level, there are significant differences among species in terms of vulnerability, as finfish species (pangasius and tilapias) are more resistant and normally affected by bacterial diseases while shrimp species are also affected by viruses. Next, intensification, which is captured by different factors in the framework, such as stocking density, use of inputs and resources, has been considered a key factor of water pollution leading to disease outbreaks in absence of adequate environmental management, and application of technology (Cong, 2017). The level of intensification and characteristics were found to vary among species and even within the same type of species. Pangasius and white-leg shrimp systems were found to be more intensive and produced in monoculture; tilapia production is mixed, with use of polyculture with other native species, and black-tiger shrimp are produced in lower stocking densities and high extension, with a dominance of integrated systems such as mangrove-shrimp, and rice-shrimp. The perceived risks of diseases and technology available influence the density, with higher density used if hormones, aeration, and water treatments can be applied.

At the meso-level (Table 3): pangasius systems required a higher level of natural resources and inputs, and produce much more solid waste and wastewater compared to the other production systems, with impact on the surrounding environment. Most of the production of this species is in the Mekong Delta, at the lowest course of the river, carrying more sediments and potentially pollutants. The production of the other systems appeared to be more heterogeneous and spread out in the country, with ecological variations. Studies in Vietnam have illustrated the different directions of pollution with bacteria, residues and AMR in the aquatic environment (Harada, 2018; Braun et al., 2019; Le and Munekage, 2004)

At the macro-level (Table 4), once more, the production systems presented diverse profiles, the main differences were associated to the participation in the export and the domestic markets, as well as the ownership and characteristics of the sector, homogeneous and integrated vs heterogeneous and different modes of production. The highest output was produced by pangasius systems (1.48 million tonnes in 2021), but the value of white fish species, pangasius and tilapias, has been lower than of shrimp species. White-leg shrimp was the second species most produced, and its value (4-8 USD/kg) lower than black-tiger shrimp (10-20 USD/kg)(VASEP, 2023a). Pangasius production takes place entirely in the 10 Mekong Delta River provinces, but three of these provinces account for 75% of the production (VASEP, 2023b) This type of production system is largely homogeneous and dominated by large farms and vertical integration, with farmers that practice contract farming. This model contrasts with the production of the other species, which present very heterogeneous models with complex value chains. In white-leg shrimp production, the existence of contract farming appeared to be less common, while in black-tiger shrimp and tilapia production the predominant model of production is the smallholder commercial producer, which increasingly is associating in groups as cooperatives for shared land and production management.

In the policy and intervention dimension (Table 5), it was found that in Vietnam the implementation of the National Action Plan for Livestock and Aquaculture in 2017, triggered a series of updates in legislations and regulatory frameworks in the following years, which were applicable to all the systems. These regulations addressed the importation of veterinary medicinal products, the sale of antimicrobials as well as the usage by feed companies, or monitoring activities for export products. However, the enforcement was reported to be lacking due to resources problems.Vietnam showed a wide range of certification standards with mixed uptake by producers. Standards demanded by importers are followed by producers, while the national good agricultural practices (VietGAP) standard of voluntary character, presented multiple barriers (Sun & van der Ven, 2020). The implementation of technology and of surveillance activities was reported to be more prevalente in systems producing for export markets, due to the access to investment and monitoring requirements from processing and importing companies.

DISCUSSION

This study presents a framework to identify the various factors influencing AMR in aquaculture systems and highlights the importance of understanding the heterogeneity of these systems when designing responses. The evidence review revealed a limited number of studies addressing AMR in aquaculture from a holistic perspective, particularly in capturing the socio-ecological interactions and dynamics in Vietnam. Most scientific studies focused on biological aspects and farmer practices, with limited exploration of socio-economic influences such as market dynamics, land use, ownership, and service provision, and only one study on white-leg shrimp systems explored how farmers lacked knowledge on disease response, relied on private services due to low trust in public services, and faced challenges linked to livelihoods, globalisation, and modern technology (Chi et al., 2021).

The framework illustrated how factors related to intensification and productivity, such as using exotic, highly productive varieties of species, producing in monoculture and high stocking dentisities can influence the emergence, spread and transmission of AMR in aquaculture. A higher intensification involves increased water pollution and solids production,

that require more water use and disposal mechanisms in place, leading to higher risk of disease occurrence, which in turn, can lead to more chemical use, and requires more investment on prevention (Cong, 2017; Cassou et al., 2018). Moreover, to mitigate the impact on the environment and risk of pollution into the farming areas, investment on management and waste disposal systems and biosecurity are needed. However, considering the the policy and governance dimension, most of the investments are only feasible for systems producing fish for export markets, which tend to be large producers, with the incentive of obtaining high value in international markets. Smallholders systems, engaged in export and domestic markets and the dominant model for shrimp species and tilapia, are highly heterogeneous, facing difficulties for investment and implementation of prevention, and will need tailored policies to overcome these barriers, address disparities and mitigate the risk of AMR.

Over the past decade, international and national stakeholders have dedicated substantial efforts to better understand and mitigate the problem of antimicrobial resistance (AMR) through the investment of resources and implementation of interventions. One of the main strategies was the development of National Action Plans. However, the application in aquaculture systems has been insufficient due to the heterogeneity and complexity of systems which is not reflected in these strategies (Desbois et al., 2025). This framework can fill this gap by informing a policy analysis that identifies key risk drivers, hotspots for each production system, prioritising entry points for prevention and mitigation in different production systems and tailor interventions. Finally, this framework can inform the design of empirical research to obtain more evidence on the linkages and dynamics of the AMR-related factors in the system.

ACKNOWLEDGEMENTS

This research was funded by, and is a contribution to, the CGIAR Research Program on Agriculture for Nutrition and Health (A4NH). It was a collaboration between RVC and ILRI (Collaborative Research Agreement ref no002/2019, part of the ILRI project CRP003533). The authors thank the colleagues who kindly contributed with their knowledge and experiences.

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STAKEHOLDER ENGAGEMENT

BARRIERS AND LEVERS OF BIOSECURITY ON SMALL-SCALE POULTRY FARMS SELLING DIRECTLY TO CONSUMERS: A SYSTEMIC APPROACH

O. SACCAVINI*, F. CHARRIER, M. DELPONT, J-P. VAILLANCOURT AND M.C. PAUL

SUMMARY

For the past decade, Highly Pathogenic Avian Influenza (HPAI) outbreaks have severely impacted the global poultry industry, leading to biosecurity regulations. Small-scale poultry farms selling directly to consumers have faced challenges in implementing these measures due to their specific characteristics. Using the analytical framework of "sociotechnical system", this study has analysed qualitative data from 36 semi-structured interviews conducted in two French poultry production areas between January and October 2023. Small-scale poultry production has been characterised by heterogeneity, strong professional identity, and stability. However, recurrent HPAI epidemics and regulations have disrupted this balance, creating tensions between biosecurity guidelines and farming practices (e.g. access restrictions), values (free-range farming), workload, financial sustainability, and relations with government authorities. Various adaptations emerged in the field, although they were limited to a small proportion of producers. This suggest that biosecurity compliance on small-scale farms cannot be achieved without simultaneously addressing the social, technical, and regulatory issues.

INTRODUCTION

The emergence and spread of Highly Pathogenic Avian Influenza (HPAI) virus is a major global concern, leading to significant impacts on the economy, animal health and public health (Wille and Barr, 2022). Since 2016, France, has severely experienced five epidemic waves of HPAI virus subtype H5Nx (Scoizec et al., 2024). These recurring events have emphasised the need to improve HPAI surveillance, prevention and control strategies. Biosecurity has been recognised as the cornerstone of HPAI prevention worldwide. Biosecurity measures include day-to-day routine practices (e.g., using farm-specific clothing and equipment), the design of adapted premises (e.g., building layout which allows the separation of zones with different sanitary statuses), and the implementation of controls (e.g., monitoring the efficacy of cleaning and disinfection) (Vaillancourt et al., 2022). Rigorous biosecurity regulations and guidelines related were issued in France in 2016 (Légifrance, 2016), in response to the first HPAI epidemic wave. Since then, incentives and regulatory measures have been continuously reinforced with the objective of improving the uptake of biosecurity measures on farms. Despite significant efforts, previous results indicated that there is still substantial room for improvement, since the adoption of biosecurity measures may vary across location and farm type (Delpont et al., 2021a). Lack of on-farm biosecurity compliance has also been shown to

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be an issue for different types of production, in a wide variety of contexts (Scott et al., 2018; Damiaans et al., 2020; Rajala et al., 2024).

In poultry production, most studies on biosecurity focus on conventional (intensive) commercial farms (Van Limbergen et al., 2018). Therefore, the body of knowledge on compliance with biosecurity standards and the associated sets of perceptions and attitudes relates to these farm types. While conventional large scale poultry operations remain the mainstay for commercial production of poultry meat (Jeni et al., 2021), alternative production systems are progressively developing in response to societal demand (Reidsma et al., 2023). As opposed to conventional systems, alternative poultry production systems are diverse and it may prove difficult to define and study them (take a census, define "typical" lay-outs). Alternative systems may relate to housing characteristics (e.g., having a free-range area, decreased stocking densities or reduced annual production) or processing (e.g., on-farm slaughter) and commercialisation choices (e.g., short food supply chains) (Holloway, 2019). Therefore, in some alternative poultry farms described as "small-scale", conventional biosecurity guidelines may be difficult to comply with and more research is needed to address the reasons behind the apparent "inadequate" implementation of biosecurity measures (East, 2007; Delpont et al., 2018; Holloway, 2019).

Since biosecurity guidelines follow standardised protocols and their uniform implementation is challenging for commercial small-scale units, they require context-specific adaptation (Enticott, 2012). This study used a socio-technical system approach to identify conditions to adapt biosecurity standards to the needs and realities of small-scale farms. This concept, as defined by Geels (2004), integrates actors, rules and techniques to fulfill important societal functions. This article aimed to (1) characterise the socio-technical system of small-scale poultry farming, (2) describe practices and perceptions of stakeholders involved regarding biosecurity and sanitary risks and (3) identify the systemic barriers and levers to implementing biosecurity in this production model. These research questions were tackled through a qualitative approach, involving semi-structured interviews with stakeholders linked to the small-scale poultry industry.

MATERIALS AND METHODS

Location and sampling

The study was carried out in the northwest and southwest regions of France, two main areas of poultry farming. Sampling was designed so as to meet various stakeholders involved in small scale production in the two areas: gallinaceous or palmiped (i.e., ducks or geese) farmers, veterinarians, technical consultants, members of trade unions or veterinary services (government). Small-scale poultry farmers were selected from two internet databases. Farmers were selected based on purposive and snowball sampling, targeting: free-range production and direct commercialisation. Backyard flocks (non-commercial) were excluded. The other stakeholders were recruited through the research team's contacts and snowball sampling.

Data collection

Thirty-six semi-structured individual and group interviews were conducted between January and October 2023 and lasted 87 min on average. Most of the interviews were conducted face-to-face, excepting five participants which were interviewed by videoconference. In-depth semi-

structured interviews were conducted, so that participants could express themselves freely. Discussions were carried in French, based on interview guides for each stakeholder category, focusing on three main topics: (i) general presentation of the participant, his farm, applied biosecurity or duties within his organisation, (ii) sanitary issues in small scale production, (iii) technical and sanitary guidance of farms. All interviews were recorded and transcribed. All the participants gave written consent to have anonymised data collected from their interviews.

Qualitative research framework and data analysis

This qualitative research was based on the interpretivism's paradigm, which involves understanding and interpreting the meaning of human behavior (Grant and Giddings, 2002). A reflexive thematic analysis was carried out on the transcripts using NVivo software, following the method described by Braun and Clarke (2021). The Grounded Theory methodology was used, which consists of building theories from field data rather than predetermined hypotheses (Glaser and Strauss, 1999).

RESULTS

Structure and diversity of small-scale poultry farms selling directly to consumers

Farm's characteristics: A wide diversity of farming situations was observed among the 21 farmers interviewed. This diversity emerged across several dimensions: the species raised, the variety of farming activities, the level of autonomy in managing resources and production stages, the interactions with the customers and the farm infrastructures. Above all, free-range rearing was an integral part of this system, with birds having outdoor access from 4 or 5 weeks of age, meeting consumers' expectations for quality, animal welfare and environmental respect.

Out of 21 farmers, five produced palmipeds (ducks and/or geese), while 12 of the 16 gallinaceous farmers raised multiple species, including broiler chickens, laying hens or high-value and slow-growing poultry (e.g., guinea fowl, turkey, capon…). Small-scale poultry farmers managed all production stages. Nine farmers raised other livestock species, in addition to poultry, such as pigs, cattle, small ruminants and even horses. Fifteen farmers combined their animal production activities with cereal production, to partially feed their livestock. The farmers interviewed explained that diversification was a common practice in small-scale production to meet customer needs, or as a strategy aimed at limiting financial losses in case of a total interruption of poultry activities. Great diversity in the poultry buildings was also observed. The five palmiped farmers used fixed buildings (i.e., sheds which cannot be moved). To meet customers' weekly or monthly needs, small-scale poultry farmers had to maintain continuous production, which involved frequent setting and slaughtering of flocks. As a result, farmers managed several small flocks of different ages and species, often housed in multiple small mobiles buildings, known as huts. For other birds (mainly chickens), most of the farmers (ten out of 16) used huts, ranging in size between 45 to 60 m², with between three and 21 huts per farm. Four farmers had larger fixed buildings, while two combined both types.

Each farm reported a different level of autonomy in terms of realising or outsourcing activities, such as producing feed, slaughtering, product processing, or energy production through methanation. Seven farmers handled both slaughter and processing on-site, three had to manage only one of these two stages on their farm, while ten chose to fully outsource both stages. A few farmers were self-sufficient regarding the management of these different stages.

There are many different ways of selling products in small-scale operations. Some farmers sell directly to consumers through on-farm shops or restaurants, traditional farmers' markets, trade fairs, mail orders with home delivery, or catering services. Direct sales also involve one intermediary, such as a local distributor (rotisseries, butchers, groceries…), specialty stores (producer's stores, organic stores, food cooperatives…) and even supermarkets. In practice, eight farmers sold exclusively to consumers without intermediaries, another two relied only on one intermediary, and 11 used a mix of both sales' mode. Nineteen of these farms often received visitors and customers on site, whether through a store, restaurant, bed & breakfast located on the farm, or as part of educational vocation.

A rooted tradition: Small-scale poultry farms selling directly to consumers, deeply established in the regional landscape and heritage, typically stemmed from a family inheritance. This transmission ensured a certain stability of the system facing market changes or technological innovations, as the farmers relied on well-established techniques. This showed their confidence in traditional practices and their effectiveness.

In addition, 11 farmers produced poultry on quality or origin tags (e.g., organic farming, Protected Geographical Indication…), complying with some specific standards designed to assure product quality and traceability. Certification programs have ensured uniform practices, contributing to market stability and a coherent and sustainable production.

Finally, the strong motivations of small-scale farmers have ensured system stability. Farmers explained that independence from a producer organisation has enabled them to control the entire production chain. Most farmers mentioned that they have been guided by deep-seated convictions about animal welfare and environmental protection. Direct sales have empowered them to forge close links with consumers, who perceived their products as authentic and of superior quality, allowing producers to adapt their offers and share their passion and values.

Diversity within this socio-technical system: Small-scale poultry farming is part of a larger system, involving a complex network of stakeholders exchanging knowledge, finance, products and contracts. These interactions have shaped the overall functioning of the system. Farmers have collaborated with a set of key stakeholders essential for the production and sale of their products. Agricultural cooperatives, input suppliers (feed, animals, litter, equipment, gas, etc.), as well as slaughterhouses, have played an important role in providing the resources necessary for this small-scale farming. Interbranches organizations have also promoted sector products, ensuring fair remuneration for all stakeholders in the supply chain. Professionals providing support, research and development (i.e., interbranch organisations, trade unions, training structures, technical consultats) provided information and knowledge transfer and also cognitive and normative frameworks. Farmers have been part of a normative framework reflected societal expectations (e.g., transparency, respect for traditions such as animal welfare and free-range farming). Cognitive rules, have been based on local, empirical savoir-faire (e.g., knowledge of production cycles, of local breeds, direct-to-consumer sales techniques). Some socio-political stakeholders, such as policy makers and certification bodies, have influenced this system by establishing regulatory rules that govern the production and commercialisation of small-scale products (e.g., compliance with sanitary, food safety, traceability, and conformity).

Impact of biosecurity guidelines on small-scale poultry farming systems

Technical and operational troubles: A 2016 governmental biosecurity decree strongly advocated that poultry farms should adopt an all-in all-out production system. This meant that a given production site could only produce flocks of the same age, with a sufficient downtime between flocks at the farm-level (i.e., a period with no live poultry anywhere on the farm). However, farmers acknowledged that year-round production, a main characteristic, was at the opposite of the decree.

To prove contact with wild birds, in 2016 government authorities imposed strict confinement periods for all free-range poultry, in particular during high-risk periods (during waterfowl migration) and restricted zones. Mobile huts are usually allowed a high stocking density of poultry than fixed houses and a strict confinement would induce overdensity. According to the majority of farmers, huts were designed for night shelter only. These farmers claimed that such high stocking densities would negatively affect poultry welfare (stress, injuries, mortality…), while three associations mentioned that it affected sale prices.

Regulations on biosecurity issues in 2016 require commercial poultry owners to design up a flow management plan, mapping all types of flows within their farms. The mapping also involved to identify and delimit different areas of the farm, in order to reduce flows and associated risks. However, this flow management plan was described as in contradiction with functional environment needed by small-scale farmers to produce according to their standards. A quarter of farms acknowledged that the layout of the farms, especially those established before biosecurity rules, made farm zoning very challenging, and they lacked the financial resources to restructure their site. Moreover, because of low mortality rates due to small flock size, small-scale farmers often would fail to meet rendering requirements, leading some to practice illegally on-site burial or incineration of carcasses instead. Although self-sufficiency was perceived by the majority of those interviewed as an asset, it is threatened by regulatory and territorial requirements. The autonomy of their way of production was considered very fragile, due to the lack of specialised facilities for small-scale farms in their areas, such as hatcheries or slaughterhouses. Most farmers had no choice but ship poultry from and to others production areas, sometimes 500 kilometers away to access these services. Additionally, raising several poultry species meant farmers had to obtain birds from different hatcheries, increasing on-farm traffic. A third of farms with on-site slaughterhouses also had to stop offering services to other farmers in high-risk zones, forcing them to slaughter plants further away, increasing contamination risks and causing financial losses. Finally, the self-sufficiency of these small-scale farms is jeopardised too by the activities needed to maintain it. Indeed, a minority of farmers sold at live poultry markets, which posed biosecurity risks when unsold birds were brought back to their farms. While customer visits were essential, they also had to be limited and adapted to minimise risk. In addition, due to high costs, small-scale farmers often relied on agricultural cooperatives to pool resources and share equipment. However, some were concerned about the sanitation of shared equipment, which represented a risk for each farm. At last, the diversification of species on farms complicated biosecurity management and favored cross-species contamination, as certain species could act as a pathogen reservoir.

Farmer 5 : "Biosecurity is very complicated to implement on a farm like ours. At some point, we have to adapt to the farm and do our the best we can."

Workload and financial balance troubles: The 2016 decree to have anterooms for each barn was deemed impractical by most of farmers due to the high number of barns and the significant

investment and time needed for cleaning and changing equipment after each use, repeated several times a day. Veterinary visits for mandatory birds sampling prior to shipment to slaughterhouses, much more frequent than in conventional commercial farms, were also seen as a non-sense in terms of reducing farm traffic.

All farmers had reduced bird stocking density, in order to reconcile confinement and animal welfare, forcing them to raise the price of their products or reducing their annual income. Confinement also introduced feeding costs, as the birds could not graze outside, and straw, which had to be brought in more frequently to maintain a sufficient litter quality. Farmers faced a heavier workload, with the management of straw-mulching, and building surveillance (ventilation, bird behavior etc.). Two veterinarians acknowledged this statement.

Since 2016, farmers have been required to establish an itinerary and ensure the traceability of incoming and outgoing movements of birds, their products and by-products, inputs, equipment and people. A third of the farmers and one association member complained that traceability generated a heavy administrative burden for the farmers, with several bird shipments overs a month. Another association member questioned the utility of reporting every poultry movement, such as transfer from one building to another, when the birds remained on the same farm.

Veterinarian 5 : "In fact, the biosecurity decree is not adapted to these types of farms. Because we're not going to make 12 anterooms, the guy's not going to change clothes 30 times a day, you know."

Cultural and social troubles: Confinement, conflicting with the values and expectations of consumers and farmers about free-range rearing, had led producers to feel striped of their professional identity and integrity. One farmer recognised that confinement had brought a new type of product and a new clientele. A few farmers raised doubt about the efficacy of confinement to avoid contact with wild birds, claiming that most HPAI clusters occurred in conventional, i.e., confined farms. These farmers believed small-scale scale were not the most vulnerable to influenza infections, considering that free-range reinforces the birds' immunity. This has sometimes led to conflicts between neighbors regarding the best practices to adopt and who was most exposed to the risk of dissemination of infectious pathogens.

Trade union 1 : "I think that [...] societal expectations, the clientele we have, they don't want that model [keeping birds inside]."

The general consensus, supported by the government representative, was that the biosecurity regulations and trainings lacked consideration for specificities of small-scale farms. Additionally, no clear communication or explanation about the regulations have been provided to farmers and some reported that a few rules were not coherent with others. Farmers felt neither valued or supported, sometimes even discredited by the authorities due to a repressive and punitive approach during inspections, though this view was not shared by an association and the government representatives as well as a veterinarian. Two farmers noted that local government services understood their difficulties with biosecurity rules, but had no decision-making power.

A few farmers indicated that they lacked the power and influence within the poultry industry to voice their concerns and needs. Trade unions were created to assert the values and practices of small-scale farming. However, two farmers reported that they suffered from a negative

image, because these unions could have "extremist" ideas and demands, going beyond the biosecurity issue. This damaged their credibility and sometimes they did not recognise themselves in the unions' position.

From isolation to adaptation: new biosecurity measure strategies

The need for avice: Farmers' difficulties in adapting biosecurity requirements to their farms were mainly due to the lack of human resources and qualified staff to assist them. All study participants revealed that small-scale farmers had to seek information about avian influenza and biosecurity decrees since no entity exists in their system to provide this information. The main problem encountered by farmers was knowing where to find information and who to ask for it. All participants agreed that the type of information provided (health, news, biosecurity, technical etc.) varied from on category of stakeholders to another (veterinarians, technical consultants, associations), and based on farm location.

Farmer 13 : "In fact, we're forgotten by the system. [...] I really have to go fishing for information if I want to know anything. Otherwise, I have no information at all."

Most of the advising stakeholders, apart from associations, noted a lack of uniformity in the identification of poultry flocks, due to the lack of guidance and training, as well as the variety and complexity of existing tools. Mandatory reports on flock placements were recorded and stored in various databases managed by different organisations such as local government services, technical consultants, associations or veterinarians. Three technical consultants admitted that the databases were never cross-referenced, compromising mapping of poultry farms and flocks in a given area. This gap in the census led to a lack of guidance and information for the farmers, leaving them isolated.

A minority of farmers expressed a lack of interest in biosecurity, considering it mainly as specific of avian influenza management or being unaware of the potential risks on their farms. The government representative explained that this is partly due to the way government authorities communicated with the farmers. Some farmers focused on improving technical aspects (e.g., animal welfare), to maintain their business, rather seeking further information on biosecurity. Veterinarians noted that farmers who have established themselves during of after HPAI outbreaks were more informed about biosecurity, as well as those with prior experience in production or human and animal health.

To overcome this gap, professional organisations offered personalised services to support small-scale farmers. Some technical consultants suggested sanitary and biosecurity trainings adapted to the specific needs of each farm but the cost made it inaccessible to some small-scale farmers. They also sent out newsletters on changes in regulations or current events, but non-member farmers had no access to this information. When the associations had specialists in small-scale farming and biosecurity, they offered support on a case-by-case basis. One association organised meeting to enable farmers discuss avian influenza outbreaks and establish connections with other farmers. In reply to small-scale farmers' biosecurity challenges, the government authorities started an experiment on free-range poultry farming to identify risks specific to free-range small-scale poultry farms and adapt biosecurity. Farmers also developed networks through homemade training, trade unions membership and agricultural cooperatives, though these remained on a small scale and not fully exploited. Finally, a guidebook supported by a trade union and a technical institute was created for small-

scale and self-sufficient farms to propose specific adjustments and meet the requirements of biosecurity regulations.

Government representative 1 : "You have to find competent people [...] they're not available everywhere. Professional assistance is not organised in certain regions".

Technical, financial and regulatory adaptations: The small-scale poultry farms have chosen to unite and profesionalise to advocate for their collective interests and request exemptions from the government through trade unions and farmers groups. For instance, while free-range access is a prerequisite for some certification labels, temporary exemptions allowed farmers to maintain certification despite bird's confinement. This created yet a moral and ethical dilemma and feelings of guilt towards their values and customers. A tolerance was also introduced concerning the "all-in, all-out system", allowing farmers to keep birds on-site year-round. The main problem noticed by veterinarians and associations was the catching process, which posed a risk to the birds left behind. Moreover, an exemption authorised farms to use an anteroom dedicated to multiple (nearby) poultry production, reducing costs and time constraints. In spite of that, technical consultants, veterinarians and farmers admitted not using properly such anterooms, with personal protective equipment shared across the entire farm, leading to cross-flow situations. Later on, in 2021, government services chose to loosen the confinement restrictions for small-scale poultry farms, by implementing instead reduced grazing land and nets, after a veterinary assessment. While a few farmers perceived it as a step forward, most considered it insufficient, as poultry behavior remained limited and the risk of wild fauna exposure persisted. Also, to reduce human, vehicle, and animal flows, the idea of grouping essential farming structures (e.g., hatcheries, slaughterhouses…) closed and adapter to small-scale farms emerged. In the north-west of France, one farmer (not included in the study) was reported to have set up her own "rural hatchery", solving day-old chicks supply issues and reducing truck traffic to the bare minimum.

Mindset change: between compliance, resistance and compromise: Most of the farmers reported complying fully with the regulations, while a minority chose to do so partially, applying only what seemed reasonable to them, and consistent with their principles and professional identity. Even if this is not perfect, farmers have progressed gradually, according to resources available, according to veterinarians. Finally, a third of the farmers rejected the measures by getting around or ignoring them. For instance, a farmer in a restricted area admitted to receiving chicks' deliveries from authorised areas, determined to maintain his business despite supply challenges. One member of an association was aware of these violations of regulations.

Farmer 20 : "The whole thing made us all think about our own practices [...]. [...] We're questioning ourselves, but not with the same points of view as conventional commercial farmers."

DISCUSSION

This study characterised the organisation and operation of small-scale poultry farms selling directly to consumers, and analysed their relationships with other stakeholders in the poultry industry. Above all, it has highlighted the barriers and possible levers in the application of biosecurity within this production mode.

The recruitment process for poultry farmers sampling may have overlooked some profiles of farmers in small-scale production. Several participants have mentioned farms that, seeking to remain discreet about their hygiene practices different from those required, have not been included in the study. These farmers, often absent from online platforms and from the clientele of veterinarians, technical consultants or associations, or reluctant to participate in the project, may have been underrepresented in the study. This has also been consistent with the lack of cross-referencing between the databases of various advisory and regulatory stakeholders, limiting the exhaustiveness of the information and census in a given territory. Additionally, the lack of a clear definition of what constitutes a small-scale farm in terms of practices and techniques has considerably complicated any attempts of representativeness (Holloway, 2019). The inclusion of various non-farmers stakeholders in the study, such as veterinarians, members of technical consultants, trade unions of agricultural associations, has provided additional information on the different profiles of farmers in small-scale, offering a more objective view of actual farm practices and a better understanding of challenges faced by small-scale poultry farms, revealing aspects of norms and field realities that might have been neglected.

The socio-technical system approach, still under used in the farming sector, has proven to be particularly innovative for understanding small-scale poultry farming. It has offered a global perspective by identifying barriers and opportunities at the systemic level, going beyond strictly individual considerations. By highlighting the complex interactions between stakeholders, rules and technologies, it has provided insights to understand the system as a whole. Small-scale poultry farming has been shown to rely on a diverse and interconnected network of stakeholders. The links and flows have been shown as complex and heterogeneous, heavily influenced by local characteristics, such as available infrastructure and human expertise within a given area. However, this diversity, which is a specific feature of small-scale poultry farms, has complicated the analysis and constrained the generalisability of conclusions.

The study has revealed significant differences between conventional poultry production systems (usually farms in contract with production organisations) and small-scale farms systems involving direct sales to consumers. Farms within production organisations have often benefited from centralised structures and facilitated advising, which has positively influenced compliance with biosecurity standards (East, 2007). In contrast, small-scale farms, characterised by greater autonomy and diversity, have had to adapt these standards to more varied and specific contexts, thereby causing additional challenges.

Barriers to implementing biosecurity in small-scale poultry farm have been observed at all levels, including stakeholders, regulations and techniques. Since these components are interconnected, a dysfunction in one inevitably impacts the others. Biosecurity standards, mainly developed by policymakers and guided by public research, require a more integrated approach to optimise their application across the system (Sony and Naik, 2020; Aubert and Botta, 2022). These standards may collide with rules and techniques designed by other stakeholders. To ensure harmonised functioning, each new rule must be guided by proposals for the other system components. It is crucial to redesign standards in light of the concept of « local universality ». This principle suggests that norms or protocols can only be considered universal if they are adaptable to specific local contexts (Timmermans and Berg, 1997). Such flexibility would enhance their adoption by addressing the specific needs of each context.

The adaptations to classical biosecurity standards implemented, such as exemptions, mutual aid among farmers, or assistance from advisory stakeholders, have so far proven insufficient to ensure both biosecurity compliance and the preservation of traditions inherent to small-scale

farming. Although promising, these initiatives have remained limited, involving only a minority of farmers and thus having a restricted impact on a large scale. A major challenge has also been identified in the divergence of perceptions regarding the implementation of biosecurity measures between authorities, farmers and advisory stakeholders. A gap was identified between farmers and advisors: farmers often tended to overestimate the level of biosecurity within their own farms, either due to a lack of awareness about existing gaps or scepticism regarding the utility of some measures. In contrast, advisors, with broader experience across multiple farms and sectors, may develop a more critical and comprehensive understanding of biosecurity implementation (Laconi et al., 2023). Another divide was observed between field stakeholders (farmers, advisors) and policymakers. Field stakeholders, facing practical realities, often viewed biosecurity regulations as "top-down" disconnected from local constraints. This perception has reinforced their feeling that their needs and realities are inadequately considered in the policy-making process.

A collective organisation involving policymakers, researchers, and local stakeholders was deemed essential to manage and stabilise the system within an adapted biosecurity framework, particularly through the creation of spaces for dialogue and experimentation (Sinclair et al., 2020). To achieve this, it is crucial to identify underrepresented producers who remain outside of regulation and other systemic stakeholders. The study has revealed that many farmers are neighbors with adjacent grazing lands, and that they share equipment and facilities. These connections may be critic for the effective risk management on a territorial scale (Guinat et al., 2020). Consequently, farmers which are commonly considered as a single epidemiological entity, require a more collective approach to risk management (Vaillancourt et al., 2022).

The present study has described a progressive evolution in the attitudes, practices, and perceptions of farmers regarding avian influenza spread and biosecurity regulations. This gradual and individual process has shown significant variability among farmers. These findings can be explained by the fact that biosecurity compliance depends on several psychological factors, such as personality, attitude and beliefs (Delpont et al., 2021b).

ACKNOWLEDGEMENTS

The authors thank all the study participants for their confidence and the richness of the discussions. This work was conducted within the framework of the Chaire de Biosécurité at the Ecole Nationale Vétérinaire de Toulouse, which is funded by the Direction Générale de l'Alimentation of the French Ministry of Agriculture and Food.

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PRIORITISATION OF YOUNGSTOCK MANAGEMENT PRACTICES: COMPARISON OF EXPERT STAKEHOLDERS' ASSESSMENT OF THE SEVERITY OF IMPACT ON DAIRY CALF WELFARE

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SUMMARY

This study aimed to establish a ranking of youngstock management practices according to the severity of their impact on calf welfare using expert elicitation, as well as identify variations in stakeholder opinion. A survey using Maximum Difference Scaling was produced, where respondents were asked to select the practice they believed had the most and least severe impact on overall welfare within the question set. Responses were received from 55 veterinarians, 44 farmers and 43 agricultural representatives in the United Kingdom. Analysis identified that a lack of pain mitigation during painful procedures and suboptimal colostrum were ranked highly, whilst feeding milk from an open bucket and weaning according to age were ranked lower. Choice heterogeneity was most prominent between farmers and the remaining stakeholder groups, a reflection of their differing knowledge acquirement. These findings could allow for the better prioritisation of limited on-farm resources to achieve maximal welfare gains.

INTRODUCTION

The intensification of farming over the past century, as a means of improving efficiency and productivity (Sinclair, 2016), has been met with opposition from the consumer due to heightened concern for welfare. Consequently, the reputation of the dairy industry and its long-term sustainability are under threat due to fractures in the farmer-consumer relationship. Interest in calf health and welfare is gaining traction within the literature (Roche et al., 2023) in response to their frequent marginalisation on farms as well as their underrepresentation within UK legislation. Their lower economic value in comparison to the milking herd (Palczynski et al., 2022) causes their frequent exposure to variable management practices (Wilson et al., 2023) and poorer standards of welfare (Enticott et al., 2022). Resultingly, optimal calf management remains an industry-wide problem despite the availability of best practice guidelines (Mahendran et al., 2022).

Although clarity on the unique impact various management practices have on calf welfare has increased, an understanding of their impact relative to one another is unknown. There are limited studies that use stakeholder consensus to prioritise key welfare issues in various

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species, including horses (Horseman et al., 2016), cats (Rioja-Lang et al., 2019a), and production animals (Rioja-Lang et al., 2019b). However, there are no expert elicitation studies prioritising calf welfare issues. Within published expert consensus studies, rating scales, such as Likert scales, are frequently used which are flawed methodologically due to their susceptibility to scale use bias (Heo et al., 2022). To our knowledge, Maximum Difference Scaling (MaxDiff) remains a novel approach to eliciting expert opinion and has not yet been applied within the context of calf research.

Animal welfare is a complex and multi-dimensional concept (Doyle et al., 2024); as such, its conceptualisation varies between individuals and key stakeholders. For example, veterinarians often perceive welfare from a health-centred perspective (Sumner and von Keyserlingk, 2018) which stems from their professional obligations, whilst consumers prioritise naturalness and natural behaviour expression (Costa et al., 2019). An understanding of the level of stakeholder agreement when prioritising welfare issues is vital in identifying how welfare is conceptualised and valued, as a disconnect between viewpoints often acts as an inhibitor of effective communication when making on-farm changes (Buddle et al., 2021). Despite this, comparison of stakeholder opinions within expert elicitation is frequently omitted during analysis.

The aim of this study was to gather expert opinion from key stakeholders of the dairy industry to establish a ranking of calf management practices according to the severity of their impact on welfare, as well as make comparisons between stakeholder choices.

MATERIALS AND METHODS

Participant selection and recruitment

To be considered an expert in this study, individuals must possess substantial knowledge on calf management, health and welfare. Thus, opinions were gathered from veterinarians, farmers, academics, and industry and government representatives. Purposive sampling was conducted to gain access to the relevant expertise within the chosen stakeholder groups.

It was stipulated that veterinarians must have at least five years of clinical experience and must spend most of their workload with farm animal species. Farmers were specified to rear at least 30 calves per year, with both dairy and beef farmers being eligible to participate. Academics must have at least three years of experience in the job role and must be involved with farm animal research or teaching. Industry and government representatives must also have three years of experience in the job role and must work in an organisation that is closely affiliated with the agricultural or dairy sector.

Eligible veterinarians were identified using the RCVS 'Find a Practice' function (RCVS, 2024) to find practices specifying the treatment of farm animal species. Farmers were recruited through social media outlets and personal connections. Relevant academics were identified from published staff lists of UK veterinary schools and agricultural colleges. Industry and government representatives were identified from personal knowledge and included levy bodies, consultancy firms, and government committees. Control measures were taken within the survey to disqualify participants that did not meet the selection criteria.

A total of 193 veterinary practices, 91 academics, and 37 industry and governmental representatives/companies were contacted. It is unknown how many farmers were exposed to the survey. Participants were contacted via email on 21st May 2024, with reminder emails being sent on 22nd July 2024.

Survey design

The survey was developed using Sawtooth Software Lighhouse Studio version 9.15.4 (Sawtooth Software Inc., 2020). The survey used MaxDiff, a discrete choice methodology that is underpinned by the random utility theory (Jackson et al., 2020). This theory assumes that an individual's preference for item Y over item Z is demonstrated in how often the individual chooses item Y as more preferred than item Z (Louviere et al., 2013), with the highest and lowest ranking attributes representing the maximum difference in utility in the characteristic being investigated. The survey comprised 13 questions, with each question presenting five attributes. The respondents were asked to select which attribute they believed had the most and least severe impact on overall welfare from the presented set of management practices. A sample question can be seen in Table 1. Although each attribute appeared an equal number of times across the survey, each question set was composed of differing combinations of attributes and attributes were placed at differing positions within the set, permitting the elicitation of trade-offs and minimising context effects, respectively. Demographic information was also collected.

Table 1. Sample question: out of the management practices listed below, which do you believe has the most severe and least severe impact on overall calf welfare?

Most severe impact		Least severe impact
	Delayed access to water (from day 3 of life)	
	Feeding suboptimal colostrum	
	Feeding pooled colostrum	
	Weaning calves according to age	
	Housing calves in a poorly ventilated barn	

The survey was pilot tested with 2 veterinarians, 2 academics from the School of Veterinary Medicine at the University of Nottingham, and 2 dairy farmers before being uploaded as a web-based survey, with separate access links being used for each stakeholder group to allow for their differentiation during analysis. Paper surveys were available upon request.

Ethical approval for the study was obtained from the University of Nottingham School of Veterinary Medicine and Science Ethics Committee (no. 3852 230516).

Attributes

Thirteen pre-weaning management practices were investigated. These were (1) immediate separation of the calf from the cow at birth; (2) feeding suboptimal colostrum; (3) feeding pooled colostrum; (4) housing calves individually pre-weaning; (5) sudden removal of milk at weaning; (6) weaning calves according to age; (7) feeding waste milk; (8) feeding milk from an open bucket without a teat end; (9) feeding low quantities of milk replacer per day; (10)

delayed access to water (from day 3 of life); (11) delayed access to starter feed (from day 5 of life); (12) no local anaesthetic or non-steroidal anti-inflammatory drug (NSAID) use during painful procedures; and (13) housing calves in a poorly ventilated barn.

Statistical analysis

Responses were analysed using a hierarchical Bayesian framework, a method based on the random utility theory (Jackson et al., 2020). In discrete choice experiments, it is assumed that individuals make decisions based on the characteristics of items (an explainable component) with a degree of error (an unexplainable component) (Willis, 2014). Each choice possesses an underlying value to the respondent and, when applied to a set of items, this value allows for the identification of the item's position on an underlying subjective scale in which an individual makes their choice (Jackson et al., 2020). This is expressed in Eq.(1).

$$Ui = Vi + \varepsilon i \quad (1)$$

Where U_i is the utility for item i, V_i is the explainable component, and ε_i is the unexplainable component, which is thought to adhere to a Gumbel distribution (Shortall *et al*., 2017). As described in Jackson *et al*. (2020), the probability (P) that a respondent will select item i as best (or in this study, the most severe in impact) from a set of K items is expressed in Eq.(2).

$$P_i = \frac{e^{Ui}}{\sum e^{UK}} \quad (2)$$

Where e^{Ui} is the antilog of the utility for item i, and e^{UK} is the antilog for the utility scores of each item within the set of K items. The probability that a respondent will select item j as worst (or in this study, the least severe in impact) from a set of K items is expressed in Eq.(3).

$$P_j = \frac{e^{-Uj}}{\sum e^{-UK}} \quad (3)$$

Where e^{-Uj} is the antilog of the negative utility for item j, and e^{-UK} is the antilog for the negative utility scores of each item within a set of K items. Further, the likelihood that a respondent will select items i and j as best and worst, respectively, is the probability that the utility difference between i and j is greater than the difference in utility between all other possible pairs within a set of K items. This probability is outlined in conditional logit form (i is chosen as best and j is chosen as worst) in Eq.(4).

$$P = \frac{e^{Ui-Uj}}{\sum_{b=1}^{K}\sum_{w=1}^{K} e^{Ub-Uw-K}} \quad (4)$$

Where b is the best choice and w is the worst choice. A fit statistic was also calculated to determine the consistency in which respondents answered the question set. The minimum fit statistic is calculated as 1/c, where c is the number of items present within the question set (Shortall et al, 2017). In this study, there were five times within each question set; therefore, the minimum fit statistic was 0.20.

RESULTS

Response rate

The survey was open from May-October 2024. A total of 55 veterinarians, 44 farmers, and 43 agricultural representatives completed the survey. A response rate cannot be calculated due to the nature in which the respondents were acquired. All participants exceeded the minimum required fit statistic; thus, all responses were retained for analysis.

Relative importance of attributes within groups

The mean preference score of each attribute for each stakeholder group is outlined in Table 2. Veterinarians ranked attribute 12 (no local anaesthetic or NSAID use during painful procedures) as most severe and attribute 8 (feeding milk from an open bucket without a teat end) as least severe in impact. Farmers ranked attribute 2 (feeding suboptimal colostrum) as most severe and attribute 1 (immediate separation of the calf from the cow at birth) as least severe in impact. Agricultural representatives ranked attribute 12 (no local anaesthetic or NSAID use during painful procedures) as most severe and attribute 6 (weaning calves according to age) as least severe in impact.

Table 2. The mean preference score for each attribute within each stakeholder group.

Attribute	Item number	Veterinarians		Farmers		Agricultural representatives	
		Mean	95% CI	Mean	95% CI	Mean	95% CI
Immediate separation of the calf from the cow at birth	1	1.57	0.58-2.55	1.21	0.05-2.37	3.86	1.84-5.87
Feeding suboptimal colostrum	2	18.40[a]	17.13-19.67	21.08	19.53-22.63	16.71	14.78-18.65
Feeding pooled colostrum	3	1.40	0.94-1.86	3.76	2.30-5.22	1.22	0.56-1.88
Housing calves individually pre-weaning	4	3.45	2.19-4.71	1.80	0.92-2.68	4.26	2.61-5.90
Sudden removal of milk at weaning	5	7.44	5.78-9.09	7.01	5.43-8.59	8.95	7.29-10.60
Weaning calves according to age	6	1.65	1.07-2.24	2.62	2.00-3.23	0.87	0.55-1.20
Feeding waste milk	7	2.17	1.45-2.88	1.87	1.24-2.49	3.40	1.82-4.99
Feeding milk from an open bucket without a teat end	8	0.61	0.42-0.81	1.89	1.11-2.68	1.64	0.51-2.77
Feeding low quantities of milk replacer per day	9	16.58	15.04-18.12	17.91	16.50-19.31	16.81	15.39-18.23
Delayed access to water (from day 3 of life)	10	6.05	4.42-7.69	5.24	3.54-6.95	5.79	3.88-7.69
Delayed access to starter feed (from day 5 of life)	11	1.74	0.94-2.54	1.94	1.45-2.43	1.18	0.48-1.87
No local anaesthetic or NSAID use during painful procedures	12	20.40	19.55-21.24	13.82	11.98-15.65	20.45	19.56-21.35
Housing calves in a poorly ventilated barn	13	18.54	17.66-19.41	19.86	18.62-21.10	14.86	13.09-16.64

[a] A higher mean preference score is indicative of a higher severity rating of the management practice.

DISCUSSION

This is the first study to establish a ranking of calf management practices according to the severity of their impact on welfare, as well as the first study to compare stakeholder perspectives on prominent welfare issues. The study is also novel in its use of MaxDiff as a method of eliciting expert opinion and in the application of this method to calf research.

Inadequate pain management during painful procedures was the highest-ranking attribute for both the veterinarians and agricultural representatives. As most participating agricultural representatives possessed veterinary qualifications, its high ranking within these stakeholders is aligned with the perception that veterinary professionals prioritise welfare issues from a health-centred perspective (Sumner and von Keyserlingk, 2018). It is clear from the literature that a failure to mitigate pain during painful procedures, such as disbudding, has a highly painful, but short-term, influence on welfare (McMeekan et al., 1998; McMeekan et al., 1999) which could explain its high ranking. However, poor compliance with the use of pain management is evident amongst veterinarians (Hambleton and Gibson, 2017), and so its high-ranking in the face of this represents a knowledge-behaviour gap. The lower ranking of this practice amongst farmers is possibly due to the perception of some farmers that such medicines are not necessary for managing pain (Saraceni et al., 2021). Although this may be due to the tendency of farmers to perceive such procedures as less painful that vets (Browne et al., 2022), it may also be the consequence of their unfamiliarity with such drugs and their methods of action (Saraceni et al., 2021). Another perceived barrier to their use is their cost, with studies identifying that farmers believe calf procedures have a lower acceptable cost for analgesic treatment than cow procedures (Browne et al., 2022). That said, studies have identified that farmers are willing to increase the utilisation of analgesics during painful calf procedures (Hambleton and Gibson, 2017), and often do this in response to veterinary advice (Saraceni et al., 2021). This, coupled with their unfamiliarity of their medicines and the pain pathway of such procedures, presents an opportunity for extension agents to educate farmers to enact change.

Feeding suboptimal colostrum was the highest-ranking attribute amongst the farmers. As good colostrum management is frequently described as the single most important factor affecting calf welfare (Godden et al., 2019), it was assumed that this practice would have been ranked highest across all stakeholders. The high ranking amongst farmers is possibly reflective of their conceptualisation of welfare from a productivity angle (Buddle et al., 2021), as suboptimal colostrum increases the risk of calfhood diseases, and thus mortality, and reduces growth rates; both of which are key performance indicators during calfhood (Lopez and Heinrichs, 2022). As calf rearing is the second highest on-farm cost (Palczynski et al., 2022), prioritisation of this attribute highlights a need of farmers to optimise profitability. It can be considered that feeding suboptimal colostrum has a non-painful, but long-term, impact on welfare due to its influence on disease and production. As veterinary professionals prioritise adequate disease and pain management when considering calf welfare (Sumner and von Keyserlingk, 2018), it is plausible that these individuals perceive a management practice as more severe if a pain element is involved in its welfare impact. Thus, the ranking of this management practice may have been higher in the other stakeholder groups had it been ranked according to other parameters such as prevalence or duration (as seen in Rioja-Lang et al., 2019b).

Feeding milk from an open bucket without a teat end was the lowest-ranking attribute amongst the veterinarians. This is likely the result of its impact predominantly being the

inhibition of natural behaviour expression, such as sucking (Vasseur et al., 2010), as well as the development of abnormal behaviours, such as non-nutritive sucking (Mahendran et al., 2022). Despite evidence of potential weaning distress, weight gain losses, and post-weaning hunger (Welk et al., 2024), weaning calves according to their age was the lowest-ranking attribute amongst the agricultural representatives. Immediate separation of the calf from the cow at birth was the lowest-ranking attribute amongst farmers which is perhaps unsurprising as they often cite minimising stress at separation and reducing disease transmission as reasons to support its practice (Boaitey, 2024).

Stakeholder agreement was most frequently observed between veterinarians and agricultural representatives which is likely attributed to their similar acquirement of knowledge through a combination of formal teaching, research and on-farm experience (Martin et al., 2012). As farmers uniquely benefit from having practical experience with calf rearing, it is possible that they relied on outcomes observed directly on their farms to guide their opinions in this study, providing a possible reason for the heterogeneity seen in their choices. This is referred to as 'farmer blindness' (Mee, 2020) whereby their perception of issues are influenced by events occurring on their own farms, thus if it is not deemed an issue on their farm it may not be considered a problem within a wider context. However, variations in knowledge and background can also result in an altered perception of problem severity, known as 'aberrant hazard perception' (Mee, 2020) which may also have contributed to differences in stakeholder views.

Variation in stakeholder values may also be the result of differences in the way in which animal welfare is conceptualised. As animal welfare is a complex and multi-faceted concept (Doyle et al., 2024), it is possible that the stakeholders value certain factors more than others, causing trade-offs between attributes within the question set. Additionally, a definition for animal welfare was not incorporated into the survey, meaning it was freely interpreted by the participants which may have further driven the variation in opinion. Overall, the disconnect between the viewpoints of veterinarians and agricultural representatives with farmers could result in a failure to establish a cohesive partnership, particularly as the former often holds an advisory role on farms, as well as result in ineffective communication when making recommendations for improvement. Therefore, the misalignment in the perception of problem severity amongst stakeholders could act as a barrier to change.

Several limitations to this study are acknowledged. The use of UK stakeholders and a focus on management practices that are common to the UK dairy industry may limit the study's applicability to the wider agricultural sector. The necessary conciseness of the attribute descriptions to avoid their overcomplication may have influenced the way in which they were interpreted by participants. As MaxDiff rankings provide a relative score, the top and bottom ranking attributes are only more or less severe in impact relative to the other attributes in the survey. Consequently, the severity of their impact compared to other practices cannot be inferred from the results.

In conclusion, the study identified a ranking of calf management practices according to the severity of their impact on dairy calf welfare using expert opinion. It identified that a lack of pain mitigation during painful procedures and feeding suboptimal colostrum were the highest-ranking attributes, whilst feeding milk from an open bucket, weaning calves according to age, and immediate cow-calf separation were the lower ranking attributes. Stakeholder agreement was most frequent between veterinarians and agricultural representatives. A better understanding of the management practices posing the greatest risk to calf welfare will aid in

the better prioritisation of on-farm resources to achieve maximal welfare gains. Additionally, clarity on how problem severity is perceived by key stakeholders will aid in facilitating effective communication when making changes on farms and allowing the establishment of more cohesive advisor-farmer relationships.

ACKNOWLEDGEMENTS

The authors acknowledge the European Union's Horizon 2020 Research and Innovation programme for funding this research as part of the DECIDE project under grant agreement No. 101000494. The authors also thank the individuals who helped pilot the study, and the participants who gave up their time to complete the survey. The authors have not stated any conflicts of interest.

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COMPUTERISED MONITORING

ASSESSMENT OF THE VALIDITY OF A COMPUTER VISION SYSTEM FOR DETECTING CHRONIC PLEURISY IN FINISHING PIGS

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SUMMARY

This study evaluates a computer vision system (CVS) for detecting chronic pleurisy in finishing pig carcases compared to official inspection. Using data from 75,881 pigs slaughtered over 15 days, Bayesian latent class modelling was employed to assess diagnostic performance without a gold standard. Results show high agreement between CVS and official inspection, with both methods demonstrating high specificity (98.7% and 99.0% respectively). Official inspection had slightly higher sensitivity (83.4%) compared to CVS (76.4%). Predictive probabilities indicate that using both methods in series yields the highest positive predictive probability. Results highlights the potential of CVSs to enhance inspection efficiency and uniformity without compromising accuracy. Latent class modelling proved suitable for evaluating imperfect diagnostic tests in the absence of a true gold standard, providing a non-intrusive and reproducible assessment methodology. The discussion highlights the need for performance criteria. Moreover, legislative changes are required before a fully automated CVS can replace meat inspectors.

INTRODUCTION

Since the EU released a whitepaper on Food Safety in 1999 (European Commission, 1999), there has been a focus on risk-based meat safety assurance. The whitepaper was followed up in 2011, when the utility of a risk-based framework was highlighted again (Antunović et al., 2021; Blagojevic et al., 2021). One of the biggest changes being the shift from traditional inspection, involving palpation and incision, to visual-only inspection (Alban et al., 2021). The risk-based focus is increasingly relevant in the context of modern slaughter operation, exemplified by the largest abattoir in Denmark, which processes approximately 100,000 pigs per week across three lines, leaving around 10 seconds per carcase for inspection. In addition to the increasing speed of slaughter plants, a uniform way of inspection remains desirable (Steinmann et al., 2014), though uniformity has proven challenging to achieve in practice (Denwood et al., 2015; Alban et al., 2022).

The Danish meat inspection is conducted according to EU regulations and includes both an ante- and post-mortem inspection. The objective of the inspection is threefold with a focus on (1) fitness for human consumption, (2) avoiding notifiable diseases or zoonotic risks and (3) ensuring no compromises to animal welfare (Danish Ministry of Food Agriculture and

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Fisheries, 2022). In Denmark, the meat inspection is performed by official auxiliaries under the responsibility of official veterinarians, and each carcase can be assigned up to four codes, specifying findings at meat inspection, from a total of 60 available codes (Danish Ministry of Food Agriculture and Fisheries, 2022).

Combining the high speed and the inspection complexity, uniformity seems increasingly challenging in the future. However, current legislation recognises the challenge and allows for use of automated systems, like computer vision systems (CVSs), to aid the inspection process for pigs and bovines (Klottrup, 2023; Sandberg et al., 2023), while the inspection can be fully replaced by CVS in poultry (Sandberg et al., 2023). In general, CVSs analyse camera data with advanced algorithms such as neural networks, a form of artificial intelligence. In meat inspection, the systems classify lesions and contaminations detected on carcases using images. However, the adoption of CVSs has been limited by legislative challenges and performance issues, such as a high false positive rate compared to traditional methods (Sandberg et al., 2023). A recent review highlights the potential of CVSs to make carcase evaluation quicker and more uniform, while also emphasising the need for rigorous validation methods of the systems to ensure their reliability in operation (Sandberg et al., 2023).

Multiple methodologies to validate CVSs are being utilised. Some use a ground truth dataset to establish a bona-fide gold standard (Trachtman et al., 2020; Bonicelli et al., 2021), while others use inter-observer-agreement measures, such as Krippendorf's Alpha or Kappa (Blömke et al., 2020). While having a gold standard enables a simple estimate of sensitivity (Se) and specificity (Sp) for CVS (Lalkhen and McCluskey, 2008; Dohoo et al., 2014), a proper gold standard is lacking for the meat inspection, because the judgement of the meat inspectors cannot be regarded as perfect (Denwood et al., 2015; Alban et al., 2022). This hinders the simple estimate of Se and Sp (Veldhuis et al., 2021). Additionally, the inspection routine would be altered significantly if a ground truth dataset was inspected retrospectively (Stärk et al., 2014). Therefore, using evaluation methods that are not reliant on a gold standard seems prudent. One such method is Bayesian latent class modelling, which enables an estimation of Se and Sp without knowledge of the true disease status (Hui and Walter, 1980; Enøe et al., 2000; Toft et al., 2005). Latent class models use binomial data from multiple diagnostic tests and populations to estimate an unobserved or latent variable such as the presence of chronic pleurisy on the inspected pig carcase.

To evaluate whether a CVS can increase uniformity in the meat inspection process, a CVS to detect chronic pleurisy was implemented alongside the regular inspection in a commercial abattoir. This paper seeks to evaluate the diagnostic performance of the CVS compared to the official inspection, using both traditional methods of inter-observer agreement alongside latent class modelling.

MATERIALS AND METHODS

Materials

This study analysed data from a 17-day period to assess the agreement between official meat inspection findings and the assessments made by a specific CVS created by the Danish Technological Institute. In the dataset, each day represented a population consisting of pig carcases. The current version of the CVS employed a combination of red-green-blue and near-infrared cameras and specialised lightning to evaluate the carcase. The camera setup is

integrated directly into the slaughter line before the post-mortem inspection at a major Danish abattoir. The full CVS setup developed to find additional lesions on top of chronic pleurisy, consists of four towers, each equipped with three camera-sets. However, for the chronic pleurisy evaluation, only four of the camera-sets, the lower one of each tower, are used. These four cameras are angled to photograph the pleural cavity and midsection, producing four images per carcase half, yielding a total of eight images in total per carcase. The extensive setup is described in detail in Lund et al. (2024). The eight images are processed using a ResNeXt-101-based convolutional neural network (Xie et al., 2017), fine-tuned for chronic pleurisy detection. The model was trained on 17,154 professionally annotated carcase images (50% with chronic pleurisy, 50% without) and validated on 4,260 images with the same distribution.

Register data on meat inspection codes and CVS assessments for all pig carcases processed at one slaughter line of the abattoir over a 17-day period in the winter of 2024 were obtained from the Danish Technological Institute. The codes recorded during post-mortem inspection by official auxiliaries were matched with the CVS results using individual carcase IDs. The days were selected for convenience and, to the authors' knowledge, do not represent any systematic bias in system performance. Carcases marked with "major slaughter defects" during post-mortem inspection were excluded, as these carcases are routinely wrapped in blue plastic bags before the CVS judgement, because they are deemed unfit for human or algorithmic inspection. Moreover, days with fewer than 3000 pigs processed were also excluded to ensure that line speed and post-mortem inspection times were consistent with regular slaughtering operations at the abattoir.

All data used were anonymised to maintain the confidentiality of the abattoir, with each day numbered for reference. Since the CVS hardware provided unique evaluations for each of the eight images per carcase, a binary variable, was created to indicate whether the convolutional neural network identified pleurisy on any of the eight images. Another binary variable was generated to indicate if an official auxiliary recorded pleurisy. The initial dataset covering the 17-day period included 86,988 pig carcases. Next, 4,447 carcases were excluded due to the "major slaughter defects" remark. And finally, 1,630 carcases from Day 6 were also excluded due to the unusual low number of pigs slaughtered on that day. After applying these exclusion criteria, 80,911 carcases from 16 days were to be included in the study.

To reliably apply a latent class model in this study, a preliminary analysis on the raw data was conducted to ensure that the following three assumptions for the Hui-Walter model were met: (1) the diagnostic tests were assumed to be independent, (2) Se and Sp should remain constant across the populations, and (3) the prevalence of chronic pleurisy should vary between populations (Hui and Walter, 1980; Enøe et al., 2003). However, the preliminary results showed that for Day 1 (5,030 carcases), the estimates for Se and Sp in the populations with the CVS test varied compared to the estimates arising from the data from the other days and hence, Day 1 was removed from the dataset. Therefore, the final dataset used in the study was based on data from 15 days involving a total of 75,881 carcases.

Methods

The data were delivered as individual Excel files, one for each day, which then were combined and analysed in R 4.4.1 (R Core Team, 2024) using RStudio (Posit team, 2024) and the tidyverse (Wickham et al., 2019), ggplot2 (Wickham, 2016), epiR (Stevenson and Sergeant, 2024), and broom packages (Robinson et al., 2024).

Descriptive statistics were used to determine whether any of the meat inspection codes assigned by the official auxiliary was associated with an increased relative risk (RR) of the CVS finding pleurisy. Contingency tables for all codes with a higher prevalence than 0.5% was created and evaluated using a chi-square test with a threshold of $P \leq 0.05$. Significant codes were further evaluated using a 4x2 contingency table to rule out that the increased RR was caused by a concurrent presence of pleurisy alongside the other meat inspection code.

The agreement between the official auxiliaries and CVS was evaluated using the two binary variables. The agreement was first estimated by calculating Cohen's Kappa (Cohen, 1960), followed by the prevalence- and bias-adjusted kappa (PABAK) (Byrt et al., 1993). To highlight the deficiencies of the traditional methodology, Se and Sp were also calculated by treating the opposite test as the gold standard, despite neither test being recognised as a true gold standard. Afterwards, the Hui-Walter latent class model was applied to estimate the Se and Sp of each of the two diagnostic tests: the CVS and the official auxiliaries' judgement. The model was applied across the 15 populations, with data from each day representing a distinct population. The model estimated a total of 19 parameters, including Se and Sp for each test and the true prevalences in each of the 15 populations. To ensure model identifiability and facilitate parameter estimation, the model was assigned 45 degrees of freedom, calculated using the formula $S(2R - 1)$, where R represents the number of tests and S the number of populations. No covariance terms were included as the CVS evaluation of the carcase was automated and based solely on the algorithms, while the official auxiliary's assessment was based on clinical expertise, supporting the assumption of independence between the two tests.

The model was fitted using minimally informative Beta (1,1) priors for all parameters and implemented via Monte Carlo Markov Chain (MCMC) simulations, which allowed for the estimation of median values and 95% posterior credible intervals from the posterior distributions, ensuring an effective sample size greater than 400 for all parameters. To reduce run variation, MCMC simulations were conducted using same seed and initial values. Initial values were set in JAGS (Just Another Gibbs Sampler) to provide reasonable starting points, with Se and Sp reflecting plausible ranges for the two tests (e.g., high for one chain and reversed for the other), and prevalence alternating between low and high across populations. This ensured sufficient exploration of the parameter space and supported model convergence by starting chains from distinct points. The model also estimated the true prevalence of pleurisy in the populations (n=15). These estimated true prevalences together with the Se and Sp estimates were used to derive the posterior positive predictive probabilities (PPP) and negative predictive probabilities (NPP) for both tests using the methodology described by Olsen et al. (2022). These probabilities represented the likelihood of a carcase being correctly classified with pleurisy given a specific test result.

The model was run using JAGS version 4.3.1 for Bayesian analysis, along with the runjags (Denwood, 2016) and tidyverse (Wickham et al., 2019) packages. It ran for 50,000 iterations across two chains, discarding the first 5,000 iterations as a burn-in period, with the remaining iterations used for statistical inference. The efficiency of MCMC sampling and model convergence was assessed through visual inspection of trace and autocorrelation plots, as well as the Potential Scale Reduction Factor (PSRF) calculated using the Gelman-Rubin method (Gelman and Rubin, 1992; Hamra et al., 2013).

A post hoc analysis was applied using the methodology outlined by Denwood (2024), using the posterior predictive probabilities, to validate the underlying assumptions of the model, ensuring its robustness and the reliability of the estimates. The assumption of constant Se and

Sp across populations, along with varying prevalence, was evaluated by inspecting the plots for overlapping posterior credibility intervals for Se and Sp and by assessing the variation in the prevalences across the populations (the 15 days). This post-hoc analysis was also the reason that Day 1 was removed from the dataset, despite fulfilling the initial inclusion criteria.

RESULTS

A total of 11 meat inspection codes, as detected by the official auxiliaries, were individually inspected for association with chronic pleurisy (as detected by CVS). Three codes were significantly associated ($P \leq 0.05$), with pleurisy having a high relative risk (RR=18.2, $P < 0.001$) and a non-negligible prevalence (Table 1). The code "minor slaughter defects" was statistically significant but with a relative risk close to 1 (RR=1.05, P=0.02). Lastly, bile contamination, as detected by the official auxiliary, was also statistically significantly associated with chronic pleurisy detected by the CVS, but with a relative risk close to 1 (RR=0.93, P=0.04).

Table 1. Contingency table showing the statistically significant associations between individual findings by the official auxiliaries (OA) and detection of chronic pleurisy by the CVS (n = 75,881).

	OA's judgement		
CVS' Judgement	Row% (count)	Row% (count)	RR (P-value)
	Chronic pleurisy (+)	Chronic pleurisy (-)	
Chronic pleurisy (+)	76.7% (7,604)	23.3% (2,306)	RR = 18.5
Chronic pleurisy (-)	4.1% (2,733)	95.9% (63,238)	(P < 0.001)
	Minor slaughter defects (+)	Minor slaughter defects (-)	
Chronic pleurisy (+)	18.7% (1,850)	81.3% (8,060)	RR = 1.05
Chronic pleurisy (-)	17.7% (11,680)	82.3% (54,291)	(P = 0.02)
	Bile contamination (+)	Bile contamination (-)	
Chronic pleurisy (+)	10.0% (988)	90.0% (8,922)	RR = 0.93
Chronic pleurisy (-)	10.7% (7,035)	89.3% (58,936)	(P = 0.04)

The two codes "minor slaughter defects" and "bile contamination" were further evaluated in a 4x2 contingency table to ensure the higher relative risk was not caused by the presence of both pleurisy and the additional lesion (Table 2). When stratified for presence of chronic pleurisy (as detected by the official auxiliary) a significantly higher relative risk persisted when the auxiliary detected minor slaughter defects (RR=1.17, P=0.002) but not chronic pleurisy. While the sample size of carcases in which the official auxiliary detected minor slaughter defects but not chronic pleurisy is relatively low (n=464), the results still indicate a point of potential false positives.

Table 2. Contingency table, stratifying the codes chronic pleurisy and minor slaughter defects or bile contamination, showing the statistically significant associations between individual findings by the official auxiliaries (OA) and detection of chronic pleurisy by the CVS (n = 75,881).

	CVS' Judgement		
OA's judgement	Chronic pleurisy (+) Row% (count)	Chronic pleurisy (-) Row% (count)	RR (P-value)
Chronic pleurisy (+) Minor slaughter defects (+)	72.5% (1,386)	27.5% (525)	RR = 0.98 (P = 0.27)
Chronic pleurisy (+) Minor slaughter defects (-)	73.8% (6,218)	26.2% (2,208)	
Chronic pleurisy (-) Minor slaughter defects (+)	4.0% (464)	96.0% (11,155)	RR = 1.17 (P = 0.002)
Chronic pleurisy (-) Minor slaughter defects (-)	3.4% (1,842)	96.6% (52,083)	
Chronic pleurisy (+) Bile contamination (+)	71.2% (718)	28.8% (291)	RR = 0.96 (P = 0.07)
Chronic pleurisy (+) Bile contamination (-)	73.8% (6,886)	26.2% (2,442)	
Chronic pleurisy (-) Bile contamination (+)	3.8% (270)	96.2% (6,744)	RR = 1.11 (P = 0.12)
Chronic pleurisy (-) Bile contamination (-)	3.5% (2,036)	96.5% (56,494)	

The agreement between the judgements made by CVS and the official auxiliary was evaluated based on the data representing chronic pleurisy presented in Table 1. The two tests agreed on 7,600 carcases having chronic pleurisy and 63,000 did not. Each test detected either 2,300 or 2,700 carcases with chronic pleurisy, where the other test disagreed. This implies that the crude accuracy was 93.4%. Evaluating the diagnostic tests using Cohen's Kappa resulted in substantial agreement (κ=0.71), which concurred with the prevalence-and-bias-adjusted Kappa (PABAK) that indicating almost perfect agreement (κ=0.87), suggesting that the two diagnostic tests are providing similar results.

However, the Kappa statistics did not provide insights to the agreement itself, and without an estimate of the diagnostic performance of each of the two tests, they could potentially agree on a carcase having pleurisy incorrectly. The diagnostic performance can be seen in Table 3, with an estimate based on using the 15-population latent class model, and another using the opposite test as the gold standard. According to the latent class model, the official inspection was superior regarding both Se and Sp, though the CVS did not trail far behind in terms of Sp. The latent class model estimated the median true prevalence of chronic pleurisy across the populations to be 15.7% [13.9%-17.5%].

Table 3. The median Se and Sp of the CVS and the official auxiliary (OA) estimated by the latent class model (LCM) and using the opposite test as "gold standard". The 95% Posterior Credibility intervals is presented in brackets.

Population Group	CVS Sensitivity	CVS Specificity	OA Sensitivity	OA Specificity
LCM 15 days	76.4% [73.1%-81.1%]	98.8% [97.8%-99.8%]	83.3% [77.8%-89.6%]	99.4% [98.5%-100%]
"Gold Standard"	74.1%	96.5%	53.4%	96.0%

The estimates for Se and Sp together with the true prevalence estimates enabled the calculation of positive and negative predictive probabilities (PPP and NPP) for each given test outcome (Table 4). The estimates showed that when the two diagnostic tests were evaluated in a series (implying both had to be positive)), they provided an almost perfect ability to rule in chronic pleurisy (PPP=99.9%). Similarly, when both tests were negative, chronic pleurisy could be ruled out in an almost perfect way (NPP=99.3%). Neither test was perfect by itself, and the official auxiliary (PPP=85.7%) was more certain than the CVS (PPP=65.6%) when chronic pleurisy was truly present. These findings align with the Se and Sp estimates in which the official auxiliary outperformed the investigated revision of the CVS.

Table 4. Predictive probabilities for the two diagnostic test outcomes along with the 95% posterior credibility interval of the 15-day model. The "+" and "-" indicate the test result made by the official auxiliary (OA) and CVS.

Predictive probabilities	OA - CVS -	OA - CVS +	OA + CVS -	OA + CVS +
Positive (PPP)	0.7% [0.4%-1.1%]	65.6% [38.3%-93.6%]	85.7% [65.4%-100%]	99.9% [99.8%-100%]
Negative (NPP)	99.3% [98.8%-99.6%]	34.4% [6.4%-61.7%]	14.3% [0%-34.6%]	0.1% [0.0%-0.2%]

The validity of the model was assessed using the predictive probabilities. The prevalence across the populations was visually inspected and found to vary across populations, as required when using Bayesian latent class modelling. Similarly, the Se and Sp estimates, were also visually inspected for constancy across populations, where most of the estimates overlapped across populations. The exception was the Se of the CVS in Day 1, which resulted in running the latent class model without the data from this day.

DISCUSSION

This study evaluated the performance of a CVS for detecting chronic pleurisy in pig carcases compared to the official auxiliary. The results demonstrate that the CVS could easily function as a tool to support and potentially enhance the efficiency and uniformity of the meat inspection processes. The tested CVS did not show any biologically speaking (implying relative risks

close to 1) significant association with other meat inspection findings than chronic pleurisy detected by the official auxiliary.

While the agreement between the CVS and official auxiliary was high when evaluated using Cohen's and the adjusted Kappa, neither of these statistics seem adequate at evaluating imperfect diagnostic tests. Using Bayesian latent class modelling provided a more nuanced and accurate assessment of the diagnostic performance. The modelling revealed that both the CVS and official inspection performed very well in detecting chronic pleurisy, with high Sp for both methods (98.7% and 99.0% respectively). The official inspection showed slightly higher Se (83.4%) compared to the CVS (76.4%), suggesting that human inspectors may still have an edge in identifying subtle cases of chronic pleurisy. However, the CVS's performance is impressive considering the complexity of the task and the speed at which it operates. The developers of the CVS have informed the authors that newer revisions of the physical camera setup and the neural network detecting pleurisy have been developed. This will likely further improve the diagnostic performance of the CVS.

The predictive probabilities derived from the latent class model provided valuable insights into the practical application of these diagnostic methods. When both tests agreed on a positive result, the positive predictive probability was nearly 100%, indicating a very high confidence in the presence of pleurisy. Conversely, when both tests agreed on a negative result, the negative predictive probability was 99.3%. However, when only one of the two tests were negative, there was only a non-negligible probability of 14% or 34% of chronic pleurisy not being present (Table 4). These findings highlight the potential benefits of using the CVS in conjunction with official inspection to enhance overall diagnostic accuracy and improve inspection.

Although the predictive probabilities provided insight by themselves, their main purpose was to enable the post-hoc analysis, which rendered the initial model invalid due to differing Se and Sp estimates for one of the initial 16 populations. Still, the diagnostic performance estimates for both the CVS and official inspection remained largely unchanged (within a percentage point) when running the model with and without the excluded population.

To further evaluate the performance, a gold standard established by a designated expert working continuously could in theory be applied, however this comes at the expense of an increased cost of hiring extra personnel, in addition to potentially altering the judgement of the official auxiliaries. The ideal evaluation framework should be non-intrusive, reproducible and reliable, which are criteria that the latent class model fulfils. The available post-hoc analyses helped to confirm the model's representativeness across populations, allowing for the exclusion of anomalous population subsets or, at the very least, which populations that warranted further investigation.

The reproducibility of the methodology also enables the benchmarking of various iterations of the neural network as it can be rerun on the existing images provided by the CVS and the official meat inspection data. Evaluating the convolutional neural network itself is also crucial as the training process can reveal shortcomings of the final model. Ensuring that the training dataset is of high quality and diverse findings is paramount as biased datasets can lead to poor generalisation, thereby skewing the performance assessments.

Regardless of which CVS is evaluated, establishing guidelines for interpreting satisfactory diagnostic performance for CVSs is recommendable, as perfect accuracy is infeasible for both

human- and computer-based inspection systems. As elaborated on in relation to Kappa values, designing the CVS to mimic the human inspector could propagate the biases and limitations of the current inspection system. Another critical consideration regards autonomy and whether the system should operate alongside human operators or whether it could operate by itself and hereby replace the official inspection. If the long-term aim is to replace official auxiliaries with CVSs, establishing a pragmatic detection threshold for good enough performance is crucial. It should be noted that a complete transition to automated pig meat inspection would necessitate legislative amendments at the level of the European Union and maybe also require acceptance of equivalence with important trade partners outside the European Union (Klottrup, 2023).

While current CVS aims to detect chronic pleurisy and carcase contaminations (Lund et al., 2024), the systems can be developed to encompass a wide range of findings that have value for the primary producer or the abattoir, without necessarily being included in the official code set for meat inspection. The gradual development of new algorithms further supports the implementation of smaller scale CVSs now, to ensure stakeholder and end user familiarisation and confidence.

In conclusion, this study provides evidence that CVS can perform comparably to official inspection in detecting chronic pleurisy in pig carcases. The associated diagnostic performance was very close to being comparable to the performance of the official inspection. The similarity in performance between the two methods and the complementary nature of their strengths suggests that integrating CVS into meat inspection processes could enhance efficiency and uniformity without compromising diagnostic accuracy.

ACKNOWLEDGEMENTS

The authors acknowledge the assistance of Troels Suhr Skovgård and Jeppe Seidelin Dam, employed at the Danish Technological Institute, for their valuable support in data collection and for providing technical insights related to the CVS.

Funding for the development of this manuscript was provided by The Pig Levy Fund 2024

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BIOSECURITY COMPLIANCE WHILE ENTERING AND LEAVING POULTRY FARM BUILDINGS BASED ON VIDEO MONITORING

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SUMMARY

Biosecurity compliance on poultry farms, with a recent history of highly pathogenic avian influenza infection, was monitored using 24/7 video surveillance at entry and exit of farm buildings by farmers and visitors. Two farms of different production types (broiler, layer, breeder chickens, but one duck farm) were selected. Observations of entry and exit from the anteroom revealed low compliance in maintaining the separation between clean and potentially contaminated areas. Handwashing with soap and water or using disinfectant lotion was rarely observed. Footwear was not changed or disinfected during egg shipping, and the egg-storage room was not disinfected after transport by the farmer. Similarly, footwear and trolley wheels were not disinfected when introducing young broilers or ducklings. In contrast, veterinary practitioners demonstrated excellent biosecurity practices. This study shows a need for an engaging awareness and training campaign for poultry farmers, their families, and transporters to promote good practices in biosecurity compliance.

INTRODUCTION

Biosecurity is the first line of defence against an introduction of pathogens. An effective biosecurity plan for poultry farms is based on two main concepts: exclusion and containment (Dewulf and Immerseel, 2018). Exclusion or external biosecurity refers to preventive measures that reduce risks of introduction of infectious agents to non-infected flocks. Containment or internal biosecurity comprises measures to prevent transmission to different poultry units within the poultry farm or to other poultry farms when the pathogen is introduced into the farm. Effective biosecurity measures depend on the formation of a successful barrier between the farm and the outside environment. In practice this can be difficult to implement. People, equipment, materials and vehicles may frequently enter poultry farms. In addition, it is impossible to completely prevent the access of vermin to poultry houses.

In the last decade, Europe has suffered devastating highly pathogenic avian influenza (HPAI) epidemics with thousands of outbreaks on poultry farms and an unprecedented number of dead wild birds (Adlhoch et al., 2023). During periods with enhanced risk of HPAI virus introduction into poultry farms, e.g., in autumn–winter periods when HPAI virus (HPAIv)-infected wild avian species might be present in the environment, all poultry farms with free-range in the Netherlands are ordered to house the poultry inside.

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Despite the fact that direct contact between poultry and infected wild avian species is prevented when poultry is housed indoors, many poultry farms became infected without conclusive evidence about the route of entry.

Direct observation of how biosecurity measures are actually practised has many advantages. The use of human observers however has several drawbacks like being time consuming, expensive, limitations with respect to inter-observer reliability and differences in auditor skills. A presumed weakness of direct observation is the possible occurrence of an undesired observer effect (Hawthorne effect): the phenomenon where individuals participating in research studies alter their behavior because they are aware they are being observed (Adair, 1984). Various electronic methods including video-monitoring have been suggested as alternatives for human observers in order to limit a possible observer effect (McKay et al., 2021). In the veterinary domain, Racicot et al. (2011) were the first to report on detailed biosecurity errors on Canadian poultry farms observed by video monitoring. At the time, they used hidden cameras to observe application of biosecurity measures by farm workers. Nowadays, the use of hidden cameras is impossible taking into account the European legislation with respect to protection of privacy. So it is a challenge to use overt video-camera monitoring and deal with a possible observer effect.

The aim of this study was to investigate and record the application of biosecurity measures at the entrance and exit of poultry farm buildings with a recent history of HPAIv infection to determine biosecurity compliance.

MATERIALS AND METHODS

Poultry farms of different production type (two broiler chicken (broiler), two laying hen (layer), one meat duck (duck) and two broiler breeder (breeder) farms) were selected from a list of all poultry farms infected with HPAIv of subtype H5Nx in recent years in the Netherlands. Potential poultry farms were contacted via their veterinary practitioner for participation. Farms received detailed information about the objectives of the study, what was needed from them, what measurements and investigations were planned on the farm, and what the study would provide, e.g. a comprehensive biosecurity assessment of their operation and a tailor-made advice to overcome shortcomings. Participants received a compensation of €500. An intake visit on site was organized, to evaluate the baseline quality of on-farm biosecurity using a combination of several scoring tools, including Biocheck.Ugent™, the Dutch AVINED hygiene scan and additional avian influenza-related risk factor questions. Participants gave signed consent to data collection, data management and storage, and dissemination of results in anonymised form.

The anteroom is the area between entrance to the poultry building and the entrance to the poultry unit within the poultry building where poultry is housed. Within the anteroom, most of the time a small kitchen is present. Furthermore, between the entrance of the poultry building and the entrance to the poultry unit, a physical barrier is formed in the form of a bench (where you can sit and properly change footwear) as a separation between the potentially contaminated area connected to the outside of the building and the semi-clean area after the barrier and where anyone entering or leaving the farm must follow specific hygiene protocols, including changing clothes and footwear, washing and disinfection of hands and equipment and preferably a shower where you shower in and out when entering and leaving the building. Instead of the

aforementioned hygiene lock, poultry farms might use a provisional (simple) barrier using small wooden boards on the floor for demarcation.

Video-cameras were installed in- and outside the poultry building. Furthermore, a laminated poster was attached on the wall and at doors in the area were video-camera monitoring was executed. The text on the poster called for attention that temporarily video-camera recording was taking place for research purposes in that specific area. In the following critical areas within the poultry building, video-cameras were installed: a) at the crossing between outside of the building and entering the anteroom, where a first separation between the potentially contaminated outside and the semi-clean anteroom is made; b) in the anteroom which connects with a second separation between a semi-clean and clean area to the entrance of the poultry units where poultry is housed; c) for breeder and layer farms: the area where eggs are stored and which the egg transporter enters from outside the poultry building to collect the eggs stored to move them to the transport vehicle; d) for broiler and duck farms: outside the building covering the door through which young broilers and ducklings are introduced to the poultry unit and through which fresh bedding material is transported into the poultry unit.

Video-cameras (VC) 4Mp were used for monitoring. The VCs were positioned on the ceiling of the anteroom (beaming towards the ground). VCs were connected to a video recorder with a 12 Tb hard disk for storage. Recording of movement of people and material in the critical areas was done at 4 frames/s, and under low-light conditions was facilitated using infra-red LEDs. Recording was done 24 h per day and 7 days per week for a consecutive period of 4 to 5 weeks. Video recordings were replayed on a monitor and initiations of target behaviour were scored for each occurrence in an observational session.

Specified movements of people or material were entered into a MS Excel database: farm identification; farm type; visitor or material identification; identification of critical area within the poultry building; time of event; specified biosecurity breaches observed. A study period of four to six weeks was planned in order to ensure that at least in the last week(s) of the recording period ‘the normal behaviour’ of the farmer or family helpers could be anticipated (not being aware anymore of the presence of the video cameras) (Stackelroth et al., 2015). This long recording period made it possible to compare the proportion of biosecurity breaches in last week with that in the first week of observation.

The following definitions of biosecurity breaches were used:

Enter/exit anteroom

Desired biosecurity behaviour: proper change of footwear. Breaches: a) not changing footwear; b) no footwear available in anteroom, move around in anteroom on socks; c) not properly changing footwear: step out of footwear and touch the ground with socks (in dirty or semi-clean area) before changing into other footwear.

Washing/disinfection of hands (enter/exit anteroom)

Desired biosecurity behaviour: wash hands with water and soap and preferably subsequently rub hands with disinfectant lotion upon entering and leaving the anteroom. Breach: not washing hands with water and soap OR if not washing hands with water and soap, not rubbing hands with a disinfectant lotion.

Use of coverall

Desired biosecurity behaviour: put on a clean coverall (made available by the farm) over normal clothes in the anteroom before entering the poultry unit where the poultry is housed. Breach: not putting on a coverall before entering the poultry unit.

Enter/exit the poultry unit

Desired biosecurity behaviour: change footwear in a proper way. Breaches: a) not changing footwear; b) not properly changing footwear: step out of footwear and touch the ground with socks (in semi-clean or clean area) before changing into other footwear.

Egg storage

Desired biosecurity behaviour: properly change or disinfect footwear when entering from outside the egg storage room to ship-off eggs; disinfect wheels of forklift or egg tray trolley when entering the egg storage room from outside. Breaches: a) not changing footwear; b) not properly changing or disinfecting footwear; c) not disinfecting wheels of the forklift.

Supply of young stock and bedding to poultry unit

Desired biosecurity behaviour: change or properly disinfect footwear when entering the poultry unit with young stock or when bedding material is brought in; disinfect wheels of trolley with crates of young stock when entering the poultry unit from outside. Breaches: a) not changing footwear; b) not properly changing or disinfecting footwear; c) not disinfecting wheels of the trolley.

The rate of disregarding biosecurity compliance per movement for each of the above defined breaches was estimated, by fitting regression models with a poisson distribution. In addition, these models were used to compare differences in rate of biosecurity breaches between the first week and the last week of the observation period to test the hypothesis of presence of an observer effect (Hawthorne effect). Furthermore, differences in the rate of biosecurity breaches between the morning and afternoon of observation days were tested.

RESULTS

During the recruiting process, the following number of poultry farms were approached but indicated no willingness to participate: 7 layer farms, 8 broiler farms, and 5 duck farms. Reasons for turning down the invitation were: not interested, having problems with video-camera observation.

Only one breeder farm was equipped with a proper hygiene lock, the other farms in the study used provisional separation of the semi-clean and potentially contaminated area using small wooden boards for demarcation (Table 1). One broiler farm did not have separate footwear available in the anteroom to change to when entering the anteroom from outside (Table 1).

Table 1. Poultry farm and biosecurity characteristics (results based on video-monitoring) of participating farms

Farm	Farm type	Flock size	Separation clean-dirty area at entrance poultry building	Footwear available in anteroom to make foot-wear change at entrance of the building	Change into coveralls available in anteroom before entering poultry unit
1	Breeder	45,500	Hygiene-lock	Yes	Yes
3	Breeder	46,000	Provisional	Yes	Yes
2	Layer	44,000	Provisional	Yes	Yes
6	Layer	24,000	Provisional	Yes	Yes
4	Broiler	75,000	Provisional	Yes	Yes
5	Broiler	47,500	Provisional	No	Yes
7	Duck	21,000	Provisional	Yes	Yes

Rubber boots to change into were available for all farms when entering the poultry unit from the anteroom (Table 2). In one of the broiler farms, mice were observed by video-monitoring to be present in the anteroom (Table 2). In the duck farm it was observed that the dog of the owner once in a while entered the poultry unit when the farmer was moving or reshuffling bedding material into the poultry unit (Table 2). Only two out of four of the egg producing farms were equipped with a physically separated egg storage room (Table 2).

Table 2. Some biosecurity characteristics (results based on video-monitoring) of participating farms

Farm	Footwear available in anteroom to change boots at entrance of the poultry unit	Mice/rats present in anteroom	Pets (dog/cat) occasionally entering poultry unit	Separate storage room for eggs
1	Yes	No	No	No
3	Yes	No	No	Yes
2	Yes	No	No	No
	Yes	No	No	Yes
6	Yes	No	No	-
4	Yes	Yes	No	-
5	Yes	No	Yes	-
7				

There were no indications for a possible Hawthorne effect: not a higher proportion of biosecurity breaches was observed in the last week of the observation period compared to the first week of the study period. The proportion of biosecurity breaches during daily movements was significantly ($p=0.007$) higher in the afternoon compared to the morning.

Anteroom movements

The mean number of daily movements by persons when entering and/or leaving the anteroom for the breeder and layer farms was at least twice as high compared to those of the broiler and duck farms (Fig. 1A). With respect to proper change of footwear at this first barrier, there was one broiler farm that had no footwear available to change into, making that a high risk (Fig. 1C). On both breeder farms, one of the layer farms and the duck farm persons showed a high proportion of not changing the footwear in a proper way when entering and leaving the anteroom (Fig. 1B). On only one farm (broiler farm with Farm ID 4), persons showed the highest level of desired proper footwear change when entering or leaving the anteroom (Fig. 1B and 1C).

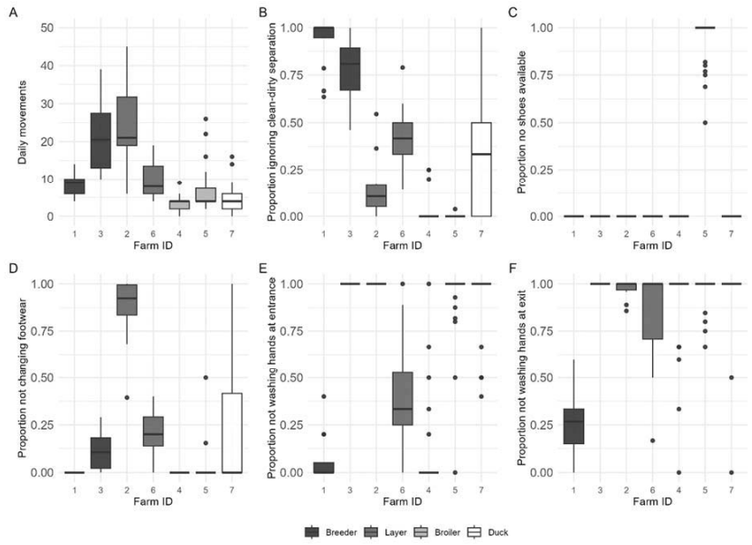

Figure 1. Distribution of daily movements and biosecurity breaches when entering and leaving the anteroom at poultry farms by farm identification and farm type (thick dark line in the box: median; lower end of the box: 25% quantile; higher end of the box: 75% quantile; highest bullet or high end of the vertical line coming out of the box: highest value; lowest bullet or low end of the vertical line coming out of the box: lowest value).

There was one layer farm where persons did not change footwear at all (Fig. 1D). On four out of seven farms (one layer, one breeder, one broiler and one duck farm) persons showed a high proportion of not washing their hands with soap or disinfecting the hands when entering the anteroom (Fig. 1E). The poultry farmer and family workers on only one breeder farm (Farm

ID 1) and one broiler farm (farm ID 4) washed their hands at the entrance of the anteroom (Fig. 1E). When leaving the anteroom to the outside premises (after visiting the poultry unit), the proportion of persons ignoring hand washing is very high for most of the farms, except for the breeder farm (farm ID 1) (Fig. 1F).

Poultry unit movements

The mean number of daily movements of persons entering and/or leaving the poultry unit from the anteroom is for the breeder and layer farms at least twice as high compared to those of the broiler and duck farms (Fig. 2A). With respect to properly changing footwear at this second barrier to separate the semi-clean anteroom area from the clean area of the poultry unit, the highest level of compliance was observed for both broiler farms (Fig. 2B). Most of the poultry farmers and visitors show a high level of compliance with respect to putting on a coverall before entering the poultry unit except for one of the breeder and one of the layer farms (Fig. 2D).

Egg storage room

When storing eggs in the egg storage room by the poultry farmer and/or family co-workers or when eggs are shipped off the farm by a transporter, no proper footwear change or not changing footwear at all is observed in 100% of the cases. Wheels of the forklift or wheels of the trolleys with egg trays were not disinfected when entering the egg storage room (100% breaching). Furthermore, during the observation period on each farm, the egg storage room or area where they are stored was not cleaned and disinfected after each shipping of eggs off the farm.

Introduction of young stock to poultry unit

When young stock (duck and broiler farms) is delivered to start a new production round, the poultry farmer, (family) co-workers and transporters showed a very low level of compliance with respect to properly changing or disinfecting footwear (100% breaching). Trolleys with trays with young stock enter the poultry unit without disinfection of the wheels (100% breaching).

Pathway analysis of biosecurity breaches

A pathway analysis of possible biosecurity breaches when entering the anteroom from outside concerning changing footwear (Breach 1), washing hands with water and soap and/or using disinfection lotion on the hands in the anteroom (Breach 2), changing into a coverall (Breach 3) and changing footwear in the anteroom before entering the poultry unit (Breach 4) indicates that (over all farms) the probability of Breach 1 is 0.42 (95% confidence interval (CI): 0.19 – 0.73) per movement; 0.36 (95% CI: 0.17 – 0.65) per movement for Breach 2; 0.04 (95% CI: 0.001 – 0.37) per movement for Breach 3, and 0.36 (95% CI: 0.13 – 0.74) per movement for Breach 4. The probability that farmers, co-workers and visitors show a lack of compliance for all four potential breaches was 0.002. However, the probability that they comply to all the biosecurity measures in these four steps is 0.23.

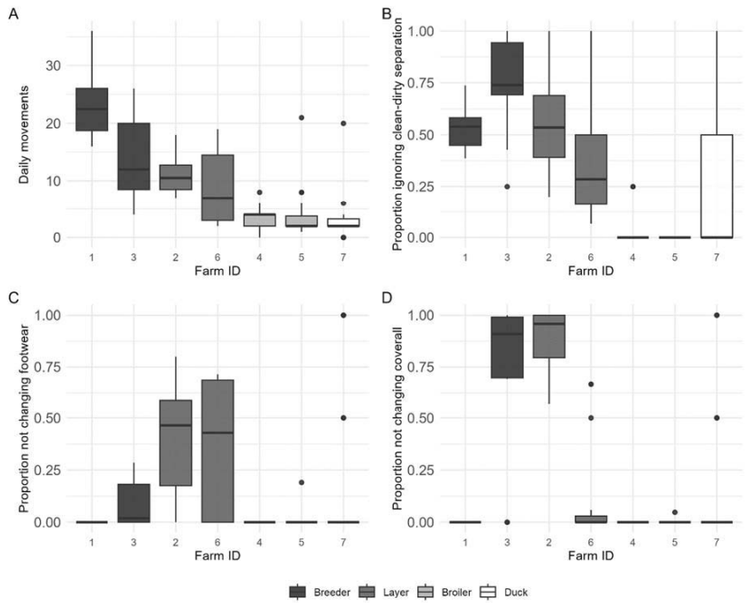

Figure 2. Distribution of daily movements and biosecurity breaches when entering and leaving the poultry unit where poultry is housed by farm identification and farm type (thick dark line in the box: median; lower end of the box: 25% quantile; higher end of the box: 75% quantile; highest bullet or high end of the vertical line coming out of the box: highest value; lowest bullet or low end of the vertical line coming out of the box: lowest value).

DISCUSSION

The good news is that it is actually possible to have a high level of biosecurity compliance in practice, as shown by one broiler farm in this study. But at the same time the study results show that there is serious work to be done for a large group of poultry farms: a low level of biosecurity compliance by farmers, assisting family members and farm personnel in the anteroom was observed. Furthermore, a low level of biosecurity compliance was observed by external transporters, especially during egg loading on egg-producing farms and placement of young stock inside the poultry unit (broilers, ducks). Transport companies should work according to prescribed procedures, and auditing should help to keep them on track. But it would also help if poultry farmers take responsibility and clean and disinfect e.g. egg storage rooms every time eggs are shipped off the farm by a transporter.

This is a sober observation, but it is also expected that the level of biosecurity compliance failure on poultry farms as observed in this study in the Netherlands will probably be comparable to other countries (Racicot et al., 2011). Given the worldwide problems with HPAI

introductions in poultry, it is relevant that a high level of biosecurity is in place and that a low level of compliance puts the industry at risk for HPAI introductions that can seriously impact livestock health, welfare and production and public health.

To have insight into the application of biosecurity measures by farmers, biosecurity surveys have been developed over several decades in the past (Nespeca et al., 1997; Van Steenwinkel et al., 2011; Souillard et al., 2024). They typically consist of a list of questions (yes/no answers) to determine whether a preventive measure is available or would be applied (self-reported). The hygiene check of the Dutch poultry industry (Avined, 2021) uses a comparable procedure. The weak point with these questionnaires is that respondents may be motivated to give the answer that is most desirable, regardless of whether that answer is accurate (Nespeca et al., 1997). More importantly, it has nothing to do with how poultry farmers actually apply biosecurity measures on a day-to-day basis. The study results obtained by direct observation endorse the notion that these questionnaires have very strong limitations in actually assessing the level of biosecurity and in particular day-to-day compliance.

Insight into the actual application of biosecurity measures by poultry farmers can be obtained by direct observation. Direct observation of participants in observational research may improve compliance to biosecurity measures unintentionally as a consequence of the awareness of being observed (McKay et al., 2020). But the use of video-cameras instead of human observers may limit the observer effect (McKay et al., 2021). An observer effect may be reduced with time, i.e. habituation may take place during a long observation period (Robson, 2024). That was the reason why an observation period of four or more weeks was used and with that the ability to test the hypothesis of a possible observer effect. Comparison of the proportion of biosecurity breaches at the beginning and at the end of this study period revealed no indications for presence of an observer effect. This reassured that the study method with the video cameras is producing valid results. It should be mentioned that the use of video-camera monitoring raises privacy concerns: concern about being monitored; fears that the results derived from electronic compliance monitoring systems could subsequently be used in a pejorative manner; concerns about punishment, dismissal or negative performance review; concerns about showing video footage to third parties (McKay et al., 2021). Therefore, the use of video-cameras in observational studies requires extensive preparation, information and communication resulting in a clear and legally sound informed consent from the participants.

The poultry farmer, family co-workers, personnel and visitors can potentially cause the highest biosecurity risk to any poultry farm: due to their frequent movement around poultry units, inherent management tasks and potential ignorance, indifference and/or carelessness, they are the most likely cause of disease introduction and spread (Collett and Smith, 2020). People can carry contaminations on footwear, clothes and on their hands (Van Meirhaeghe et al., 2018), and when entering poultry buildings, measures must be taken to decrease the likelihood to accidently introduce pathogens by complying to stringent biosecurity measures. This study shows a need for an engaging awareness and training campaign for poultry farmers, their families, and transporters to promote good practices in biosecurity compliance.

ACKNOWLEDGEMENTS

Participating poultry farmers for their willingness to cooperate.

Willem Dekkers and Jan de Rond (Animal Health Service, Deventer, The Netherlands) and Yasmin Bakhshi and Sara Burt (University of Utrecht) UU) for help with selection of - and preparing visits to - the poultry farms.

This research was conducted within the framework of the One-Health for Food (1H4F) public–private cooperation project Avian flu risk: Relative role of introduction routes and biosecurity on and around poultry farms (grant number LWV 19081), and was funded by the Dutch Ministry of Agriculture, Fisheries, Food Security and Nature and the Dutch poultry foundation Avined.

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CANINE EPIDEMIOLOGY

QUANTITATIVE RISK-ASSESSMENT OF *BRUCELLA CANIS* INTRODUCTION TO FRANCE THROUGH COMMERCIAL IMPORTATION OF BREEDING DOGS

P. MVUMBI*, G. CROZET AND G. ZANELLA

SUMMARY

Canine brucellosis, caused by *Brucella canis*, is an underdiagnosed zoonotic disease that leads to reproductive failure in dogs and economic losses for kennels. Since 2020, cases in mainland France have been on the rise, with most attributed to the importation of dogs from Eastern Europe. However, the risk of introducing *B. canis* into mainland France through the importation of breeding dogs from worldwide sources remained unclear. To address this gap, a quantitative risk assessment was conducted using a stochastic scenario tree model incorporating the region of origin of the imported dogs. The results showed that unregulated importations of breeding dogs represent a substantial risk for introducing *B. canis* into mainland France. This risk was particularly high for imports originating from the USA, Eastern Europe, North Africa and Latin America. These findings highlight the need for targeted management measures to prevent the introduction of *B. canis* into France.

INTRODUCTION

Canine brucellosis is an underdiagnosed chronic zoonotic disease caused by *Brucella canis (B. canis)*, primarily affecting dogs. Humans can be infected with *B. canis*, which typically causes mild, non-specific symptoms. Individuals at higher risk of exposure include those who handle breeding dogs in kennels or are exposed to their reproductive tissues and fluids, such as veterinarians and laboratory personnel (Santos et al., 2021). In dogs, transmission can occur through the oral, nasal or conjunctival mucosae, via sexual contact, or vertically, through the uterus (Carmichael, 1976; Carmichael and Joubert, 1988; Wanke, 2004; Santos et al., 2021; Djokic et al., 2023). It is a major animal health concern, particularly in canine breeding populations, due to the resulting severe reproductive problems. Infected female dogs are prone to developing metritis, placentitis, and late-term abortions, while males often suffer from epididymitis, orchitis, and prostatitis (Carmichael, 1976; Wanke, 2004; Hollett, 2006; Santos et al., 2021; Djokic et al., 2023). Both sexes may experience infertility, which can lead to significant economic losses in breeding kennels where reproductive success is critical (Makloski et al., 2011).

Several methods are available to diagnose *B. canis* infection, including serology, blood and genital bacterial cultures, and PCR. However, current serologic tests are particularly limited by their low sensitivity and specificity (Wanke, 2004; Djokic et al., 2023).

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The burden of this infection on dog breeding operations is exacerbated by the lack of effective treatment options. Treatment of infection requires prolonged administration of multiple antimicrobials over several months. However, there is a high risk of relapse following only a brief remission (Hollett, 2006; Santos et al., 2021; Djokic et al., 2023). Moreover, infected dogs remain a potential source of infection for other dogs and for humans for several years (Wanke, 2004). Preventing the introduction of *B. canis* into breeding facilities is therefore essential to avoid the significant economic costs associated with managing this infection. The combined impact on canine reproductive health, human health, and kennel economics highlights the need for monitoring and control measures, especially in breeding environments.

Canine brucellosis is well established as an enzootic disease in certain regions worldwide, including the southern United States, Central and South America, Asia, and Eastern Europa (Wanke, 2004; Buhmann et al., 2019; Santos et al., 2021). However, the true prevalence of this infection in these regions remains unknown. Recently, it has emerged as a growing threat in Western European countries, including France, where seropositive dogs have been detected since 2020 in ten breeding kennels across various regions. Most of these cases have been linked to online purchases of dogs from Eastern European countries. In 2022, the High Council of Public Health ("Haut Conseil de la santé publique– HCSP") conducted the first risk assessment in France, focusing on the risk to humans exposed to infected dogs. This assessment followed several reported cases in dogs imported from Eastern Europe (Russia, Belarus and Romania) and the United States since December 2021 (Haut-Conseil de la Santé Publique, 2022). However, it focused exclusively on the risk to humans, using a qualitative approach, and did not consider the exposure risk to dogs from the importation of infected animals. Therefore, the main objective of this study was to quantitatively assess the risk of introducing *B. canis* into mainland France through worldwide commercial importation of breeding dogs.

MATERIAL AND METHODS

A quantitative risk assessment (QRA) was conducted using a stochastic scenario tree model (Vose, 2010) that took into account the region of origin of the imported dogs.

Country grouping

To gain insights into the risk associated with different regions of the world involved in the commercial dog trade to France, countries were grouped into 12 clusters. These clusters were defined based on geographical proximity, the epidemiology of canine brucellosis, and the organization of veterinary services. Each cluster included only countries from which France imported dogs for commercial purposes between 2021 and 2023, as recorded in the French database of domestic carnivores (I-CAD). Table 1 shows the 12 clusters of countries considered in the QRA.

Risk pathways

A schematic diagram of the model structure was constructed, detailing the sequential events necessary for the entry of *B. canis*. The scenario tree model presented in Fig. 1 outlines two potential pathways for the introduction of *B. canis* into mainland France, starting with the selection of a dog in the country of origin. It details the sequence of events that could lead to the importation of an infected dog.

Table 1. Cluster of countries considered in the quantitative risk assessment.

Cluster of countries	Countries
Western Europe	Austria, Belgium, Germany, Luxembourg, Monaco, Netherlands, Switzerland
Eastern Europe	Albania, Belarus, Bulgaria, Czech Republic, Croatia, Hungary, Moldavia, Poland, Romania, Russia, Slovakia, Slovenia, Ukraine
Northern Europe	Denmark, Estonia, Finland, Iceland, Ireland, Latvia, Lithuania, Norway, United Kingdom, Sweden
Mediterranean Region of Europe	Andorra, Vatican City, Greece, Gibraltar, Italy, Malta, Portugal, Spain
South Asia	Afghanistan, Armenia, Cyprus, United Arab Emirates, Israel, Iraq, Iran, Lebanon, Oman, Pakistan, Qatar, Syria, Turkey
East Asia 1	Cambodia, China, India, Indonesia, Malaysia, Thailand, Vietnam
East Asia 2	Japan, Hong-Kong, Republic of Korea, Taiwan
Sub-Saharan Africa	Cameroon, Cape Verde, Gabon, Kenya, Madagascar, Mauritius, Nigeria, Senegal, South Africa
North Africa	Algeria, Egypt, Morocco, Mauritania, Tunisia
Latin America	Argentina, Bolivia, Brazil, Chile, Ecuador, Mexico, Peru, Puerto Rico, Venezuela
USA	United States of America
Canada	Canada

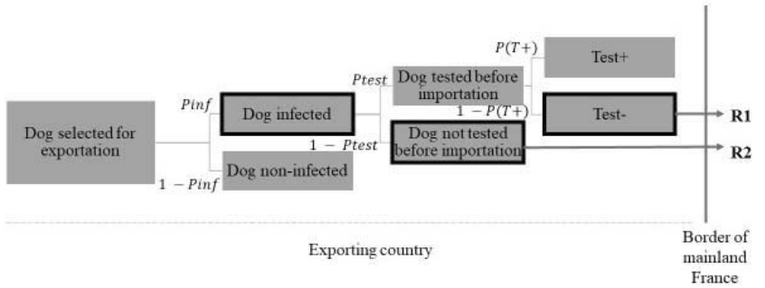

Figure 1. Risk pathways (scenario tree) for the entry of *Brucella canis* into mainland France following the importation of breeding dogs. Where, *Pinf* = Probability of infection, *Ptest* = Probability of testing a dog selected for importation and *P(T+)* = Probability of a positive test outcome in an infected dog. The boxes outlined in thick black represent the adverse events leading to the risk. R1 represents a scenario where a dog is tested but not identified as infected due to the test's limited sensitivity, allowing its entry into mainland France. R2 depicts the introduction of an infected dog into mainland France without any testing.

Model parameterization

Probability of infection: The true seroprevalence of *B. canis* infection (i.e. seroprevalence adjusted for sensitivity and specificity of the diagnostic test used) in each cluster of countries was used as an estimate of the probability of infection, assuming it remained stable and accurately reflected the current epidemiologic status. This seroprevalence was estimated using data gathered from two sources: literature on *B. canis* seroprevalence published since 2000 and test results from the European reference laboratory for *B. canis* (Buhmann et al., 2019). The European laboratory data were specifically used for clusters of European countries due to the lack of surveys directly measuring apparent seroprevalence in this region.

To address the limited availability of seroprevalence surveys and the imperfections of diagnostic tests, Bayesian Latent Class Analysis (BLCA) was used to estimate the true seroprevalence for each country cluster (Branscum et al., 2005; Kruschke, 2015; Cheung et al., 2021). In this approach, a dog's true infection status was the latent variable; in other words, it was considered unobserved. The posterior distribution of the probability of *B. canis* infection was estimated through a combination of observed diagnostic test outcomes (observed data), prior information on the sensitivity and specificity of the serological test(s) and prior information on the disease seroprevalence by country cluster. Practically, the following steps were followed:

i) Specification of prior distributions for true seroprevalence, test sensitivity and test specificity.

The prior distribution for the true seroprevalence was a uniform beta distribution (Beta (1, 1) meaning that all seroprevalence values were equally probable. Conversely, informative Beta prior distributions were used to account for the uncertainty in the sensitivity and specificity of the different *B. canis* serological tests. Published data on the sensitivity and specificity of *these* tests, including their minimum and maximum values (Hollett, 2006; Djokic et al., 2023), were used to estimate the parameters for their prior Beta distributions. The fifth percentile values for these estimates were derived from findings in the relevant literature. The parameters for these beta prior distributions were then calculated using the Epi Beta Buster application (available at https://shiny.vet.unimelb.edu.au/epi/beta.buster/).

ii) Specification of the likelihood function for the observed data.

The methods described by Branscum et al. (2005) were used to define the likelihood functions for the observed data. In the studies selected, either a single test or two tests were used to estimate the apparent seroprevalence within a single population. For the one-test, single population case, the likelihood function y_1 was defined in Eq. (1) as a binomial distribution:

$$y_1 \sim binomial(n, p) \quad (1)$$

where n is the number of dogs randomly sampled from a population and p the probability of a dog testing positive ($P(T+)$) defined in Eq. (2) as:

$$p = P(T+) = (\pi \times Se) + ((1 - \pi) \times (1 - Sp)) \quad (2)$$

with π, the true seroprevalence, *Se*, the sensitivity, and *Sp* the specificity of the test.

For the two-test, single population case, a Bayesian conditional-dependence model was implemented which accounts for the covariances between the two tests (Dendukuri and Joseph, 2001), as the tests measure the same biological process: the presence of anti-*B. canis* antibodies. The likelihood function y_2 defined in Eq. (3), follows a multinomial distribution:

$$y_2 \sim multinomial\left(n, (p11, p12, p21, p22)\right) \quad (3)$$

where p_{11} is the probability that the results for both tests (T_1 and T_2) are positive, p_{12} the **probability that T_1**'s result **is positive and T_2**'s is **negative**; p_{21} the **probability that T_1**'s result **is negative and T_2**'s **positive** and p_{22}, the **probability that both test results are negative**.

iii) Calculation of posterior estimates for true seroprevalence, test sensitivity and test specificity

A Bayesian analysis was conducted to estimate the posterior distributions of the true seroprevalence surveys, tests sensitivity and test specificity. For country clusters with multiple posterior distributions, these were combined using a discrete distribution that assigned equal weight to each posterior distribution.

Probability of testing a dog selected for importation: The probability of testing a dog selected for importation (Ptest) (Fig. 1) was set at *0.01*. This conservative estimate reflects the minimum probability, as testing for *B. canis* is not currently required prior to importation.

Probability of a positive test outcome: Two serological tests, the 2-mercaptoethanol microscopic agglutination test (MAT-2ME) and lateral flow immunochromatographic assay (LFIA) were assumed to be used to screen dogs prior to importation, following the protocol of the French National Reference Laboratory for Brucellosis. These tests were interpreted as parallel tests, meaning a dog was considered *B. canis* seropositive if at least one test was positive. The posterior distributions of test sensitivity for these tests derived from the BLCA, were incorporated into the model to estimate the probability of a positive screening outcome.

Average annual number of dogs imported: Data from the French database of domestic carnivores for the 2021-2023 were used to calculate the average annual number of dogs imported ($NIMP_C$) for commercial purposes into mainland France from a country cluster (c).

Risk assessment

Annual number of infected dogs imported: Based on the defined scenario tree (Fig. 1), the probability of occurrence of the risk pathways R1 and R2 was calculated respectively as in Eq. (4) and Eq. (5), for each country cluster (c) by considering the probabilities of the events within both pathways:

$$R1_C = PINF_C \times Ptest \times \left(1 - P(T+)\right) \quad (4)$$

and

$$R2_C = PINF_C \times (1 - Ptest) \quad (5)$$

where $PINF_C$ represents the probability of infection in country cluster (c); Ptest is the probability of testing a dog selected for importation and P(T+) denotes the probability of a positive test outcome for an infected dog.

The probability that a dog imported from a country cluster (c) is infected ($RIMP_C$) was calculated as in Eq. (6):

$$RIMP_C = R1_C + R2_C \quad (6)$$

The annual number of infected dogs imported into mainland France was then calculated by country cluster ($NINF_C$) as in Eq. (7):

$$NINF_C = RIMP_C \times NIMP_C \quad (7)$$

where, $RIMP_C$ is the probability that a dog imported from a country cluster (c) is infected and $NIMP_C$ represents the average number of dogs imported annually into mainland France for commercial purposes from a country cluster (c). Finally, the global risk, or the annual number of infected dogs imported into mainland France from across the world (NINF), is described in Eq. (8):

$$NINF = \sum_{C=1}^{12} NINF_C \quad (8)$$

where, $NINF_C$ is the annual number of infected dogs imported into mainland France from country cluster (c).

Sensitivity analysis

A sensitivity analysis using Spearman's correlation, a rank-based correlation measure, was conducted to identify the input parameters whose uncertainty or variability has the greatest impact on the uncertainty or variability of the output parameter NINF.

BLCA, QRA and sensitivity analysis implementation

The BLCA, QRA model and sensitivity analysis were implemented in R (version 4.4.1) (R Core Team, 2024) using R Studio (Posit team, 2024). For the BLCA, the software JAGS software was also employed, along with the "rjags" and "runjags" R packages. The "mc2d" R package (Pouillot and Delignette-Muller, 2010) was used to define the distributions in the QRA model and run simulations (n = 100,000). Output convergence was assessed graphically using the "converge" function to ensure that a sufficient number of simulations were performed. Sensitivity analysis was conducted using the tornado function. Results are presented as the mean and median of the posterior distribution for true seroprevalence, along with its 95% probability interval, corresponding to the 2.5th and 97.5th percentiles of the Markov Chain Monte Carlo (MCMC) output.

RESULTS

Estimation of true seroprevalence of *Brucella canis* infection by country cluster

Overall, the posterior estimates of the true seroprevalence of *B. canis* infection by country cluster were low with very wide 95% probability intervals (PIs) (Fig. 2). Four country clusters exhibited higher median posterior estimates: the Latin American cluster (median value = 0.04; 95% PI: 5.1 × 10−5 − 0.66), the South Asian cluster (median value = 0.01; 95% PI: 1 × 10−5−

0.20), the North African cluster (median value = 0.01; 95% PI: 0.0003 − 0.06) and the USA (median value = 0.004; 95% PI: 0.0002 − 0.03).

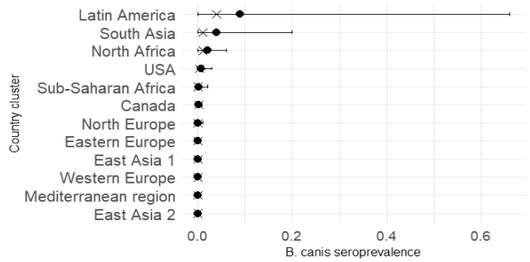

Figure 2. Posterior estimates of medians (crosses) and means (dots) for *Brucella canis* seroprevalence by country cluster along with their 95% probability intervals.

Risk assessment

Figure 3 shows the results of the risk assessment by country cluster. Four country clusters provided a high annual number of *B. canis* infected dogs into mainland France: the USA (median value = 3.70; 95% PI: 0.14 − 30.50), the Eastern European cluster (median value = 5.80; 95% PI: 0.13 − 25.40), the North African cluster (median value = 1.40; 95% PI: 0.05 − 7.80) and the Latin American cluster (median value = 0.93; 95% PI: 0.001 − 17.70). The global risk (NINF) was high with a median value of 41.60 (95% PI: 5.12 − 8784.00).

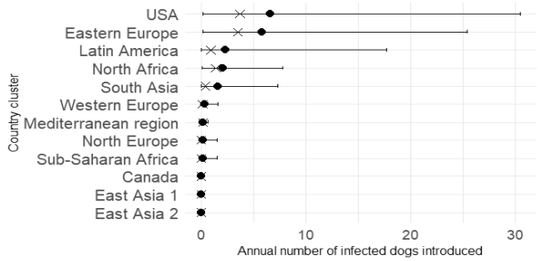

Figure 3. Estimates of the median (crosses) and mean (dots) of annual number of *Brucella canis* infected dogs imported into mainland France by country cluster along with their 95% probability intervals.

Sensitivity analysis

Uncertainty in seroprevalence by country cluster had the greatest impact on the annual number of *B. canis* infected dogs introduced into mainland France (NINF) from worldwide, with a strong positive correlation (Fig. 4). The sensitivities of MAT-2ME and LFIA and

specificity of MAT-2ME showed weakly positive correlations, while LFIA specificity exhibited a weak negative correlation with the output parameter.

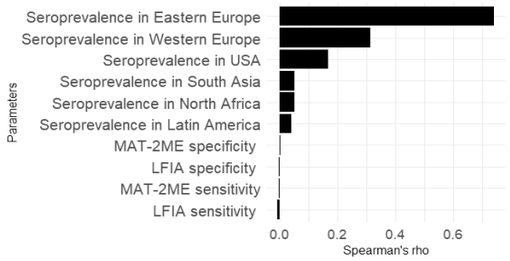

Figure 4. Results of the sensitivity analysis based on Spearman's correlation.

DISCUSSION

This study presented the first QRA addressing the risk of *B. canis* introduction into mainland France through the global importation of breeding dogs. The findings indicate that unregulated importation of breeding dogs is highly likely to introduce *B. canis* into mainland France, particularly from specific country clusters, including the USA, Eastern Europe, North Africa and Latin America.

The USA appears to be the primary source of *B. canis* for mainland France, with an estimated four infected dogs potentially being introduced annually. This may be attributed to the high prevalence of the disease in the country as supported by the surveys conducted, *B. canis* infection remains enzootic, particularly in the southern regions, and there is a presence of a large, minimally regulated dog industry (Brower et al., 2007; Hubbard et al., 2018). Carmichael and Joubert (1988) had already highlighted that canine brucellosis in the USA was more common in large kennels and areas with high densities of free-roaming dogs, with higher infection rates in commercial kennels lacking control measures. Other authors also agree that *B. canis* is one of the most important *Brucella* species in the USA in terms of One Health relevance (Brower et al., 2007; Hensel et al., 2018; Hubbard et al., 2018; Whitten et al., 2019; Pinn-Woodcock et al., 2023). Additionally, a significant annual number of dogs were imported into mainland France from the USA between 2021 and 2023 according to the records in the French database of domestic carnivores.

For the Eastern Europe cluster, although the posterior *B. canis* true seroprevalence was low, it represented the second largest source of infected dogs in the risk assessment. This outcome is probably due to the high number of imported dogs from this region. Data on dog imports from Eastern Europe to France indicate an increasing trend since 2020, which may have contributed to the rise in detected cases. Remarkably, before 2020, only two cases of *B. canis* infection were reported. However, between 2020 and 2022, 26 infected dogs were identified, most of which were imported from Russia, Belarus, and Romania through online purchases (Haut-Conseil de la Santé Publique, 2022). If this import trend persists, the future introduction of infected dogs from Eastern Europe into France cannot be ruled out. Other studies have also identified Eastern Europe as the primary source of *B. canis*-infected dogs in several European

countries, including the Netherlands, Germany, Sweden (Holst et al., 2012; Buhmann et al., 2019), the United Kingdom (Escauriaza et al., 2021), as well as Belgium, Italy, Austria, Switzerland, Finland, Denmark, and Norway (Buhmann et al., 2019).

Regarding the Latin American cluster, the study indicates a higher posterior *B. canis* true seroprevalence within this cluster. This likely explains its ranking as one of the top four sources of the pathogen for mainland France, with an estimated one infected dog potentially imported annually. A distinctive feature of this cluster is the abundance of available seroprevalence surveys (Aguiar et al., 2005; Cavalcanti et al., 2006; Agudelo-Flórez et al., 2012; Silva et al., 2012; Tuemmers et al., 2013; Galarce et al., 2020), used to estimate the true posterior seroprevalence. Furthermore, several of these studies highlight the absence of canine brucellosis control and prevention regulations in the region, a factor that can contribute significantly to the risk of international spread of *B. canis*.

Finally, the results also showed that the risk of introducing *B. canis* through the importation of dogs from other clusters of EU countries (Western Europe, Mediterranean region, North Europe) was very low but not null. Residual risk exists, that could be increased by less stringent sanitary controls for dogs imported from these countries since they belong to the European Union. Additionally, the permeability of borders for dogs coming from North Africa, particularly in the Mediterranean EU countries, further increases the risk of introduction.

The findings must be interpreted with caution, as they rely on certain assumptions due to limited consistent data availability on *B. canis* seroprevalence. Specifically, identical seroprevalence of *B. canis* infection within each cluster was assumed, although regional variations likely exist. Most clusters were geographically large, often spanning entire continents, and the available samples were not always representative of the study population. Additionally, previously published data on apparent seroprevalence were obtained in situations where none of the tests used was a perfectly accurate gold standard. To address these limitations, a BLCA was applied, allowing to account for uncertainty in seroprevalence data and to obtain estimates of true seroprevalence by cluster despite the use of imperfect diagnostic tests. BLCA was a useful component of the QRA because of its ability to integrate different data sources by estimating true prevalence values, even when the data sources themselves were heterogeneous. This method enhanced the robustness and accuracy of the model by accounting for uncertainties and the limitations of imperfect diagnostic tests. Future studies should prioritize the generation of more reliable and representative seroprevalence data, especially from high-risk regions, as this would greatly improve the precision of risk estimates.

There is an urgent need to enhance global surveillance and establish harmonized diagnostic protocols for *B. canis* infection. This need arises from the lack of specific clinical manifestations in infected dogs and variability in testing procedures and result interpretation across countries (Mateu-de-Antonio et al., 1994; Wanke, 2004; Djokic et al., 2023). Furthermore, unlike other *Brucella* species, *B. canis* is not currently classified as a notifiable disease by the World Organization for Animal Health (WOAH) or the World Health Organization (WHO). In France, although canine brucellosis is classified as a regulated and, therefore notifiable disease, specific surveillance and management measures have yet to be implemented (Haut-Conseil de la Santé Publique, 2022).

The significant reproductive disorders of *B. canis* infections in dogs, especially those used for commercial breeding purpose and its zoonotic potential underscores the urgency for controlling its introduction into mainland France. This study contributes to the understanding of the role

of unregulated dog trade movements in the introduction of the pathogen into mainland France and provides a flexible model designed to be easily updated with new data as it becomes available. This could allow animal and public health managers to generate up-to-date results that could guide the implementation of *B. canis* surveillance and control measures.

ACKNOWLEDGEMENTS

This work is part of an ongoing PhD thesis funded by the Réseau Doctoral de Santé Publique (RDSP), managed by the School of Advanced Studies in Public Health in Rennes (EHESP). We are very grateful for the support provided by the following institutions: RDSP/EHESP, École Doctorale de Santé Publique (EDSP), Université Paris-Est Créteil (UPEC), and the French Agency for Food, Environmental and Occupational Health & Safety (Anses), particularly the research team of the EPIMIM Unit. We also thank the French Society for the Identification of Domestic Carnivores (I-CAD) for providing records of the number of dogs imported into France from worldwide sources used in this study.

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EPIDEMIOLOGY OF HAEMANGIOSARCOMA IN DOGS UNDER PRIMARY VETERINARY CARE IN THE UNITED KINGDOM: FREQUENCY, DIAGNOSIS AND RISK FACTORS

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SUMMARY

This study aimed to estimate the frequency of haemangiosarcoma diagnosis and identify its risk factors in dogs. A nested case-control study on clinically diagnosed haemangiosarcoma was conducted within the cohort of dogs under veterinary care in 2019 at VetCompass practices, United Kingdom and logistic regression was used to identify risk factors in those dogs aged five years or older. The annual incidence risk of canine haemangiosarcoma across all ages was 0.036% (95% confidence interval: 0.033-0.038%). In the risk factor analysis, 788 clinical cases were compared to 1,064,187 controls. Ten breeds had increased odds of haemangiosarcoma diagnosis compared to crossbreed dogs. Breeds with the highest odds were the Dogue de Bordeaux, Flat Coated Retrievers and German Shepherd Dogs. Additional risk factors included increasing age, neuter status, clinic deprivation and urban clinic location. Breed is a key risk factor for haemangiosarcoma diagnosis, while environmental risk factors may also play a role.

INTRODUCTION

Canine haemangiosarcoma is a malignant neoplasia that originates from vascular endothelial cells or haematopoietic progenitor cells in the bone marrow (Mullin and Clifford, 2020). In a cohort of insured UK dogs, an incidence of 25 cases per 100,000 dogs was estimated (Dobson et al., 2002). Haemangiosarcoma has been reported to account for, 40-64% of primary splenic disease (Johnson et al., 1989; Day et al., 1995; Davies and Taylor, 2020; Schick and Grimes, 2023), 46% of canine cardiac tumours (Ware and Hopper, 1999) and 2-8% of cutaneous masses (Villamil et al., 2011; Tompkins et al., 2020). Except for cutaneous haemangiosarcomas, the biological behaviour of these tumours is generally aggressive (Mullin and Clifford, 2020), having a severe welfare impact on affected dogs.

Age and breed have been consistently reported in the literature as important demographic risk factors for canine haemangiosarcoma but there is conflicting information about sex, neutering and bodyweight. Risk of haemangiosarcoma increases with increasing age (Prymak et al., 1988; Ware and Hopper, 1999; Carnio et al., 2020), though dogs over the age of 15 years

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have been observed at lower odds compared to their middle-aged counterparts for cardiac and cutaneous haemangiosarcoma (Ware and Hopper, 1999; Villamil et al., 2011). Breed predisposition also plays an important role in risk with Retrievers, Shepherds and Boxers particularly susceptible, and Golden Retrievers and German Shepherd Dogs showing the highest risk overall (Robinson et al., 2020). However, breed predispositions vary depending on tumour location (Ware and Hopper, 1999; Pirie et al., 2006; Villamil et al., 2011; Yamamoto et al., 2013; Tompkins et al., 2020) with cutaneous haemangiosarcoma associated with a higher risk in Jack Russell Terriers (Tompkins et al., 2020), Boxers, Greyhounds, Airedale Terriers, Golden Retrievers and English Pointers (Villamil et al., 2011).

While sex has not been identified as a significant risk factor for canine haemangiosarcoma in most studies (Prymak et al., 1988; Hargis et al., 1992; Ware and Hopper, 1999; Pirie et al., 2006; Carnio et al., 2020), one retrospective study of a population sourced from 27 US academic hospitals reported males at increased risk, after matching cases and controls by age and time period of diagnosis and adjusting for neuter status and breed (Robinson et al., 2020). Evidence of the effects of neuter status is mixed, but spaying in females has been more consistently associated with haemangiosarcoma than castration in males (Ware and Hopper, 1999; Pirie et al., 2006; Zink et al., 2014; Grüntzig et al., 2016; Carnio et al., 2020; Robinson et al., 2020). Dogs diagnosed with haemangiosarcoma are typically larger (Sherwood et al., 2016; O'Byrne and Hosgood, 2019; Carnio et al., 2020) though the effect of bodyweight is unclear due to inconsistent weight categories used. Additionally, its correlation with breed complicates the interpretation of these associations (Fleming et al., 2018).

Few studies have evaluated environmental risk factors for the disease. No association was found between indoor-outdoor living conditions or estimates of pollution exposure and visceral haemangiosarcoma in an Italian study (Carnio et al., 2020) and to date, no known studies have reported on socioeconomic risk factors associated with haemangiosarcoma in dogs. Whilst little is recorded about how levels of socioeconomic deprivation directly relate to risk of haemangiosarcoma in dogs, it is reasonable to hypothesise that factors such as owner employment status, income, education and living environments affect husbandry and pet care decisions, and consequently, the risk of diagnosis.

Generalising the results of previous epidemiological studies on haemangiosarcoma to the wider UK dog population is restricted by the common use of insured and referral populations in research (O'Neill et al., 2014), as well as diverse geographical locations across studies where breeding programmes and environmental conditions are likely to differ. This current study aimed to estimate the frequency of haemangiosarcoma diagnosis, the frequency of associated clinical signs and the frequency of tests associated with haemangiosarcoma diagnosis in dogs of all ages under primary veterinary care in the UK. Additionally, the study aimed to identify demographic and environmental risk factors for haemangiosarcoma diagnosis in the subset of study dogs aged five years or older.

MATERIALS AND METHODS

A cohort study of dogs under veterinary care in 2019 at participating practices in VetCompass was conducted to estimate the annual incidence risk and 12-month period prevalence of haemangiosarcoma diagnosis for dogs of all ages, while a nested case–control study within the 2019 cohort was used to assess demographic and environmental risk factors for this diagnosis in dogs aged five years or older. Ethical approval for using VetCompass data

was granted by the Social Science Research Ethical Review Board at the Royal Veterinary College (SR2018-1652). VetCompass collects de-identified electronic health record (EHR) data from UK primary care veterinary practices for epidemiological research (VetCompass, 2024). The study included all dogs under primary veterinary care at participating VetCompass clinics in 2019. These were defined by at least one EHR (clinical note, treatment, or bodyweight measurement) during the year.

A haemangiosarcoma case was defined by the presence of a final recorded clinical diagnosis of haemangiosarcoma, with or without histopathological confirmation. Prevalent cases included all cases first diagnosed before January 1, 2020, with at least one EHR during 2019. Incident cases included those first diagnosed between January 1, 2019, and December 31, 2019 (inclusive). To identify potential haemangiosarcoma cases, the clinical free-text field for all 2,250,417 dogs under primary veterinary care in 2019 within VetCompass was searched using search terms related to haemangiosarcoma (haemangios*, hemangios*, HSA, angiosa*, haemangiosarcoma~1, hemangiosarcoma~1, hameangios*). The 4,997 candidate cases identified were randomly ordered and manually reviewed to confirm any dogs meeting the case definition. Confirmed haemangiosarcoma cases had additional clinical information extracted and dogs for which haemangiosarcoma was listed only as a differential diagnosis were excluded. Dogs not identified as candidate cases were considered controls in the risk factor analysis. The risk factor analysis focused on dogs aged either five years or older at diagnosis (cases) or as of June 30, 2019, (controls) because dogs diagnosed with haemangiosarcoma cases were generally older than dogs not diagnosed with haemangiosarcoma. June 30, 2019, was chosen to calculate the age of controls as it was the mid-point of the study period. Cases and controls from all the study clinics contributed to overall frequency estimates, but cases and controls from one practice group with insufficient cases (n=2) were excluded from the risk factor analysis. Since all non-haemangiosarcoma diagnosed dogs were included as controls, the final case: control ratio was approximately 1: 1,350. For cases and controls, data regarding possible risk factors were either extracted directly from the EHR or derived from data linked to the veterinary clinics' postcodes (Table 1). Age was extracted as a continuous variable and categorised into 2-year intervals (Table 1). The variable for breed included individual pure breeds and designer hybrids that each had over 7,000 dogs in the overall population or at least 5 diagnosed haemangiosarcoma cases overall, along with a group for all crossbreeds and a single group for all remaining breeds.

Data were checked and cleaned using Microsoft Excel (Microsoft Corporation, 2018) and analyses were performed using R version 4.4.1 (2024-06-14 ucrt) (R Core Team, 2023). For risk factor analysis, approximately 700 cases and 2,800 non-haemangiosarcoma controls were required to identify breeds as risk factors with an odds ratio of at least 2 compared to crossbreeds (95% confidence interval (CI), 80% power, 2% breed exposure among controls, 1:4 case ratio)
(Fleiss et al., 1980; Dohoo et al., 2009; Dean et al., 2011). These estimates were based on findings that German Shepherd Dogs had a four-fold increase in odds of haemangiosarcoma (Prymak et al., 1988; Carnio et al., 2020) and accounted for approximately 2.1% of the 2019 UK dog population (O'Neill et al., 2023).

For risk factor analyses binary logistic regression was employed to assess univariable associations between potential risk factors and incident cases in 2019. Factors whose association with a haemangiosarcoma diagnosis had a p-value < 0.2 in univariable analysis were considered for multivariable evaluation. Individual variables taken forward for evaluation in the multivariable analysis were evaluated for collinearity using the chi-square test. Final

model selection followed a manual backward stepwise elimination process, and pairwise interaction effects were assessed for the variables in the final model (Dohoo et al., 2009). The clinic was evaluated as a random effect, and practice group was included as a fixed effect to account for data clustering at these levels. Model quality and discrimination were assessed using the area under the receiver operating characteristic (ROC) curve and the Hosmer-Lemeshow test (Hosmer and Lemeshow, 1980; Dohoo et al., 2009). The intraclass correlation coefficient (ICC) measured clinic-level clustering in the mixed-effect model. Statistical significance was set at $p < 0.05$.

Table 1. Summary of the extraction and categorisation of potential risk factor variables for haemangiosarcoma diagnosis for dogs five years or older under primary veterinary care during 2019 in the VetCompass Programme in the United Kingdom.

Variable	Method of data extraction and categorisation
Breed	Recognised purebreds and designer crossbreeds extracted from electronic health records (EHRs), cleaned and mapped to a VetCompass breed list derived from the VeNom Coding breed list (The VeNom Coding Group, 2023).
Reproductive status	For cases, reproductive status in the EHR at the time of first diagnosis. For controls, reproductive status defined by the final EHR value.
Age (years)	The age at the date of first diagnosis for cases and as of June 30, 2019, for controls. Age was categorised as 5 to < 7, 7 to < 9, 9 to < 11, 11 to < 13, 13 to < 15, and ≥ 15.
Practice group	1-5 based on the practice group extracted from the EHR.
Clinic Indices of Multiple Deprivation (IMD)	Veterinary clinic postcodes from VetCompass linked to Lower Super Output Areas (LSOAs) or the devolved nation equivalent. Each LSOA was assigned a ranked IMD value from open-access governmental databases for England, Northern Ireland, Scotland, and Wales (Ministry of Housing, Communities and Local Government et al., 2020). IMD ranks for LSOA areas of each devolved nation were divided into quintiles and categorised from quintile 1 (most deprived) to 5 (least deprived).
Clinic location	Veterinary clinic postcodes from VetCompass linked to LSOAs or the devolved nation equivalent. LSOAs classified by urban-rural location status as defined by the Office for National Statistics (Department for Environment Food and Rural Affairs and Office for National Statistics, 2016).
Clinic identification number	Extracted directly from the EHR.

RESULTS

Prevalence and incidence

From a study population of 2,250,741 dogs under primary veterinary care in 2019, 1,030 dogs were confirmed as haemangiosarcoma cases at any date up to January 1, 2020. Of these, 229 (22.23%) were pre-existing diagnoses from before January 1, 2019, and the rest (77.77%) were incident cases. In 2019, the 12-month period prevalence of haemangiosarcoma was 0.046% (95% CI: 0.043-0.049%) and annual incidence risk was 0.036% (95% CI: 0.033-0.038%).

From 1,066,381 dogs aged five years or older in the study population, 999 dogs were confirmed as haemangiosarcoma cases. Of these, 209 (20.92%) were pre-existing diagnoses from before January 1, 2019, and the rest (79.08%) were incident cases. For dogs aged five years or older, the estimated frequencies of canine haemangiosarcoma diagnosis were approximately twice the overall frequencies with a 12-month period prevalence of 0.094% (95% CI: 0.088-0.099%) and annual incidence risk of 0.074% (95% CI: 0.069-0.079%).

Clinical presentation and diagnosis of overall incident cases

Of 801 cases diagnosed during 2019, 773 (96.50%) had at least one clinical sign in the EHR associated with haemangiosarcoma. These signs were described using 63 distinct clinical terms. The most frequently recorded clinical signs among these cases were lethargy (38.58%), hyporexia/anorexia (35.83%), abdominal distension (24.59%), abdominal pain (24.09%), haemoabdomen (23.85%) and pale mucous membranes (21.47%). The most frequently recorded diagnostic modalities associated with cases were abdominal ultrasound (68.29%) and blood analyses (60.55%). Biopsy (incisional or excisional) with histopathology to support a more definitive diagnosis, was reported in 277 dogs (34.58%).

Twenty-nine different primary tumour locations were recorded among the 801 incident cases, with the most common being the spleen (60.17%), liver (17.35%), cutis (9.86%), heart (9.49%), subcutis (4.99%) and muscle (2.87%). The most common tumour size was 5 to 10 cm in 93/241 (38.59%) of measured tumours. Metastatic lesions were recorded in 177 (22.10%) cases, with 82 (10.24%) in the liver, 40 (4.99%) in the lung and 24 (3.00%) in the omentum. The majority of metastatic haemangiosarcomas were diagnosed based on clinical criteria alone (90.96%) with diagnosis by histopathology in just 9.04% of cases.

Risk factor analysis in dogs aged five years and older

In the 788 incident cases aged over five years (from the five large veterinary groups) used in the risk factor analysis, the median age of first haemangiosarcoma diagnosis was 10.5 years (interquartile range (IQR): 9.0 to 12.0 years, range: 5.0 to 17.1 years). The most common types of dogs among these 788 cases were crossbreeds (22.59%), Labrador Retrievers (12.18%), German Shepherd Dogs (10.66%) and Staffordshire Bull Terriers (5.46%). There were 360 females (45.69%) and 424 males (53.81%). At the time of diagnosis, 580 dogs (73.60%) were neutered and 208 (26.40%) were entire with four dogs having unrecorded neuter status (0.51%). The median adult bodyweight of cases was 26.4 kilograms (kg) (IQR: 16.8 to 33.6 kg). Among 1,064,187 controls used in risk factor analysis, the median age on June 30, 2019, was 8.7 years (IQR: 6.7 to 11.1 years, range: 5.0 to 24.5 years) and the most common types of dogs among were crossbreeds (24.47%), Labrador Retrievers (7.85%), Jack Russell Terriers

(6.49%) and Staffordshire Bull Terriers (5.43%). There were 513,764 females (48.2%) and 546,661 males (51.3%). Neutered dogs accounted for 663,892 (62.3%) of controls and entire dogs for 396,533 (37.2%). Reproductive status was unrecorded in 4,957 dogs (0.5%) and the median adult bodyweight of controls was 14.4 kgs. (IQR: 8.6 to 25.2 kgs.).

All study variables had a p-value < 0.2 in univariable analyses and were taken forward for consideration in multivariable analyses. The final mixed effects multivariable model retained six risk factors: breed, reproductive status, age, clinic index of multiple deprivation (IMD) quintile, clinic location and practice group (Fig. 1), with clinic ID included as a random effect. The final fixed effects model showed acceptable model-fit and discrimination (Hosmer-Lemeshow test statistic: p = 0.733, area under the ROC curve: 0.784). For the final mixed effects model, the ICC was 0.24 (95% CI: 0.17-0.25), indicating little clustering of haemangiosarcoma diagnosis at the individual clinic level.

After accounting for the effects of the other variables in the final model, 10 breeds showed increased odds of haemangiosarcoma diagnosis compared with crossbreed dogs (Fig. 1). Breeds with the highest odds included Dogue de Bordeaux (OR = 9.56; 95% CI: 4.17-21.91), Flat Coated Retriever (OR = 8.33; 95% CI: 4.03-17.20), German Shepherd Dog (OR = 6.35; 95% CI: 4.86-8.29), and Hungarian Vizsla (OR = 5.49; 95% CI: 2.95-10.22). Six breeds showed reduced odds of haemangiosarcoma diagnosis compared with crossbreeds (Fig. 1). Neutered females (OR = 1.28; 95% CI: 1.01-1.64) and neutered males (OR = 1.49; 95% CI: 1.17-1.90) showed increased odds compared to entire females. Compared to dogs aged 7 to less than 9 years old, dogs aged 11 to less than 13 years old were at higher odds of haemangiosarcoma diagnosis (OR = 2.83; 95% CI: 2.31-3.48). Dogs in the youngest age category (5 to less than 7 years old), had reduced odds of haemangiosarcoma diagnosis compared to dogs aged 7 to less than 9 years (OR = 0.22; 95% CI: 0.15-0.31) and dogs brought to clinics in the least deprived areas (quintile 5) of the UK had the highest odds of haemangiosarcoma diagnosis compared to those attending clinics in the most deprived areas (quintile 1) (OR = 1.66; 95% CI: 1.18-2.34). Dogs attending clinics in mixed urban/rural areas were at reduced risk of haemangiosarcoma diagnosis compared to those attending clinics in urban areas (OR = 0.71; 95% CI: 0.57-0.89). Odds of haemangiosarcoma diagnosis significantly varied depending on the veterinary practice group attended (Fig. 1).

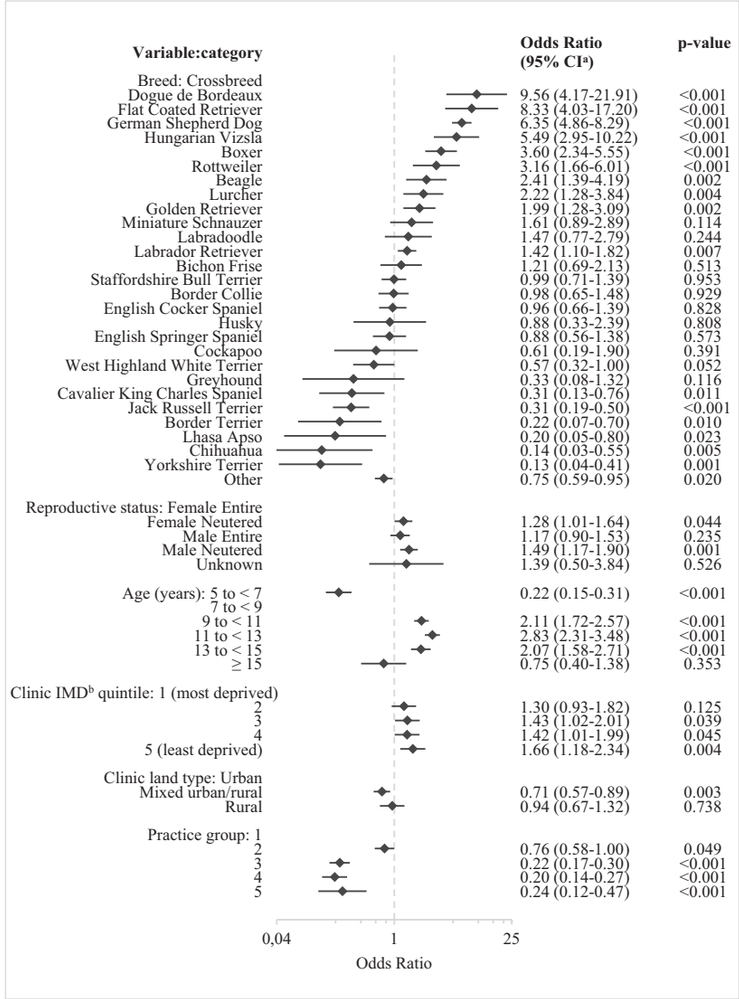

Fig. 1 Multivariable logistic regression results for associations between breed, reproductive status, age, deprivation, clinic location and practice group and haemangiosarcoma diagnosis in dogs five years or older under primary veterinary care during 2019 in the VetCompass Programme in the United Kingdom.

[a] Confidence interval. [b] Index of Multiple Deprivation.

DISCUSSION

The current study is the first to report the incidence and prevalence of haemangiosarcoma diagnosis in dogs under primary veterinary care in the UK as well as associated risk factors. Utilising a large population of 2,250,741 dogs, the study identified 1,030 diagnosed haemangiosarcoma cases and identified breed, reproductive status, age, clinic-associated deprivation and clinic location as risk factors.

The annual incidence risk of 0.036% was greater than the previously reported 0.024% by Dobson et al. (2002) and 0.007% by Robinson et al. (2020). This is likely due to those other studies using data from academic hospitals or insurance claims. Although referral centre data offers greater diagnostic confidence, such populations are subject to selection bias, with overrepresentation of owners wishing to pursue diagnostic workups or facing fewer financial concerns, limiting generalisability to wider primary care populations (Bartlett et al., 2010; O'Neill et al., 2014). Potential misclassification of haemangiosarcoma cases in this study, due to diagnoses made without histopathological confirmation, may have inflated case numbers. However, this reflects typical primary care diagnostic practices, enhancing the findings' relevance to the broader UK population and similar settings.

Breeds previously reported to show a strong association with diagnosis of haemangiosarcoma include Golden Retrievers, German Shepherd Dogs and Boxers (Robinson et al., 2020). The current study confirmed these associations and additionally identified the Dogue de Bordeaux, Flat Coated Retrievers, Hungarian Vizslas, Rottweilers, Beagles, Lurchers and Labrador Retrievers as at increased odds. Identifying breeds at increased risk of haemangiosarcoma diagnosis helps owners and veterinarians by aiding clinical suspicion and guiding diagnostic testing, especially for visceral haemangiosarcoma where early clinical signs may be vague or intermittent (Mullin and Clifford, 2020). The current study also identified breeds unreported in US populations, potentially reflecting genetic differences across geographical locations (Quignon et al., 2007), which could inform future research into genetic variations affecting haemangiosarcoma diagnosis risk.

Neutered females and neutered males were at a slightly increased risk of haemangiosarcoma diagnosis compared to entire females, partially aligning with findings from an Italian study of visceral haemangiosarcoma (Carnio et al., 2020). The current study did not find entire males at increased risk compared to entire females, unlike the Italian study. The role of sex hormones in the pathogenesis of haemangiosarcoma is unclear in animals. Research on humans suggests that sex hormones may influence cancer development by modulating inflammatory pathways, but their exact role remains unclear (Lawrence et al., 2007). The odds of haemangiosarcoma diagnosis increased with age from 5 to 13 years but declined slightly in dogs over 13 years. This non-linear trend aligns with previous findings regarding cutaneous and cardiac haemangiosarcoma (Ware and Hopper, 1999; Villamil et al., 2011). The apparent reduced risk in geriatric dogs may result from underdiagnosis, as owners might attribute clinical signs of haemangiosarcoma to aging and delayed veterinary visits (Wallis et al., 2024). Veterinarians should stress vigilance for haemangiosarcoma clinical signs in senior dogs, especially those over 11 years old.

Lower clinic deprivation levels were associated with higher odds of haemangiosarcoma diagnosis. This finding may reflect that in less deprived areas, owners may have higher disposable income which, due to greater affordability of diagnostic testing and more frequent veterinary care, increases the likelihood of haemangiosarcoma diagnosis. Dogs from clinics in

mixed urban/rural areas had lower odds of haemangiosarcoma diagnosis compared to urban clinics, a novel finding that warrants further investigation into environmental and social factors that differ between locations.

This study had some limitations; data were not collected primarily for research purposes and relied on final diagnoses of haemangiosarcoma recorded in the EHR. Diagnoses without histopathology could also have been influenced by confirmation bias based on previously reported risk factors (Brown et al., 1985; Ward et al., 1994; Villamil et al., 2011; Dobson, 2013; Yamamoto et al., 2013; Grüntzig et al., 2016; O'Byrne and Hosgood, 2019; Carnio et al., 2020; Robinson et al., 2020; Tompkins et al., 2020). However, in primary care, many diagnoses are additionally based on clinical criteria due to financial and/or practical constraints, reflecting real-world practice. Risk factor analysis for less common breeds may have been underpowered, and restricting the analysis to dogs aged five years and older reduces the generalisability of findings to younger dogs.

In conclusion, this study estimates the annual incidence risk of haemangiosarcoma diagnosis in dogs of all ages in primary care practice to be 0.036%, while the incidence in dogs aged five years and older was 0.074%. The Dogue de Bordeaux, Flat Coated Retrievers, and German Shepherd Dogs showed the highest breed predisposition. Older age was strongly associated with haemangiosarcoma diagnosis, while neutering, lower clinic deprivation and urban clinics appear to be less strongly associated with diagnosis. These findings highlight the need for further research into previously unidentified environmental and genetic factors that may play a role in the diagnosis of canine haemangiosarcoma. Future studies could examine the role of individual socioeconomic measures, potentially clarifying the role of deprivation in haemangiosarcoma diagnosis risk. High-risk breeds require proactive monitoring, and veterinarians should remain vigilant for haemangiosarcoma signs in senior dogs to facilitate early intervention.

ACKNOWLEDGEMENTS

The authors are grateful to Morris Animal Foundation for their funding of this research. Thanks to Noel Kennedy (RVC) for VetCompass software and programming development. We acknowledge the Medivet Veterinary Partnership, Vets for Pets/Companion Care, Goddard Veterinary Group, CVS Group, IVC Evidensia, Linnaeus Group, Beaumont Sainsbury Animal Hospital, PDSA, Blue Cross, Dogs Trust, Vets Now and the other UK practices who collaborate in VetCompass. We are grateful to The Kennel Club Charitable Trust, Agria Pet Insurance and The Kennel Club for supporting VetCompass.

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ENVIRONMENTAL RISK FACTORS AND CANINE LEPTOSPIROSIS IN EASTERN COASTAL AUSTRALIA: A REGIONAL STUDY

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SUMMARY

By analysing clinical canine leptospirosis in southern New South Wales (NSW), Australia (October 2021 – May 2023), the associations between environmental factors and the local occurrence of canine leptospirosis were examined. Poisson models (log-transformed canine population offset) of landscape and socioeconomic factors, and Cox Proportional Hazards models of cumulative temperature and precipitation were developed. During the 2022 winter, a significant cluster of canine leptospirosis was identified near lakes, overlapping with the heavily flooded areas in March 2022. The risk of canine leptospirosis was increased by the presence of nature conservation areas and reduced by the presence of recreational areas within the relevant neighbourhood. Extreme climate events (high temperature and heavy precipitation) over the preceding three-month period significantly increased disease hazards. The re-emergence of canine leptospirosis in southern NSW was multifactorial, with hydrological events likely the main driving factor. Increased pet owner awareness during and following such events is advocated.

INTRODUCTION

Leptospirosis is a zoonotic disease that causes enormous fatalities and economic losses globally (Ellis, 2015; Haake and Levett, 2015). The pathogenic *Leptospira* species of subclades P1 and P2 are mainly transmitted by direct contact with reservoir hosts (e.g., rodents and cattle), or indirect exposure to environmental contamination as the bacteria are shed in urine (Ellis, 2015; Vincent et al., 2019). Consequently, human and canine leptospirosis outbreaks have often been associated with certain spatial attributes (rural farms, socioeconomic disadvantaged areas infested by rodents or having open drainage systems) and meteorological events (heavy rainfall and flooding) (Costa et al., 2014; Garvey et al., 2014; Casanovas-Massana et al., 2018; Chadsuthi et al., 2021).

In New South Wales (NSW), Australia, leptospirosis has been rarely reported and long neglected. Starting with the re-emergence of canine leptospirosis in urban Sydney in December 2017 (Griebsch et al., 2022), outbreaks of canine and human leptospirosis have been reported in various locations spontaneously (Katelaris et al., 2020; Australian Veterinary Association, 2022). As epidemiological studies on leptospirosis in Australia are rare, and elsewhere are limited to temperate, developed countries, an exploratory risk factor study of canine

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leptospirosis in urban Sydney was performed (Lu et al., 2023). Surprisingly, the Sydney cases occurred independently of the local climate (temperature and rainfall) and were not predicted by community socioeconomic status. Whilst further investigation of the unique traits that has led to the re-emergence of canine leptospirosis in urban Sydney are ongoing, it is important to confirm whether these traits are shared across NSW environments, and therefore associated with specific *Leptospira* strains.

Shoalhaven, a peri-urban council area in southern coastal NSW (Villaseñor et al., 2015), is another location that has suffered from canine leptospirosis outbreaks. Shoalhaven possesses rich natural and farming assets and no canine leptospirosis cases had been reported until late 2021. However, during the winter of 2022 a massive surge of clinical canine leptospirosis cases was reported in this region, coinciding with heavy precipitation a few months previously (Bureau of Meteorology, 2024a).

This current risk factor study describes the clustering of clinical canine leptospirosis cases in Shoalhaven during 2021–2023, and the effects of environmental factors (elevation, land use, community socioeconomic status, and climate) on canine leptospirosis in the region were explored. A major objective of this study was to determine if extreme meteorological events facilitate the hazards of canine leptospirosis in non-metropolitan NSW.

MATERIALS AND METHODS

Study area and case definition

The current study area included the council area of Shoalhaven, NSW, and residential Statistical Areas Level 1 (SA1s) of Jervis Bay Territory (JBT). JBT is a small Australian Commonwealth Territory (65 km^2) managed by multiple governing bodies, that includes the Shoalhaven Council (Australian Government, 2014). To avoid confusion, the study area is referred to as "the Shoalhaven region". The ambient temperature in the study area ranges from 1.6 – 40.3 °C (monthly mean temperature 12.6 – 21.6 °C). The local mean annual precipitation is 1207.3 mm [the highest annual precipitation recorded in 2022 (2588 mm)] (Bureau of Meteorology, 2024b).

Canine leptospirosis is a non-notifiable disease in NSW, so the case inclusion process relied on voluntary reporting from the local primary-care and referral veterinarians (Griebsch et al., 2022). The included cases presented local veterinary services with typical clinical signs and clinicopathological abnormalities, and were diagnosed with leptospirosis by PCR, Microscopic Agglutination Testing (MAT) or a combination of both diagnostic tests. The cases were de-identified prior to the study, without accessible signalments and medical history. The study period was from 1st October 2021 to 31st May 2023.

Mapping and cluster scanning

The residential coordinates (decimal degrees) of the cases were manually geo-coded using Google Earth (Google, 2023). Mapping of the SA1s was conducted in ArcGIS Pro 2.5.0 (Esri Inc., 2020; Australian Bureau of Statistics, 2021), using the Geocentric Datum of Australia 2020. Non-residential SA1s (defined by no residential household recorded in the 2021 Census) in the study area were excluded (Australian Bureau of Statistics, 2022). The underlying canine population size was estimated by multiplying count of households per SA1 by 0.624 (Animal

Medicines Australia, 2022). Significant spatio-temporal clusters (with monthly precision and up to 50% of the study area) of the cases were identified using the scan statistic (SaTScan v10.1.2) (Kulldorff and Information Management Services Inc., 2023).

Statistical analysis of landscape and socioeconomic factors

Elevation of the Shoalhaven region was extracted from the national Digital Elevation Model (5 m resolution) (Geoscience Australia, 2015) and a mean value of elevation per SA1 was calculated. The other landscape characteristics were adapted from Version 1.5 of the 2017 NSW Landuse dataset (NSW Government, 2023a). The Index of Economic Resources (IER) and Index of Education and Occupation (IEO) were used to simulate the SA1-specific socioeconomic status (Australian Bureau of Statistics, 2023). All modelling was conducted using R Version 4.2.2, via the RStudio interface (2023.09.1+494) (Posit Team, 2023).

A merged spatial buffer (geodesic radius of 2 km) radiating from SA1s with at least one case was created. This buffer was used to select a subset of adjacent SA1s and minimise spatial heterogeneity. Poisson models of all SA1s and SA1s within the buffer (buffered SA1s) were tested respectively. A log-transformed dog population offset was added to all Poisson models. Univariable Poisson models of all variables were tested prior to stepwise development of the multivariable models. Although the significance of IER as an individual predictor variable at overall and buffered levels was assessed, it was also added to the final multivariate models regardless of the univariable results to address its anticipated confounding effects on the landscape–reported clinical cases association.

Three variables (proximity, presence and area in km^2) of each land use factor (water body, nature conservation area, grazing pasture, commercial area and recreational area) were assessed using univariable models. When multiple variables of the same factor were statistically significant at a univariable level, the variable with the lower Akaike information criterion (AIC) was selected for inclusion in the stepwise model. All continuous variables were converted to categorical variables with four (defined by the lower quartile, median, and upper quartile) or two (defined by the median) levels for modelling. In addition, types of residential infrastructure within the SA1 (urban, rural, and mixed) were also assessed as independent predictors.

Statistical analysis of meteorological factors

Daily climate data (precipitation, maximum temperature and minimum temperature) recorded at the Sanctuary Point and Jervis Bay Airfield stations were retrieved from the Climate Data Online database (Bureau of Meteorology, 2024b). A survival time series of canine leptospirosis cases was generated in RStudio, in which each diagnosis was considered an event, and the week of 12th October 2021 (week of the first diagnosis) was set to be the starting time. Lagged climate variables of three temporal resolutions (weekly, monthly and cumulative) were then calculated using this data.

Days of extreme meteorological events (e.g., extremely high daily maximum temperature, extreme rainfall) were calculated for all temporal resolutions by identifying all extreme records ($\geq$ the monthly 90% percentile of all years, or $\leq$ the monthly10% percentile value of all years). The percentiles were calculated from data available at the two observatory stations (daily precipitation records from April 2000 to July 2024, daily temperature records from October 2018 to July 2024).

Univariable Cox proportional hazards (Coxph) models were developed using the lagged climate data and the survival time series. The mean value of concordance indices (C-indices) of a variable at all lags was calculated and used to determine the precipitation and temperature variables in the multivariable models. Therefore, three multivariable mixed effect models (weekly, monthly and cumulative variables) were built. The model with the best predictive quality was selected by assessing C-indices and the prediction residuals (i.e., the differences between prediction values and the observed events) at significant lags.

RESULTS

From 12th October 2021 to 26th May 2023 a total of 23 clinical canine leptospirosis cases living in 17 residential SA1s (17/285 or 5.96% of all SA1s) were diagnosed in the Shoalhaven council area and Jervis Bay (Fig.1). A significant spatio-temporal cluster of canine leptospirosis (radius 5.75km) was detected in seven suburbs (Huskisson, Tomerong, Vicentia, Erowal Bay, St George Bain, Basin View, and Sanctuary Point) during late Autumn and Winter (May – September) of 2022 (Fig. 1). The cluster was located adjacent to lakes (Fig. 1), with

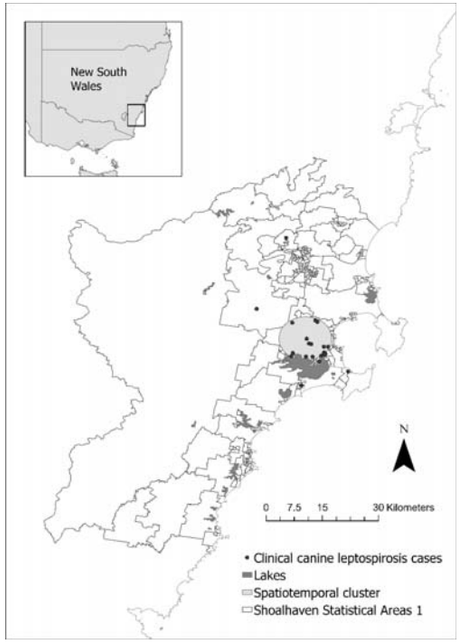

Figure 1. Clinical canine leptospirosis cases (n = 23) diagnosed in the Shoalhaven region (2021–2023). A significant spatio-temporal cluster of cases was present from 1st May to 30th September 2022 (grey circle).

the most prominent being St George Basin, the largest lake south of the spatio-temporal cluster. Half (i.e. 8/16) of the clustered cases were diagnosed in June 2022.

When all SA1s (n = 285 in the univariable models, n = 280 in the final model due to missing IER values) were included in the Poisson models with log-transformed canine population offset, no significant association was detected between elevation and the occurrence of canine leptospirosis. In addition, socioeconomic factors (IER and IEO) as independent predictors did not show a significant impact on the disease. The IER was retained in the final multivariable model as it had confounding effects on other land use factors (e.g., living in a strict urban SA1, living in proximity to lakes) and canine leptospirosis. The dichotomous presence of recreational areas was included in the multivariable model due to its lower AIC score (AIC 156.61 versus AIC 171.46 for the proximity univariable model). The final multivariable model of all 280 SA1s (Table 1) indicated a positive association between proximity to lakes and canine leptospirosis, and a protective effect of the presence of recreational area in the SA1 on the disease occurrence Relative Risk (RR) = 0.16 (95% CI: 0.05 – 0.41, $p < 0.001$).

Table 1. Relative Risks (RRs) and 95 % Confidence Intervals (CIs) for the association between environmental factors and canine leptospirosis in the Shoalhaven region (2021-2023), obtained from the multivariable Poisson regression model adjusted for the Index of Economic Resources.

Variables	Levels	Case count	Case Statistical Areas Level 1 (SA1s) (Total SA1s)[a]	RR (95% CI)	p-value
Recreational areas in the SA1?	No	18	12 (95)	Ref	
	Yes	5	5 (185)	0.2 (0.1 – 0.4)	< 0.001[b]
Within 2 km to the nearest lake?	No	12	9 (214)	Ref	
	Yes	11	8 (66)	4.5 (1.8 – 11.4)	0.001[b]
Index of Economic Resources	≤ 952	3	3 (69)	Ref	
	953 - 999	6	4 (70)	1.3 (0.3 – 6.2)	0.72
	1000 - 1048	4	3 (70)	1.1 (0.2 – 5.7)	0.89
	≥ 1049	10	7 (71)	3.2 (0.9 – 15.1)	0.09

[a]Case SA1s are the number of SA1s with at least one clinical canine leptospirosis patient and Total SA1s are the total number of SA1s at a given level of the variable. There were multiple canine patients lived on the same SA1 and therefore the count of Case SA1s diffed from the count of canine patients.

[b]Statistical significance ($p < 0.05$).

Table 2 summarises the multivariable Poisson model of all buffered SA1s (n = 83), adjusted for IER. The presence of recreational areas in the SA1 had a similar negative association with canine leptospirosis (RR = 0.13; 95% CI: 0.04 – 0.34, $p < 0.001$). Living in an SA1 with nature conservation areas also increased the risk of canine leptospirosis (RR = 2.47; 95% CI: 1.0 – 6.06, $p = 0.047$). Proximity to lakes did not affect the risk within the 2 km spatial buffer.

Table 2. Relative Risks (RRs) and 95% Confidence Intervals (CIs) for the association between environmental factors and canine leptospirosis in a merged 2-km spatial buffer in The Shoalhaven region (2021-2023), obtained from the multivariable Poisson regression model adjusted for the Index of Economic Resources.

Variables	Levels	Case count	Case Statistical Areas Level (SA1s) (Total Buffered SA1s)[a]	RR (95% CI)	p-value
Recreational areas in the SA1?	No	18	12 (27)	Ref	
	Yes	5	5 (56)	0.1 (0 – 0.3)	< 0.001[b]
Nature conservation areas in the SA1?	No	13	10 (65)	Ref	
	Yes	10	7 (18)	2.5 (1.0 – 6.1)	0.047[b]
Index of Economic Resources	≤ 995	8	6 (41)	Ref	
	> 995	15	11 (42)	1.0 (0.4 – 2.7)	0.97

[a]Case SA1s are the number of SA1s with at least one clinical canine leptospirosis patient, and Total Buffered SA1s are the total number of SA1s at a given level of the variable, within the spatial buffer. There were multiple canine patients lived on the same SA1, and therefore the count of Case SA1s diffed from the count of canine patients.

[b]Statistical significant ($p < 0.05$).

In terms of Coxph models incorporating the lagged local climate data, the cumulative models were selected for their higher C-indices and less sparse prediction residuals (Table 3).

Table 3. Concordance indices (C-indices) and prediction residuals for the final multivariable mixed effect Cox Proportional Hazards models of three temporal resolutions (weekly, monthly, cumulative).

Temporal resolution	Significant lags ($p < 0.05$)	C-index	Prediction residuals
Weekly	–1 week	0.782	–3.04 – 0.97
	–3 weeks	0.739	
	–9 weeks	0.761	
	–10 weeks	0.613	
Monthly	–2 months	0.761	–3.18 – 1.0
	–3 months	0.782	
	–6 months	0.664	
Cumulative	–3 weeks	0.798	–1.87 – 1.0
	–11 weeks	0.891	
	–12 weeks	0.920	

A significant ($p < 0.05$) interaction effect of cumulative precipitation and days with high temperature was detected at the temporal lags of –2, –3, –4 and –12 weeks (Table 4). For both climate variables, a significant protective effect during the three-week period prior to the event (Hazard Ratio (HR) = 0.41) and significant detrimental effect over the longer period preceding the event (around 12 weeks, i.e., 3 months) (days with extremely high maximum temperature HR = 2.7, cumulative precipitation HR = 78.63) were identified.

Table 4. Hazard Ratios (HRs) and 95% Confidence Intervals (CIs) for the association between cumulative (cum.) climate variables and canine leptospirosis cases (n = 23) in Shoalhaven (2021-2023) at different lag weeks (wks), obtained from the multivariable Cox Proportional Hazards model.

Lags (wks.)	Cum. days with extremely high daily maximum temperature		Cum. precipitation (/100 mm)		Cum. high temperature days - Cum. precipitation interaction	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
–2	0.5 (0.3 – 1.1)	0.08	0.7 (0.1 – 3.8)	0.70	2.4 (1.1 – 5.4)	0.04[a]
–3	0.4 (0.2 – 0.8)	0.01[a]	0.4 (0.2 – 1.0)	0.04[a]	2.4 (1.4 – 3.9)	0.001[a]
–4	0.7 (0.4 – 1.1)	0.11	0.7 (0.2 – 1.6)	0.27	1.4 (1.0 – 2.0)	0.04[a]
–11	1.5 (0.9 – 2.4)	0.11	6.9 (1.4 – 34.4)	0.02[a]	1.0 (0.9 – 1.0)	0.23
–12	2.7 (1.2 – 6.3)	0.02[a]	78.6 (3.0 – 2089.0)	0.01[a]	0.8 (0.7 – 1.0)	0.03[a]

[a] Statistical significant ($p < 0.05$).

DISCUSSION

In regional south New South Wales, Australia, the re-emergence of canine leptospirosis was markedly influenced by the environment, specifically landscape and extreme climate events. The effect of extreme precipitation and high temperature appeared biphasic: within a short duration (≤ 3 weeks), such conditions limited the occurrence of canine leptospirosis, whereas over the longer term (≤ 3 months), the extreme meteorological events facilitated the risk of canine leptospirosis. Spatially the cases resided in areas adjacent to lakes, in which the risk of canine leptospirosis was further increased by the presence of nature conservation areas (e.g., strict nature reserves, national parks, and other conserved areas) in the residential SA1. Combining all evidence gathered, the re-emergence of canine leptospirosis in the Shoalhaven region was potentially multifactorial, and essentially driven by hydrological events.

From Winter 2020 to Summer 2023, Australia was in the co-occurring phase of La Niña within the El Niño Southern Oscillation cycle, and negative Indian Ocean Dipole (Rathore et al., 2021; Lawrence et al., 2022; Bureau of Meteorology, 2024c,d). Consequently, vast regions of Australia, including the Shoalhaven region, experienced extreme precipitation anomalies. During the initial re-emergence and the most intense phase of canine leptospirosis in 2021–2022, the following events were observed in the Shoalhaven: high precipitation and floods in March and May 2021, a dry winter in 2021, extremely high precipitation from January to October 2022, and severe flooding in March and July 2022) (Shoalhaven City Council, 2021a,b, 2022a,b; Bureau of Meteorology, 2024a). While a challenge in the current study was the lack of availability of detailed flooding records in the study area, the heavily flooded suburbs listed in the council-issued evacuation orders (Sanctuary Point, Old Erowal Bay and St George Basin) (Shoalhaven City Council, 2022a) matched the suburbs included in the 2022 spatio-temporal cluster of canine leptospirosis. Although complete hydrological records in Shoalhaven are needed for a more specific study investigating the role of precipitation and transient floods on canine leptospirosis re-mergence, the contribution of extreme flooding in March 2022 to the canine leptospirosis outbreak during the following winter (approximately three months later) was evident.

Based on the current findings, two flood-related transmission pathways of leptospirosis in Shoalhaven can be hypothesised: environmental spillover via riverine floods and soil erosion (Davignon et al., 2024; Sato et al., 2024; Thibeaux et al., 2024), and interspecies spillover via altered wildlife activity due to loss of shelter or food resources in natural habitats, or accumulated waste in residential areas post-flooding (Shoalhaven City Council, 2022b). The significant association between the presence of nature reserves and canine leptospirosis may indicate that wildlife dwelling in the nature conservation areas were more important reservoir hosts, but previous ecological studies did not find a strong edge effect along the urban-forest boundary in the study area (Villaseñor et al., 2014; Villaseñor et al., 2015). The study area (more specifically, the area within the 2 km buffer from case SA1s) features a mosaic landscape of urban settlements, grazing livestock farms and heavily vegetated conservation areas. Local wild animals frequently travel across the habitat edges, which presents considerable difficulty in tracing the sources of pathogenic *Leptospira*. Therefore, a role of pastoral wildlife populations in transmitting *Leptospira* in Shoalhaven should not be fully rejected until further field studies are performed.

The protective effect of neighbouring recreational areas on canine leptospirosis at both spatial scales was an interesting finding. A similar effect of recreational area (OR = 0.26, p = 0.053) has also been detected in Sydney, the capital city of NSW (Lu et al., 2023). This effect is likely common in the state of NSW, as on-leash dogs are allowed in most public spaces (including recreational parks, and public pavements) (NSW Government, 2023b). Those who live in SA1s without convenient access to parks may have to walk longer distances to access these amenities, and thus are exposed to greater risks of exposure during such trips.

Overall, the long-term relationship between hydrological events and canine leptospirosis that was found in this study is consistent with previous reports of human leptospirosis outbreaks in endemic countries (Cunha et al., 2022; Douchet et al., 2022), and potentially canine leptospirosis in developed countries such as the United States and Switzerland (Ward, 2002; Major et al., 2014). In contrast, conflicting reports regarding the impact of high maximum temperature have been documented in multiple studies (Desvars et al., 2011; Chadsuthi et al., 2012; Chadsuthi et al., 2021; Cunha et al., 2022). The effect of temperature needs to be discussed in specific context as it varies dramatically in different local climate zones, and it is also affected by the variables selected for statistical analysis (e.g., monthly maximum temperature *versus* monthly days with high maximum temperature). The variable used in the multivariable model (cumulative days with extremely high maximum temperature) represented the higher extremity of the relative maximum temperature. It also partially neglects the influence of the naturally occurring seasonal temperature variation, because only a relatively short time period (20 months) was analysed, and the limited data was suboptimal for assessing seasonality. It needs to be acknowledged that the local climate of Shoalhaven (humid temperate) is dissimilar to the tropical areas where previous studies have been conducted (Cunha et al., 2022; Bureau of Meteorology, 2024e). The vegetation-abundant, peri-urbanised landscape also greatly differs from the City of Sydney, where canine leptospirosis was less climate-dependent (Lu et al., 2023).

In conclusion, this study offers novel and valuable evidence of the risk factors contributing to canine leptospirosis in a temperate, non-metropolitan region of Australia. Canine leptospirosis in Shoalhaven demonstrated different dynamics compared to the same disease in urban Sydney. This knowledge suggests that tailor made information should be developed for pet owners to reduce the risk and impact of canine leptospirosis within specific areas.

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BLUETONGUE DISEASE

HOW TO MANAGE FUTURE BLUETONGUE SEROTYPE 3 OUTBREAKS IN CATTLE AND SHEEP FARMS: PREVALENCE AND RISK FACTORS IDENTIFIED FROM THE 2023 OUTBREAK IN THE NETHERLANDS

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SUMMARY

After the 2023 bluetongue virus serotype 3 (BTV-3) epidemic, there was a need to evaluate the prevalence and associated risk factors in preparation for 2024. Bulk milk samples of 12,756 dairy herds and 13 blood samples in 387 sheep flocks were tested for BTV antibodies. A questionnaire related to management, BTV-3, and animal movement data was distributed. In 64% (95% CI: 63-65) of dairy cattle herds BTV antibodies were found with an average within-herd prevalence of 36% (95% CI: 35-37). Factors associated with lower odds for BTV antibodies included keeping cattle indoors, large barn ventilation openings and mechanical ventilation. Factors associated with higher odds included purchase of cattle from BTV-3 high risk areas. Sheep-flock prevalence was 47% (95% CI: 41-52) with an average within-flock prevalence of 23% (95% CI: 5-54). Keeping sheep indoors and shearing in late winter/early spring 2023 was identified as protective. Our findings provide measures that may reduce the impact of BTV-3.

INTRODUCTION

Bluetongue (BT) is an infectious, non-contagious arthropod-borne disease of mainly ruminants. BT is caused by bluetongue virus (BTV), a segmented double stranded virus belonging to the genus Orbivirus from the Reoviridae family. BTV is almost exclusively transmitted by midges of the genus Culicoides (Maclachlan et al., 2009 & 2015). BTV causes haemorrhagic disease in ruminants and is characterised by high fever, depression, mucosal lesions in mouth and nose, congestion of the coronary band, hypersalivation, nasal discharge, lameness and mortality. BTV affects most species of wild and domestics ruminants, but clinical signs and mortality are more severe in domestic sheep, compared to cattle and goats. The first outbreak of BTV in the Netherlands was identified in 2006 and was determined to be BTV serotype 8 (Elbers et al., 2006). After a combination of acquirement of natural immunity and a successful vaccination campaign, the virus stopped spreading and the Netherlands regained its official free status in February 2012. On September 3 2023, two veterinary practices notified BT-like symptoms in five sheep farms located in close proximity in the centre of the

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Netherlands. Two days later, BTV was confirmed by the national reference laboratory (WBVR, Lelystad, The Netherlands), and on September 8, whole genome sequencing identified serotype 3 as the causal serotype (Holwerda et al., 2024). In the subsequent weeks, bluetongue virus serotype 3 (BTV-3) spread rapidly. During this first epidemic year, more than five thousand sheep and cattle farms were infected by BTV-3 (Van den Brink et al., 2024). The impact was substantial, with severe clinical signs in both cattle and sheep farms (van den Brink et al., 2024) and particular high mortality in sheep flocks (Santman-Berends et al., 2024; Van den Brink et al., 2024; Van den Brink et al., 2025).

In preparation for the vector season of 2024, there was a need to evaluate antibody prevalences in cattle and sheep to obtain insight into the proportion of the sheep and cattle population that would be protected in 2024. As case fatality rates were much higher in sheep than in cattle, it was assumed that the prevalence in one species could not be extrapolated to the other. Therefore, it was decided to study both sheep and cattle populations. Additionally, it was unclear whether farmers could take precautionary measures to protect their animals from the virus during the next vector season, especially as it was unknown at that time if vaccination would become possible in the near future. Consequently, it was decided to combine both prevalence studies with a risk factor evaluation.

The primary aim of this study was to evaluate the farm-level, within-farm level and animal-level BTV antibody prevalence in sheep and cattle following the 2023 BTV-3 epidemic in the Netherlands. Secondly, risk factors associated with BTV antibody positivity at the farm-level and a high within-farm prevalence, were identified.

MATERIALS AND METHODS

Study population

Cattle: Bulk tank milk (BTM) samples are a convenient and low-cost sample type to estimate BT seroprevalence in dairy herds (Mars et al., 2010). In the Netherlands, BTM samples are collected and tested for milk quality after each milk collection (two or three times per week) by Qlip laboratories (Zutphen, the Netherlands). For several disease control programmes (Santman-Berends et al., 2021), monthly milk samples are automatically sent to Royal GD (Deventer, the Netherlands). This automatic flow of milk samples enabled the selection of a BTM sample from each dairy herd. Given BT's vector borne nature, it was assumed that dairy herds are representative for the target population (all cattle herds). Therefore, the study population consisted of almost all dairy cattle farms in the Netherlands. All dairy farmers were informed that they would be anonymously included in this study in April 2024 and asked i) to reply if they did not agree with participation, ii) to indicate whether they wanted their herd-specific result (free of charge) and iii) to state if they wished to participate in a short survey investigating risk factors for BTV infection. If they agreed to participate in the survey they were immediately redirected to the online survey.

Sheep: In the Netherlands, a random sample of 1,475 small ruminant farms with >10 animals are selected each year to submit serum samples of 13 animals >1 year of age for the national *Brucella melitensis* monitoring programme (van den Brom et al., 2012). From these, 500 sheep farms were randomly selected for this study, stratified to the 20 regions (i.e. 25 flocks/region) that are distinguished based on geographic boundaries as proposed in Commission Decision 2005/393/EC (Fig. 1). In April 2024, farmers were asked whether they

wanted to participate in this study. Farmers that agreed to participate filled in a short survey and gave permission to use the collected sheep samples for BTV antibody evaluation. A total of 374 farmers agreed to participate and from each flock the 13 serum samples submitted between December 2023 and April 2024 were included in this study. Thirteen samples per flock is deemed sufficient to detect presence of BTV in a free situation without vaccination according to EU regulation 1108/2008/EC, article 2.2.2.

Diagnostic evaluation

Cattle: To evaluate the herd and within-herd BTV antibody status, samples were tested using the ID Screen Bluetongue milk indirect ELISA (IDVet®) (Mars et al., 2010). This ELISA detects antibodies against all BT serotypes and has a 100% (95% CI: 83-100) sensitivity and >97.8% (95% CI: 92-100) specificity to detect >1% BTV prevalence in milk. The test was subsequently adapted and validated in-house at Royal GD to enable an indication of a semi-quantitative BTV within herd prevalence (Table 1). Farmers were informed about their test result on May 15, 2024.

Sheep: the Idexx® Bluetongue competition antibody test, that is accredited under ISO17025 at Royal GD, was used for antibody evaluation of each sampled individual sheep. This ELISA detects antibodies against all BT serotypes and its sensitivity relative to the test of the national reference laboratory was 100% (95% CI: 97.5-100). The specificity was 99.83% (95% CI: 99.69-99.97).

As the antibody tests used in this study are sensitive to all BT serotypes, antibodies against BTV-8 induced by vaccination could interfere with the study results. Therefore, data from farms that vaccinated against BTV-8 in the previous five years were excluded from the study. Whether or not farmers vaccinated was determined based on the survey results (sheep farmers) or via the national database on compulsory registration of veterinary medicines (MediRund) (cattle farms).

Questionnaires

A short survey was developed that included questions about the herd's BTV-3 status, observed clinical signs of BT in 2023 and/or confirmation of infection by PCR, administration of BTV serotype 8 vaccines in the previous five years and questions related to farm management in 2023. Management questions included questions that could potentially be associated with BT or that were found in previous studies as being associated with BT. These questions included grazing (who, when and how long), size of ventilation openings in the barn, use of mechanical ventilation, wind break curtain, use of insecticides, moment of shearing sheep and presence of other BTV susceptible species on the farm. All selected farmers were contacted by email or post on 16 April 2024. On 23 April a reminder was send and on 10 May, the survey was closed to ensure that the replies were not influenced by the BTV antibody results. The surveys for cattle and sheep are available (in Dutch) upon request.

Available data

Data from three sources were available for analysis. Diagnostic results were available from all participating cattle herds and sheep flocks. Survey results were available and were combined with the diagnostic data at farm level. Identification and registration data (RVO, Assen the Netherlands) were available to determine herd size and animal movements during the BTV-3 epidemic, i.e. between September and December 2023.

Analyses

Prevalence estimation: For cattle the farm-level prevalence was defined as the percentage of herds with a positive BTM result. The within-herd prevalence was estimated for the group of cattle herds with an antibody positive test result and was first estimated at the farm level based on the BTM outcome (Table 1). The herd-level results were subsequently averaged over all herds. In sheep, the farm-level prevalence was defined as the percentage of sheep flocks in which at least one out of 13 sampled sheep tested BTV antibody positive. For calculation of the within-herd prevalence, only BTV antibody positive flocks were included. The within-flock prevalence was subsequently calculated as percentage of sampled sheep that tested antibody positive relative to the 13 sampled sheep per flock.

For calculation of the animal level prevalences in the cattle and sheep populations, within-herd prevalences of sampled herds were corrected for herd size (Eq. (1)), to adjust for the fact that larger farms will contribute more to the overall animal-level prevalence than smaller farms. Additionally, for sheep flocks, the animal prevalence was also corrected for clustering of sampled sheep within the same flock.

$$ANprev_j = \frac{\sum_{j=0}^{n} prevalence_j * animals_j}{\sum_{j=0}^{n} animals_j} \quad (1)$$

where:

ANprev$_j$: Animal-level BTV-3 prevalence in farm *j*
prevalence: Estimated within-farm prevalence of farm *j*
animals: The number of adult cattle (>2 year) or the number of sheep (>1 year) at farm *j*

Risk factor evaluation: Stata® 17 (Stata, 2022) was used for data validation, descriptive statistics and multivariable analyses. First, diagnostic results were combined with the identification data and the survey results. For both cattle and sheep, two types of models were developed: 1) a model to identify risk factors for presence of BTV antibodies at farm level, and 2) a model to identify risk factors for (high) within-herd spread of BTV on antibody positive farms. For each model, regression analysis was performed, using appropriate distribution functions depending on the nature of the dependent variable.

Cattle: To evaluate risk factors associated with BTV cattle herd antibody status, herds were classified as either BTV BTM antibody positive or antibody negative. In the cattle within-herd model, a high BTV antibody positive cattle herd was defined as having a highly or very highly positive BTM result. Herds with a low positive or positive result were classified as having a low BTV within-herd prevalence. Logistic regression was applied (xtlogit) to quantify the association between potential risk factors and the probability of i) being BTM BTV antibody positive or ii) having a (very) high positive BTM result. Risk factors were expressed as odds ratios (OR) with 95% confidence intervals (CI).

Sheep: To evaluate risk factors associated with BTV sheep flock serostatus, flocks were classified as either BTV antibody positive, meaning at least one out of 13 sheep tested positive for BTV antibodies, or BTV seronegative. Risk factors were expressed as odds ratios (OR) with 95% CI. Given the very high mortality rates in sheep flocks in 2023, the within-herd prevalence that resulted from the diagnostic evaluation was likely an underestimate of the true proportion of sheep that became BTV-3 infected in 2023 (Santman-Berends et al., 2024). This

hampered the identification of risk factors for high within-flock spread in antibody positive flocks. Therefore, it was decided to combine the within-herd prevalence of antibody positive flocks with the number of sheep that died in that herd between September and December 2023. For the within-flock spread risk evaluation, these mortality-adjusted percentages of affected sheep per flock were log-transformed and subsequently analysed via linear regression (regress). Risk factor coefficients were back-transformed and are therefore expressed as incidence rate ratios (IRR).

In each model for sheep and cattle, BTV antibody status or level of the farm was included as dependent variable. Potential risk factors were included as explanatory variables. In both cattle and sheep models an explanatory variable was included that controlled for the geographical location of the farm related to the initial outbreak area (low, medium or high risk, Fig. 1). This was done to ensure that regional differences between farm management were not wrongfully assigned as being associated with the farm's BTV antibody status.

Figure 1. The Netherlands subdivided in risk areas for BTV-3 in 2023 based on the number of BTV notifications per area. Source: NVWA, Utrecht, the Netherlands.

Additionally, the following explanatory factors were evaluated:

Cattle: grazing (number of hours/day in September, October and November, and number of days in each month), ventilation openings at the side of the barn or an open front barn, presence of wind break curtain, use of mechanical ventilation, use of insecticides or repellents in autumn 2023, herd size and whether other precautionary measures were taken. Additionally, a categorical interaction term representing on-farm movement of cattle between Sept-Dec 2023 (yes/no) and the origin of those cattle (low/medium/high-risk area, Fig. 1) was included. Furthermore, presence of sheep on the farm and whether or not they showed clinical signs of BTV in 2023 was evaluated.

Sheep: preventive indoor housing of sheep, use of mechanical ventilation when sheep are kept indoors, use of insecticides or repellents in autumn 2023, most recent month of shearing, flock size and whether other precautionary measures were taken. Similar to the analyses for cattle an interaction term between on-farm movement of sheep between September and December 2023 and risk profile of location of origin was included (Fig. 1).

Given the limited number of variables in the models, it was unnecessary to conduct an univariable pre-screening and all variables were directly included in the full multivariable models. For final model selection a backward selection and elimination method was used. Each round, the variable with the highest *p*-value was excluded based on the likelihood ratio test until all variables were significantly (*p*-value<0.05) associated with the BTV antibody status. During this process confounding was monitored by the change in the coefficient of a variable after removing another variable. If the change of the estimates exceeded 25%, the removed variable was considered a potential confounder and was re-entered in the model. In the final model, all biological logical two-way interactions were evaluated and included if significant. Normality of linear model residuals was checked using normal probability plots and the normality assumption was met for the within-flock prevalence after log transformation. The goodness-of-fit of the logistic models was assessed using Pearson's goodness of fittest (estat gof) and visual inspection of diagnostic plots of deviance residuals. Statistical dependence between independent variables, potentially leading to multicollinearity, was monitored by examining pairwise correlation coefficients prior to multivariable analysis. A maximum of 0.50 between two variables was set.

RESULTS

Prevalence of BTV-3 antibodies after the 2023 epidemic

A BTM antibody result was available for 12,756 dairy herds that agreed to participate in this study and that did not vaccinate against BTV serotype 8 in the five preceding years. In total, 64% (95% CI: 63-65) of all dairy herds tested positive for BTV antibodies. Twenty percent of the BTM samples tested high or very high positive (Table 1). The regional herd prevalence of BTV antibodies in cattle varied between 5% of the farms being positive (southern part of the country) to 99% of the farms being positive (centre part of the country) (Fig. 2). Within antibody positive cattle herds, it was estimated that on average 36% (95% CI: 35-37) of the milking cows tested antibody positive. The overall animal-level prevalence was estimated at 23% (95% CI: 22-24), with again a high variation between regions within the country (Fig. 2).

Table 1. Number and percentage of Dutch dairy cattle farms per bulk tank milk bluetongue antibody class after the BTV serotype 3 epidemic in 2023.

Bluetongue result	Herd-level interpretation	Number of herds	Percentage herds (95% confidence interval)
Negative	No antibodies detected	4,613	36% (35% - 37%)
Low positive	>0 to ≤25% of the cattle antibody positive	3,335	26% (25% - 27%)
Positive	>25 to ≤50% of the cattle antibody positive	2,241	18% (17% - 19%)
High positive	>50 to ≤75% of the cattle antibody positive	2,328	18% (17% - 19%)
Very high positive	>75% of the cattle antibody positive	239	2% (1,8% - 2,3%)

In total, 4,947 sheep test results from 387 sheep flocks were available of which 527 tested BTV antibody positive. In 47% of the sheep flocks (95% CI: 41-52) at least one of the sheep

tested antibody positive. Similar to the results of cattle, highest prevalences were found in the area were BTV-3 was first detected (93% of tested flocks) and declined with increasing distance from the initial outbreak area (Fig. 1 & 2). Within BTV antibody positive flocks 23% of sheep tested antibody positive (95% CI: 5-54). Overall, at animal level 10% (95% CI: 8-13) of the examined sheep tested antibody positive (Fig. 2).

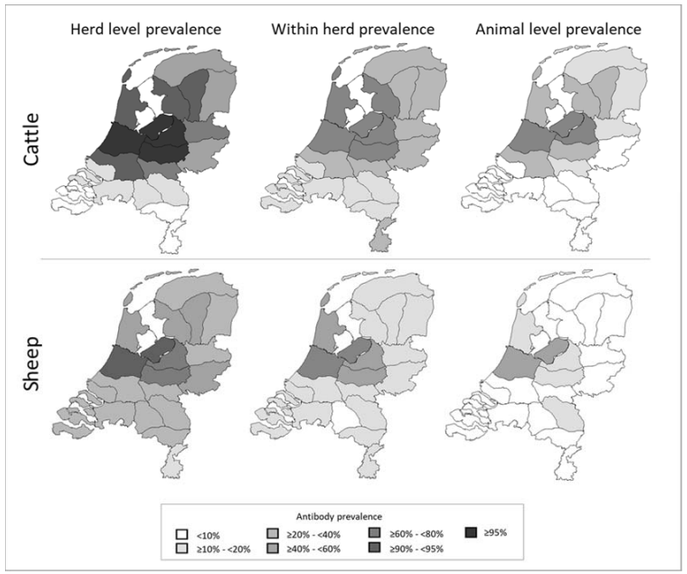

Figure 2. Herd-level, within-herd and animal-level bluetongue antibody prevalence per compartment in 12,576 cattle herds and 387 sheep flocks after the BTV serotype 3 epidemic in the Netherlands in 2023

Risk factors associated with bluetongue serotype 3 antibodies in cattle

In total, 5.253 dairy farmers who had not vaccinated against BTV-8, participated in the survey and provided a mandate to use their diagnostic BTM result for risk factor evaluation. Herd-level antibody prevalence in this group was slightly higher compared to the complete dairy population (68% vs 64%, respectively). Participating farmers indicated that BT-like clinical signs in 2023 were most often observed in cattle >1 year (58%) and less often in cattle <1 year (21%).

Eleven variables that were potentially associated with herd-level BTV antibody status, were included in the multivariable logistic model. Only records from dairy herds with complete data were entered in the model, and after model selection, six variables were retained in the final model (Table 2; Model 1). Grazing time was associated with 4.4 times (≤360 grazing hours in autumn of 2023) or 7.5 times higher odds (>360 hours) of being BTV antibody positive compared to keeping the cattle indoors (Table 2). Farmers that introduced cattle during the

BTV-3 epidemic period had a significantly higher risk (3.7 times) of testing BTM antibody positive if the introduced animal originated from a BT high risk area, compared to no introduction at all. Other factors associated with the presence of BTV antibodies in cattle herds included having sheep with clinical signs of BT and being located in a medium or high BT risk area (Table 2). Also, herds with large ventilation openings in their barns tested 1.3 times less often BTV antibody positive compared to herds with small ventilation openings (OR=0.76, Table 2). Additionally, always applying mechanical ventilation when cattle were in the barn was associated with 1.3 times lower odds of testing BTV antibody positive (OR=0.75).

Data from 2,888 dairy herds were included in the final multivariable model that evaluated risk factors associated with an indication of a high BTV within-herd prevalence i.e. BTM high or very high positive. Similar to the herd level model, the location of the farm, grazing during the BTV epidemic, and presence of sick sheep were associated with a higher odds for high within-herd prevalence (Table 2; Model 2). Mechanical ventilation and large ventilation openings with or without windbreak curtains were associated with a lower odds of having a high within-herd prevalence. Finally, larger farms with higher numbers of cattle had a lower odds to test high positive compared to smaller farms (Table 2). Factors that were not associated with the herd nor the within-herd BTV status included amongst others the use of insecticides.

Risk factors associated with bluetongue serotype 3 antibodies in sheep

To evaluate the risk factors associated with BTV flock status, sheep flocks were classified as either BTV antibody positive, meaning at least one out of thirteen sheep tested positive for BTV antibodies (n=180), or all 13 sheep were BTV seronegative (n=207). Complete data were available for 384 flocks, which were included in the multivariable analysis. Of the seven risk factors assessed (location, shearing, use of insecticides, flock size, keeping sheep indoors, ventilation and other measures taken to prevent BTV), three were significantly associated with the herd-level BTV antibody status (Table 3; Model 3). Flocks of farmers who kept sheep indoors preventively were four times less likely to be BTV antibody positive (OR: 0.25), compared to flocks that were kept outside. Furthermore, shearing sheep in spring tended to be associated with a higher odds to test BTV antibody positive compared to shearing in winter/early spring (OR: 1.6). Furthermore, farms located in medium or high BTV-3 risk areas were associated with a higher odds to test BTV antibody positive (Table 3; Model 3).

Within infected flocks (N=186), shearing between July-September was associated with a higher within-flock prevalence compared to shearing between January-March (IRR: 0.48) (Table 2; Model 4). Also, largest flock size (>99 sheep) was associated with a lower within-flock prevalence (IRR: 0.51). Flocks that were kept indoors preventively tended to have lower within-flock prevalence than those that were kept outside (IRR: 0.61). Ventilation openings in the barns and the use of insecticides were not significantly associated with the flock level nor the within-flock level BTV status.

Table 2. Risk factors associated with presence of BTV (Model 1) or with high versus low within-herd prevalence (Model 2) in Dutch dairy cattle herds after the BTV-3 epidemic in 2023 based on multivariable logistic regression models, with odds ratio (OR), 95% confidence interval (CI) and *p*-value. Associations with a *p*-value <0.05 are displayed in bold.

Parameter	**Model 1** Herd level BTV status (positive/ negative) N=4,286				**Model 2** Within-herd BTV status (high/ low prevalence) N=2,888			
	OR	95% CI		*p*-value	OR	95% CI		*p*-value
Grazing during the BTV-3 epidemic in 2023								
None	Ref.				Ref.			
Yes, but limited (>0-≤360 hours)	**4.41**	**3.51**	**5.54**	**<0.01**	**4.42**	**2.80**	**7.00**	**<0.01**
Many hours (>360 hours)	**7.54**	**5.78**	**9.84**	**<0.01**	**10.66**	**6.65**	**17.1**	**<0.01**
Presence of large (horizontal) ventilation openings in the barn								
No, ventilation openings are <30cm	Ref.				Ref.			
Yes, large horizontal ventilation openings (≥30cm) or an open front	**0.76**	**0.58**	**1.00**	**0.05**	n.a.			
Yes, large horizontal ventilation openings (≥30cm) or an open front, without windbreak curtain	n.a.				**0.67**	**0.50**	**0.89**	**<0.01**
Yes, with windbreak curtain (small openings)	n.a.				**0.55**	**0.40**	**0.75**	**<0.01**
Yes, with windbreak curtain (large openings, >10mm)	n.a.				**0.46**	**0.33**	**0.63**	**<0.01**
Did you use mechanical ventilation when the cattle were indoors?								
No	Ref.				Ref.			
Sometimes	0.92	0.72	1.17	0.48	**0.73**	**0.55**	**0.97**	**0.03**
Yes	**0.75**	**0.62**	**0.90**	**<0.01**	**0.78**	**0.62**	**0.98**	**0.03**
Are sheep present at your farm?								
No	Ref.				Ref.			
Yes, but they did not show clinical signs of bluetongue	1.06	0.81	1.39	0.65	**0.42**	**0.28**	**0.64**	**<0.01**
Yes, and they showed clinical signs of Bluetongue	**4.34**	**2.64**	**7.13**	**<0.01**	**1.79**	**1.40**	**2.31**	**<0.01**
Have you introduced cattle from other herds between Sep-Dec 2023?								
No	Ref.							
Yes, from BTV-3 low risk areas	1.50	0.81	1.44	0.09				n.s.
Yes, from BTV-3 medium risk areas	0.95	0.77	1.16	0.59				
Yes, from BTV-3 high risk areas	**3.65**	**2.25**	**5.93**	**<0.01**				
Location of the farm								
Low BTV-3 risk area	Ref.				Ref.			
Medium BTV-3 risk area	**11.9**	**9.0**	**15.9**	**<0.01**	**24.0**	**3.3**	**176**	**0.02**
High BTV-3 risk area	**114**	**76.3**	**170**	**<0.01**	**178**	**24.2**	**1309**	**<0.01**
Herd size (adult cattle)								
Smallest (≤51 cows)					Ref.			
Smaller (>51-≤101 cows)				n.s.	**0.60**	**0.44**	**0.81**	**<0.01**
Larger (>101-≤191 cows)					**0.30**	**0.22**	**0.42**	**<0.01**
Largest (>191 cows)					**0.19**	**0.12**	**0.32**	**<0.01**

n.a. = not applicable: in Model 1 the interaction term was not significant and was thus not included in the model (only the main effect of ventilation openings was significant). In model 2 the interaction term was significant and therefore the main effect is not presented. ns. = not significant

Table 3. Risk factors associated with presence of BTV (Model 3) or increasing within-herd prevalence (Model 4) in Dutch sheep flocks after the BTV-3 epidemic in 2023 based on multivariable regression models, with odds ratio (OR) or incidence rate ratio (IRR), 95% confidence interval (CI) and *p*-value. Associations with a *p*-value <0.05 are displayed in bold.

Parameter	**Model 3** Flock level BTV status (positive/ negative) N=384				**Model 4** Within-flock prevalence N=186			
	OR	95% CI		*p*-value	IRR	95% CI		*p*-value
Did you put your sheep indoors to prevent them from acquiring BTV-3								
No	Ref.				Ref.			
Yes	**0.25**	**0.09**	**0.67**	**<0.01**	0.61	0.34	1.10	0.09
When was the last time you sheared your sheep in 2023								
January-March	Ref.				Ref.			
April-June	1.59	0.95	2.66	0.08	1.05	0.81	1.37	0.69
July-September	0.76	0.31	1.85	0.54	**2.22**	**1.27**	**3.86**	**<0.01**
October-December	3.03	0.77	11.9	0.11	1.56	0.85	2.85	0.15
Location								
Low BTV-3 risk area	Ref.				Ref.			
Medium BTV-3 risk area	**2.03**	**1.10**	**3.75**	**0.02**	1.32	0.88	1.98	0.18
High BTV-3 risk area	**8.39**	**4.24**	**16.6**	**<0.01**	**3.30**	**2.23**	**4.88**	**<0.01**
Flock size (adult sheep)								
Smallest flocks (0-9 sheep)					Ref.			
Smaller flocks (10-23 sheep)				ns	1.10	0.73	1.66	0.63
Larger flocks (24-99 sheep)					1.10	0.70	1.59	0.80
Largest flocks (>99 sheep)					**0.51**	**0.30**	**0.86**	**0.01**

DISCUSSION

In this study the prevalence of BTV antibodies after the BTV-3 epidemic in the Netherlands in 2023 and associated risk factors were studied. The estimated antibody prevalences provided an indication of the proportion of the sheep and cattle population that would be protected against BTV-3 in 2024. Beef cattle herds were not included in this sample and it was assumed that dairy, who are mostly grazed in the Netherlands are representative for all cattle. Furthermore, the results are likely an underestimate of the true spread of BTV-3 in 2023. Mortality during the outbreak in 2023 was estimated to be 1.7 times higher than normal in dairy cattle farms (Van den Brink et al., 2025). In sheep flocks an increased mortality of 55 thousand sheep was observed during the epidemic, and at flock-level estimated to be five to 15 times higher than normal (Santman-Berends et al., 2024). Mortality during the 2023 outbreak has undoubtedly decreased the proportion of antibody positive animals and farms this study. This posed a challenge for the risk factor evaluation because we aimed to evaluate factors associated with the spread in 2023, not to the proportion of infected animals that survived until spring 2024. To correct for this, the estimated within-herd prevalence in sheep flocks was combined with recorded sheep mortality in September to December 2023. This may in turn have overestimated within-herd prevalence in sheep flocks to some extent, yet it was assumed that this did not bias the identification of risk factors or their magnitude. As BTV-3 related mortality in cattle herds was less excessive and the fact that the within-herd prevalence was analysed as being either high or low, it was decided to not correct the within-herd BTV prevalence in cattle for mortality. The major increase in mortality in the sheep population may also be one of the reasons why a lower flock and animal-level BTV antibody prevalence was found in sheep compared to cattle.

The risk factor evaluation in both sheep and cattle indicated that keeping animals indoors was associated with a lower odds of testing BTV antibody positive. This result agrees with previous findings related to other vector borne diseases in the Netherlands, i.e. BTV-8 and Schmallenberg virus (Santman-Berends et al., 2010; Veldhuis et al., 2014). It is suggested that cattle that are kept indoors are less likely to be bitten by midges of the *culicoides* species compared to cattle kept outdoors (Baylis et al., 2010). In addition, a protective effect of a higher amount of air movement in the barn has been found during the BTV-8 epidemic (Santman-Berends et al., 2010). For sheep, shearing in late winter or early spring was associated with a lower herd prevalence and lower within-herd prevalence compared to shearing in (late) summer. This may be due to fleece regrowth in sheep that were sheared early preventing sheep from being bitten during the outbreak, but needs further investigation.

In conclusion, the results indicated that a large proportion of the Dutch dairy herds and sheep flocks encountered BTV-3 after the first epidemic year in 2023. The results gave an indication of the proportion of the population that would be protected against BTV-3 in 2024, rather than the amount of BTV-3 spread in 2023. The within-herd and within-flock prevalences show that the majority of both populations are still susceptible for BTV-3 in 2024. This emphasises the importance to obtain knowledge on preventive measures against BTV-3 infection and within-herd spread, in addition to vaccination. Since May 2024, three BTV-3 vaccines are available that are expected to reduce the risk and impact of BTV-3 in the Netherlands.

ACKNOWLEDGEMENTS

This project was financed by the Ministry of Agriculture, Fishery, Food Security and Nature (LVVN, The Hague, The Netherlands). We would further like to acknowledge the producer organisation for dairy (ZuivelNL, The Hague, The Netherlands), the Dutch agriculture organisation (LTO, The Hague, The Netherlands) and all participating farmers for their input in the project.

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THE IMPACT OF THE BLUETONGUE SEROTYPE 3 EPIDEMIC ON CATTLE HEALTH IN THE NETHERLANDS IN 2023

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SUMMARY

In September 2023, bluetongue virus serotype 3 (BTV-3) was identified in sheep and cattle in the Netherlands infecting thousands of farms. The aim of this study was to quantify the impact of the BTV-3 outbreak in 2023 on cattle health using routinely collected census data. The results showed that the short-term impact of BTV-3 epidemic was associated with increased cattle mortality in both dairy and non-dairy herds, increased levels of premature births, decreased milk production and decreased udder health in dairy herds. Additionally, antimicrobial usage was clearly increased in non-dairy herds. This considerable impact on cattle health highlights the need for protective measures such as vaccination.

INTRODUCTION

In September 2023, bluetongue virus serotype 3 (BTV-3) was identified in sheep and cattle in the Netherlands for the first time in history (Holwerda et al., 2024). The virus spread rapidly, affecting over 5,000 farms during the 2023 epidemic. Although sheep show more severe clinical signs and higher mortality rates, cattle play an important role in the transmission of BTV-3. Additionally, during the BTV-3 epidemic, severe clinical signs among cattle such as lameness, decreased milk production and increased mortality were also reported in infected cattle herds (Van den Brink et al., 2024).

In the Netherlands, as part of the national cattle health surveillance system, quarterly trend analyses (TASC) on cattle health indicators are conducted using routinely collected census data. The aim of TASC is to monitor trends and developments in cattle health in time (Santman-Berends et al., 2016). The data collected as part of TASC offered the opportunity to quantify the impact of the 2023 BTV-3 outbreak in Dutch cattle herds. In this study, the associations between the BTV-3 outbreak in 2023 and mortality, abortions, premature births, milk production, udder health and antimicrobial usage in Dutch cattle herds were quantified.

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MATERIALS AND METHODS

Data availability and classification of herds to BTV-3 status

For this study, cattle census data were available from 1 January 2020 until 31 December 2023. The data included cattle movement records (Dutch enterprise agency RVO, Assen), carcass collections (Dutch rendering facility Rendac, Son), milk production and milk quality data (Qlip laboratories, Zutphen), bulk milk delivery data (milk processing companies), data on antimicrobial usage (ZuivelNL, the Hague), herd health statuses (Royal GD, Deventer) and test-day data (Royal Dutch Cattle Syndicate CRV Holding BV, Arnhem and milk control association Nijland, Nijland). Data were validated, combined and aggregated on herd-month level into various key monitoring indicators (Santman-Berends et al., 2016).

In addition to the TASC data, BTV-3 notification data were available from the Dutch Food and Consumer Product Safety Authority (NVWA) for all herds of which the farmers notified clinical signs of BTV-3. These data included the unique herd identification number (UHI), the animal species in which clinical signs were observed, whether the report was solely a clinical notification or notified and confirmed by PCR and the date and location of the report. Based on these notification data, all cattle herds were assigned a BTV-3 status for each herd-month:

1) Herds that were notified: herds of which the farmer notified clinical signs of BTV-3 to the authorities
2) Herds located in BTV-3 infected areas: herds of which the farmer did not notify clinical signs to the authorities but were located in two digit zip code areas where presence of BTV-3 was confirmed
3) Herds located in BTV-3 free areas. This was the category for all herds before September 2023 and after that for those herds located in two digit zip code areas for which no clinical signs of BTV-3 were notified to the authorities during the outbreak.

For this paper, dairy (N=~13,700) and non-dairy herds were included. Non-dairy herds included suckler herds (N=~2,500 herds with >20 heads of cattle and births), beef herds (N=~1,300 herds with >20 heads of cattle and no births) young stock rearing herds (N=~2,100 herds with >20 heads of cattle, no births and percentage cattle under 2 years old ≥80%) and small-scale herds (N=~10,600 herds with ≤20 heads of cattle).

Cattle health indicators

The impact of the BTV-3 outbreak on mortality, abortions, premature births, milk production, udder health and antimicrobial usage was quantified for dairy herds. Calf mortality indicators were defined according to the definitions that were developed in previous research (Santman-Berends et al., 2019). The cattle mortality rate (≥1 year old) was calculated as the number of dead cattle relative to the number of cattle in this specific age category present in the dairy herd in the same period (corrected for the time present in the herd). The number of dead cows during the first 60 days of lactation relative to the number of cows present in this period was defined as the mortality rate in the first 60 days of lactation. The percentage of abortions was defined as the number of calvings with gestation length between 180-260 days relative to the number of births. Additionally, the percentage of premature births was defined as the number of calvings with a gestation length between 260-265 days relative to the number of births.

The average milk yield per lactating cow per day was calculated on herd-month level and based on the amount of delivered milk to the dairy plant relative to the average number of dairy cows present in the herd (assuming 10% of dairy cows were in the dry-period). For each dairy herd, the average of all bulk milk somatic cell count (BMSCC) test results within a month was determined. Additionally, for herds that joined a regularly test-day scheme (~85% of Dutch dairy herds) the average percentage of new high SCC (>150,000 c/ml for primiparous cows and >250,000 c/ml for multiparous cows) was determined. The animal defined daily dose of antimicrobial use ($DDDA_F$) was calculated at the herd-month level based on the total amount of antimicrobial supplies relative to the average number of animals present in a herd (SDa, 2020). Subsequently, antimicrobial usage above or below the median animal defined daily dose in 2020 (>0.11 $DDDA_F$ per month) was defined as key indicator.

For suckler herds, beef herds, young stock rearing herds and small-scale herds the impact of the BTV-3 outbreak on mortality and antimicrobial usage was quantified. Mortality parameters were calculated similarly to those calculated for dairy herds. Mortality was determined in three different age groups for suckler herds: perinatal calves (non-ear tagged), ear tagged calves <1 year old and cattle ≥1 year old. Additionally, total cattle mortality was determined for beef herds and young stock rearing herds, whereas occurrence of cattle mortality per herd-month (yes/ no) was determined for small-scale herds. For each of the non-dairy herd types, antimicrobial use was defined as the animal defined daily dose, which were determined in the same way as for dairy herds. The use of antimicrobials (yes/no) was defined for each herd-month as key indicator.

Data validation and analysis

All data suppliers sent their data to an external encryption company (IntoFocus Data Transformation Services, IDTS, Deventer) to have their data encrypted to prevent traceability of results to individual farms or animals. Each dataset was encrypted with the same encryption key to enable combining different datasets. Each dataset was validated using standardised scripts in SAS 9.4® (SAS Institute Inc, 2013) to check for duplicate entries, extreme values and data completeness. After validation, the datasets were aggregated and combined with each other at the herd-month level. Based on the notification data, the week of the initial BTV-3 notification for each two-digit zip code area was determined and combined at the two-digit zip code-month level. Additionally, farm-level notification data were combined at the herd-month level.

Descriptive statistics were applied on the number of reported dead cattle for dairy, suckler, young stock rearing and small-scale herds in the years 2020-2023 in the Netherlands. For each month in 2023, the difference between the number of reported dead cattle per month with the average number of reported dead cattle in the corresponding month in the years 2020-2022 was quantified. Additionally, the difference in the average milk production per cow per day for each month in 2023 and the years 2020-2022 was analysed in the same way for Dutch dairy herds.

Multivariable Population-Averaged-Generalized-Estimating-Equation (PA-GEE) models in Stata® version 17 (StataCorp, 2021) were used to quantify the association between the BTV-3 outbreak and cattle health indicators as described by Santman-Berends et al. (2016). The mortality parameters, premature births, abortions and milk production were included as dependent variables. For each dependent variable, the most appropriate distribution and link function was chosen. In all models an independent correlation structure was assumed. Mortality, premature births and abortions were analysed using a Poisson distribution with a log

link function or a negative binomial distribution (in case of overdispersion of residuals). The natural logarithm of the number of cattle at risk corrected for the time at risk was included as exposure variable. For the analysis of mortality in small-scale herds, a binominal distribution with a logit link function was chosen. Milk production levels were analysed using a Gaussian distribution and an identity link function.

The BTV-3 herd status was included as an independent variable of interest. Data of herds classified as 'BTV-free' were considered as the reference. In addition, herd size, milk production level, geographical location, growth in herd size, replacement rate, milk prices and meat prices, season, trend in time and herd health statuses (for BVD, IBR, salmonellosis, paratuberculosis and leptospirosis) were included as independent variables in the models when considered relevant. Results are presented as incidence rate ratios (IRR), odds ratios (OR) or estimates depending on the distribution of the dependent variable. Results were considered statistically significant at a *P*-value ≤ 0.01. Model fit was evaluated using the quasi-likelihood under the independent model criterion (QIC) (Pan, 2001; Cui, 2007) and the amount of variance explained by the model (R^2).

RESULTS

Descriptive results

The difference in the number of dead cattle per age category reported by Dutch dairy, suckler, young stock rearing and small-scale herds for each month of the year between 2023 and 2020-2022 is shown in Fig. During the BTV-3 outbreak in the period September-December 2023, increased mortality numbers were particularly observed in cattle ≥1 year, with an additional 6,217 dead cattle. Additionally, 596 more perinatal calves, 1,197 more calves ≤14 days and 325 more calves between 56 days and 1 year died as compared to the average mortality numbers in the same months in 2020-2022. No increased mortality figures were observed in calves between 15 and 55 days old (-433 calves fewer died during the BTV-3 outbreak in 2023).

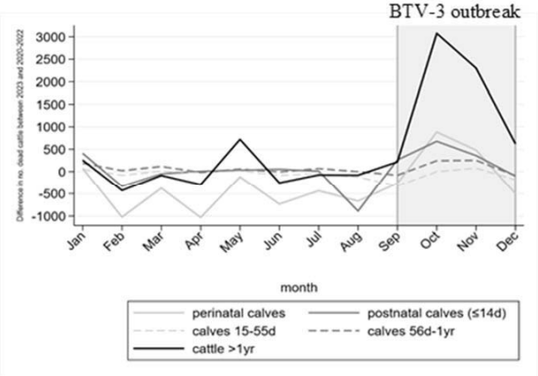

Figure 1. Difference in the number of dead cattle for each month of the year between 2023 and 2020-2022 in Dutch cattle herds

The average milk production per cow per day was higher in the months January until August 2023 compared to the previous years (2020-2022, Fig. 2). During the BTV-outbreak, the milk production level in 2023 dropped below the average milk production level in the years 2020-2022.

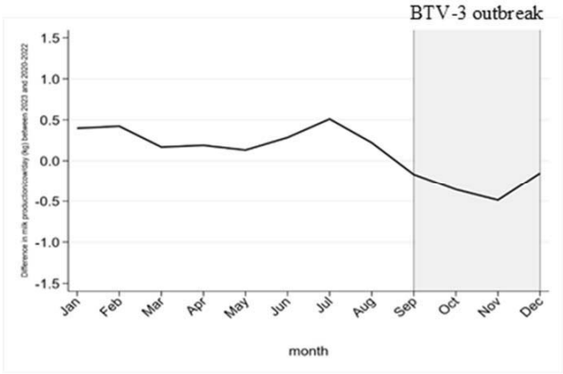

Figure 2. Difference in the average milk production per cow per day for each month of the year between 2023 and 2020-2022 in Dutch dairy herds

Multivariable results

During the BTV-3 outbreak, mortality in cattle (>1 year) increased 1.78 times in dairy herds of which the farmer notified clinical signs and 1.20 times in dairy herds that were located in BTV-3 infected areas compared to dairy herds in BTV-3 free areas (Table 1). Mortality of non-ear tagged calves increased 1.11 and 1.07 times in dairy herds that were notified and dairy herds that were located in infected areas, respectively. This increase may partly be explained by the 1.39 times increase in premature births in dairy herds that were notified during the BTV-3 outbreak compared to dairy herds in BTV-3 free areas. (Table 1). No significant increase in ear tagged calf mortality was observed during the BTV-3 outbreak in 2023 in dairy herds as compared to earlier years (Table 1).

Suckler herds, beef herds and young stock rearing herds of which the farmer notified clinical signs, showed a clear increase in mortality during the BTV-3 outbreak compared to herds in BTV-3 free areas (Table 1). Additionally, there were 2.23 times more small-scale herds of which the farmer notified clinical signs and 1.35 times more small-scale herds located in BTV-3 infected areas that experienced cattle mortality compared to small-scale herds in BTV-3 free areas (Table 1).

Milk production decreased -1.15 kg and -0.74 kg per cow per day in dairy herds that were notified and dairy herds located in BTV-3 infected areas, respectively (Table 1). This means for example that the milk production loss associated with BTV-3 infection in a herd with 110

lactating cows in which cows showed clinical bluetongue signs during a 10 week period, would be on average ~-9,000 kg. Whereas the percentage of new high SCC cows was higher in both dairy herds that were notified (+0.43%) and dairy herds located in BTV-3 infected areas (+0.26%), BMSCC was only higher in dairy herds that were notified (+3,700 c/ml) compared to dairy herds located in BTV-3 free areas (Table 1).

Dairy herds that were notified had 1.14 times more often an antimicrobial usage (AMU) above 0.11 $DDDA_F$ per month compared to dairy herds located in BTV-3 free areas. The use of antibiotics in notified non-dairy cattle herds was significantly higher in all non-dairy herd types of which the farmer notified clinical signs, whereas AMU was not significantly higher in dairy and non-dairy herds located in BTV-3 infected areas (Table 1).

DISCUSSION

This study showed that the BTV-3 outbreak had severe adverse consequences on mortality, premature births and milk production in 2023. In dairy herds, the BTV-3 outbreak was associated with a significantly higher cow mortality rate (≥1 year), a higher perinatal mortality risk (including late abortions, stillbirths and calves born alive that died before ear tagging) and increased premature births. Additionally, cattle mortality was increased in suckler, beef, young stock rearing and small-scale herds. Previous studies also have shown that BTV infections in cattle are associated with increased mortality and stillbirths (Santman-Berends et al., 2011; Rushton and Lyons, 2015). The abortion rate in dairy herds was not significantly associated with BTV-3. This result is consistent with the BTV-8 outbreak in 2006-2008, where no clear effect of BTV-8 infection on abortions was observed (Santman-Berends et al., 2010a). The fact that perinatal mortality and premature births in dairy herds were increased during the BTV-3 epidemic, may indicate that more stillbirths and weak calves have been born during the acute phase of the epidemic. Short gestation lengths were also seen during the Schmallenberg virus (SBV) epidemic in 2011 in the Netherlands. Fever followed by BTV and SBV infections may have accelerated parturition (Veldhuis et al., 2016).

In both dairy and suckler herds, ear tagged calf mortality up to one year old was not significantly increased during the BTV-3 outbreak in 2023. Most Dutch dairy herds keep their calves indoors in fall/winter, which may have reduced their exposure to the virus during the BTV-3 outbreak in 2023. Keeping cattle indoors has previously shown to have a protective effect against vector-borne diseases (Veldhuis et al., 2014; Santman-Berends et al., 2010b). In contrast to calves, the vast majority of lactating cows and young stock have daily access to pasture, which may have led to increased risk of exposure to the virus and consequently increased cattle mortality rates (>1 yr) in dairy herds during the BTV-3 outbreak in 2023. This theory is supported by the fact that increased cattle mortality rates were observed in suckler, beef, young stock rearing and small-scale herds, in which cattle is mainly kept outdoors. However, the BTV-3 outbreak had more severe consequences on cattle mortality (>1 yr) in dairy herds compared to the BTV-8 outbreak in 2007 (IRR: 1.8 versus IRR 1.4). A similar result was observed in sheep, during the 2023 epidemic (Santman-Berends et al., 2024). It is known that the impact of bluetongue varies between serotypes due to among other differences between vector competence, virulence and density and susceptibility of the host population.

Table 1. Results of multivariable population averaged regression models in all Dutch dairy and non-dairy cattle herds based on census data from 1 January 2020 until 31 December 2023. Results are presented as incidence rate ratio (IRR), odds ratio (OR)[1] or coefficient[2]. Bold numbers represent a significant difference compared to herds that were located in BTV-3 free areas (reference category, *P*-value ≤ 0.01)

Cattle health indicator	**Herds that were notified** IRR/OR[1]/coef.[2] (95% CI)	**Herds in BTV-3 infected areas** IRR/OR[1]/coef.[2] (95% CI)
Dairy herds		
Cattle mortality (>1 yr)	**1.78** (1.69-1.87)	**1.20** (1.17-1.23)
Cattle mortality (<60 days of lactation)	**1.93** (1.79-2.09)	**1.30** (1.25-1.35)
Ear tagged calf mortality		
≤14 days	1.03 (0.95-1.11)	1.01 (0.98-1.05)
15-55 days	0.99 (0.90-1.10)	**0.92** (0.88-0.96)
56 days-1 yr	1.16 (1.02-1.31)	1.02 (0.96-1.08)
Non-ear tagged calf mortality	**1.11** (1.06-1.16)	**1.07** (1.05-1.09)
Abortions (gestation length 180-260 d)	1.10 (1.02-1.20)	1.04 (1.00-1.07)
Premature births (gestation length 260-265 d)	**1.39** (1.26-1.54)	1.01 (0.95-1.07)
Milk production (kg/cow/day)	**-1.15** (-1.29- -1.01)[2]	**-0.74** (-0.81- -0.67)[2]
New high somatic cell count cows (%)	**+0.43** (0.26-0.60)[2]	**+0.26** (0.18-0.34)[2]
Bulk milk somatic cell count (c/ml)	**+3,700** (1,530-5,870)[2]	+600 (-480-1,680)[2]
Antimicrobial usage >0.11 $DDDA_F$/month	**1.14** (1.07-1.21)[1]	1.00 (0.97-1.03)[1]
Suckler herds		
Cattle mortality (>1 yr)	**2.62** (1.86-3.69)	**1.32** (1.15-1.52)
Ear tagged calf mortality (≤1 yr)	1.08 (0.73-1.61)	1.15 (1.02-1.28)
Non-ear tagged calf mortality	0.79 (0.56-1.11)	0.95 (0.81-1.13)
Use of antimicrobials	**1.72** (1.45-2.04)[1]	1.04 (0.96-1.13)[1]
Beef herds		
Cattle mortality (all ages)	**2.86** (1.27-6.47)	1.06 (0.88-1.29)
Use of antimicrobials	**3.08** (1.60-5.95)[1]	1.06 (0.88-1.28)[1]
Young stock rearing herds		
Cattle mortality (all ages)	**1.61** (1.12-2.32)	1.02 (0.87-1.19)
Use of antimicrobials	**1.66** (1.24-2.21)[1]	0.96 (0.87-1.05)[1]
Small-scale herds		
Presence of cattle mortality (all ages)	**2.23** (1.71-2.91)[1]	**1.35** (1.23-1.49)[1]
Use of antimicrobials	**2.26** (1.76-2.91)[1]	0.88 (0.79-2.98)[1]

This study also showed a clear reduced milk production level in both herds of which the farmer notified clinical signs and herds in BTV-3 infected areas relative to herds in areas in which no BTV-3 was notified. During the BTV-8 outbreak also a clear drop in milk production

of 59 kg per infected lactating cow was observed (Santman-Berends et al., 2011). Increased SCC was clearly observed in dairy herds that were notified. The associations found between BTV-3 and udder health were quite similar to the associations found at the time BTV-8 circulated in the Netherlands in 2006-2008 (Santman-Berends et al., 2011).

There was especially a clear increase in the number of non-dairy herds with AMU during the BTV-3 outbreak. It is possible that antibiotics have occasionally been used on non-dairy farms to support sick animals and to treat and prevent secondary infections.

Since May 2024, multiple BTV-3 vaccines are available. Vaccination is voluntary in the Netherlands. The results of this study may support the decision to vaccinate. Of course, vaccination results in substantial costs for the farmer. However, if vaccination can prevent or reduce the losses related to BTV-3 infection regarding increased mortality and reduced milk production, it will probably be economically beneficial for the individual farmer to vaccinate cattle. It remains unclear to what extend farmers decided to vaccinate their livestock against BTV-3 in 2024. Nevertheless, since July 2024 massive spread of BTV-3 was observed in a second year of the epidemic. To what extend vaccination reduced the impact is currently investigated.

ACKNOWLEDGEMENTS

This work was conducted as part of the national Cattle Health Surveillance System which is supported and financed by the Ministry of Agriculture, Fisheries, Food Security and Nature and producer and interbranch organisations for dairy and veal in the Netherlands.

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EPIDEMIOLOGICAL METHODS

EVALUATION AND APPLICATION OF A BAYESIAN HIDDEN MARKOV MODEL TO THE SURVEILLANCE OF BVDV IN FINISTERE, FRANCE

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SUMMARY

A Bayesian Hidden Markov Model (HMM) was developed to estimate test characteristics and infection dynamics from longitudinal disease surveillance data. The model was validated using simulated datasets, demonstrating robust performance with minimal bias and high precision across diverse epidemiological scenarios. The model was applied to data from the Bovine Viral Diarrhoea Virus surveillance programme in Finistère, France, which involved quarterly bulk tank milk testing from 2011 to 2020 using two ELISA tests. The HMM provided reliable estimates of Gaussian mixture parameters and infection dynamics during early programme years. However, declining seroprevalence impacted parameter estimation, underscoring the need for adaptive strategies such as incorporating informative priors in later stages. This approach is particularly valuable for estimating test characteristics in the absence of a gold standard by leveraging the temporal correlation in longitudinal data. The findings highlight its potential to enhance disease surveillance systems in dynamic epidemiological contexts.

INTRODUCTION

Surveillance of cattle diseases often relies on bulk tank milk (BTM) testing using ELISA assays at regular intervals. This approach is widely used to monitor infections by Bovine Viral Diarrhoea Virus (BVDV), *Salmonella*, *Mycobacterium avium* subsp. *Paratuberculosis*, or *Neospora caninum* aiming to identify infected herds. ELISA test results are measured as sample-to-positive ratios (S/P%), but herds are typically classified as positive or negative based on predefined thresholds.

The absence of a gold standard complicates the evaluation of such tests. Latent class models (LCMs) are commonly used to estimate test characteristics and disease prevalence under these conditions (Branscum et al., 2005). In surveillance programmes, herds are tested repeatedly over time. The temporal correlation between the results of these tests can be incorporated using a Hidden Markov Model (HMM), in which the disease status at time *t* is assumed to depend solely on the latent status at time *t-1*.

Building on earlier work (Madouasse et al., 2022), we developed a Bayesian HMM to model BVDV herd status. Unlike conventional LCMs that dichotomise continuous test results, our

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model retains the full information from continuous test values by representing them as mixtures of normal distributions: one for the uninfected status and the other for the infected status. Maintaining continuous data and incorporating temporal correlations improve the estimation of test characteristics and disease dynamics.

BVDV is a significant cattle disease, and surveillance programmes are widely implemented to control or eradicate the infection (van Roon et al., 2020). In Brittany, France, a BVDV surveillance programme has been in place since 1998 (Joly et al., 2005). All herds are tested on BTM four times a year using two different ELISA antibody tests, with a single threshold applied to classify herds as positive or negative. For this study, we analysed continuous BTM test data collected from Finistère, a sub-region of Brittany, between 2011 and 2020.

This study has three objectives: first, to evaluate the bias and standard deviation of model parameter estimates using simulated data; second, to estimate the parameters of the distributions associated with seronegative and seropositive herds for the two ELISA tests used; and third, to analyse the dynamics of seroprevalence in Finistère over time.

MATERIALS AND METHODS

Hidden Markov Model for Disease Surveillance

This study employed a Bayesian HMM to analyse longitudinal test data for the surveillance of disease dynamics in cattle herds. The HMM is a stochastic process that models a latent categorical variable (herd status) evolving over time. At any given time, the latent state (Z_t) is determined from an observed variable (Y_t), such as a test result, which is considered an imperfect observation of the true status. Consequently, two distributions for (Y_t) are required to describe the two latent states ($Z_t = 0$) and ($Z_t = 1$).

Continuous Test Results

Unlike models that dichotomise test results, the evaluated HMM models test outcomes as continuous variables. The observed test results (Y_t) are assumed to follow a mixture of two normal distributions (Eq. 1):

$$Y_t \sim (1-\pi)N(\mu_0, \sigma_0^2) + \pi N(\mu_1, \sigma_1^2) \quad (1)$$

where μ_0 and μ_1 are the means of the test results for latent state negative and latent state positive herds, respectively; σ_0 and σ_1 are their standard deviations; and π is the probability of infection determined from the latent state dynamic (see below). These parameters are estimated directly from the data.

Latent State Dynamics

The latent state Z_t represents the true infectious or serological status of a herd at time t, classified as either negative ($Z_t = 0$) or positive ($Z_t = 1$). This status evolves over time according to a Markovian dynamic, characterised by:

- π_1: the initial probability of a herd being a latent state positive.
- τ_1: the monthly probability of a herd transitioning from latent state negative to latent state positive.

- τ_2: the monthly probability of a herd remaining to latent state positive.

The HMM assumes time homogeneity, meaning that these probabilities remain constant over time.

Model Inference

The likelihood of the HMM is given by Eq. 2:

$$P(Y_{1:T}, Z_{1:T}|\theta) = P(Z_1)\prod_{t=2}^{T} P(Z_t|Z_{t-1})\prod_{t=1}^{T} P(Y_t|Z_t, \mu_0, \sigma_0, \mu_1, \sigma_1) \quad (2)$$

where $\theta = \pi_1, \tau_1, \tau_2, \mu_0, \sigma_0, \mu_1, \sigma_1$ denotes the model parameters. These parameters can be estimated in a Bayesian framework. We utilised the Markov Chain Monte Carlo (MCMC) algorithm as implemented in the Stan software (Stan Development Team, 2024) to draw samples from the posterior distribution of θ.

Evaluation of Parameter Estimation

The performance of the model in estimating its parameters was evaluated using simulated datasets. Continuous test results were generated for a single herd over 500 time periods across 6 scenarios (see Table 8). The scenarios were designed to reflect varying levels of disease prevalence, infection dynamics, and overlap between the Gaussian components of the mixture model. For each scenario, 1500 repetitions were performed to ensure robust evaluations.

For each scenario, the mean and the standard deviation of the posterior distribution were calculated for all model parameters, including the dynamic parameters (π_1, τ_1, τ_2) and the parameters of the Gaussian mixture ($\mu_0, \sigma_0, \mu_1, \sigma_1$). The accuracy of the estimate was assessed using bias, defined in Bayesian inference as the difference between the true parameter value and the mean of its posterior distribution. The standard deviation of the posterior distribution was computed to evaluate the precision of the parameter estimates. These metrics were used to evaluate the robustness and reliability of the model across different epidemiological scenarios.

Table 8: Parameters used in the six simulation scenarios. In all scenarios, σ_0 and σ_1 were fixed at 15.

Scenario	τ_1	τ_2	μ_0	μ_1
1	0.1	0.85	25	75
2	0.1	0.85	25	50
3	0.1	0.85	30	50
4	0.1	0.85	25	45
5	0.2	0.7	25	75
6	0.4	0.4	25	75

Application to Surveillance Data

The model was applied to data from the Bovine Viral Diarrhoea Virus (BVDV) surveillance programme in Finistère, a *département* in the Brittany region of France. This programme, operational since 1998, conducts quarterly bulk tank milk (BTM) testing for dairy herds, alternating between two ELISA antibody tests. Continuous S/P% test results from both tests collected between 2011 and 2020 were used for this analysis.

For each herd, the model estimated:

- The parameters of the Gaussian mixture for the two ELISA tests.
- The dynamics of seropositivity over time (τ_1 and τ_2).

To account for changes in test characteristics over time, the model was run sequentially for 28 overlapping periods, with each run incorporating three years of historical data and predicting the latent state of each herd for the last month of data. Predictions were made for four months per year over a span of seven years (7 years x 4 predictions / year = 28 periods), covering the period from 2014 to 2020. The first predicted month was April 2014 and the last one December 2020.

RESULTS

Evaluation of Parameter Estimation

The performance of the model was evaluated using simulated datasets generated under various scenarios of infection dynamics and test result distributions. Across all scenarios, the model demonstrated robust parameter estimation, with low bias and standard deviation for both the dynamic parameters (π_1, τ_1, τ_2) and the Gaussian mixture parameters ($\mu_0, \sigma_0, \mu_1, \sigma_1$). Figure 4 shows the bias for μ_0, μ_1, τ_1 and τ_2.

The bias of the dynamic parameters was close to zero in scenarios with stable infection processes, indicating reliable estimation of initial prevalence (π_1), infection acquisition (τ_1), and persistence (τ_2). In scenarios characterised by more unstable dynamics, with higher probabilities of new infections and lower probabilities of persistence, the model maintained acceptable levels of bias, though standard deviations of the estimates were slightly larger.

For the Gaussian mixture parameters, the means and variances of the distributions for seronegative (μ_0, σ_0) and seropositive herds (μ_1, σ_1) were accurately estimated. The model showed higher precision in estimating the parameters for seronegative herds, reflecting their larger representation in most scenarios. The overlap between the Gaussian components influenced the precision of the seropositive parameters, with greater overlap leading to increased standard deviation of the estimates. Overall, the model performed well across the simulated scenarios, confirming its suitability for analysing real-world surveillance data.

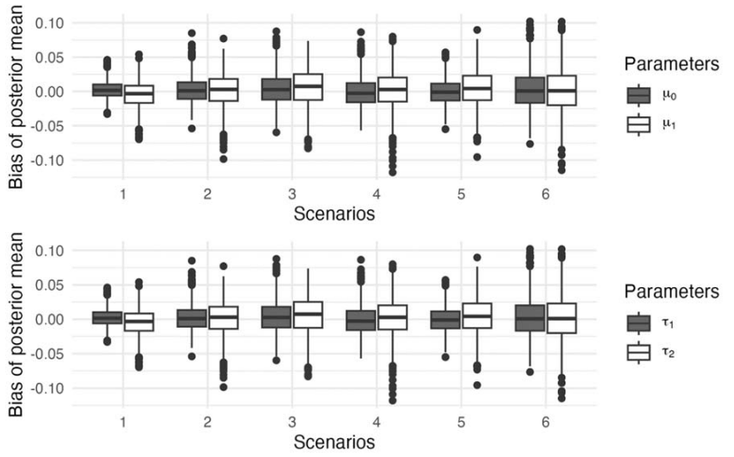

Figure 4. Boxplots showing the bias of Bayes estimates for the parameters estimated by the model across scenarios 1 to 6, as defined in Table 1.

Application to Surveillance Data in Finistère

The HMM was applied to longitudinal BTM test results from the BVDV surveillance programme in Finistère, spanning the period from 2011 to 2020. The model provided estimates of the Gaussian mixture parameters for the two ELISA tests, the dynamics of seropositivity, and the probability of seropositivity for individual herds over time.

Gaussian Mixture Parameters: The estimated Gaussian mixture parameters for the seronegative and seropositive distributions revealed notable trends over the study period (Figure 5 and Figure 6). The mean of the seronegative distributions (μ_0) remained relatively stable for both ELISA tests, reflecting consistency in baseline test results for uninfected herds. In contrast, the mean of the seropositive distribution (μ_1) showed a significant decline, particularly after period 17. For the first ELISA test (BTMab1), the mean of the seropositive distribution fell below the threshold of 35 used to classify herds, indicating reduced test sensitivity in identifying seropositive herds.

The standard deviation for the seronegative distribution for the BTMab1 test decreased gradually from period 1 to 28. For the BTMab2 test, the standard deviation for the seronegative distribution decreased gradually until period 16, dropped more sharply between periods 16 and 21, and then stabilised at a lower level. In contrast, the standard deviations for the seropositive distribution of BTMab1 and BTMab2 increased over time, with a notable jump from approximately 22 to 30 between periods 17 and 30.

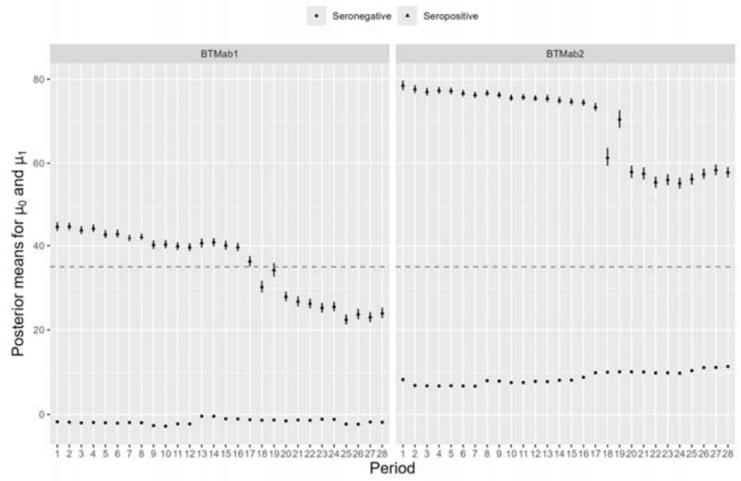

Figure 5. Bayes estimates and 95% credible intervals (vertical bars) for the means of the Gaussian mixture parameters (μ_0 and μ_1) for the two tests, BTMab1 and BTMab2, across 28 periods.

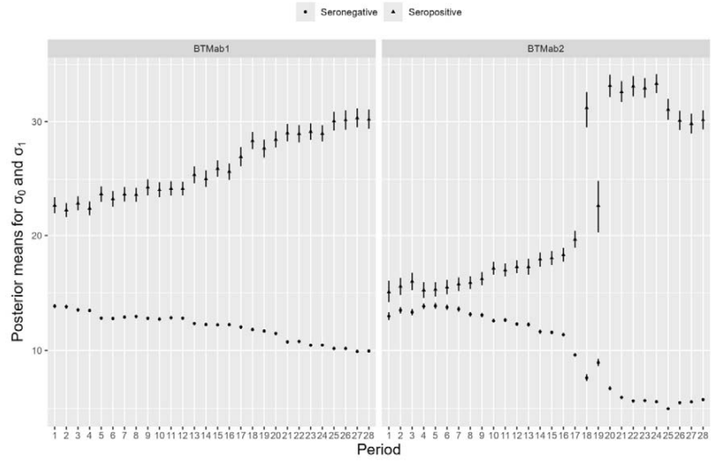

Figure 6. Bayes estimates and 95% credible intervals (vertical bars) for the standard deviations of the Gaussian mixture parameters (σ0 and σ1) for the two tests, BTMab1 and BTMab2, across 28 periods.

Dynamics of Seropositivity: The monthly probability of seroconversion estimated by the model (

Figure 7) remained between 0.006 and 0.008 from period 1 to period 17. It then increased to 0.011 in period 22 before gradually decreasing to 0.009 by period 28. Based on these values, the probability of a seronegative herd becoming seropositive over the course of a year is 8.1% when τ_1 is 0.007 and 12.4% when τ_1 is 0.011.

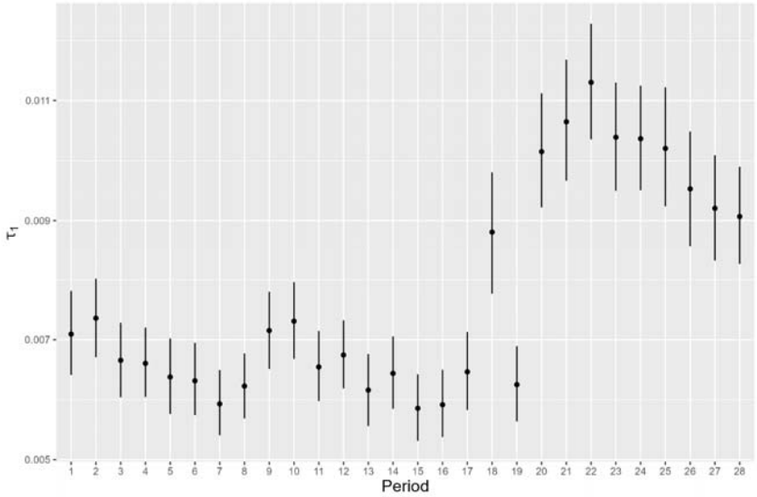

Figure 7. Bayes estimates and 95% credible intervals (vertical bars) for the monthly probability of seroconversion (τ_1), across 28 periods.

Probability of seropositivity for individual herds over time: The estimated probability of remaining seropositive (τ_2) remained consistently above 95% across the 28 periods, but decreased significantly between period 17 and period 22 (see

Figure 8).

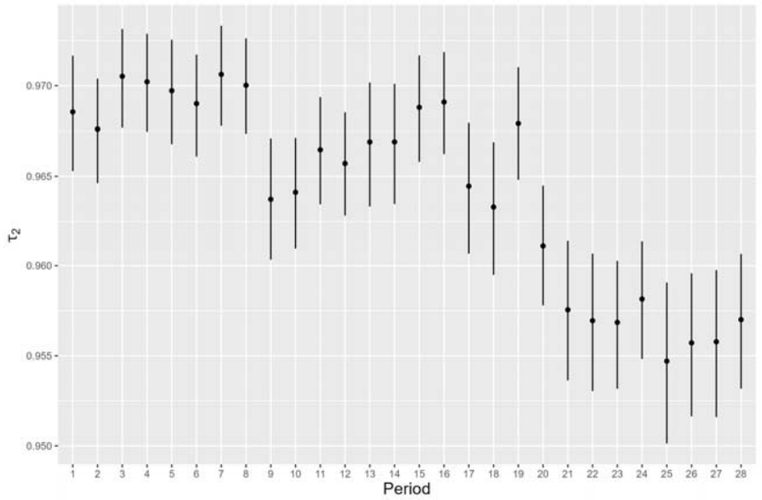

Figure 8. Bayes estimates and 95% credible intervals (vertical bars) for the monthly probability of remaining seropositive (τ_2), across 28 periods.

DISCUSSION

This study proposed an HMM to estimate test characteristics and the probabilities of infection or seropositivity from longitudinal surveillance data. Using simulated datasets, the model demonstrated high accuracy and precision, with only slight bias and low standard deviations in the estimates of all parameters. When iteratively applied to surveillance data from a BVDV control programme, the model produced consistent parameter estimates during the early years of the programme. However, during a specific period (period 17), a marked deviation in all estimated parameters was observed. In the absence of a gold standard, it is challenging to determine the true underlying situation. It can be hypothesised that the gradual decline in seroprevalence over time reduced the model's ability to reliably estimate parameter values.

After period 17, the estimated parameters suggested an increased probability of new infections, a decreased probability of remaining seropositive. Simultaneously, the model shifted the S/P% values associated with seropositivity closer to those of seronegativity. This shift indicates that values previously considered indicative of seropositivity became less distinct, aligning more with the seronegative distribution.

This issue could potentially be mitigated by using stronger priors on the parameters of the Gaussian distributions during the later stages of the surveillance programme. These priors could be informed by results obtained in earlier years, when the seroprevalence was higher. Using informative prior distributions was the approach used by van Roon et al. (2022) to

estimate the probability of freedom from infection using an HMM under low prevalence conditions. Such an approach would allow for the estimation of dynamic parameters even when the probability of seropositivity is low. However, when test characteristics are well established, seropositivity probabilities at both the individual and population levels can be estimated without relying on a Bayesian HMM.

The assumption that test characteristics are independent of the population being tested is often implicit in test evaluation. However, this assumption is particularly unlikely to hold for group tests, such as those performed on BTM. In herds composed entirely of seronegative animals, test results can reasonably be expected to follow a consistent distribution. Conversely, in seropositive herds, the distribution of BTM S/P% values is influenced by within-herd prevalence, which may vary depending on the epidemiological context. Additionally, as herd sizes increase, the contribution of each cow to the BTM sample diminishes, potentially shifting the distribution of test results for seropositive herds towards lower values.

Because the proposed model uses data collected within the surveillance programme to estimate the parameters associated with the distributions for the states of interest, it is well-suited to the local context of the programme. However, progressive changes in variables associated with the distributions for infection/seropositivity and infection dynamics necessitate periodic re-estimation of model parameters. As discussed, when the disease becomes too rare, the parameters associated with the infected states must either be replaced with strong priors for the mixture model parameters or an alternative method should be used to predict the probabilities of infection from the assumed test characteristics.

It has long been recognised that dichotomising continuous test results leads to a loss of information. Test results near the threshold are more uncertain than those farther away, but this nuance is lost during dichotomisation. Thurmond et al. (2002) addressed this issue by proposing a probability diagnostic assignment method that uses continuous test values to estimate the likelihood of infection. Their approach relied on access to a gold standard and modelled test results as a mixture of an inverse gamma distribution for seronegative animals and a Weibull distribution for seropositive animals.

Subsequent developments introduced Bayesian mixture models, which were able to accommodate a wider range of distributions for continuous test results. Erkanli et al. (2006) introduced a semi-parametric framework where test results were modelled as mixtures of multiple distributions, each weighted using a Dirichlet process as a prior, assuming the availability of a gold standard. Branscum et al. (2008) extended this approach by proposing a semi-parametric model that does not require a gold standard, enabling test characteristic estimation even in its absence. However, most later research in test evaluation without a gold standard has focused on dichotomised test results, even when the objective was to determine the optimal test cut-off (Olsen et al., 2022).

Recently, there has been renewed interest in using mixture models for continuous test results (Yang et al., 2022; Chen et al., 2024; Wang et al., 2024). The approach presented here differs by exploiting temporal correlation in longitudinal surveillance data to improve model identifiability. Unlike models that could be applied to cross-sectional data, this method relies on the sequential nature of the data to infer the underlying true state of infection. This framework also allows for the incorporation of results from multiple diagnostic tests. Additionally, although not implemented in the current study, the model could be extended to

include risk factors for new infections, as illustrated by Madouasse et al. (2022). This highlights the potential of this approach for enhancing disease surveillance in dynamic contexts.

ACKNOWLEDGEMENTS

The authors would like to thank Innoval for funding the work of M. Trotreau and for providing the data.

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DEVELOPMENT AND VALIDATION OF A SURVEILLANCE SYSTEM FOR THE FINISHING PHASE USING MORTALITY PATTERNS TO EARLY DETECT CHANGES IN HEALTH AND PRODUCTIVITY

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SUMMARY

This study aimed to improve the early detection of health and productivity challenges in swine finishing sites by developing mortality benchmark curves to identify lots with deviations. The weekly mortality data was used to develop syndromic surveillance. Lots were classified as a case or included as a benchmark based on available diagnostic results, the status of the sow site source for PRRSV and PEDV, and the median absolute deviation of weekly mortality. Different statistical methods, including frequentist, resampling, and Bayesian techniques were tested for creating benchmark curves. The Bootstrapping resampling method demonstrated the highest early detection rate (56.76%), and the Bayesian methods showed similar detection rates compared to Bootstrapping. The study highlights the effectiveness of mortality-based surveillance for identifying health and productivity challenges in finishing swine sites.

INTRODUCTION

The swine industry faces ongoing challenges in maintaining herd health and maximizing productivity, especially during the wean-to-finish phase (Fablet et al., 2018; Magalhães et al., 2022). Surveillance systems designed to detect outbreaks and manage potential threats to swine populations early are crucial. Surveillance based on production data is a key aspect of this approach to detect outbreaks early (Mandl et al., 2004). The core of the proposed approach is using weekly mortality routinely collected by the farm staff to systematically monitor possible challenges in production and herd health. This data can be used to detect outbreaks early, allowing swine producers to take proactive measures such as initiating diagnostic investigations, administering medications to reduce clinical and production impacts, adjusting animal flow, and enhancing biosecurity practices between interconnected sites (e.g., employees, trucks, supplies, and maintenance). These interventions can minimize the disease's impact on the site and prevent its spread to other sites.

Research studies have highlighted the effectiveness of surveillance using production data in swine breeding sites using statistical process control charts to monitor the number of abortions,

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enabling the detection of deviations associated with Porcine Reproductive and Respiratory Syndrome virus (PRRSV) outbreaks up to four weeks earlier compared to weekly diagnostic tests (Silva et al., 2017; Pedro Mil-Homens et al., 2024). Concerning wean-to-finish sites, a study tracked mortality and associated mortality higher than expected (out-of-control) to events at the site, such as vaccination and disease outbreaks (Rademacher, 2004). Another study used cumulative sum control charts to detect deviations in mortality in sows and piglets using the mortality rate (Bono et al., 2014). These studies showed that production indicators routinely collected at the sites can aid in monitoring herd health. However, these indicators are not being analyzed daily or weekly to implement surveillance systems. Disease surveillance during the wean-to-finish phase primarily involves observing clinical signs and conducting diagnostic testing of biological samples as needed (Prickett and Zimmerman, 2009, Gomes Neto et al., 2015; Hernandez-Garcia et al., 2017). Depending solely on farm staff to recognize the need for diagnostics when observing clinical signs is a challenge for early detection.

The objective of this study was to develop and validate a surveillance system that utilizes mortality benchmark curves in swine finishing sites to enable the early detection of health and productivity deviations. By taking advantage of underutilized data in the swine finishing sites, the authors aim to provide the decision-makers with a tool that can be applied routinely at the sites, aiding in anomaly detection.

MATERIALS AND METHODS

Project overview

A health challenge was defined as being diagnosed with PRRSV, *Actinobacillus pleuropeumoniae*, Influenza A virus, enterohemorrhagic *Escherichia coli*, or *Pasterurella multocida*. Diagnosis was established by the herd veterinarian based on clinical and diagnostic data available.

Benchmark mortality curves were built with weekly data from finishing lots not having health-challenges assigned throughout the growth period. Then, mortality curves of finishing lots known to have assigned health events were built, and contrasted with the benchmark curves from non-health-challenged flows.

Different methodologies were used to assess the ability to early detect health events based on changes in the mortality of the affected flows.

Study design and eligibility criteria

A retrospective study utilized weekly data from swine finishing lots from one swine production system in the United States Midwest. The information available for lots included weekly mortality (count of dead animals per week and inventory), report of health challenges with documented diagnostic investigation (diagnostic results from tissue submission), PRRSV and porcine epidemic diarrhea virus (PEDV) status for the sow farm, and vaccination status for PRRSV. To be included in the study, the finishing sites provided data on weekly mortality and the results of tissue submissions for diagnostic testing. They also had to originate from sow farms that weaned negative pigs for PRRSV and PEDV.

Selection of benchmark and case lots

Lots with positive results of tissue submissions were selected as cases (health-challenged flows). The tissues were positive for diseases like PRRSV, *Actinobacillus pleuropneumonia,* or Influenza A virus.

Lots without tissue submissions or reports of outbreaks from the veterinarian were pre-selected for the benchmark curve (non-challenged flows). The median absolute deviation (MAD) was calculated for each lot and each week in the finishing phase to refine the benchmark lot selection to build the mortality curve. The MAD is the deviation of mortality for a lot for a specific week compared with the median of all groups for the same week. This method is preferred over the average because it deals better with non-normal distributions and outliers. If a lot had a mortality value below or equal to the overall median plus MAD, that lot was included in the benchmark curve. A total of 99 lots were identified for the benchmark curve, and 37 were identified as cases.

Statistical analysis

Frequentist methods: Different approaches were tested to build the benchmark curves. The average and cumulative average (CUM) with a 95% confidence interval (95%CI) and several standard deviations (1 SD, 1.5 SD, 2 SD, 2.5 SD, and 3 SD) were used to build different benchmark curves with thresholds.

Resampling methods: In addition to the previous methods, two resampling techniques were tested to create the benchmark curve and thresholds. Bootstrapping (BOOT) is a resampling technique used to estimate the variability of survival estimates and derived statistics such as confidence intervals. It involves repeatedly sampling with replacement from the original dataset to create multiple bootstrap samples from which survival curves are generated to assess uncertainty in the survival analysis results (Kumar and Srivistava, 2012). For this method, the number of bootstrap samples was set to 1000, and the bootstrapping for each row was implemented using the R package boot (Canty and Ripley, 2024) . After the bootstrapping, the 95%CI was calculated. The other resampling technique was the Jackknife (JK). This is a resampling method that involves systematically leaving out one observation at a time from the original dataset to create multiple jackknife samples. In this approach, the number of jackknife samples was the same as the number of observations in the dataset.

Bayesian methods: Finally, Bayesian statistics was also implemented to build the benchmark curves using Markov Chain and Monte Carlo (MCMC) models. One of the MCMC models used Stan implementing Bayesian inference techniques, specifically Hamiltonian Monte Carlo (HMC) and its variant, the No-U-Turn Sampler (HMC-NUT), to sample from the posterior distribution of the model. This method effectively explores the parameter space by utilizing gradient information, which enhances convergence and reduces autocorrelation among samples. The Stan model was defined to analyze observed data `y`, which was assumed to follow a normal distribution with parameters `mu` (mean) and `sigma` (standard deviation). The other MCMC model used JAGS, which employs Bayesian inference through Gibbs sampling and Metropolis-Hastings (MCMC-MH) sampling to estimate parameters in a model with unknown mean and variance. The defined model assumed that the observed data `y` followed a normal distribution, where the likelihood was specified by the normal density function `dnorm(mu, tau)`. The model incorporated a prior for the mean `mu`, drawn from a normal distribution with a mean of 0 and a very small variance (0.0001), while the prior for the

precision (inverse of the variance) `tau` followed a gamma distribution with shape and rate parameters set to 0.1. Additionally, the standard deviation `sigma` was computed as the inverse square root of `tau`.

For each method tested, the mortality curve for the case sites was individually juxtaposed with the benchmark curve to evaluate deviations. Deviations from the benchmark were determined by assessing if the case mortality was above the upper limit of the expected 95% confidence interval or above the tested standard deviations (Fig. 1).

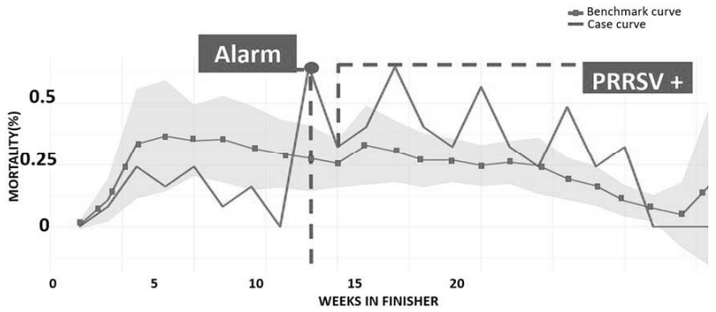

Figure 1. Mortality curve of a PRRSV positive case juxtaposed with the average reference and 95% confidence interval of the benchmark curve.

The deviations above the upper limits were further validated with the disease diagnosis tissue submission result. Performance was assessed by calculating the detection rates, the percentage of early detection two weeks before, one week before, and the percentage of detection one week after the positive tissue submission (Table 1). The analysis was done in the R© Statistical Software (v4.4.0; R Core Team 2024) and Microsoft Excel©.

Table 1. Metrics evaluated in the study.

Metric	Calculation
Early detection rate	$\frac{Deviation\ 1\ or\ 2\ weeks\ before\ Tissue\ submission\ report}{All\ Tissue\ submission\ reports}$
Detection rate	$\frac{Deviation\ on\ the\ week\ of\ Tissue\ submission\ report}{All\ Tissue\ submission\ reports}$
Late detection rate	$\frac{Deviation\ 1\ week\ after\ Tissue\ submission\ report}{All\ Tissue\ submission\ reports}$

RESULTS

The benchmark lots (n=99) had an average of 1,185 animals per lot and an average final mortality rate of 2.5%. In contrast, the case lots (n=37) had an average of 1,157 animals and an average final mortality rate of 5.9%. The case lots had four positive results of *Actinobacillus pleuropneumoniae,* two positive cases of enterohemorrhagic *Escherichia coli*, ten positive results of Influenza A virus, five positive results of *Pasteurella multocida*, nine positive results of PRRSV and nine positive results of non-specificed agent that caused systemic disease.

The two-week early detection rate was higher using the Bootstrapping method (56.7%). It was followed by the MCMC-MH and HMC-NUT methods (51.35%). Concerning the detection rate, this was 91.89% for the BOOT, 86.49% for the HMC-NUT, and 83.78% for the MCMC-MH and the Jackknife. The late detection rate was 56.76% for BOOT, JK, HMC-NUT, and MCMC-MH (Table 2).

Table 2. Early detection rate, detection rate, late detection rate, sensitivity and specificity for the different methods used in the mortality curves.

Method	Early detection rate two weeks (%)	Early detection rate one week (%)	Detection rate (%)	Late detection rate (%)
95%CI	45.95	54.05	81.08	54.05
1 SD	45.95	48.65	59.46	48.65
1.5 SD	40.54	43.24	48.65	45.95
2 SD	29.73	40.54	45.95	29.73
2.5 SD	27.03	40.54	37.84	27.03
3 SD	18.92	37.84	35.14	18.92
CUM 95%CI	13.51	16.22	13.51	10.81
CUM 1 SD	29.73	37.84	43.24	40.54
CUM 1.5 SD	24.32	29.73	29.73	29.73
CUM 2 SD	13.51	18.92	21.62	16.22
CUM 2.5 SD	5.41	10.81	13.51	13.51
CUM 3 SD	5.41	8.11	8.11	10.81
JK	48.65	56.76	83.78	56.76
BOOT	56.76	56.76	91.89	56.76
MCMC-MH	51.35	56.76	83.78	56.76
HMC-NUT	51.35	56.76	86.49	56.76

DISCUSSION

Monitoring mortality rates in finishing swine sites is essential for assessing herd health and detecting the introduction of pathogens. Constant vigilance allows producers to identify abnormal increases in mortality, which could indicate the presence of a new infectious agent. By maintaining systematic records and analyzing trends over time, it's possible to identify patterns correlating with disease outbreaks, enabling timely interventions. Early detection of disease-associated clinical signs is crucial for controlling and preventing outbreaks in swine

sites. Recognizing symptoms promptly can lead to quicker diagnostic procedures, allowing for immediate treatment and increased biosecurity measures to contain the spread and minimize impact, reducing the spread among the population. Implementing such early warning systems supports herd health maintenance and may improve the animals' welfare and the herd's longevity and productivity (Engler et al., 2009; Silva et al., 2017; Merca et al., 2022; Pedro Mil-Homens et al., 2024).

Several studies have investigated the utility of monitoring production indicators, including mortality, to inform management decisions in various swine production stages. Bono et al., (2014) showed the application of control charts to detect alarms in mortality rates, and although it lacked site-specific information to distinguish false alarms from true alarms, it was possible to observe that the pattern of sow mortality was very stable in contrast with the pattern of mortality in stillbirths and pre-weaning piglets. In this study, an attempt was made to mitigate the impact of variance in the mortality curves by getting the best average per week in the finishing sites from the benchmark lots with low mortality and without tissue submissions. That provided an expected weekly value of what should be considered normal mortality compared with the cases.

There is little information in the literature on implementing this methodology in finishing sites. Although there are publications that focus on the same purpose, that is, to take advantage of the data that is being collected routinely at the site to create a surveillance system. Detection of deviations in production indicators can be implemented by applying different methods. One approach used cumulative curves, to detect deviations in water intake in sick calves (Knauer et al., 2018), other approaches used moving averages to detect deviations in production indicators, such as abortions, prenatal losses, and dead sows, to detect deviations associated to PRRSV outbreaks (Silva et al., 2017; Merca et al., 2022; Pedro Mil-Homens et al., 2024). Kaplan-Meier survival curves were used to estimate the probability of swine getting infected with classical swine fever by using indicators such as herd size and pig density (Benard et al., 1999), and proportional hazard with Weibull and Cox time-dependent models as baseline, threshold models and sequential threshold models were tested to predict piglet preweaning survival based on mortality (Cecchinato et al., 2010). In these studies, it was observed that deviations possibly related to the disease were observed weeks before the diagnosis of the disease. For this study the results were similar, even though the methods were different. It was observed that using mortality to implement a surveillance system raised deviations weeks before the positive result of tissue submission.

The Bootstrapping method outperformed others, achieving a two-week early detection rate of 56.7% and an overall detection rate of 91.89%. This high performance may be attributed to its resampling approach, which effectively captures variability in the data and enhances sensitivity. The Bootstrapping method also has the benefit of not requiring assumptions about the data (Kumar and Srivastava, 2012). In contrast, the MCMC-MH and HMC-NUT methods demonstrated lower early detection rates (51.35%) and overall detection rates (83.78% and 86.49%, respectively). The Jackknife method mirrored the MCMC-MH in performance, suggesting that both Bayesian and resampling methods can yield similar outcomes but with different underlying mechanics. While these Bayesian methods exhibited a high detection rate, they can be prone to false positives, which occur because the MCMC will base their posterior distribution on the priors. The posterior thresholds might be too restricted if the prior distribution has low mortality values. One benefit of using these methods is, however, their ability to handle missing data (Columbia University, 2016).

Conversely, methods employing weekly cumulative averages showed low detection rates (below 50%), highlighting their inadequacy in detecting deviations. Cumulative average-based methods seem to compromise the ability to identify actual mortality changes effectively. This suggests that for timely and accurate mortality surveillance, methods like Bootstrapping, which leverage resampling techniques, are more effective in detecting deviations.

Implementing continuous surveillance using routinely collected mortality data at finishing sites offers significant benefits for swine health management, especially given the labor shortages in these operations. Relying solely on already stretched staff to monitor clinical signs across multiple sites may not provide the accuracy or reliability needed for timely disease detection. Producers can quickly identify deviations by continuously tracking mortality rates, triggering early interventions, and minimizing health impacts. Building benchmark curves to compare ongoing lots with historical data enables early detection of anomalies, ensuring a more efficient response. This proactive approach improves biosecurity, enhances decision-making, and helps maintain herd productivity, particularly during the critical wean-to-finish period.

It's crucial to acknowledge the limitations of this study, which encompassed only finishing sites. The study may overlook finer subtleties and variations with data collection constrained to weekly intervals. Furthermore, the research was limited to sites originating from sow sites with PRRSV status 2, 3, or 4, which could influence the generalizability of the findings. With mortality as the sole parameter considered, the study's scope does not extend to other potential site health indicators.

This research has successfully demonstrated the applicability of various methodologies to monitor mortality in finishing sites, pinpointing deviations that could be indicative of disease. Future studies should explore these methodologies with daily data and other indicators to comprehensively assess herd health dynamics. The inclusion of daily analysis offers a more granular perspective, capturing transient or rapidly evolving health issues that weekly data might not reveal. Furthermore, expanding the focus beyond mortality to include additional indicators such as feed consumption could yield a multifaceted understanding of animal well-being and productivity.

In conclusion, this study underscores the importance of employing robust and sensitive methodologies for the early detection of deviations in mortality rates within finishing swine sites. Among the methods evaluated, the Bootstrapping approach emerged as particularly effective, demonstrating high early detection rates. These methods not only facilitate the timely identification of health challenges but also enhance the ability of producers to implement prompt and targeted interventions, thereby mitigating the impact on herd health and productivity. The findings also highlight the critical role of continuous monitoring and systematic analysis of mortality data as foundational elements of an effective disease surveillance strategy in swine production.

ACKNOWLEDGEMENTS

This study was funded by the U.S. Department of Agriculture - National Institute of Food and Agriculture (USDA-NIFA) #2023-68008-39860. The authors would thank the production system for collaborating in the study.

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APPLICATION OF A QUALITATIVE RISK OF BIAS ASSESSMENT TOOL IN VETERINARY MEDICINE: EXAMPLE OF CANNABIDIOL USED FOR TREATMENT OF EPILEPSY IN DOGS

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SUMMARY

Biases can affect the validity of scientific studies if they are not appropriately considered throughout the research process. Still, the risk of bias is not routinely and systematically assessed in veterinary medicine. A qualitative tool was tested to appraise evidence in three randomised controlled trials (RCTs) evaluating the effect of cannabidiol (CBD) used as a supplementary treatment for dogs with epilepsy. Despite all three studies being RCTs, high on the evidence pyramid, the studies showed moderate to high risk of bias, and this was not systematically reported in the studies. This oversight along with selective reporting may compromise findings, particularly in outcome assessment, confounding, and statistical analyses. The results highlight the importance of considering biases and using bias visualisation tools to support study assessments.

INTRODUCTION

Potential biases in a study can affect the quality of evidence and, consequently, how the results are interpreted and used. Unlike in human medicine, risk of bias assessment is not systematically implemented in veterinary sciences. Several tools and guidelines for risk of bias assessment exist (Higgins et al., 2011; Sterne et al., 2016, 2019), but the majority are focused on human study populations and are not designed specifically for evaluating biases relevant to animal studies. Some guidelines have been designed for animal studies (Hooijmans et al., 2014), but a tool designed for animal study populations housed outside of a laboratory, for example production or companion animals, is warranted. Additionally, existing tools are often in a comprehensive and lengthy format. Hence tools to do rapid, but still systematic assessments using comprehensive methods are needed.

To meet the need for systematic and transparent guidelines for risk assessors, the German Federal Institute of Risk Assessment (BfR) developed a tool for rapid assessment of risk of bias, called raRoB (Plate et al., 2024). The tool consists of seven domains or categories of bias, namely selection, exposure/intervention, outcome assessment, confounding, loss to follow-up, analysis and selective reporting. It includes a total of 15 items with a series of questions,

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referred to as signalling questions, which help indicate if the risk of bias should be evaluated as “high”, “some concerns” or “low” in each domain.

The objective of this study was to demonstrate the use of the above-mentioned raRoB tool to assess the risk of bias in selected studies within veterinary science. Since the use of CBD in veterinary medicine has gained attention in recent years, while the available evidence remains limited, the authors chose to test the raRoB tool on randomised controlled trials (RCT) evaluating the effect of CBD used as a supplementary treatment for dogs with refractory idiopathic epilepsy. The RCTs were used as case studies to apply the raRoB tool, and here the process of conducting a risk of bias assessment using the tool, signalling questions, and reaching an agreement between assessors is reported.

MATERIALS AND METHODS

Systematic review of studies to include in risk of bias assessment

Using the PRISMA guidelines (Page et al., 2021), a systematic review was conducted of RCT studies evaluating the efficacy and adverse effects of CBD as a supplementary treatment for dogs with refractory idiopathic epilepsy. The literature search was performed in the databases PubMed, Web of Science, Embase, Google Scholar and ProQuest in December 2023, and only English publications were eligible for inclusion. The search included three compartments, 1) cannabidiol, 2) epilepsy and 3) dogs, as defined in the following search string: dog OR dogs OR canine OR canines OR canis (All Fields) AND epilep* OR IGE OR seizure* OR fit OR fits (All Fields) AND cannabi* OR CBD OR phytocannabinoid* OR endocannabinoid* OR tetrahydrocannabinol* OR THC OR Sativex OR epidiolex OR epidiolex (All Fields). Additionally, the MeSH database was used in the search string in PubMed.

Risk of bias assessment tool

The qualitative raRoB tool was *a priori* selected for rapid assessment of the risk of bias in the included studies. In an Excel file, the tool provided different sheets to guide the assessment: a cover sheet, where information about the study under evaluation could be inserted; a template sheet for the individual assessments of each item in every bias domain; an explanation sheet consisting of signalling questions to guide the judgements; and a result sheet where a summary of the assessment was available. The template sheet included a drop-down menu and a comment section for each item to be evaluated.

Resolving disagreements between assessors

Three of the authors of this paper (HAJ, AO, SSN) evaluated the risk of bias separately and inserted their assessments in the raRoB Excel file for each evaluated study. The three authors will be referred to as “the assessors” hereafter. After completion of the individual assessments, the assessors compared their results by discussing their comments and the judgements of risk of bias for each evaluated item. Any disagreements in the individual evaluations between the assessors were resolved by discussing the rationale behind the assessments. When resolving disagreements, the seniority of the assessors was considered.

RESULTS

Studies eligible for inclusion

A total of three studies out of 105 records from the literature search were considered eligible and hence included for risk of bias assessment. In this paper, the studies are referred to as Study 1 (McGrath et al., 2019), Study 2 (Garcia et al., 2022) and Study 3 (Rozental et al., 2023). The trials in each study included epileptic dogs in a treatment group, which was treated with CBD-infused oil as a supplement to antiepileptic medication, and a control group, which was treated with a placebo oil designed to mask the resemblance of the CBD oil as a supplement to antiepileptic medication. The outcomes of the trials were efficacy and adverse effects in the treatment and control groups. The results of the RCTs are not summarised in detail in this paper. However, some of the study characteristics relevant to the assessment of risk of bias are summarised in Table 1.

Table 1. Characteristics of randomised controlled trials (RCT) included for assessment.

Study ID	Design	Blinding	Sample size
1	RCT, parallel	Vets, owners, statisticians	16
2	RCT, cross-over	Owners	14
3	RCT, cross-over	Owners	51

Selection bias

In the domain concerning selection bias, the eligibility criteria, comparability of intervention and control groups, non-response rate and recruitment time frame were evaluated. The assessors agreed that Study 1 had a high risk of selection bias, while some concerns were reported for Studies 2 and 3. Particularly one of the signalling questions provided by the raRoB tool gave rise to concern in the studies, namely “*Was the selection process likely to select participants that were representative of the target population under investigation?*” (Explanation sheet in raRoB tool, Plate et al., 2024). The assessors addressed their concerns specifically related to the risk of self-selection bias, which was present in all studies. The studies recruited dog owners for the study in different centres and animal clinics; however, it was unclear which owners allowed enrolment of their dogs and from which centres they originated (Studies 1 and 3). The cross-over design of Studies 2 and 3 accounted for the comparability of the groups, while no balancing techniques were used in the parallel design of Study 1. Additionally, due to the parallel group design in Study 3 and the small heterogenous sample of dogs, the assessors evaluated that a balancing of the groups would have been necessary to support the comparability of the treatment and control groups. In general, the reasons for the exclusion of dogs otherwise eligible for inclusion into the studies were unclear and compromised the representativity of the study populations. For example, 90% of dogs in Study 1 were excluded, but it was not clear if this was done based on owner decision or due to lack of eligibility. Therefore, the assessors judged the risk of selection bias to be high in Study 1, while some concerns were also raised for Studies 2 and 3.

Differential misclassification of intervention

In the domain concerning biases related to the intervention, the accuracy and validity of intervention measurements, and differential misclassification of intervention were evaluated. The assessors had some concerns about the blinding of involved parties in the trials. The

concerns were linked to the signalling question *"Is there evidence that outcome assessors were adequately blinded to the groups?"* (Explanation sheet in raRoB tool, Plate et al., 2024). In particular, concerns were raised for Studies 2 and 3, where some blinding was allegedly done, but it was either not stated if the researchers were blinded or that the researchers were unblinded due to observing the results of blood tests performed to monitor the study participants.

Differential misclassification of outcome

In the domain concerning biases related to the outcome, the accuracy and validity of outcome measurements, and differential misclassification of outcome were evaluated. One of the signalling questions formed the basis of judgements of high risks of bias in all three studies, namely *"Are objective, suitable and standard criteria used for measurement of the health outcomes?"* (Explanation sheet in raRoB tool, Plate et al., 2024). Similarly, for all the evaluated studies, the recording of adverse effects was inconsistent and lacked a standardised approach. Furthermore, some of the reported adverse effects from CBD treatment, including ataxia and changes in clinical pathology, were not discussed.

Confounding bias

In the domain concerning confounding bias, it was evaluated whether confounding was accounted for and how. This was formulated in two of the signalling questions in the tool, *"Have the authors taken potential confounding factors in the design and/or analysis into account, and are the confounders in each group to be compared clearly described?"* (paraphrased from Explanation sheet in raRoB tool, Plate et al., 2024). Potential confounding was not discussed in the three RCTs, and the assessors of the present study discussed the relevance of confounders in RCT study designs. The process of randomly allocating dogs into treatment and placebo groups should be undertaken in a way that allows for control of potential confounding factors. That said, when dealing with small and heterogeneous samples as represented in the three studies, confounding could potentially be present. This is especially the case for dogs, where the number of dog breeds included in the study was huge compared to the sample size, and the "standard" epilepsy treatments were highly variable. This can easily lead to procedural confounding, which is a result of the situational characteristics of the two heterogenous populations. Due to the parallel design in Study 1, the risk of confounding was higher than in Studies 2 and 3, which had cross-over designs. After a discussion among the assessors, they reached agreement that the risk of confounding bias was high in Study 1 and moderate in Studies 2 and 3.

Loss to follow-up

In the domain concerning loss to follow-up, the length of the follow-up and the management of loss to follow-up were evaluated. The assessors addressed some concerns in the answer to the signalling question *"Were withdrawals and drop-outs reported in terms of numbers and/or reasons per group?"* (Explanation sheet in raRoB tool, Plate et al., 2024). The evaluated studies each had 2-10 dogs lost to follow-up or withdrawn from the trials. Some of the dogs that dropped out could be due to adverse effects from the treatment, but this was not discussed in detail. Furthermore, drop-outs could be due to lack of blinding, where owners of dogs in the placebo groups may realise the lack of effect over a long trial period. However, this was also not discussed.

Bias in data analysis

The statistical analysis and handling of missing values were assessed using the signalling questions *"Are the statistical methods appropriate for the study type, design and/or the question, and were appropriate statistical methods used to account for missing data?"* (Explanation sheet in raRoB tool, Plate et al., 2024). The assessors found the three studies to lack statistical power, and the studies did not discuss the risk of statistical Type 2 errors. Furthermore, some analyses were inappropriate for their purpose. For example, Study 3 compared the number of baseline epileptic seizures with the number of seizures during treatment without applying an appropriate method for analysis of count data like Poisson regression. None of the studies handled the missing data resulting from drop-outs. The concerns about the appropriateness of statistical analyses and not handling missing data formed the basis of a high risk of bias assessment in the data analysis.

Selective reporting bias

Selective reporting of study outcomes was evaluated by addressing the signalling questions *"Are any measured outcomes missing, and do the conclusions reach beyond observed results?"* (Explanation sheet in raRoB tool, Plate et al., 2024). As the assessors already pointed out in the outcome assessment, some outcome measures, such as ataxia and changes in clinical pathology, were not reported in a systematic way and discussed rigorously. Moreover, Studies 1 and 3 had inconsistent reporting of the number of dogs in the study population, while Study 2 described the adverse effects as minimal, despite reporting multiple and some with a prevalence of 60% in the treatment group. If they were "minimal" even with a prevalence of 100%, this should have been explained, but it was not, and therefore the conclusions were not transparent. Based on these findings, the assessors judged the studies to contain a high risk of selective reporting bias.

DISCUSSION

Through this study, the applicability of the recently developed raRoB tool was demonstrated on studies including animal populations outside of a laboratory setting. The tool was integrated within a systematic review to assess the risk of bias in three RCTs testing the efficacy and safety of CBD as a supplementary treatment for dogs with refractory idiopathic epilepsy. Based on small and heterogeneous study populations, the trials suggested that CBD is an effective supplement to reduce epileptic seizures in dogs, but several adverse effects should be investigated further. In the present assessment, it was found that the validity of the results was influenced by moderate to high risk of biases, particularly in the domains evaluating outcome assessment, confounding, analysis and selective reporting.

The authors of the present study selected a qualitative risk of bias assessment tool designed for observational studies and applied it to experimental studies due to the lack of a tool that covered potential biases in experimental studies with a study population consisting of companion animals. Since the authors used a tool not specifically targeting the RCT study design, relevant biases could have been missed. However, the assessors found the raRoB to provide clear and helpful signalling questions that covered the potential biases in the RCT studies, despite of the tool being designed for observational studies. Additionally, qualitative risk of bias assessment tools designed for RCTs often do not include the potential risk of confounding under the assumption that randomisation accounts for confounding. This may not

be the case if the study population is small and heterogeneous as was the case in the Studies 1-3 evaluated here.

Previous reviews have assessed at least two of the clinical trials and arrived at a different conclusion, wherein they highlighted a promising effect of CBD while downplaying the reported adverse effects (Morrow and Belshaw, 2020; Lima et al., 2022; Potschka et al., 2022). Only one of the three existing reviews had included a risk of bias assessment (Lima et al., 2022). Contrary to the findings in this study, the previous review found a low risk of bias in four of five domains in their assessment, which was not reported in detail. The present study indicates that relevant biases and limitations were overlooked, even though RCTs and systematic reviews are at the top of the evidence pyramid and considered to provide high-quality evidence. There is a need for a systematic and transparent approach to assess the risk of bias in veterinary sciences. This study can be used to promote and develop the use of a risk of bias assessment tool to accommodate relevant study designs and reduce bias.

ACKNOWLEDGEMENTS

The authors would like to thank Associate Professor Anette Boklund from the Department of Veterinary and Animal Sciences, Section for Animal Welfare and Disease Control, for contributing valuable comments to the conference abstract.

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RISK QUANTIFICATION

ECONOMIC EVALUATION OF POTENTIAL PREVENTIVE VACCINATION STRATEGIES FOR HIGHLY PATHOGENIC AVIAN INFLUENZA IN AUSTRIAN POULTRY FARMS

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SUMMARY

The ongoing global epidemic of highly pathogenic avian influenza (HPAI) has led to the culling of millions of birds across the European Union and beyond. In response, vaccination has been increasingly considered alongside existing measures to control HPAI in poultry. This study evaluates the economic and logistical implications of a potential preventive vaccination program for HPAI in Austrian poultry farms across multiple scenarios, considering different animal and production types, vaccination areas, and vaccination periods. Results show that targeting high-density poultry areas, specific farm types, and seasonal risks can optimize resources and reduce costs. Total cost per vaccinated animal varies from €1.52 to €4.06. Sensitivity analysis identifies vaccine costs and post-vaccination diagnostics as key cost drivers. The findings of this study serve as a basis for policymakers to conduct a cost-benefit analysis, supporting informed decision-making on implementing a vaccination program in Austria for the effective and economic prevention of HPAI outbreaks.

INTRODUCTION

Highly pathogenic avian influenza (HPAI) is considered one of the most concerning animal diseases of the 21st century, with a notable global increase in the number of outbreaks over the past few years (Adlhoch et Baldinelli, 2023; Xie et al., 2023). Since 2020, multiple HPAI virus subtypes have co-circulated worldwide and the virus has been increasingly detected in wild birds, poultry, mammals and occasionally in humans (Adlhoch et al., 2023; Graziosi et al., 2024). In the European Union (EU) alone, the current HPAI epidemic has led to more than 14 thousand reported outbreaks and the culling of over 96 million farm-reared birds (Adlhoch et Baldinelli, 2023). This lead to significant economic losses for farmers and governments. These serious consequences have spurred debate and a rethink about the prevention and control of outbreaks and the role of vaccination alongside other interventions (Kalthoff et al., 2010; Germeraad et al., 2023; Rautenschlein et al., 2023; Shi et al., 2023).

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In 2023, the EU established a legal framework to facilitate vaccination against HPAI under certain conditions (Delegated Regulation (EU) 2020/687; Delegated Regulation (EU) 2023/361; Regulation (EU) 2016/429), while calling upon its member states to intensify efforts in developing vaccination strategies for prevention and control, and encouraging them to conduct economic assessments of potential vaccination programs (Council Conclusion 2022/C 245/03).

Like other EU countries, Austria has experienced numerous HPAI outbreaks in both wild birds and poultry (Adlhoch et al., 2023; AGES, 2023, 2024b). These outbreaks have led to a considerable financial burden resulting from the implementation of control measures and efforts to prevent the further spread of the virus. In response, Austrian veterinary authorities have initiated a comprehensive evaluation of a potential vaccination campaign to assess the feasibility and economic impact and to better prepare and protect the poultry industry against possible future large-scale outbreaks.

The aim of this study is to evaluate the cost and logistical implications of a preventive vaccination program against HPAI in Austria. By analyzing various hypothetical vaccination scenarios, the study seeks to identify optimal strategies to reduce the economic burden of HPAI on the poultry industry.

MATERIALS AND METHODS

Poultry population and structure in Austria

Data on Austrian poultry farms, used to calculate the financial impact of vaccination scenarios, were extracted from the Austrian Animal Husbandry Register (2023–2024). The dataset initially included 19,815,210 susceptible animals across 4,612 farms. Only farms with more than 50 animals, classified as commercial under Austrian legislation, were included in the economic assessment, representing 77.5% of farms and 99.9% of poultry (Table 1). Commercial poultry farming in Austria is dominated by laying hens (56.9% of farms) and broiler chickens (23.2%), with smaller shares for turkey farming (4.3%) and waterfowl (2.9%). Hatcheries, rearing, and parent stock farms account for less than 12.8%, though hatchery throughput is substantial.

Vaccination scenarios

Based on Austrian poultry industry structure and population data, identified risk factors, international experience, scientific literature, and consultations with Austrian veterinary authorities, three primary vaccination strategies were developed tailored to animal type and production systems:

- Vaccination of parent stock farms in both free-range and indoor systems (S1),
- Vaccination of parent stock farms in both free-range and indoor systems, and all free-range waterfowl farms (S2),
- Vaccination of all free-range farms, excluding broiler chicken farms (S3).

To explore the economic differences resulting from various regional approaches, each strategy was evaluated across three regional variations:

- Austria-wide vaccination program (AT),

- Vaccination program in the officially designated “very high-risk areas” during spring 2023 (VHRA 2023),
- Vaccination program in the officially designated “very high-risk areas” during spring 2024 (VHRA 2024).

In free-range systems, poultry has regular access to outdoor areas where they can roam freely, whereas in barn-raising systems, poultry is kept indoors in large, open spaces within a barn, without access to outdoor areas.

Table 1. Classification of poultry farms with more than 50 birds by species, production type, and husbandry system. The data are provided for the Austria-wide population (AT), the very high-risk area for 2023 (VRHA 2023) and the very high-risk area for 2024 (VRHA 2024).

Species	Production type	Husbandry	Number of farms			Average flock size (AT)
			AT	VHRA 2023	VHRA 2024	
Duck	Parent stock	Barn raising	3	3	1	3,500
		Free-range	2	2	-	1,788
	Hatchery	-	1	1	-	3,500
	Fattening	Barn raising	25	19	1	2,613
		Free-range	22	20	3	4,645
Goose	Parent stock	Barn raising	-	-	-	-
		Free-range	2	2	1	788
	Hatchery	-	-	-	-	-
	Fattening	Barn raising	36	27	3	1,039
		Free-range	19	10	3	562
Chicken	Parent stock	Barn raising	140	134	14	9,762
		Free-range	99	90	3	9,859
	Hatchery	-	10	9	1	7,983
	Rearing	Barn raising	133	115	21	10,545
		Free-range	65	54	5	10,923
	Laying hens	Barn raising	598	415	73	4,848
		Free-range	1,435	1,011	119	2,889
	Fattening	Barn raising	413	363	119	9,548
		Free-range	315	231	74	7,224
	Fattening - Young roosters	-	102	89	15	9,431
Turkey	Parent stock	Barn raising	-	-	-	-
		Free-range	-	-	-	-
	Hatchery	-	-	-	-	-
	Rearing	Barn raising	1	1	-	150
		Free-range	-	-	-	-
	Fattening	Barn raising	118	89	40	6,294
		Free-range	37	17	1	1,627

Both VHRAs were defined temporarily by Austrian veterinary authorities based on poultry, captive birds and wild bird HPAI outbreaks and other epidemiological risk factors, covering 75.6% (VHRA 2023) and 13.9% (VHRA 2024) of all commercial poultry farms in Austria (Table 1, Fig. 1). VHRAs are subject to strict restrictions on the movement and containment of poultry and other captive birds. Farms with more than 50 birds are required to permanently house them in structures such as barns or enclosed facilities, ensuring at least overhead coverage to prevent contact with wild birds and their feces (Geflügelpestverordnung, 2007).

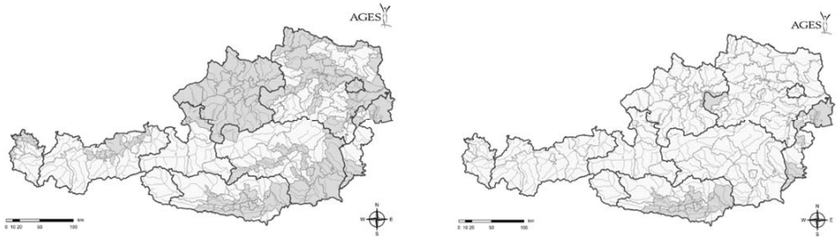

Figure 1. The distribution of very high-risk areas (VHRAs) (shaded areas) as defined by the national veterinary authorities based on the number of poultry and wild bird outbreaks and other epidemiological factors.

Additionally, each scenario was assessed for both year-round vaccination (12 months) and seasonal vaccination (September to March; 7 months), resulting in a total of 18 different vaccination scenarios evaluated in economic and logistical terms.

Cost assessment and operational considerations

The assumptions for vaccination performance and costs were consistent across scenarios, accounting for the varying lifespans of commercial farm animal species. Each animal was assumed to receive two vaccinations in the first four weeks of life, followed by additional vaccinations at intervals based on its lifespan. Vaccinations were conducted by veterinarians and included clinical flock examinations (Nielsen et al., 2023).

In accordance with Delegated Regulation 2023/361, both active and passive post-vaccination surveillance were considered in the calculations. It was assumed that active post-vaccination surveillance would occur every four weeks following the vaccination phase, under veterinary supervision. The duration of this monthly monitoring was assumed to last for one year in the year-round approaches across all scenarios. In the seasonal approaches, due to the shorter vaccination campaign, the monitoring was assumed to continue only until the last animals vaccinated were slaughtered, in accordance with their lifespans. During this surveillance, all vaccinated animals undergo clinical examinations, and based on a representative sample size, virological testing. The collection of representative samples for laboratory surveillance must be carried out according to a sample size that shall be calculated to detect a within-establishment infected animal prevalence of 5%, with a 95% confidence

(Delegated Regulation (EU) 2023/361). Additionally, serological testing of 20 animals at the end of the batch in each farm was included in the calculations. It was further assumed that passive post-vaccination surveillance would be conducted weekly by farmers, with virological testing of 3% of their animals, as this value represents a threshold for the normal weekly abortion rate in poultry (Prock, 2021).

Costs were categorized into vaccination and post-vaccination phases. Vaccination costs included personnel (application, catching, handling, clinical examinations), materials (vaccines), and other expenses (certificates, travel). Surveillance costs covered personnel, diagnostics, and logistical expenses. Vaccine costs were estimated using Nobilis® RT + IBmulti + ND + EDS due to the lack of specific HPAI vaccine data.

Logistical parameters, such as the number of vaccinated animals and farms, vaccination doses, and visits, were included in the analysis. A comparative cost structure analysis was conducted to identify differences across scenarios.

Sensitivity analysis

Given the uncertainty of cost parameters in economic assessments, a one-way sensitivity analysis evaluated their impact on economic outputs. The following costs were analyzed: (i) vaccine, (ii) application, (iii) clinical examination, (iv) sample collection, and (v) serological testing. Each parameter was adjusted by ±20%, keeping all others constant. Changes in total costs were compared to the original, highlighting factors with the most significant economic impact. As year-round vaccination across Austria (scenarios S1–S3) was expected to incur the highest costs, the analysis focused on these scenarios, representing the sensitivity range of all others.

RESULTS

This study examines the cost and logistical implications of various HPAI vaccination strategies, highlighting differences in the number of vaccinated farms, animals, vaccine doses, farm visits, and associated costs across regional approaches and scenarios. As expected, the Austria-wide year-round strategy had the largest coverage, with scenario S3 vaccinating 1,681 farms, significantly more than S1 (445 farms) or S2 (486 farms). However, S3 vaccinated fewer animals (~7.9 million) compared to S1 (~8.7 million) and S2 (~9.1 million), as it focused on smaller free-range farms with longer-lifespan animals. Correspondingly, S3 required fewer vaccine doses (~15.8 million) than S1 (~17.4 million) or S2 (~18.3 million) (Fig. 2).

The results further indicate that the number of farm visits required for both the vaccination phase and post-vaccination surveillance varies substantially across scenarios. For example, scenario S3 in the Austria-wide year-round approach necessitates the highest number of visits (3,978 vaccination phase and 20,172 post-vaccination surveillance), reflecting the extensive coverage of free-range farms (Fig. 2).

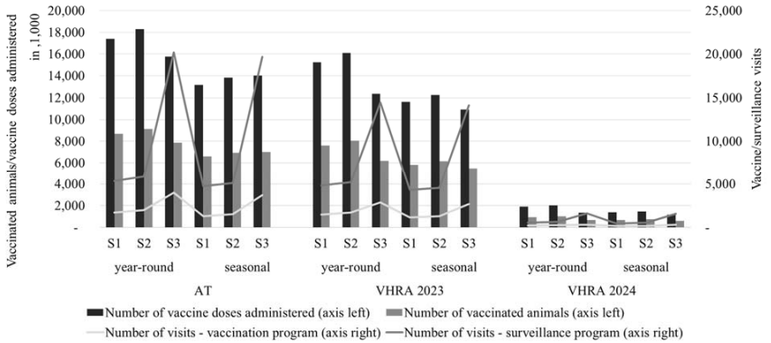

Figure 2[a]. Operational and logistical results of the vaccination program, including the vaccination phase (VP) and post-vaccination surveillance (PV-S).

[a] S1: Vaccination of parent stock farms in both free-range and indoor systems; S2: Vaccination of parent stock farms in both free-range and indoor systems, and vaccination of all free-range waterfowl farms; S3: Vaccination of all free-range farms, excluding broiler chicken farms. AT: Austrian-wide vaccination program; VHRA 2023: Vaccination program in official very high-risk areas in spring 2023; VHRA 2024: Vaccination program in official very high-risk areas in spring 2024 (VHRA 2024).

The economic analysis reveals that total costs for year-round vaccination are higher than for seasonal approaches. Both vaccination and post-vaccination costs were lower in seasonal scenarios, as fewer animals were vaccinated and the duration of post-vaccination surveillance is shorter, reflecting the animals' lifespan. Among Austria-wide programs, the year-round scenario S3 incurred the highest cost (€29.7 million), followed by S2 (€14.7 million) and S1 (€13.7 million). Regional variations showed lower costs, with VHRA 2023 amounting to €21.8 million in S3 and VHRA 2024 to €2.4 million (Fig. 3).

The total cost per vaccinated animal varied between €1.52 and €4.06, with year-round S3 costing €3.77 per animal, compared to €1.57 for S1 and €1.60 for S2. Seasonal scenarios followed the same trend but had higher per-animal costs. This pattern was consistent across VHRAs due to fewer vaccinated animals in S3 relative to the number of vaccinated farms. Figures 2 and 3 illustrate that the economic and logistical implications of the three main strategies (S1–S3) are comparable across regions and periods, with Austria-wide, year-round scenarios showing the highest economic and logistical demands.

Cost distribution

The analysis of the cost distribution shows that across all regional and seasonal approaches, the total costs of the vaccination phase in scenarios S1 and S2 were approximately the same as those of post-vaccination surveillance (48–54% of the total cost). In scenarios S3, on the other hand, the share of costs for post-vaccination surveillance is substantially higher (75–78% of the total cost). These comparatively high costs are a result of the high number of farms vaccinated and subsequently monitored. The analysis further shows that both the costs for the vaccination phase and for post-vaccination surveillance are relatively comparable across the

scenarios within a regional approach. The exception is scenario S3, where the costs for post-vaccination surveillance are approximately twice as high as in scenarios S1 and S2. Vaccines consistently account for the largest expenditure within the vaccination phase across all scenarios (77–84%), followed by vaccine administration, travel costs, and clinical examinations. Within post-vaccination surveillance, the highest costs are for diagnostics (52–53%), followed by personnel for sample collection, travel costs and personnel for clinical examinations (Fig 3).

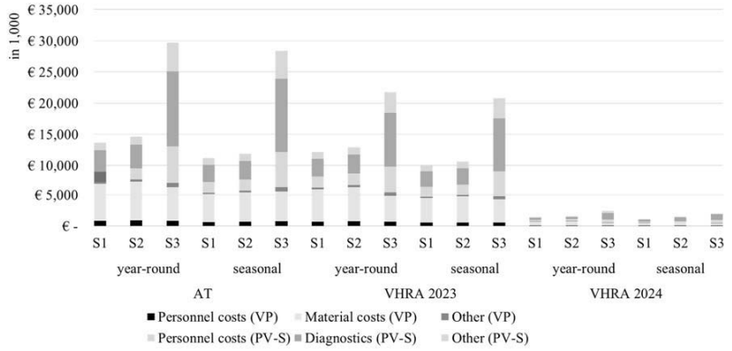

Figure 3[a]. Cost distribution of the total cost across all analyzed vaccination strategies. The costs are aggregated as follows: Vaccination phase (VP) – personnel costs (costs for clinical examinations and vaccine application); material costs (costs of vaccines); and other costs (travel costs and costs for issuing certificates). Post-vaccination surveillance (PV-S) – personnel costs (costs for clinical examinations and sample collection); diagnostic costs (costs for serological and virological testing); and other costs (travel and shipping costs).

[a] S1: Vaccination of parent stock farms in both free-range and indoor systems; S2: Vaccination of parent stock farms in both free-range and indoor systems, and vaccination of all free-range waterfowl farms; S3: Vaccination of all free-range farms, excluding broiler chicken farms. AT: Austrian-wide vaccination program; VHRA 2023: Vaccination program in official very high-risk areas in spring 2023; VHRA 2024: Vaccination program in official very high-risk areas in spring 2024 (VHRA 2024).

Sensitivity analysis

Adjusting five cost parameters by ±20% revealed their impact on total costs. Sensitivity analysis shows that vaccine cost had the greatest impact in scenarios S1 and S2 (±8.6% and ±8.5%), which involved the highest vaccine usage. Diagnostics were the second most influential factor (±8.1%). In scenario S3, where the most farms were vaccinated and tested, diagnostics had the largest impact (±8.1%), followed by vaccine costs (±3.6%). Costs for vaccine application, clinical examinations, and sample collection had minimal influence on total costs.

DISCUSSION

This study assessed the economic and logistical implications of a preventive HPAI vaccination program in Austria, focusing on costs and operational activities across multiple scenarios.

Among all scenarios analyzed, the Austria-wide year-round vaccination strategy S3 had the highest vaccination coverage with 1,681 farms and approximately 7.9 million animals. However, it also resulted in the highest total costs of around € 29.7 million. Implementing vaccination programs in VHRAs only substantially reduced the total costs compared to an Austria-wide approach, and the seasonal approaches always resulted in lower total costs compared to year-round strategies. However, it is important to note that the cost reductions in VHRA scenarios are accompanied by a corresponding reduction in coverage. For instance, the coverage rate of farms in VHRA 2023 compared to the Austria-wide approach is 90% for S1, 89% for S2, and 72% for S3, while for VHRA 2024, it is 10% for S1, 10% for S2, and 8% for S3. These percentages are also reflected in the shares of total costs for each scenario in relation to the total cost of Austria-wide strategy. Given the interdependence between costs and coverage, the VHRA scenarios can still be considered more cost-effective as they provide a better balance between the total costs incurred and the coverage achieved. However, which scenario is chosen depends on what trade-off between coverage and cost savings is deemed acceptable. Policymakers must carefully evaluate whether the reduced coverage in the VHRA scenarios adequately meets the goals of HPAI prevention in Austria. The regions of the VHRA 2023 are among those with the highest poultry density in Austria and have been predominantly affected by outbreaks in both hobby flocks and commercial farms in previous years (AGES, 2023, 2024b). This suggests that a higher risk of HPAI outbreaks can also be expected in these regions in the coming period, and due to the high poultry density, there may be greater economic damage in the event of a HPAI outbreak. As such, the targeted approach in VHRA scenarios may offer a strategic advantage by concentrating resources where they are most needed.

The results of this study underscore the high costs of post-vaccination surveillance, particularly in scenarios S3, which involve a large number of vaccinated farms. Surveillance costs in these scenarios were nearly double those of S1 and S2, driven mainly by diagnostics and personnel expenses for clinical examinations and sample collection. These findings emphasize the economic challenges of extensive surveillance, especially over prolonged periods. To address this, strategies such as optimizing surveillance frequency, integrating it into routine veterinary activities, or adopting cost-effective diagnostic technologies should be explored to reduce costs without compromising effectiveness.

The selection of poultry production types in vaccination scenarios significantly influenced the study results. Parent stocks, with longer lifespans and economic importance, were prioritized to ensure production continuity and mitigate HPAI risks (Capua et al., 2009). Waterfowl and free-range poultry, at higher risk due to exposure to wild birds, and laying hens, economically vital in Austria, were also included (Hesterberg et al., 2009; Agrarmarkt Austria, 2024). Broilers were excluded due to their short production cycle (between 5 to 7 weeks), making vaccination impractical and economically unfeasible (van der Goot et al., 2005).

The sensitivity analysis highlights vaccine costs and post-vaccination surveillance diagnostics as key cost drivers, particularly in scenarios S1 and S2 with the highest vaccine usage. Due to a lack of public pricing data for HPAI vaccines, the study used the price of

Nobilis® RT + IBmulti + ND + EDS, introducing some uncertainty. Vaccine costs vary by type, manufacturer, and regional pricing strategies (Plotkin et al., 2017; Hussain et al., 2020; Sanodat AIT2, 2024), emphasizing the need for favorable pricing and cost-effective options. While this study assumes injection-based vaccination by veterinarians, alternative methods like in ovo, spray, or water vaccinations could reduce costs and logistical demands (Vermeulen et al., 2002). Involving farmers and technical workers under veterinary supervision, as done in France, could further enhance cost-efficiency (Ministère de l'agriculture et de la souveraineté alimentaire, 2024).

Deciding for or against a vaccination program in Austria largely depends on the country's favorable epidemiological situation, with only a few reported HPAI cases in commercial poultry since 2020 and relatively low numbers of cases in wild birds and backyard flocks compared to neighboring countries (Adlhoch et al., 2023; AGES, 2023, 2024b; WOAH, 2024). Austria's effective surveillance system and high farm biosecurity have been instrumental in preventing outbreaks (AGES, 2024a). However, the high costs of a vaccination program must be weighed against its benefits, such as reducing the risk of HPAI spillover to mammals, including humans, which poses significant public health concerns. Vaccinating poultry reduces virus circulation, thereby lowering the likelihood of mutations and cross-species transmission (Webster et al., 2014; Hill et al., 2022; CDC, 2024). While vaccination costs are quantifiable, the benefits remain uncertain without simulation modeling, which is currently unavailable for HPAI in Austria. Effective outbreak prevention also depends on achieving adequate population coverage, determined by virus characteristics and the basic reproduction number (R_0).

ACKNOWLEDGEMENTS

We hereby acknowledge the assistance, expertise and data provided by the Austrian Agency for Health and Food Safety and the Federal Ministry of Social Affairs, Health, Care and Consumer Protection, and the financial support received from the VetAustria Cooperation.

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IDENTIFYING HOTSPOTS FOR EPIZOOTIC HEMORRHAGIC DISEASE VIRUS INTRODUCTION IN EUROPE BY THE TRADE AND THE WIND DISPERSAL PATHWAYS

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SUMMARY

A comprehensive assessment of the risk of further Epizootic Hemorrhagic Disease Virus (EHDV) introduction in Europe was conducted considering both the wind dispersal of vectors and the trade of live animals as potential pathways. The study quantified this risk spatially and temporally and examined the respective contribution of each pathway. The findings revealed a wide risk zone across Europe, with total risk levels varying significantly among countries. The wind dispersal pathway emerged as the primary risk factor, particularly affecting France, Spain, and their neighbouring countries. On the other hand, the trade of live animals presented a consistently low, yet persistent, risk even in distant countries. Notably, France stood out as the principal source of risk, irrespective of the pathway. These findings enhance our understanding of the risk of EHDV expansion, as well as potential control measures.

INTRODUCTION

Epizootic Hemorrhagic Disease (EHD) emerged in southern Europe (Italian Islands, southern Spain) by the end of 2022, spread over the Iberian Peninsula and Portugal within one year, and reached France in September 2023. The virus responsible for the disease was identified as serotype 8 (EHDV-8). In domestic and wild ruminants, the virus can cause severe and painful clinical signs, leading to death or at least impairment of domestic cattle productivity (Zientara et al., 2024). Moreover, the disease is listed as a regulated disease. Introduction of EHDV into new areas is a major threat for farmers, livestock industry and more broadly for food sustainability. Transmissibility of EHDV from an infected animal to a susceptible is mediated by a small biting midge of genus *Culicoides* spp. Both the wind dispersal of vectors and the movement of infected animals can trigger new introductions.

Quantitative Risk Assessments (QRAs) are crucial for estimating disease introduction risk, but previous QRAs have primarily focused on trade routes, often overlooking meteorological factors (Simons et al., 2019) . This study aims to identify European hotspots at high risk of EHDV-8 introduction by considering both trade in live animals and wind dispersal of vectors,

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enhancing previous QRA frameworks (Bibard et al., 2024) to provide a comprehensive understanding of the risk.

MATERIALS AND METHODS

To estimate the risk of further EHDV expansion, a dual-pathway risk assessment framework was developed which integrates wind dispersal of *Culicoides* vectors and transboundary trade of live domestic ruminants, for which the respective risk estimates W_{Sj} and T_{Sj} were calculated as shown in Eq.(1) and Eq.(2):

$$W_{Sj} = R_{0_j} \sum_{i \in S}^{n_i} \sum_{v \in \{1,2\}} V_{inf_{v_i}} \cdot U \cdot H_{ij} \cdot P_{surV_{vj}} \quad (1)$$

$$T_{Sj} = R_{0_j} \sum_{i \in S}^{n_i} \sum_{h \in \{c,r\}} Prev_{hi} \cdot Q_{ijh} \cdot (1 - P_{det}) \cdot P_{surH_h} \quad (2)$$

With c and r for 'cattle' for 'small ruminants' (the latter encompassing sheep and goats), the two host species h , and 1 and 2 for '*C. imicola'* and *'C. obsoletus/scoticus'* respectively, the two vector species v included in the model.

The biological interpretation of W_{Sj} was defined as the number of secondary cases that are expected from all grid cells i of country S in a given destination j in a month, knowing the number of *Culicoides* vectors that have been successfully infected ($V_{inf_{vi}}$) , uplifted in the air mass (U), passively dispersed by the wind (H_{ij}), remained alive ($P_{surV_{vj}}$), and further transmitted the virus to other individuals (R_{0_j}). The wind dispersal pathway has been inspired from previous risk assessment involving Bluetongue virus, another *Culicoides*-borne disease (Bibard et al., 2024), and used atmospheric trajectory simulations by HYbrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) at the large scale of Europe. The parameters used to filter the atmospheric simulations, such as the start time and the frequency per day, were specifically adjusted to the current knowledge around midges' flight activity, period, and meteorological limits (temperature, altitude) assumed during flight of *Culicoides*.

The biological interpretation of T_{Sj} was defined as the number of secondary cases that are expected from all grid cells i of country S at a given destination j in a month, knowing the probability for a host to be infected ($prev_{hi}$), the quantity of animals transported from i to j (Q_{ijh}), evading detection by a PCR test ($1 - P_{det}$), remaining infectious until destination (P_{surH_h}), and transmitting the virus to other individuals at destination (R_{0_j}). The official transboundary imports movements reported to UN Comtrade were used to calculate Q_{ijh} as a weighted average over the 4 past years (2020-2023).

For both pathways, the model considered the potential disease transmissibility of EHDV at the destination using the Basic Reproduction Number for vector-borne diseases, R_0, a metric that estimates the average number of secondary cases produced by one infected individual introduced into a population of susceptible individuals. All disease transmission parameters to calculate the R_{0_j} at destination were retrieved from the literature.

The final monthly risk of introduction in grid cell j, IR_j, was estimated by summarizing the risk outputs from the two pathways and over the n_S source countries contributing to the risk, for a given month as shown in Eq.(3):

$$IR_j = \sum_{S=1}^{n_S} W_{Sj} + T_{Sj} \quad (3)$$

The respective monthly risk for wind and the trade pathway in j, were also defined as $W_j = \sum_{S=1}^{n_S} W_{Sj}$ and $T_j = \sum_{S=1}^{n_S} T_j$.

While the finest temporal and spatial unit provided by the model is the month and the 25° grid cell respectively, the final risk outputs analysed here were averaged over the 12 monthly estimates and summed over the number of destination cells included in the destination country D, leading to risk estimates expressed as $\overline{T_D}$, $\overline{W_D}$ and $\overline{IR_D}$, respectively for the trade, wind and combined pathways. In this case, $\overline{IR_D}$ was expressed as the average number of secondary infections expected within a month in a given country when considering the two pathways of introduction.

To analyze the contribution of each source country, the risk outputs were not added over the source countries like in Eq.(3) but expressed as a combination between source S and destination countries D. Consequently, $\overline{W_{S,D}}$, $\overline{T_{S,D}}$ expressed the mean monthly risk in D provided by country S only, due to the wind or trade pathway respectively.

The model was applied considering the EHVD-8 outbreaks reported from 2022 to August 2024 by each country in Europe (Fig. 1). For this, the outbreak's locations were retrieved from WAHIS database for France and Italy, from a national database for Spain and manually for Portugal from maps in the French surveillance platform (ESA, 2024). The official number of outbreaks within the period declared by each affected country was used to estimate the disease prevalence within the source infected zone.

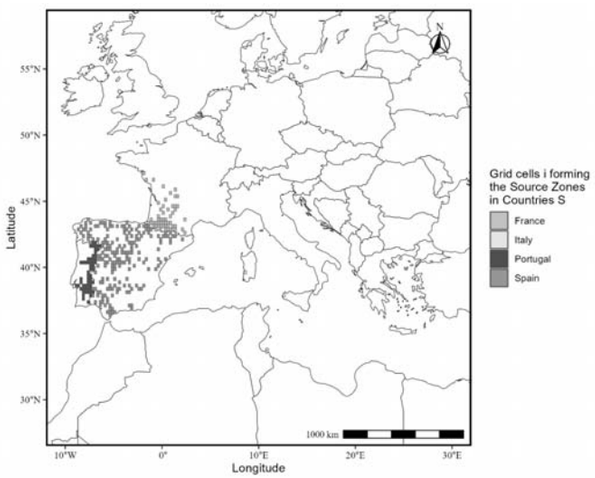

Figure 1. Definition of the source zones within the source countries S encompassing all the grid cells i where at least one EHDV-8 outbreak has been reported between January 2022 and August 2024.

RESULTS

The total risk zone encompassing countries with $\overline{IR_D}$> 0 was broadly distributed over 42 countries, including 30 European and 12 non-European countries (Fig. 2). The total surface of this zone covers 541 million hectares. However, the level of risk per country was highly variable ranging from ~8.7 10^{-10} to 747 potential secondary infections per month on average (Fig. 3). The countries that showed higher risk estimates were France, Spain, Italy, Portugal and Germany ($\overline{IR_D} \in [1;747]$), followed by Belgium, Switzerland, Portugal, United Kingdom, Luxembourg and the Netherlands ($\overline{IR_D} \in [0.1;1]$) (Fig. 2). Interestingly, the North African countries that are close to the source countries exhibited similar risk as Denmark. As expected, the Eastern countries and the northernmost country, Sweden, belonged to the lowest risk category (Fig. 2).

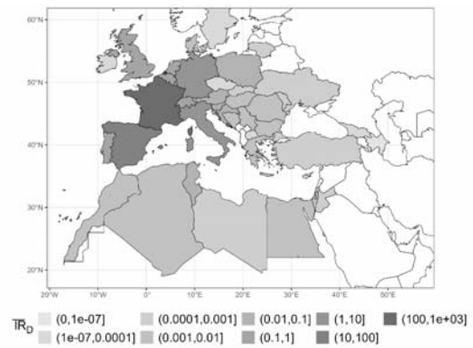

Figure 2. Spatial distribution of $\overline{IR_D}$, the mean monthly risk of EHDV-8 introduction per destination country D. The risk output considers here both the wind and the trade pathway cumulatively. In grey are countries with $\overline{IR_D}$, =0 (no risk).

Figure 3 depicts separately the level of risk per country due to the Wind, $\overline{W_D}$ and due to the Trade, $\overline{T_D}$.

In considering solely the wind pathway (Fig. 3A), the total risk surface ($\overline{W_D}$ > 0) encompasses 176 million hectares, covering 20 countries, with 16 in Europe and 4 outside (Tunisia, Algeria, Morocco, and Libya). The countries with the highest $\overline{W_D} \in]10; 10^{+3}]$, were indeed some source countries, such as France, Spain, and Italy. Interestingly, Germany, a country free from the disease and neighboring the source countries, exhibited a risk category equivalent to Italy. The lowest risk category ($\overline{W_D}$ <10^{-3}) was observed in the countries most distant from the source countries, such as Ireland, Croatia and Libya.

Regarding the trade pathway (Fig. 3B), the total surface of risk ($\overline{T_D}$>0), covers 529 million hectares, covering 40 countries, with 30 in Europe and 10 outside. Countries with the highest level of risk were those with $\overline{T_D} \in]10^{-1};1]$, such as Italy and Spain. Croatia and Slovenia shared the second highest risk category with $\overline{T_D} \in]10^{-2}; 10^{-1}]$.

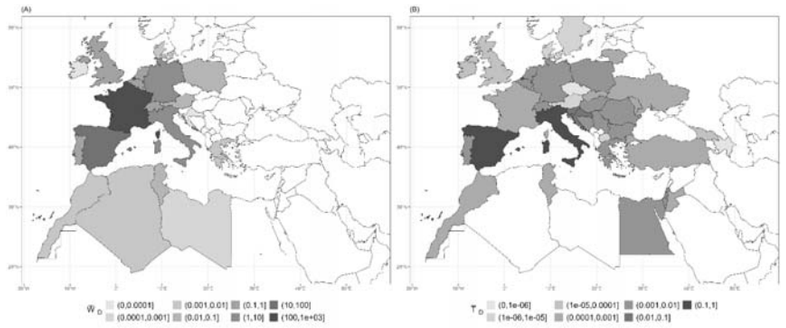

Figure 3. Spatial distribution of ($\overline{W_D}$) (A) and ($\overline{T_D}$) (B), the respective Wind and Trade mean monthly risk per destination country D.

Figure 4 represents a heatmap of risk highlighting the contribution of each source country S to the risk in a destination country D, regarding either the Wind pathway (A) or the Trade pathway (B) independently.

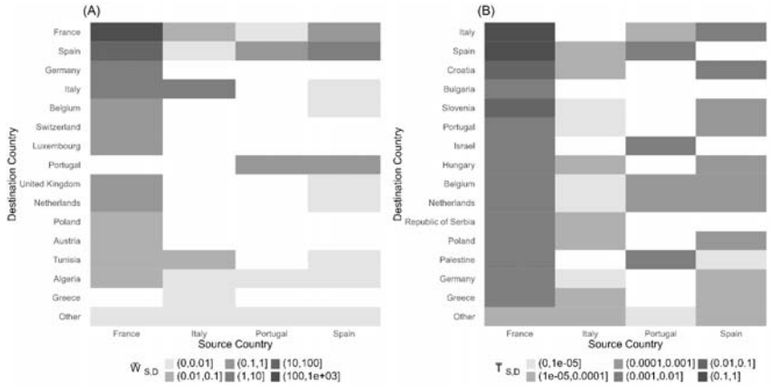

Figure 4. Heat maps showing $\overline{W_{S,D}}$ (A) and $\overline{T_{S,D}}$ (B), the monthly risk estimates between each combination of source S and destination D countries, considering independently and respectively the Wind Pathway or the Trade Pathway. For the sake of clarity, only the 15 destination countries with the highest risk estimates were depicted in each panel and the others summed under "Other" category.

Regardless of the pathway, France was the primary source country contributing to a large proportion of risk across a wide range of destination countries, including itself. France is also the unique source that contributes to a risk in Germany, Switzerland, Luxembourg and for the

most part to Belgium, United Kingdom and Netherlands. Conversely, Portugal presented a minimal risk contribution to a limited number of countries, particularly when considering the wind pathway. Interestingly, Portugal contributed to a risk in distant countries by the trade pathway, such as Israel and Palestine. Italy contributed to a trade risk in a wide range of destination countries but to a limited range of countries in addition of itself when considering wind risk. Spain, significantly contributed to the risk in France and Portugal, primarily through the wind pathway, and was also responsible for risk in Eastern European countries such as Croatia and Slovenia through the trade pathway.

DISCUSSION

This study's findings revealed that the spatial distribution and level of EHDV-8 risk were significantly influenced by the nature of the introduction pathway considered.

The wind dispersal pathway was identified as the primary contributor to the overall risk level, which aligns with expectations given the established role of wind-borne transmission in the spread of *Culicoides*-borne diseases. In consideration of this pathway, the countries deemed to be the most at-risk were the source countries, or neighbouring countries, such as Germany. This aligns with previous observations that *Culicoides* can passively be blown by the wind over long distances, between 500 to 700 kms over sea (Ducheyne et al., 2011; Eagles et al., 2012, 2014) and over land (García-Lastra et al., 2012), under suitable conditions.

In contrast, the trade pathway had a minimal impact on the overall risk level. This may be attributed to the rigorous preventive measures that are in place for the transboundary movement of animals or to the relatively low number of animals that are transported. Although this pathway contributes minimally to the overall risk level, it nevertheless extends the risk zone to more distant countries, including those in the east of the continent and countries outside Europe. Italy and Spain were identified as the two countries with the highest risk levels associated with the trade pathway, in alignment with their respective rankings as the top one and three importers of cattle in Europe (Rossi, 2020). It must be noted that the Netherlands, which is the second-largest cattle importer in the EU (Rossi, 2020), demonstrated a reduced risk in the model. This is largely attributed to the fact that the animals imported to the Netherlands typically originate from countries other than the three primary infected source countries (France, Spain, Portugal). Instead, they are imported from countries such as Germany and Belgium. It is therefore likely that risk of EHDV-8 incursion of the Netherlands would drastically increase in the situation where either of these two countries were to be infected.

France was identified as the primary source country contributing to risk, irrespective of the pathway. France's central and strategic geographical position within Europe, which places it at the crossroads of various wind and trade pathways, may be a contributing factor. Furthermore, France is the foremost cattle exporter in Europe, particularly to Italy, where the market for weanlings is particularly strong (Rossi, 2020). Considering these findings, the implementation of preventive measures in France, such as vaccination, would not only protect the French herds but more broadly the whole European livestock population.

ACKNOWLEDGEMENTS

This work was funded by the French Government through the ‘Plan de Relance’ initiative (ANR-21-PRR-0075-01) and by Boehringer Ingelheim Animal Health France.

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HIGH-RESOLUTION GENOMIC ANALYSES OFFER UNIQUE INSIGHTS INTO THE EMERGENCE OF A BACTERIAL PATHOGEN OF UNGULATES IN THE CANADIAN ARCTIC

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SUMMARY

A single lineage of the bacterium *Erysipelothrix rhusiopathiae* has been associated with muskox mortalities and population declines across the Canadian Arctic Archipelago since 2010. This study analyses the spread of this Arctic clone using genome-wide single nucleotide polymorphism data from over 200 isolates collected across five Arctic islands over the past 14 years. Samples were collected from various tissues, soil and faeces of muskoxen and other species, revealing phylogenetic clustering by island during early outbreaks (2010-2017) and extensive mixing of isolates between two islands more recently (2021-2023). The bacterium appears to have spread north-eastward over 1,000 km in under 15 years, potentially facilitated by long-ranging carnivores. The study shows the bacterium's extended environmental persistence and emphasizes the need for continued monitoring to understand its long-term effects on muskox populations and the broader ecosystem.

INTRODUCTION

Muskoxen (*Ovibos moschatus*) are one of the most significant resident herbivores in Arctic ecosystems, and a critical food and economic resource for Arctic Indigenous communities, including Inuit people (Cuyler et al., 2020). In recent years, muskox populations have faced several challenges, including declining numbers associated with emerging infectious diseases (EIDs) (Cuyler et al., 2020), which pose a particular threat to Arctic wildlife that have evolved in relative isolation, with limited exposure to new pathogens (Dobson, 2009). Muskox populations also have very low genetic diversity, which may decrease their resilience to EIDs (Prewer et al., 2020). Moreover, the rate of warming in the Arctic, nearly four times the global average since the mid-20th century, is exacerbating these problems (Rantanen et al., 2022).

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Erysipelothrix rhusiopathiae (ER) is an emerging bacterium associated with widespread muskox mortality in. In 2012, approximately 150 dead muskoxen were found on Banks Island (BI), Northwest Territories (NWT) and ER was isolated from tissue samples (Kutz et al., 2015). Subsequent analyses of archived samples from BI and Victoria Island (VI), Nunavut (NU) confirmed the presence of ER in muskox carcasses dating back to 2010-11. Mobilisation of Indigenous Knowledge held by local Inuit harvesters also indicated that die-offs happened in several consecutive summers on VI between 2009 and 2013, affecting more muskoxen than originally thought (Tomaselli et al., 2018). The pathogen has also been detected in other North American wildlife, including caribou (*Rangifer tarandus*), moose (*Alces alces*), and Arctic foxes (*Vulpes lagopus*) (Forde et al., 2016a; Spraker and White, 2017).

Erysipelothrix rhusiopathiae is a zoonotic Gram-positive, rod-shaped bacterium causing erysipelas in domestic animals, particularly pigs, and erysipeloid in humans (Wang et al., 2010). In pigs, ER is commonly found in the tonsils, Peyer's patches, and other lymphoid tissues, with up to 50% of healthy pigs serving as carriers (Opriessnig and Coutinho, 2019). Transmission typically occurs through ingestion of contaminated feed, water, or faeces, or through skin wounds. More importantly, ER is associated with carriage and/or disease in a wide range of domestic and wild host species around the world (Forde et al., 2016a).

The introduction of ER to Arctic muskoxen populations likely dates back several decades. Seroprevalence studies in muskoxen in Alaska and the Canadian Arctic from 1976 to 2017 suggest that the bacterium was present in the region as early as 1976 (Mavrot et al., 2020). Isolates of ER fall into three broad phylogenetic clades, with most isolates from North America falling within clade 3 (Forde et al., 2016a). Phylogenetic analyses have revealed that most muskox mortality events across the Canadian Arctic Archipelago (CAA) have been associated with a single clonal lineage of ER within clade 3 (Forde et al., 2016b; Seru et al., 2024) known as the 'Arctic clone'. This strain has been associated with multiple mass mortality events in muskox populations across the Arctic, with reports spanning over a decade and covering distances of more than 1,000 kilometres across the CAA. These have occurred on BI (2012–2013), VI (2010–2011), and Prince Patrick Island (PPI) (2017), as well as a recent mass mortality event on Ellesmere Island (EI) in 2021 (Forde et al., 2016b; Seru et al., 2024).

Understanding evolutionary rates and timeframes can provide useful insights into pathogen evolution and spread. High-resolution whole genome sequences (WGS) associated with known time points at which samples were collected are widely used to estimate molecular clock rates for a wide range of pathogens (Biek et al., 2015), providing invaluable information on spatiotemporal patterns of spread.

In the light of the emergence of ER in muskoxen on the CAA, this study aimed to:

1. Use WGS data from ER isolates collected from across the CAA to explore their fine-scale spatiotemporal relationships and identify potential transmission routes.
2. Use longitudinal sequence data on isolates from the same locations to gather evidence for potential bacterial survival/replication in carcasses and the wider environment.
3. Explore evidence for a molecular clock in ER.

MATERIALS AND METHODS

Sequencing, bioinformatics and phylogenomic analyses

This study included previously described sequences of the Arctic clone of ER from past outbreaks on Victoria, Banks, Prince Patrick and Ellesmere Islands (2010-2021) (Forde et al., 2016b; Seru et al., 2024) as well as sequences of more recent PCR-confirmed ER isolates from EI and Axel Heiberg Island (AHI) and mainland Canada (2022-2023; Fig. 1). These latter isolates, as well as a subset of isolates from 2010–2021 which were re-sequenced to obtain higher depth of coverage (Table 1), were sequenced at MicrobesNG (Birmingham, UK) on an Illumina NovaSeq 6000 platform, generating 250 base-pair, paired-end reads with a minimum average coverage depth of 30X. Sequences were adapter trimmed with Trimmomatic v0.30 (Bolger et al., 2014) with a sliding window quality cutoff of Q15. Kraken (Wood and Salzberg, 2014) was used to confirm bacterial species identity.

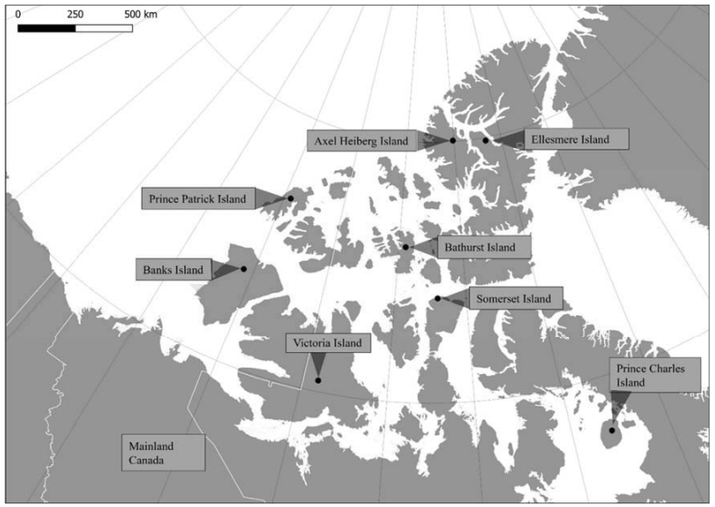

Figure 1. Map of the Canadian Arctic Archipelago indicating all locations from which *Erysipelothrix rhusiopathiae* has thus far been isolated from Arctic wildlife species.

To situate newly sequenced ER isolates in a global phylogeny, WGS data from 100 GenBank datasets were accessed, generating a core-genome alignment using Parsnp v1.2 (Treangen et al., 2014). For Arctic clone genomes, read mapping and variant calling were conducted on the CLIMB platform for microbial genomics (Connor et al., 2016), using a pipeline available at https://github.com/tlforde/OHRBID. A newly assembled complete genome of the Arctic clone isolate AMX7 (from a muskox from BI) was used as a reference. This genome was assembled using the hybrid assembly service of MicrobesNG (Birmingham, UK), with short reads having 30X minimum average coverage depth using the same Illumina platform as above and long reads having 50X minimum coverage depth using Oxford Nanopore Technologies-based services on a GridION with R10.4.1 flowcells. For all other sequences, trimmed reads were aligned to this AMX7 reference genome using bwa-mem v0.7.17. Picard v2.27.4 (http://broadinstitute.github.io/picard) was used to mark/remove duplicates, add read group info and index BAM files. VarScan v2.4.4 was used to detect SNPs with parameters of minimum read depth 8, base quality ≥20, and variant allele frequency ≥0.8.

A maximum-likelihood phylogeny was estimated from concatenated SNPs with IQ-TREE v2.2.2.3. Pairwise SNP differences between isolates were calculated with snp-dists v0.6 (https://github.com/tseemann/snp-dists), and subsequent comparative analyses were carried out in R v4.4.1 (R Core Team, 2024).

Table 1. *Erysipelothrix rhusiopathiae* isolates (n = 186) newly sequenced in this study.

Location	Year isolated	Host species (# unique individuals)	# isolates (# resequenced)	# isolates Arctic Clone (# included in molecular clock analyses)
Axel Heiberg Island	2022-2023	Arctic fox (1), Arctic wolf (5), Collared lemming (1), Unidentified bird (2), Muskox (10)	70 (0)	67 (31)
Banks[a] Island	2013	Muskox (1)	1 (1)	1 (1)
Bathurst Island		Muskox (1)	1 (0)	0 (0)
Ellesmere Island	2021-2023	Arctic fox (2), Arctic wolf (5), Muskox (36), Unidentified bird (2)	88 (2)	84 (42)
Prince Patrick Island	2017	Muskox (6), Peary caribou (3), Arctic fox (1)	16 (16)	16 (14)
Victoria Island	2015	Muskox (2), Ringed seal (1)	7 (7)	7 (3)
Mainland Canada	2022-2023	Bison (1), Caribou (1)	3 (0)	0 (0)

[a]Sequence AMX7 used as the reference sequence

Molecular clock analyses

The alignment of concatenated SNPs was used for molecular clock analyses after excluding a subset of identical or highly similar sequences; these were excluded using a cut-off value based on the mean pairwise SNP differences between isolates obtained from a single carcass in a single year (further details in Results). The remaining alignment was reduced to contain only variable sites, separately recording the number of non-varying sites involving each nucleotide base based on the AMX7 reference genome to allow correction of mutation rates during phylogeographic reconstruction. The most appropriate substitution model determined using IQ-TREE version 2.3.6 (Kalyaanamoorthy et al., 2017) was a General Time Reversible (GTR) model with empirical base frequencies. Finally, a discrete phylogeographic reconstruction (Lemey et al., 2009) was performed using BEAST version 1.10.4 (Suchard et al., 2018), allowing for asymmetric rates of movement between islands. This analysis used a Bayesian SkyRide demographic prior (Drummond et al., 2002; Minin et al., 2008) along with a log-normal uncorrelated relaxed molecular clock, and the GTR substitution model. Since the mutation rate of ER is unknown, the mean clock rate was given a broad uniform prior between 10^{-8} and 10^{-5}, broadly in line with values seen for *E. coli* (Williams, 2014). The reconstruction was performed across two independent MCMC chains of 50 million steps each, saving values every 5000 steps. After discarding the first 10% of samples and visually confirming convergence and appropriate effective sample sizes in each chain with Tracer version 1.7.2

(Rambaut et al., 2018), the combined posterior set of phylogenies across both chains was summarized as a maximum clade credibility tree, with clade ages summarized using the common ancestor algorithm in treeannotator version 1.10.4 (Suchard et al., 2018). Phylogenies were visualized using ggtree version 3.14.0 (Yu, 2022) in R version 4.1 (R Core Team, 2024).

RESULTS

Phylogenetic relationship of new *E. rhusiopathiae* isolates with those previously characterised

A total of 186 ER isolates were sequenced as part of this study, of which 160 had not been previously sequenced (Table 1). All newly sequenced isolates from mainland Canada were genetically distinct from the Arctic clone – these included two tissue isolates (spleen and tonsil) from a wood bison (*Bison bison*) from the Dehcho region (NWT) and a bone marrow isolate from a caribou from Bathurst inlet (NU) (all identified as belonging to clade 2). From the CAA, 157 ER isolates were newly sequenced, cultured from a range of samples from muskox carcasses, including organs, faeces and bone marrow, as well as non-carcass samples (hereafter defined to include samples from carnivores like Arctic fox and Arctic wolf (*Canis lupus arctos*), other species (unidentified birds, Northern collared lemming (*Dicrostonyx groenlandicus*)), carcass-associated carrion fly larvae and soil) (Table 1). Nearly all (149/157, 95%) ER isolates from the CAA belonged to the Arctic clone of ER. Eight non-Arctic clone isolates were found from clades 1 and 3 of ER. Clade 1 isolates (n=6) were from a muskox bone marrow sample (1) and soil samples (2) from AHI; and muskox bone marrow (2) and a carrion fly larva (1) from EI. Non-Arctic clone Clade 3 isolates were from a bone marrow sample from muskoxen on each Bathurst Island (2022) and EI (2023).

In total, sequences from 234 Arctic clone isolates that satisfied sequence quality thresholds were included in the SNP pipeline, including previously sequenced isolates from past outbreaks. The mean number of pairwise SNP differences was 13.17 SNPs (median 11, range 0 – 44). The largest SNP difference of 44 SNPs was observed between a 2023 muskox bone marrow isolate from EI and isolates from two different carcasses from 2010 on VI.

The mean pairwise SNP differences between islands (15.8, 95% CI: 15.68, 15.91) was significantly higher than within islands (7.22, 95% CI: 7.12, 7.32, $p < 0.005$). Isolates from past outbreaks on VI (2010-2011), BI (2012) and PPI (2017), and those from individual mortality events from VI (2015) were closely related by geographic origin and clustered together phylogenetically, except a muskox bone marrow isolate obtained from PPI in 2017 which clustered closely with a group of 2015 VI isolates. In contrast, isolates from EI and AHI were more intermixed, with isolates from one island phylogenetically clustering with isolates from the other, suggesting movement of ER between the two islands (Fig. 2).

Long-term persistence of ER

Between 2021 and 2023, ER was successfully isolated from 48 and 16 unique carcass sites on EI and AHI respectively, with several sites resampled over multiple years. Genetically identical, or closely related, ER isolates (pairwise SNP distances of 0-2) were obtained from three sites on EI in all three years, and from 17 sites in two (sometimes non-consecutive) years, indicating long-term persistence of ER within carcasses and in the environment for at least two years, without evidence of bacterial replication or sequence evolution.

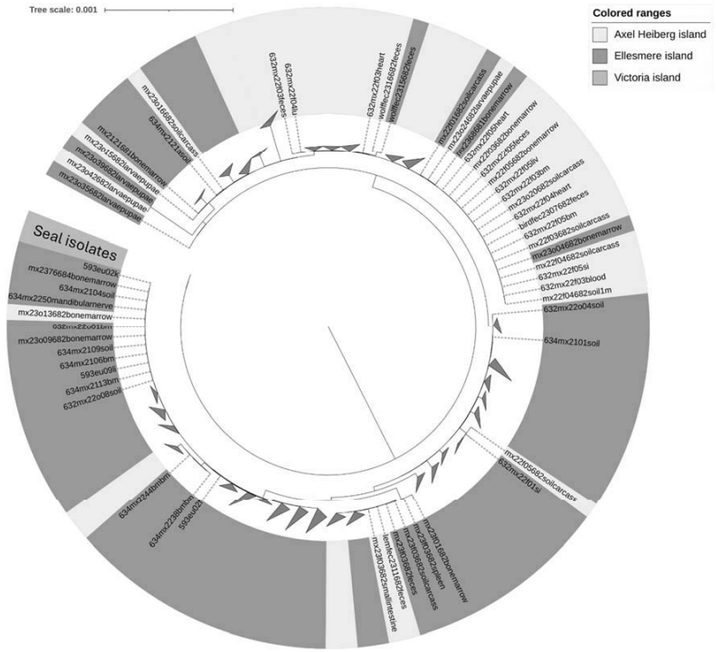

Figure 2. Maximum-likelihood phylogenetic tree of *Erysipelothrix rhusiopathiae* Arctic clone isolates based on high-quality single nucleotide polymorphisms, obtained from Ellesmere and Axel Heiberg Islands (2021 – 2023), rooted to the most closely related seal isolates from Victoria Island (labelled), and coloured by their geographic origin of isolation. Multiple clades have been collapsed to facilitate representation of >150 unique isolates.

Genetic diversity of *E. rhusiopathiae* within and between carcasses

Between 2010 and 2023, there were 23 unique carcasses across all CAA islands with at least two ER 'carcass isolates' obtained in a single year. The mean pairwise SNP difference between isolates within carcasses was 2.65 (95% CI 2.23-3.08) SNPs (median - 2; range - 0 to 24 SNPs). While most carcasses (15, 65%) had mean pairwise SNP differences of ≤ 2 SNPs between their various isolates, five had mean SNP differences of ≥ 3 SNPs, with some isolates differing by 11, 12 or 24 SNPs. These differences suggest that animals can occasionally be infected by multiple variants of ER simultaneously. Between-carcass diversity (mean pairwise SNP difference of 5.44 (95% CI 4.99 – 5.89), median - 4; range – 0-26 SNPs) was significantly higher than within-carcass diversity ($p < 0.001$). In one case, isolates obtained the same year from carcasses situated only 333m apart differed by 4-7 SNPs, suggesting that despite being found in close proximity, they may not have been infected from a common source.

Epidemiological clusters

After excluding non-carcass isolates, there were 11 unique epidemiological clusters where ER isolates were genetically identical among two or more carcasses (at least one pair of isolates with SNP difference of 0). At least one epidemiological cluster was identified on every island where the Arctic clone was detected, with 10 clusters of two or three unique carcasses each, with distances between carcasses ranging from 0 to 53 km. The largest epidemiological cluster was on EI, with 8 carcasses sampled from 2021-2023 over 163 km^2. Additionally, isolates from this latter cluster were genetically identical to an isolate found at a carcass site at least 114 km away on AHI. Similarly, isolates from the sole epidemiological cluster identified on AHI, involving three unique carcasses, were genetically identical to an EI isolate from a site ~63 km away, providing clear evidence for inter-island movement of bacteria by infected animals.

Host-mediated spatial spread of *E. rhusiopathiae*

There was substantial evidence of ER exchange between EI and AHI. Carcass isolates from five muskoxen and carcass-associated soil and fox faecal isolates that were sampled on EI were genetically related to isolates from AHI. Similarly, carcass isolates from two muskoxen and associated soil and scavenger faecal isolates sampled on AHI phylogenetically clustered with EI isolates. In both instances, some carcass sites were located >100km apart on either island.

Isolates of the Arctic clone were obtained from Arctic fox (4), Arctic wolf (10), collared lemming (1) and bird (4) faeces and tissues associated with 15 unique carcass sites on three different islands (2017-2023). At five sites, these isolates were genetically identical (SNP difference of 0) to one or more carcass isolates from the same or a different year, indicating ER survival without replication in the digestive tracts of carnivores and scavengers.

There was also evidence for carnivore mediated movement of ER within and between islands. Two wolf faecal isolates that were genetically identical to or differed by only 1 SNP from a soil isolate were sampled at carcass sites ~ 4.5km and ~50km away, respectively, from the carcass site where the soil isolate was obtained. Crucially, all three isolates – along with a closely related bird faecal isolate and a carrion fly larva isolate – were sampled in 2023 on AHI but phylogenetically clustered with 2022 isolates from EI. In another instance, two wolf faecal isolates obtained in 2023 from sites ~ 131km apart on EI and AHI differed by only 1 SNP.

Molecular clock analyses

Based on observed within-carcass sequence diversity and the mean pairwise SNP difference of 2.65 SNPs between tissue isolates from the same carcass, the number of unique sequences used for the molecular clock analyses was refined to 130 sequences (including the AMX7 reference genome) as follows:

1. For each animal with multiple tissue isolates / sequences, all sequences with 3 or more SNPs differences compared to other sequences from the same animal were included. Where multiple sequences from the same carcass had < 3 SNPs difference, one sequence was selected arbitrarily. Where possible bone marrow isolate sequences were prioritised, as bone marrow was the most common tissue source of isolates in the study dataset.
2. Where carcasses were sampled in multiple years (2 or more years between 2021 and 2023, which may not be consecutive), only isolates from the first sampling year were included.

3. For sequences from soil, scavenger faeces or carrion fly larvae samples, all sequences with < 3 SNPs differences compared to sequences chosen above were excluded.

The most recent common ancestor (MRCA) for all included cases was dated to 2006 (95% highest posterior density [HPD]: 2004 – 2008), four years before the first confirmed (by bacterial isolation) outbreak on VI in 2010. Although this outbreak's origin is uncertain, with roughly equal posterior probabilities for BI (0.51) and VI (0.49), there was clear evidence of independent transmission (monophyletic clusters with a posterior probability for location assignment > 0.98) on VI by 2008 (HPD: 2007 – 2009) and BI by 2010 (HPD: 2009 – 2010). Most cases observed on PPI could be confidently linked to a single origin on BI, with an MRCA dated to 2014 (HPD: 2012 – 2015) shared by all but one sequence from PPI. Similarly, all cases on EI and AHI appear to derive from a single introduction from VI, estimated to have arrived at EI around 2016 (HPD: 2015 – 2017). The location of this introduction is uncertain, with AHI receiving a non-trivial amount of support as an alternative introduction point (posterior probability = 0.25, vs. 0.74 for EI); this uncertainty is driven by frequent observations of exchange between these two islands. Nevertheless, largely independent transmission cycles are evident from at least 2017 (EI, HPD: 2016 – 2018) and 2019 (AHI, HPD: 2018 – 2020).

DISCUSSION

Microbial WGS has emerged as a powerful epidemiological tool, enabling the study of pathogen emergence, outbreak investigations, and transmission pathways (Biek et al., 2015; Forde et al., 2016b, 2022). Using WGS data from over 200 ER isolates collected across five islands of the CAA from 2010 to 2023, potential transmission pathways at multiple spatial scales were examined.

These results suggest that ER spread north-eastward from VI or BI to EI, covering >1000 km in just over a decade since the first detected outbreaks in 2010. While the mode of this spread is unclear, WGS data suggest a few possibilities. During field sampling on EI and AHI, Arctic wolves and foxes were frequently observed scavenging on old muskox carcasses. Microbiological and phylogenomic analyses confirmed that ER survives in scavenger faeces without necessarily replicating. Identical or near-identical isolates from faeces and carcasses, sometimes separated by hundreds of kilometres between EI and AHI, suggest scavengers play a key role in pathogen dissemination. Arctic wolves are known to travel between EI and AHI (Mech and Cluff, 2011), likely aiding ER's long-distance spread. Arctic foxes, known for nomadic movements across the CAA and into Greenland (Tarroux et al., 2010; Lai et al., 2017) may also facilitate this spread. Additionally, all isolates from EI and AHI were most closely related to a 2015 seal isolate from VI, suggesting marine mammals may play a role in transmission, either through direct inter-island movement (Ogloff et al., 2021) or indirectly when scavengers like foxes feed on seal remains and shed ER in faeces (Lai et al., 2017).

Muskoxen are generally considered a sedentary species with limited annual movement within their home ranges (Schmidt et al., 2016). While it is unclear how extensively muskoxen move between EI and AHI, epidemiological clustering based on genetically identical ER isolates between the two islands suggests the possibility of some movement, although this cannot currently be distinguished from movement of ER by carnivores or scavengers. For instance, muskox bone marrow isolates from AHI were genetically identical to carcass isolates found over 100 km away on EI. Conversely, muskox carcass and scavenger faecal isolates from EI clustered phylogenetically with isolates from AHI, indicating inter-island linkages.

Isolates from muskox carcasses found in different years on EI and AHI were also genetically linked, suggesting potential point-source infections from environmental reservoirs like soil at mortality sites. ER's survival in carcasses and the environment for at least two years over multiple freeze-thaw cycles supports this hypothesis, aligning with studies showing ER survival for four years at -5°C (Belova, 1972). Carcass sites, enriched by nutrients from decomposition and scavenger faeces, often exhibited lush vegetation within a year of animals' death and for many years thereafter (unpublished data), which can attract herbivores. Such site fidelity (Ferraro et al., 2024) at ER "hotspots" may promote repeated exposure to ER, enabling environmentally driven infection cycles. Carcass sites also harbour diverse ER genotypes, as seen in the SNP variation among soil isolates and carcasses with multiple tissue isolates.

Globally, ER exhibits high genomic diversity, and this study reflects that variation. Non-Arctic clone isolates were identified on EI and AHI in 2023, representing major clades 1 and 3, and expanding on similar findings from Somerset and Prince Charles Islands (Seru et al., 2024). However, Arctic clone isolates continue to dominate, comprising 95% of newly sequenced CAA isolates, highlighting the threat posed by this emerging pathogen for muskoxen, whose populations have declined by up to 85% in some areas (Cuyler et al., 2020).

This study enhances understanding of how a single clone of ER, typically a commensal of animals, can dominate across a vast area exceeding one million km^2. The findings suggest potential roles for various hosts, including carnivores and marine mammals, in its spread. The study underscores the importance of ongoing monitoring to assess the impact of this pathogen on muskox populations and determine whether this already-stressed species can recover.

ACKNOWLEDGEMENTS

This work was supported by funding from the Canada – Inuit Nunangat – United Kingdom (CINUK) Arctic research programme; the Government of Nunavut; Canadian Polar Continental Shelf Program; Canada North Outfitting; Polar Knowledge Canada; Van Sloun Foundation; Arthur French Family Foundation and Weber Arctic.

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SOCIETY FOR VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

PAST VENUES AND ORGANISERS OF ANNUAL MEETINGS

Year	Venue	Organiser(s)
1983	Southampton	Davies & Thrusfield
1984	Edinburgh	Thrusfield
1985	Reading	Thrusfield
1986	Edinburgh	Thrusfield
1987	Solihull	Thrusfield
1988	Edinburgh	Thrusfield
1989	Exeter	Howe
1990	Belfast	McIlroy
1991	London	Jones
1992	Edinburgh	Thrusfield
1993	Exeter	Howe
1994	Belfast	Menzies
1995	Reading	Paterson
1996	Glasgow	Reid
1997	Chester	Clarkson
1998	Ennis, Ireland	Collins
1999	Bristol	Green
2000	Edinburgh	Thrusfield & Mellor
2001	Noordwijkerhout	van Klink
2002	Cambridge	Wood & Newton
2003	Warwick	Green
2004	Martigny	Stärk
2005	Nairn	Gunn
2006	Exeter	Peeler
2007	Dipoli	Virtala & Alban
2008	Liverpool	Pinchbeck & Robinson
2009	London	Verheyen & Pfeiffer
2010	Nantes	Fourichon & Hoch
2011	Leipzig	Thulke & Lange
2012	Glasgow	Parkin & Others
2013	Madrid	Martínez-López & Vargas Alvarez
2014	Dublin	More & Collins
2015	Ghent	Mintiens & Dewulf
2016	Elsinore	Nielsen & Halasa
2017	Inverness	Gunn & Reeves
2018	Tallinn	Viltrop
2019	Utrecht	van Schaik
2020	Online virtual conference	Barrett
2021	Online virtual conference	Vergne
2022	Belfast	Menzies
2023	Toulouse	Vergne
2024	Uppsala	Frössling

PAST PRESIDENTS

1982-’83	G. Davies
1983-’84	P.R. Ellis
1984-’85	G. Gettinby
1985-’86	R.W.J. Plenderleith
1986-’87	M.V. Thrusfield
1987-’88	K.S. Howe
1988-’89	A.M. Russell
1989-’90	S.G. McIlroy
1990-’91	J.E.T. Jones
1991-’92	J.M. Booth
1992-’93	E.A. Goodall
1993-’94	R.G. Eddy
1994-’95	J.T. Done
1995-’96	G.J. Gunn
1996-’97	M.S. Richards
1997-’98	J.D. Collins
1998-’99	F.D. Menzies
1999-’00	K.L. Morgan
2000-’01	S.W.J. Reid
2001-’02	A.D. Paterson
2002-’03	L.E. Green
2003-’04	J.L.N. Wood
2004-’05	E.G.M. van Klink
2005-’06	D.J. Mellor
2006-’07	E. J. Peeler
2007-’08	J. R Newton
2008-’09	L. Alban
2009-’10	D.U. Pfeiffer
2010-’11	L.A. Kelly
2011-’12	C. Fourichon
2012-’13	T.D.H. Parkin
2013-’14	K.L.P. Verheyen
2014-’15	K. Mintiens
2015-’16	H.H. Thulke
2016-’17	A. Lindberg
2017-’18	L.R. Nielsen
2018-’19	M.L. Brennan
2019-’21	K.M. McIntyre
2021-’22	G. van Schaik
2022-’23	P.A. Robinson
2023-’24	D. Brodbelt

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F. Menzies, K. Morgan, A. Paterson, S.W.J. Reid, A.M. Russell, M.V. Thrusfield,
H.-H. Thulke, J. Wilesmith

PLENARY TALKS

Year	Gareth Davies Lecture	Conference Opening Plenary
2025	Timm Harder A virological evergreen with new beats: Influenza viruses in birds and mammals	Katharina Stärk The role of science at times of crisis
2024	Lis Alban The Danish model for collaboration in animal health and food safety	Thomas Schön On the state of machine learning research – where are we today
2023	Francois Roger Contribution of epidemiology to the sustainable development of the Global South	Marius Gilbert Sciences at the policy-communication nexus during the COVID pandemic
2022	Julie Fitzpatrick Science based policy – Not *just* about animal disease!	Robert Huey The role of vets on the national stage
2021	Christine Fourichon Controlling infectious diseases: how does the interplay between field problems and epidemiological research lead to success?	John Edmunds Modelling to support policy in real time: insights from the COVID-19 pandemic
2020	Jonathan Rushton How GBADs will link to clinical practice and veterinary epidemiology	Matthew Stone The policy perspective and science in an evidence-based policy structure Sam Thevasagayam A funder's perspective of disease studies
2019	Simon More Perspectives from the science-policy interface	Marion Koopmans To jump or not to jump: viruses at the human-animal interface
2018	Klaus Depner African swine fever: Lessons learned about the epidemiology, politics and practical implementation of control measures	Päivi Rajala-Schultz Veterinary epidemiology at the intersection of livestock disease, production and animal welfare
2017	Theresa Bernardo TRENDS: Technology, Research, Epidemiology, Networks, Data & Surveillance	Tine Hald Source attribution: Translating science into public health action
2016	Bernhard Url The foundation of science-based risk assessment for decision support on food safety and animal health in EU	Mirjam Nielen Evidence-based veterinary medicine needs clinical epidemiology

2015	Piet Vanthemsche Preventive Veterinary Medicine as an essential part of sustainable animal production	Crawford Revie Hype and Hysteria: Should veterinary epidemiologists really care about Big Data?
2014	Ian Gardner Bridging the gap in infectious disease epidemiology between aquatic and terrestrial food animals: challenges and future opportunities	Nils Toft Confessions of a wannabe Bayesian
2013	Andreas Hensel Dioxins, EHEC and strawberries: Risk assessment and risk communication in practice	José Manuel Sánchez-Vizcaíno The Spanish experience on the control and eradication of infectious diseases: from the old to the current system
2012	Stuart Reid Evidence-based prevention: well done or rare	Didier Boichard Genomic selection: an opportunity for improving health of farm animals
2011	Karin Schwabenbauer From science to policy - the case of classical swine fever (CSF) control	Dominic Mellor The trouble with epidemiology: the tyranny of numbers
2010	David Waltner-Toews Beyond one world, one health and ecohealth…what's out there?	James Wood From pathogen adaption to host ecology: epidemiological and experimental contributions to the understanding of emerging infectious diseases
2009	Jørgen Westergaard The interaction between veterinary science, legislation and management in animal disease control in the European Union	Katharina Stärk Food safety challenges in a global market – are we ready?
2008	Paul Fine Infectious disease eradication – meanings and implications	Kenton Morgan For the benefit of Mr Kite
2007	Yrjö Gröhn Food supply veterinary medicine: Modelling of production, health and food safety	Laura Green Improving Animal Health
2006	David Galligan From partial budgets to real options - concepts in animal health economics	Nigel French Understanding human exposure to zoonoses from food and the environment: The application of molecular tools and modeling
2005	Bill Reilly From TB to VTEC: The changing epidemiology of foodborne zoonoses	Simon More Towards eradication of bovine tuberculosis in Ireland: A critical review of progress
2004	Ulrich Kihm BSE and the stable to table concept	Gary Smith Spatial models of infectious disease in the USA: a crisis of conference and confidentiality
2003	Sir David Cox The current state of statistical science	Ynte Schukken Molecular and mathematical epidemiology of bovine mastitis
2002	George Gettinby Informatics and epidemiology – the first 400 years	Bryan Grenfell Deterministic and stochastic influences on the dynamics and control of infectious diseases
2001	Will Houston Science politics and animal health policy: epidemiology in action	Mart de Jong Design and analysis of transmission experiments
2000	Jim Scudamore Surveillance – past, present and future	Dirk Pfeiffer Spatial analysis – a new challenge for veterinary epidemiologists
1999	Aalt Dijkhuizen The 1997/98 outbreak of classical swine fever in the Netherlands: lessons learned from an economic perspective	Mark Woolhouse Understanding the epidemiology of scrapie
1998	Wayne Martin Art, science and mathematics revisited: the role of epidemiology in promoting animal health	

SOCIETY FOR VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

APPLICATION FOR MEMBERSHIP

Name .....

Address .....

.....

.....

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Telephone: .....

Fax: .....

E-mail: .....

Signed ..... Date .....

Please enclose the membership fee (£60 sterling to cover two years' membership) along with this application form. Overseas members without British bank accounts are requested to pay 2 - 3 years in advance. Cheques should be in £ sterling and drawn from a British bank. British members should pay future dues by standing order (forms are available from the Secretary or Treasurer). Payment can also be made by credit card; the appropriate form is available from the Society's website, http://www.svepm.org.uk/, or from the Secretary or Treasurer.

Please send this form to the Society's Treasurer:

Dr Egil A.J. Fischer
Department of Population Health Sciences
Faculty of Veterinary Medicine
Utrecht University, Yalelaan 7, 3584 CL Utrecht
The Netherlands

TEL +31 30 253 1119
Email: treasurer@svepm.org.uk

Please turn over

INTEREST GROUPS

Please tick appropriate boxes to indicate your interests:

- ☐ Analytical Epidemiology (Observational Studies)
- ☐ Quantitative Epidemiology & Statistical Techniques (Incl. Modelling)
- ☐ Herd/Flock Level Disease Control Strategies
- ☐ National/International Disease Control Policy
- ☐ Sero-Epidemiology
- ☐ Herd Health and Productivity Systems
- ☐ Disease Nomenclature and Epidemiological Terminology
- ☐ Economic Effects of Disease on Animal Production
- ☐ Veterinary Public Health and Food Hygiene
- ☐ Computing, including data logging
- ☐ Computer Programming *per se*
- ☐ Population and Animal Disease Databases
- ☐ Information System Design
- ☐ Geographical Information Systems (GIS)
- ☐ Risk Analysis

CONSTITUTION AND RULES

NAME

1. The society will be named the Society for Veterinary Epidemiology and Preventive Medicine.

OBJECTS

2. The objects of the Society will be to promote veterinary epidemiology and preventive medicine.

MEMBERSHIP

3. Membership will be open to persons either actively engaged or interested in veterinary epidemiology and preventive medicine.

4. Membership is conditional on the return to the Honorary Treasurer of a completed application form and subscription equivalent to the rate for two calendar years at first application or subsequent application following an elapsed subscription. Subsequent annual subscriptions fall due on the first day of May each year.

5. Non-payment of subscription for six months will be interpreted as resignation from the Society.

OFFICERS OF THE SOCIETY

6. The Officers of the Society will be President, Senior Vice-President, Junior Vice-President, Honorary Secretary and Honorary Treasurer. Officers will be elected annually at the Annual General Meeting, with the exception of the President and Senior Vice-President who will assume office. No officer can continue in the same office for longer than six years.

COMMITTEE

7. The Executive Committee of the Society normally will comprise the officers of the Society and not more than five ordinary elected members. However, the Committee will have powers of co-option. Elected officers and ordinary members of the Committee have normal voting rights at committee meetings but co-opted and ex-officio members (e.g. the proceedings editors) do not

ELECTION

8. The election of office bearers and ordinary Committee members will take place at the Annual General Meeting. Ordinary members of the Executive Committee will be elected for a period of three years. Retiring members of the Executive Committee will be eligible for re-election. Members will receive nomination forms with notification of the Annual General Meeting. Completed nomination forms, including the signatures of a proposer, seconder, and the nominee, will be returned to the Secretary at least 21 days before the date of the Annual General Meeting. Unless a nomination is unopposed, election will be by secret ballot at the Annual General Meeting. Only in the event of there being no nomination for any vacant post will the Chairman take nominations at the Annual General Meeting. Tellers will be appointed by unanimous agreement of the Annual General Meeting.

FINANCE

9. An annual subscription will be paid by each member in advance on the first day of May each year. The amount will be decided at the Annual General Meeting and will be decided by a simple majority vote of members present at the Annual General Meeting.

10. The Honorary Treasurer will receive, for the use of the Society, all monies payable to it and from such monies will pay all sums payable by the Society. The Treasurer will keep account of all such receipts and payments in a manner directed by the Executive Committee. All monies received by the Society will be paid into such a bank as may be decided by the Executive Committee of the Society and in the name of the Society. All cheques will be signed by either the Honorary Treasurer or an elected Committee member.

11. Two auditors will be appointed annually by members at the Annual General Meeting. The audited accounts and balance sheet will be circulated to members with the notice concerning the Annual General Meeting and will be presented to the meeting.

MEETINGS

12. Ordinary general meetings of the Society will be held at such a time as the Executive Committee may decide on the recommendations of members. The Annual General Meeting will be held in conjunction with an ordinary general meeting.

GUESTS

13. Members may invite non-members to ordinary general meetings.

PUBLICATION

14. The proceedings of the meetings of the Society will not be reported either in part or in whole without the written permission of the Executive Committee.

15. The Society may produce publications at the discretion of the Executive Committee.

GENERAL

16. All meetings will be convened by notice at least 21 days before the meeting.

17. The President will preside at all general and executive meetings or, in his absence, the Senior Vice-President or, in his absence, the Junior Vice-President or, in his absence, the Honorary Secretary or, in his absence, the Honorary Treasurer. Failing any of these, the members present will elect one of their number to preside as Chairman.

18. The conduct of all business transacted will be under the control of the Chairman, to whom all remarks must be addressed and whose ruling on a point of order, or on the admissibility of an explanation, will be final and will not be open to discussion at the meeting at which it is delivered. However, this rule will not preclude any member from raising any question upon the ruling of the chair by notice of motion.

19. In case of an equal division of votes, the Chairman of the meeting will have a second and casting vote.

20. All members on election will be supplied with a copy of this constitution.

21. No alteration will be made to these rules except by a two-thirds majority of those members voting at an annual general meeting of the Society, and then only if notice of intention to alter the constitution concerned will have appeared in the notice convening the meeting. A quorum will constitute twenty per cent of members.

22. Any matter not provided for in this constitution will be dealt with at the discretion of the Executive Committee.

Laid down April, 1982
Revised March, 1985; April, 1988; November 1994, March 2014
Corrected January 1997; April 2002