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SURVEILLANCE

USING FEEDING, MOVEMENT, AND SOCIAL BEHAVIOURS FROM SENSOR DATA TO PREDICT RESPIRATORY DISEASE IN PRE-WEANED CALVES

E.V. BUSHBY*, M. THOMAS, J.A. VÁZQUEZ-DIOSDADO, F. OCCHIUTO AND J. KALER

SUMMARY

This study aimed to predict BRD onset in pre-weaned calves using machine learning algorithms and 229 novel and previously researched feeding, movement, and social behavioural features. Data for 172 group housed calves were collected using automatic milk feeding machines and ultrawideband location sensors. Health assessments were carried out twice weekly using a modified Wisconsin scoring system in which a calf with a score of five or above and/or a rectal temperature of 39.5ºC or higher was classed as sick. Moderate to high performance metrics (accuracy: 0.773; precision: 0.766; sensitivity: 0.625; specificity: 0.872; *F*1-score: 0.689) were achieved with a gradient boosting machine classification algorithm. Movement behaviours were the most important feature for model prediction, whereas feeding and social features aided minimally. These results highlight the predictive potential but the need for improvement in model performance before behavioural changes can be used to reliably predict the onset of BRD in pre-weaned calves.

INTRODUCTION

Disease and poor health significantly impact all aspects of livestock production from animal welfare to farm economics (Dawkins, 2017). Of significant concern, bovine respiratory disease (BRD) is the most common cause of poor performance and mortality in calves under ten months of age as well as having a large economic impact on the UK cattle industry per year (Johnson et al., 2011; Agriculture and Horticulture Development Board, 2023). Identifying the disease early is key to timely treatment and reducing disease transmission, consequently reducing the impact on the animal, herd, and farm.

Sickness behaviours, or behavioural changes in response to disease, can often precede clinical signs (Hart, 1988; Millman, 1997; Johnson, 2002; Dantzer and Kelley, 2007; Tizard, 2008; Sepúlveda-Varas et al., 2016) and identifying these changes in behaviour can be vital to detecting a health event early on. These often-subtle changes in behaviour can be identified more easily and accurately with the use of precision livestock technologies (Miguel-Pacheco et al., 2014; Barkema et al., 2015; Schillings et al., 2021). Previous research has used wearable accelerometers to show that calves with BRD are less active than healthy individuals (Bowen et al., 2021a; Costa et al., 2021; Gardaloud et al., 2022). Automatic milk feeding machines have also been utilised to identify changes in feeding behaviours which in calves with BRD

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can change up to five days prior to clinical signs (Johnston et al., 2016; Knauer et al., 2017; Sutherland et al., 2018; Duthie et al., 2021a; Cantor and Costa, 2022). However, to the authors knowledge, only a handful of studies have used feeding and activity-based behaviours with machine learning algorithms to predict BRD in pre-weaned calves (Bowen et al., 2021b; Cantor and Costa, 2022; Ghaffari et al., 2022; Casella et al., 2023; Perttu et al., 2023). These studies used a range of machine learning algorithms and typically produced moderate to high performance values, indicating the potential in this area. However, due to the limited amount of research, there is need for further studies to identify how best this type of behavioural data can be utilised in early disease prediction in calves. It should also be noted that only a small number of feeding variables have been explored: typically, total milk consumption, numbers of rewarded and unrewarded visits to the feeder, and feeding speed (Morrison et al., 2021). Despite data being automatically collected from the milk feeders, the wealth of data produced is underutilised and a wider range of feeding behaviour features could be engineered and explored. Furthermore, other types of behaviours that are impacted by poor health and disease are yet to be explored, such as social behaviours that have been shown to be impacted by disease (Stockmaier et al., 2020; Vázquez-Diosdado et al., 2023) and can have implications for disease transmission (Craft, 2015; Sosa et al., 2021).

Therefore, this study aimed to predict bovine respiratory disease events in pre-weaned calves using a range of previously researched and novel behavioural features (feeding, movement, and social) with machine learning classification algorithms. To the authors' knowledge this is the first study to use movement and social behaviours to predict BRD onset, as well as to use the combination of all three feeding, movement, and social features.

MATERIALS AND METHODS

This study was approved by the Ethical Committee at the School of Veterinary Medicine and Science, University of Nottingham (reference number: 1481150603) and was performed in accordance with relevant guidelines and regulations of the School of Veterinary Medicine and Science, University of Nottingham.

Animals and Housing

This study took place at the Centre for Dairy Science Innovation (CDSI) at the University of Nottingham, UK, and all calves were managed according to standard procedures for the CDSI. All calves were fed four litres of pasteurised colostrum within two hours of birth and removed from the dam within four hours. As soon as possible post-birth, all animals were ear tagged with an RFID ear tag. Following this they were vaccinated for respiratory disease at nine days old (Rispoval RS+Pi3 IntraNasal; Zoetis) and disbudded from two weeks old. Initially, all calves were reared in pairs in a straw bedded pen (paired housing pen: 1.5m × 3.5m), until six to eight pairs of calves were present, creating a cohort of 12 to 16 calves. Once the youngest individual in the cohort was a minimum of two weeks old, the cohort was moved into a larger straw pen as a group (group housing pen: 6m × 10m) where they remained up to 12 weeks. Throughout both housing stages, all calves had ad-lib access to water, concentrates and chopped straw, as well as 10 litres of milk replacer (Milkivit Energizer ECM, Trouw Nutrition GB) per day from the automatic feeder (Forster-Technik, COMPACT smart), allocated via RFID recognition. Each allocation was given at two-hour intervals, and once the allowance had been consumed the feeder would not dispense another allowance for two hours. If the calf did not consume the whole allowance, then it was kept available for the whole two-hour interval before the entitlement restarted at two litres. Regardless of age, from day 36 after moving to the group housing the milk entitlement was reduced by 400ml per day until day 56 when the calves were completely weaned. Throughout the study the calves were checked twice daily by the farm staff.

Data for this study was collected for 11 cohorts of up to 16 calves during the group housing stage between April 2021 to July 2022 for. Four calves were removed from the cohorts for health or management reasons, resulting in data collected for a total of 172 animals. 168 calves were female Holstein-Friesian however, one male Holstein-Friesian, one female Aberdeen Angus X Holstein-Friesian and two male Aberdeen Angus X Holstein-Friesian calves were also included in the study.

Data Collection, Pre-Processing, and Feature Engineering

R software (R Core Team, 2019) was used for all data pre-processing and analysis. The first two days after moving to the group pen were removed to account for the calves' acclimatisation to the new environment. Likewise, the weaning and post-weaning period were removed from the dataset to remove any change of behavioural change due to weaning. In total, this resulted in thirty-three days of data per cohort.

Health Assessment: During the group housing, each calf was assessed for bovine respiratory disease twice weekly, three to four days apart, by one of three scorers. This was done using a modified version of the Wisconsin health scoring system (Mcguirk and Peek, 2014), where the eye, nose, ear, cough, and rectal temperature were scored from zero to three. These scores were then combined to create a total Wisconsin score for each calf. For this study a calf was classed as sick using the Wisconsin health scoring system corrected for pyrexia, as defined as a rectal temperature of 39.5ºC or above. For example, a calf with a score of five or more would be classed as sick, and likewise a calf that had a score lower than five but a rectal temperature of 40ºC would also be classed as sick on that day.

Feeding Behaviours: The computerised milk feeder (Forster-Technik, COMPACT smart) automatically collected a range of data each time a calf visited the feeder including calf ID, date, visit start time, visit end time, total milk consumed, drinking speed, actual entitlement at that time (ml), time the next entitlement is due, visit with entitlement, and visit without entitlement. These data were exported from the SD card to create feeding features.

Movement Behaviours and Social Interactions: Each calf wore a collar with a mounted ultra-wideband sensor (Tag Leonardo Personal, Noldus, Wageningen, the Netherlands) which recorded the relative (x, y) coordinates of the animal. These were set to a frequency of 1Hz, and the location data collected was processed and filtered as in Occhiuto, et al., (2022). This allowed for the calculation of the following movement features: walking distance, mean turning angle autocorrelation, mean residency time, and sinuosity.

Social features were also computed using the location data from the ultrawideband sensors worn by the calves. For the social features interactions were defined as "*proximity interactions below a threshold distance for a minimum duration of time between a pair of calves*" (Vázquez-Diosdado et al., 2023). Full details of how movement and social interaction data were collected and pre-processed can be found in Occhiuto, et al., (2022) and Vázquez-Diosdado, et al., (2023), respectively. The following social features were computed: mean contact duration and number of proximity encounters.

Feature reduction: Correlations between all feeding, movement, and social features were assessed and any features with a correlation above 0.7 or below -0.7 were removed. This was done based on how often they correlate with other features (i.e if one feature correlates with lots of features it was removed instead of the feature that didn't correlate with as many) and relevance based on previous literature. This resulted in 21 features, and a total of 229 features once delta features were created.

Classification Algorithm

Using the caret package in R (Kuhn, 2023), a gradient boosting machine classification algorithm was employed. For training, the dataset was balanced into a 40% sick and 60% healthy split. Firstly, the optimal hyperparameters (the number of trees, the number of splits in each tree, learning rate, and the minimum number of observations in the trees terminal node) were found using a grid search and an 80:20 data split for training and testing, respectively. Secondly, the model was implemented using a 5-fold cross validation technique and all 229 features. Accuracy, specificity, sensitivity, precision, and *F*1-score were used to evaluate the algorithm performance and the *varImp* function from the caret package (Kuhn, 2023) was used to assess feature importance. Finally, to find the optimal number of features, the feature ranking was used to implement the classification algorithm using an increased number of features added according to their importance. This model performance was used to select the final optimal number of features.

RESULTS

Overall, the optimal number of features was found to be 30 which resulted in the gradient boosting machine algorithm producing moderate to high performance (Table 1). The original 229 features consisted of 168 feeding features (73.79%), 38 movement features (17.46%), and 20 social features (8.73%). In the final dataset of 30 features movement features made up the highest proportion with 15 features (50%), feeding with 12 features (40%), and social features with 3 (10%).

Table 1. Performance metrics for the gradient boosting machine algorithm for the model containing all 229 features and the model only containing 30 features ranked as most important

Metric	Performance 229 Features	Performance 30 Features
Accuracy	0.764	0.773
Precision	0.775	0.766
Sensitivity	0.580	0.625
Specificity	0.887	0.872
*F*1-score	0.663	0.689

DISCUSSION

To the authors knowledge, this is the first study to include both movement and social behavioural features in combination with feeding behavioural features and machine learning algorithms with the aim of predicting bovine respiratory disease in pre-weaned calves.

Movement features made up 50% of the final dataset, highlighting that movement behaviours, were where the largest behavioural changes occurred in BRD positive calves. This is in line with the results of previous literature which, although did not use movement features, showed that measures of activity (calculated using accelerometers rather than location sensors), such as lying time (Duthie et al., 2021a) and the number of steps taken daily (Swartz et al., 2017), to be of high importance in the early detection of respiratory disease.

Previously there has been a large focus on feeding behaviours to predict bovine respiratory disease (Johnston et al., 2016; Duthie et al., 2021b; Morrison et al., 2021; Cantor and Costa, 2022), yet only a small number of feeding behaviour features have been explored (Ghaffari et al., 2022; Perttu et al., 2023). To explore the wealth of data produced by automatic milk feeders and to ensure all aspects of this behaviour were assessed, commonly employed feeding variables and new ones were included. Of the final 30 top features, 18 were based on these novel feeding features. Also included in the final 30 features were features, or variations of features, that have been explored in previous studies however, despite the inclusion of novel feeding variables in the present study and focus on feeding behaviours in the previous literature, changes in feeding behaviour contributed minimally to BRD prediction. This is in contrast with two previous studies that used feeding behaviour with machine learning algorithms. One demonstrated high performance values (sensitivity: 0.826; specificity: 0.790; accuracy: 0.800; negative predictive value: 0.970) except for the positive predictive value (0.365) using a convolutional neural network approach (Ghaffari et al., 2022). The second achieved high precision (0.814), sensitivity (0.740), and $F1$-score (0.775) (Perttu et al., 2023) using a gradient boosting machine algorithm however, using a different dataset the performance was much lower (precision: 0.484; sensitivity: 0.219; $F1$-score: 0.301), and again performance varied greatly when the authors employed random forest or generalised linear models. How a calf is classed as sick may explain some of the differences between this study and the two previous studies as both used more frequent health scoring and lower Wisconsin score thresholds (Ghaffari et al., 2022; Perttu et al., 2023). It may also be that feeding is an essential behaviour which is maintained as much as possible even during disease periods (Weary et al., 2009), explaining why these behaviours were not found to be overly important in our model and the variability in performance found by Perttu, et al., (2023). Consequently, based on current research, feeding behaviours are not consistently a good predictor of BRD status in calves.

On the other hand, social behaviours are less likely to be essential and therefore less likely to be maintained when the calf is in a diseased state. In both diseased cattle and other species, a higher level of social isolation, or a reduction in social interactions, has been demonstrated (Proudfoot et al., 2012; Townsend et al., 2020). As expected in this study, the model used social features to determine if a calf was BRD positive, and three of these were included in the final 30 features. Although, these social features, like the feeding features, still only contributed minimally to the model prediction in comparison to movement features. As only a small number of social behaviours were explored here, future research should aim to expand on this to enable a better understanding of how other social metrics could be used to predict bovine respiratory disease.

Overall, the gradient boosting machine algorithm produced moderate values for all performance metrics (accuracy: 0.773; precision: 0.766; sensitivity: 0.625; $F1$-score: 0.689), except specificity which was higher (0.872; Table 1). Previously Bowen and colleagues' (Bowen et al., 2021b) also achieved a similar accuracy (0.75), but higher specificity (0.95) and lower sensitivity (0.54) than this study, when using a moving average and random forest combined model. Similarly, our model's accuracy (0.773) was comparable to that achieved by

Casella, et al. (2022) who reported an accuracy of between 0.70 and 0.90, depending on the budget (the authors' estimated cost of on-farm implementation of the framework). In contrast Cantor, et al. (Cantor et al., 2022) achieved much higher performance values with accuracy, precision, sensitivity, and $F1$-score values of 0.99 each. Although, it should be noted that the individual score for each area (nose, eye, cough etc.) were included as features in Casella, et al. (2022) and Cantor, et al. (2022)'s models. Furthermore, it is difficult to compare this study with Casella, et al. (2022) as they used a different way of categorising and labelling the data. In the published literature the thresholds vary greatly when classifying a calf as BRD positive (or sick), highlighting the difficulty of identifying the disease and the need for more understanding of the most accurate way to do so. In the current study it was only possible to health score twice weekly however, daily scoring may improve the performance by ensuring the ground truth data were as specific to the data as possible. Moreover, there is some evidence to show that combining thoracic ultrasound scoring with Wisconsin scoring may improve the sensitivity of BRD identification (Buczinski et al., 2014, 2015; Rhodes et al., 2021). However, as BRD can present with a range of symptoms in varying severity (McGuirk, 2008; Ferraro et al., 2021), by correcting for pyrexia when health scoring in this study, it may have ensured that calves with BRD but with few clinical signs were identified correctly as sick.

In conclusion, to the authors knowledge, this is the first study to include social and movement features in combination with feeding behaviours and machine learning algorithms to predict BRD status in pre-weaned calves. Overall, model performance was moderate to high when using the combination of feeding, movement, and social behaviours collected using automatic milk feeders and location sensor data. The best indicator of a change in BRD disease status was changes in movement behaviours. In contrast feeding and social features only aided in the model's prediction minimally. These results highlight the predictive potential of this area, but also the need for further improvement before feeding, social, or movement behavioural changes that occur pre-clinical signs can be used to reliably predict BRD status in pre-weaned calves.

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EVALUATING SURVEILLANCE STRATEGIES FOR HIGH PATHOGENICITY AVIAN INFLUENZA IN LIVE BIRD MARKETS IN SOUTHEAST ASIA

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SUMMARY

Avian influenza viruses (AIVs) pose an on-going threat to domestic poultry and wild birds. Live bird markets, prevalent throughout southeast Asia where AIVs are endemic, have been identified as ideal locations for conducting surveillance. Using multiple years of surveillance data from Cambodia, Laos, Myanmar, and Vietnam, Bayesian latent class analysis was employed to determine the effectiveness of chicken, duck, and environmental sampling strategies. In Vietnam, for H5N1 H5N6 and H5N8 subtypes, duck samples outperformed environmental samples which were more sensitive than chicken samples. In Laos, similar though less pronounced trends emerged. In Myanmar, H5N1 was best detected by environmental samples, whereas H5N6 and H5N8 saw environmental samples as the least sensitive. In Cambodia, duck samples outperformed chicken samples for the H5 subtypes. Observed differences reflect the varied dynamics between countries and between years, highlighting the necessity of situation-specific surveillance tailored to the needs of each country.

INTRODUCTION

In Southeast Asia, live bird markets (LBM) are cultural institutions that play a central role in poultry trade (Fearnley and Zheng, 2023; Nguyen et al., 2017). However, they have also been established as hotspots of avian influenza virus (AIV) circulation and amplification (Cardona et al., 2009; Indriani et al., 2010; Coker et al., 2011; Fournié et al., 2012; Rimi et al., 2019; Islam et al., 2023). Early detection of circulating AIV subtypes is a critical component of AIV control strategies, though current prescribed surveillance measures—using live bird sampling during periods of market activity—are not without contention. The perception of ill-health and subsequent decreases in the economic value of live birds can make both market vendors and buyers averse to live bird sampling strategies (Indriani et al., 2010; Leung et al., 2007; Zeynalova et al., 2015; Vergne et al., 2019). In contrast, environmental sampling has been championed for many of its qualities including non-invasiveness, cost-effectiveness, and benefits to animal welfare (Indriani et al., 2010; Zeynalova et al., 2015; Bui et al., 2019; Hood et al., 2021).

Multiple studies have examined environmental sampling, with a myriad of sample types represented: including samples from cages, floors, air, and water and feed sources (Hood et al.,

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2021). Fresh fecal samples have been of particular interest, as they have been shown to have similar or better sensitivity than live bird samples, with the caveat that only H7N9 was assessed among LBMs in China and the other studies examined fresh feces from wild birds (Busquets et al., 2010; Pérez-Ramírez et al., 2010; Chen et al., 2014; Hood et al., 2021).

Previous research in Vietnam for the 2014–2015 period, which included both oropharyngeal live bird samples and multiple types of environmental samples, had shown that AIV detection accuracy varies by sample type, and that sample-type sensitivity is subtype dependent (Vergne et al., 2019). However, only two subtypes (H5N1 and H5N6) were assessed across one 14-month period, and it is uncertain if the recommendations and conclusions of sampling strategy effectiveness can be extrapolated across years and/or national borders. Here, we expand on this research through analyzing national surveillance data from multiple Southeast Asian countries over multiple years. The objective was to quantify the effectiveness of different sampling strategies, estimating both the prevalence of detected AIV subtypes and the sensitivity of different sample types for each represented country.

MATERIALS AND METHODS

Data across multiple time periods was provided by the Food and Agriculture Organization of the United Nations (FAO) from national AIV surveillance projects in collaboration with national animal health departments from Cambodia (2020–2022), Laos (2017–2022), Myanmar (2017–2019) and Vietnam (2016–2022). Sample collection, testing protocols, and types of samples collected varied by country and by year. Surveillance consisted of the collection of chicken and duck oropharyngeal samples and fresh fecal environmental samples in Vietnam, Laos, and Myanmar. In Cambodia, surveillance consisted of tracheal and cloacal samples from chickens and ducks.

Chicken, duck, and environmental samples were collected from vendors and either pooled into groups of five (Vietnam, Myanmar, and Laos 2017–2018) or tested individually (Laos 2020–2022) for the influenza A matrix gene. Following a positive result, samples were subtyped by hemagluttin (H) and neuraminidase (N) proteins. Samples were tested for the H5, H7, and H9 proteins, with positive H5 results subsequently tested for N1, N6, and N8 proteins, positive H7 results tested for N9 proteins, and positive H9 results tested for N2 proteins.

Sample populations were constructed of all represented combinations of sampling strategies, with a sampling strategy defined by the number of each type of sample collected per market visit. Sample populations were excluded from analysis if all sample types were not represented in the population. Given the high turnover of poultry at an LBM, market visits were assumed independent as long as they occurred at least 10 days apart, as AIV is unlikely to persist in the environment beyond such a period of time (Kurmi B et al., 2013; Shortridge et al., 1998). Market visits less than 10 days from a previous visit were excluded from analysis. A market was considered contaminated if at least one of a given sample type was detected as positive for an AIV subtype.

Latent class analysis

Bayesian latent class analysis (BLCA), used to evaluate test accuracy when the true epidemiological status of tested individuals is unknown, is an established methodology endorsed the by World Organization for Animal Health (WOAH) (Branscum et al., 2005; Cheung et al., 2021; Joseph et al., 1995; World Organisation for Animal Health (WOAH), 2019). Here, we estimate both the prevalence of AIV subtypes and the detection sensitivity of each sample type per AIV subtype through Markov Chain Monte Carlo methods. The detailed steps of BLCA have been comprehensively outlined in other sources (Branscum et al., 2005; Joseph et al., 1995).

For scenarios of three imperfect surveillance protocols (as in Laos, Myanmar, and Vietnam) there were eight combinations of results, whereas four imperfect protocols (as in Cambodia) allowed for sixteen combinations of results. A multinomial distribution was assumed to define the observed frequencies of outcome combinations and was informed by the number of sampled LBMs and the number of probability combination (eight or sixteen, depeding on national sampling scenario), with each sample population fit to its own multinomial distribution. This distribution was expressed as a function of the proportion of infected LBMs and the sensitivity and the specificity of each of the different surveillance protocols.

BLCA models were created for each subtype for each country. In Vietnam, Laos, and Myanmar, seven distinct models were used to test for positive and negative conditional dependence between sample combinations. Model performance was evaluated via the deviance information criterion (DIC). The best-fitting model was defined as the model with the fewest parameters (i.e. the most parsimonious) with a DIC value less than two points from the model with the lowest DIC (Spiegelhalter et al., 2002; Vergne et al., 2019).

Three MCMC chains were ran for 50,000 iterations for all subtypes across all countries (except H5Nx in Vietnam, which required 100,000 chains for convergence), having initially underwent 10,000 adaptation iterations followed by a burn-in period of 10,000 iterations, and thinned to every 5th value to reduce autocorrelation. Uniform priors were assumed for both prevalence and sample sensitivity parameters. Given the high specificity of rRT-PCR, a beta distribution with a median of 98% and a 5th percentile of 80% was used to define the specificity priors (Ellström et al., 2008; Vergne et al., 2019). Covariance parameters followed uniform distributions as defined in Dendukuri and Joseph (2001) and Vergne et al. (2019). Modelling and analysis was performed through the JAGS library (Depaoli et al., 2016) via the Rjags package version 4.12 (Plummer et al., 2023) in R version 4.1.3 “One Push-Up” (R Core Team, 2022).

RESULTS

Data sufficient for analysis varied by country. In Vietnam, surveillance data from December 2015 through June 2022 was included, with the exception of the period from March 2017 to November 2018 as no single markets were sampled for all three sample types at that time. In Laos, data was available for the periods of May 2017 through March 2018 and March 2022 through September 2022. In Myanmar, data was available from February 2017 through Februrary 2019, and in Cambodia from January 2017 through April 2022. Data from Vietnam was able to evaluated both en-masse and in across years.

In Vietnam, markets were visisted a minimum of 25 days apart, in Laos 14 days, in Myanmar 28 days, and in Cambodia 10 days. 12,384 samples were collected over the study period in Vietnam, 7,201 in Loas, 1,686 in Myanmar, and 1,566 in Cambodia. In Vietnam, 34 out of 1467 market visits between 24 April 2017 and 07 October 2020 were not included as they occurred less than 10 days from the previous visit. Similarly in Cambodia, 71 out of 434 market visits between January 2017 and April 2022 were excluded for occurring within 10 days of each other.

Model fitting

In almost all scenarios, the model without covariance parameters was the most parsimonious. In Vietnam, the data for the H5N8 subtype was best fit by a model that included negative conditional dependence between chicken and duck samples. In Laos, data for the H5N6 subtype was best fit when negative conditional dependence was assumed between chicken and environmental samples. This implies that non-contaminated LBMs in Vietnam that tested negative for H5N8 in chicken samples were more likely to also test negative by duck samples, and that non-contaminated LBMs in Laos that tested negative for H5N6 in chicken samples were more likely to also test negative by environmental samples.

Visual inspection of trace plots of the best fit models across all subtypes, along with Gelman-Rubin statistics less than 1.1, confirmed the convergence of MCMC chains for all parameters. Further, stability in the estimates of the limits of the 95% Highest Density Intervals for prevalence, sensitivity, and specificity was assured through obtaining effective sample sizes greater than 10,000.

Per-country prevalence and sensitivity estimates

Prevalence and sensitivity estimates varied by country and subtype (Fig.1, Table 1). In Vietnam, all H5 subtypes (including H5N1, H5N6, and H5N8) showed similar sensitivity profiles, with duck samples the having the best detection sensitivity. Among H5N1, H5N6, and H5N8 subtypes, duck samples outperformed environmental samples which outperformed chicken samples. Similar trends are weakly suggested for the untyped H7 subtypes. Only among the untyped H5 subtypes was a parity seen between chicken and environmental samples. All other AIV subtypes not including those of H5 H7 and H9 — “HxNx” — were better detected by environmental samples, followed by chicken and lastly duck samples. Prevalence was low for all H5 subtypes, ranging from 0.01 (interquartile range (IQR) 0.01–0.02) for H5N8 to 0.08 (IQR 0.06–0.09) for H5N1. Overall AIV prevalence was estimated at 0.46 (IQR 0.44–0.48).

When evaluated by year, variations in detection sensitivity can be seen for all sample types across all years (Fig.2, Table 2). Only from the 2019–2020 to the 2021–2022 seasons did sample specificity—for duck and environmental samples—remain consistent. Subtype prevalence also varied by year, with the H5N1 subtype increasing in prevalence from 2015–2016 to 2021–2022, and the H5N6 subtype increasing in prevalence from 2015–2016 to 2019–2020, but then falling to its lowest prevalence in 2021–2022.

In Laos, a clear distinction between sample type sensitivity was less evident. Indeed, H5N1 was better detected by environmental samples while duck and chicken samples performed similarly. H5N6 sensitivity was not well estimated as it was only detected in a single market by an environmental sample. Untyped H9 and all other AIV varients outside of H5 H7 and H9 (“HxNx”) suggest duck and chicken samples slightly outperforming environmental samples. Prevalence for H5N1 was estimated at 0.25 (IQR 0.21–0.30) and for H5N6 at 0.31 (0.14–0.52).

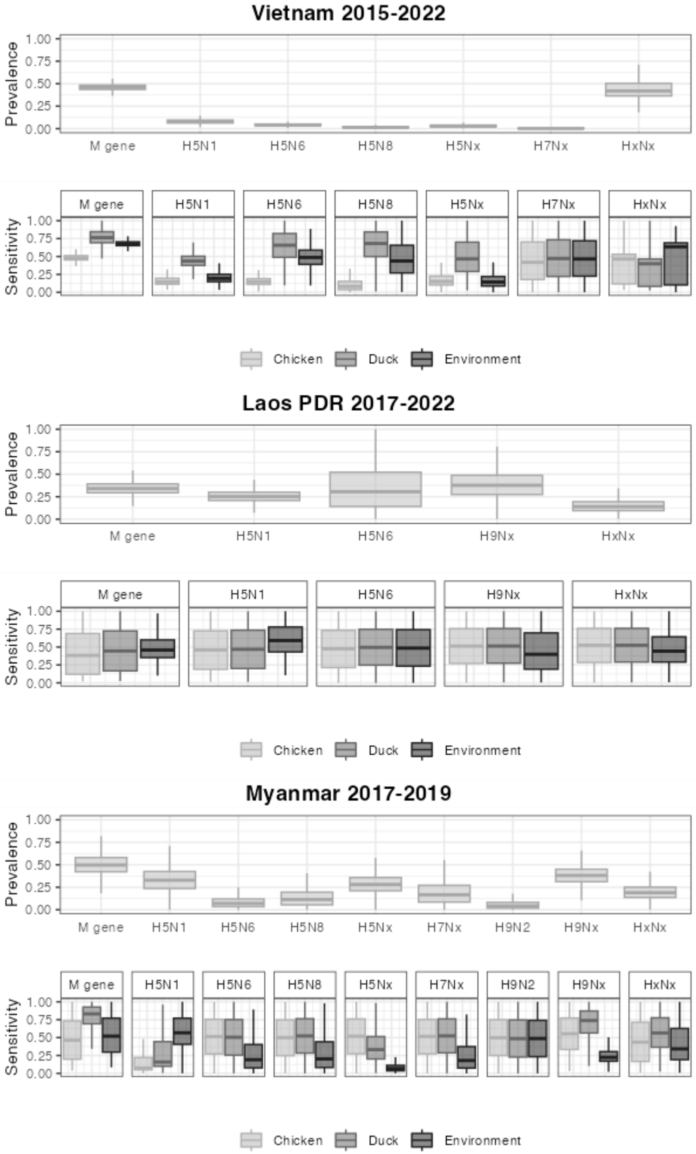

Figure 1. Estimated prevlance and sample sensitivity of subtypes by country for countries which sampled using chicken oropharyngeal, duck oropharyngeal, and fecal environmental samples

Myanmar had the greatest number of explicit subtypes detected, including H5N1, H5N6, H5N8, and H9N2. Overall AIV prevalence in Myanmar was similar to Vietnam (0.50 [IQR 0.42–0.58]). For H5N1, environmental samples outperformed duck and chicken samples, with an estimated sensitivity of 0.57 (IQR 0.41–0.77). Conversely, environmental samples performed worse than chicken and duck samples for H5N6 and H5N8 subtypes. H7N9 was not detected in any markets in Vietnam, Laos, or Myanmar, and H9N2 was not detected in any markets in Vietnam or Laos.

In Cambodia, where oropharyngeal and cloacal samples were compared amongst chickens and ducks, subtype-specific patterns in sample sensitivity were observed (Fig.3, Table 3). For H5N1 and H5N8 subtypes, duck samples outperformed chicken samples, and among H5N1-positive duck samples, oropharyngeal samples had slightly higher sensitivity than cloacal samples (0.78 [IQR 0.65–0.88] and 0.70 [IQR 0.56–0.84], respectively). Conversely, sensitivity to the H9N2 subtype was much higher in chicken than in duck samples, with chicken oropharyngeal samples having the highest sensitivity (0.8 [IQR 0.73-0.87]). Prevalence among H5 subtypes was estimated from 0 (0–0.01) for the H5N6 subtype (indeed, only one positive market was detected) to 0.06 (0.04–0.08) for H5Nx subtypes. Overall AIV market prevalence was estimated at 0.52 (0.49–0.55).

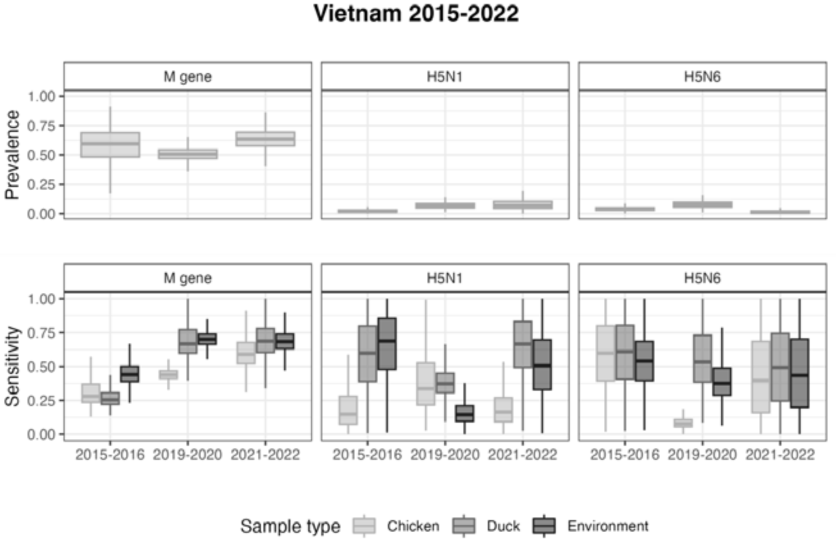

Figure 2. Estimated market prevalence and sample detection sensitivity in Vietnam by subtype across multiple biannual periods (2015–2016, 2019–2020, and 2021–2022)

Table 2. Bi-annual sensitivity and prevalence estimates (with interquartile range) comparing chicken oropharyngeal, duck oropharyngeal, and environmental samples in Vietnam

Year period	Prevalence	$Se_{chicken}$	Se_{duck}	$Se_{environment}$
M gene				
2015–2016	0.59 (0.48-0.69)	0.28 (0.24-0.37)	0.25 (0.22-0.31)	0.44 (0.39-0.5)
2019–2020	0.51 (0.47-0.54)	0.44 (0.41-0.47)	0.67 (0.6-0.77)	0.7 (0.66-0.74)
2021–2022	0.64 (0.58-0.69)	0.59 (0.52-0.68)	0.69 (0.6-0.78)	0.68 (0.63-0.74)
H5N1				
2015–2016	0.02 (0.01-0.03)	0.15 (0.07-0.28)	0.6 (0.39-0.8)	0.69 (0.48-0.86)
2019–2020	0.06 (0.05-0.09)	0.34 (0.22-0.53)	0.37 (0.31-0.45)	0.15 (0.1-0.21)
2021–2022	0.07 (0.05-0.1)	0.16 (0.09-0.27)	0.67 (0.49-0.83)	0.51 (0.33-0.7)
H5N6				
2015–2016	0.04 (0.03-0.05)	0.6 (0.39-0.8)	0.61 (0.41-0.8)	0.54 (0.4-0.69)
2019–2020	0.08 (0.06-0.1)	0.08 (0.06-0.11)	0.53 (0.39-0.73)	0.38 (0.29-0.49)
2021–2022	0.01 (0-0.02)	0.4 (0.16-0.69)	0.49 (0.25-0.74)	0.43 (0.2-0.7)

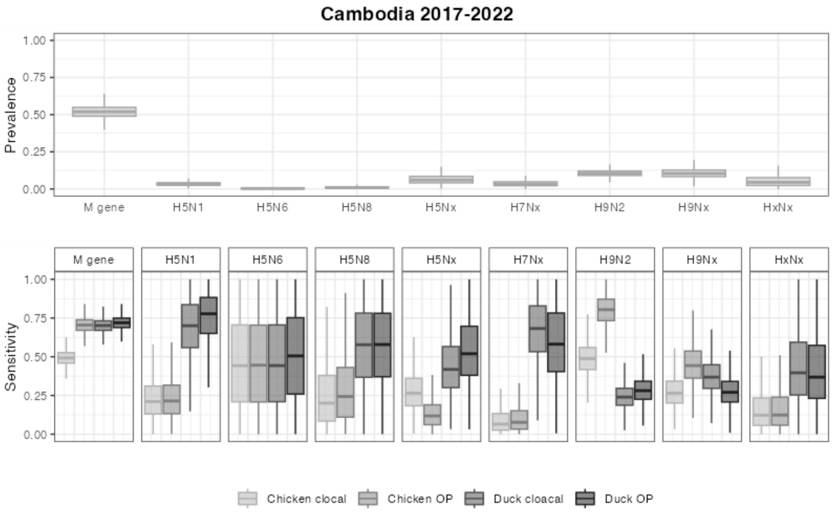

Figure 3. Estimated market prevalence and sample sensitivity in Cambodia for the period 2017–2022, comparing chicken cloacal, chicken oropharyngeal (OP), duck cloacal, and duck OP samples

Table 3. Sensitivity and prevalence estimates (with interquartile range) comparing chicken and duck cloacal and oropharyngeal samples, Cambodia 2017–2022

Subtype	Prevalence	$Se_{chicken\_cl}$	$Se_{chicken\_op}$	Se_{duck_cl}	Se_{duck_op}
M gene	0.52 (0.49–0.55)	0.49 (0.46–0.53)	0.7 (0.67–0.74)	0.7 (0.67–0.73)	0.72 (0.69–0.75)
H5N1	0.03 (0.02–0.04)	0.21 (0.13–0.31)	0.21 (0.13–0.32)	0.7 (0.56–0.84)	0.78 (0.65–0.88)
H5N6	0 (0–0.01)	0.44 (0.21–0.71)	0.45 (0.21–0.7)	0.44 (0.21–0.71)	0.51 (0.26–0.75)
H5N8	0.01 (0.01–0.01)	0.2 (0.08–0.38)	0.24 (0.11–0.43)	0.58 (0.37–0.78)	0.58 (0.37–0.78)
H5Nx	0.06 (0.04–0.08)	0.27 (0.18–0.36)	0.12 (0.06–0.19)	0.42 (0.3–0.57)	0.52 (0.38–0.7)
H7Nx	0.03 (0.02–0.05)	0.06 (0.03–0.13)	0.08 (0.03–0.15)	0.68 (0.53–0.83)	0.58 (0.4–0.78)
H9N2	0.11 (0.09–0.12)	0.49 (0.42–0.56)	0.8 (0.73–0.87)	0.24 (0.19–0.3)	0.28 (0.23–0.34)
H9Nx	0.1 (0.08–0.13)	0.26 (0.2–0.34)	0.44 (0.36–0.54)	0.37 (0.3–0.45)	0.27 (0.21–0.34)
HxNx	0.04 (0.02–0.08)	0.12 (0.06–0.23)	0.12 (0.06–0.24)	0.4 (0.25–0.59)	0.37 (0.23–0.57)

DISCUSSION

As previously shown, the sensitivity of chicken, duck, and environmental samples is dependent on the AIV subtype being detected (Choi et al., 2005; Leung et al., 2007; Amonsin et al., 2008; Indriani et al., 2010; Vergne et al., 2019). Here, such subtype-specific fluctuations in sample sensitivity are apparent across all countries and also across years. Differences in estimated sample sensitivity by country can be the result of differences in sampling procedures, including whether random or targeted sampling occurred and the technical capabilities of the field personnel, or externalities such as the presence of or adherence to end-of-day market cleaning protocols. These differences reinforce the importance of surveillance strategies adapted to the individualities of each country.

Beyond variations in sample sensitivity estimates, prevalence estimates showed wide variation between countries for the same subtypes. These country-specific differences can be due to multiple internal factors, including time since virus introduction into the country, vaccination policy, and the structure of the production systems. Indeed, in Myanmar high pathogenicity H5N1 had been previously identified in apparently healthy chickens, suggesting that vaccination, though illegal, had been occurring (Thurain et al., 2020).

Differences in prevalence and sensitivity estimates by year, as seen in Vietnam, can also reflect changes in both sampling procedures and technical capabilities, changes in local policies such as market cleaning instructions, as well as molecular changes that influence detectability among circulating subtypes. Within-market prevalence plays a large role in detectability, and levels of circulation will be dependent on market policies (e.g. regarding overnight stays, or single-day closures).

Here, through aggregating data across multiple years and including multiple sampling strategy populations, a greater number of positive results were able to be included to refine the reported credible intervals. Though annual prevalence is expected to vary, aggregation of yearly data was justified as AIV is considered endemic in many Southeast Asian countries (Eagles et al., 2009; Mellor et al., 2018).

As not all market sampling strategies reflected the prescribed sampling scenario, to be able to include all available data, models that accounted for multiple sampling strategies were required. In multipopulation BLCA models, individual covariance parameters are required for each population of sample combinations, resulting in linear scaling of the number of final parameters estimated in the model. Correspondingly, large variation in sampling strategies resulted in models with upwards of 30 covariance parameters (as would be the case with 30 distinct sampling populations). In these scenarios, the trade-offs of including such non-protocol sampling strategies will have to be weighed against the increased computational costs and complexity, and is an area for future research.

Positive conditional dependence, accounting for correlation among sensitivities of two tests, was unable to be assessed by the MCMC algorithm for multipopulation models. When sampling for the conditional dependence priors, “invalid parent values for node [x]” was a repeated point of model failure, and was tied to the estimation of covariant distribution parameters. Further investigation into the mathematical causes of the error may yield solutions that enable the inclusion of even more surveillance data, and allow for increased refinement in prevalence and sensitivity estimates.

The assumption of equal risk of AIV contamination across all markets is known to be false, as previous work identified multiple factors associated with increased risks of AIV contamination including duration of daily activity, LBM density, and number of poultry types (Gilbert et al., 2014; Wang et al., 2017). Future surveillance activity that records market metadata could be beneficial for helping to address this weakness in the model, though the increased costs to do so are not unrecognized.

Environmental sampling for AIV has been in use for 50 years—the first study having been published in 1979 (Hinshaw et al., 1979)—and continues to hold promise as an alternative means of surveillance. In spite of its advantages, multiple studies have revealed its limitations in detection, especially in scenarios of low prevalence (Hood et al., 2021). Here, we can see that the median of multiple prevalence estimates of H5N1, H5N6, H5N8, and H9N2 in Cambodia, Myanmar, and Vietnam are around or under 10%. Current environmental sampling techniques, often consisting of fecal water or waste samples, are but only some of the available sample types that could be evaluated. Exploration of additional environmental sample types, as is being explored with bioaerosols and dust samples (Bessière et al., 2023; Bui et al., 2019), is an important next step towards identifying optimal non-invasive methods that are on-par with current live bird methods.

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CATTLE

ELEVATING YOUNGSTOCK REARING PRACTICES: INSIGHTS FROM KALFOK DATA AND FARMER PERSPECTIVES IN THE NETHERLANDS

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SUMMARY

KalfOK is a data-driven tool in which a farm-level overview is created with indicators of youngstock rearing quality. After the implementation in 2018, a noticeable decline in calf mortality in Dutch dairy farms occured. However, since 2022, the decline started to level off. To continue the reduction, it is important to evaluate the current system and to gain understanding about differences between high and low scoring farms. Therefore, the aim of this study was to to assess which farm characteristics are related to obtaining a continuously high KalfOK score. Data from KalfOK indicators and the identification and registration system were available for all participating herds (2019-2022). A conditional logistic regression analysis on historic KalfOK data was performed to identify differences in characteristics between farms with a continuously high and a continuously low score. A continuously high KalfOK score was positively associated with a closed farming system and low replacement rate.

INTRODUCTION

Young stock rearing is an essential part of dairy management given that calves are raised as replacement for milking cows, and it should occur optimally to maximize health and welfare (Hultgren and Svensson, 2009; Sandgren et al., 2009; de Vries et al., 2011; Santman-Berends et al., 2021). An important indicator to assess the quality of calf rearing is the calf mortality rate (Ortiz-Palaez et al., 2008; Kelly et al., 2013), where, in general, a higher mortality rate in calves is associated with poor young stock rearing and poor animal welfare (Sandgren et al., 2009; de Vries et al., 2011). Between 2009 and 2017, a slight but steady increase in calf mortality was observed in Dutch dairy farms (Santman-Berends et al., 2014; Santman-Berends et al., 2019). Previous research showed that Dutch dairy farmers with suboptimal young stock rearing and high mortality were not aware of the fact that calf mortality was high at their farm (Santman-Berends et al., 2014). Also, they did not know how to decrease calf mortality or were not prepared to adjust their management. Based on these results several actions were initiated in the Netherlands and, amongst others, multiple data-driven tools were developed: (i) the voluntary information tool “KalfOK” (Santman-Berends et al., 2018), (ii) national surveillance of calf mortality at herd level (Santman-Berends et al., 2019) and (iii) the implementation of a

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calf tracking system for veal calves. The aim of these tools was to benchmark calf rearing by several key indicators for performance of young stock, to stimulate dairy farmers to improve young stock husbandry and to reduce calf mortality. Since the implementation of KalfOK and other initiatives in 2018, a decline in perinatal calf mortality, postnatal mortality up to 14 days, mortality in dairy calves between 15 and 56 days old and mortality in dairy calves between 56 days and two years old has been seen (Santman-Berends et al., 2021). However, the decline has become less prominent since 2022 and seems to move towards stabilisation. To continue the reduction in calf mortality, it is important to evaluate the current monitoring system and assess where it can be improved. The objective of this study was (i) to assess which farm characteristics are related to obtaining a continuously high or a continuously low KalfOK score and (ii) to assess farmers' opinions towards KalfOK and their needs for improvement.

MATERIALS AND METHODS

Study population and available data

The study population consisted of over 95 percent of dairy farms participating in KalfOK, where consent for using anonymous data in this study was obtained. Data from these herds over the period January 2019 – September 2022 was available for this study. According to the national Identification and Registration system (I&R), the total cattle population in the Netherlands in 2022 comprised of approximately 14,500 dairy herds (RVO, Assen, the Netherlands). The study population consisted of 13,343 Dutch dairy herds.

For all participating herds, data was available from KalfOK including the values obtained for the key indicators. Additionally, animal movement data was available from the identification and registration system (Dataset A). Furthermore, a random sample of 1,200 herds participating in KalfOK was selected and surveyed to obtain information for evaluation of farmers opinions towards the use of KalfOK (Dataset B). Farmers were approached via phone and email in the period February-March 2023. In the survey, questions on the following topics were included: farm characteristics, KalfOK data and farmer data. Farm characteristics included the location where a farm is situated (province), milk factory (anonymized), number of calves born in 2022 (as a proxy for farm size) and milking system (milking robot present yes/no). KalfOK data included the KalfOK score of a farm (moving average KalfOK score for 2022), whether the farmer has given their veterinarian permission to access their KalfOK report and whether KalfOK is discussed with their veterinarian (never/sometimes/regularly). Lastly data on the farmer included years of working experience, to what extent the farmer is responsible for care of the calves, whether there are people employed at the farm, and who is responsible for the youngstock.

Data analysis

All data validation and analyses to assess which farm characteristics were associated with having a high or low KalfOK score, were performed using STATA/SE version 16.1.

The aim of the multivariable analysis was to assess which farm characteristics are associated with the probability of belonging to farms with a continuously high or a continuously low KalfOK score, using a matched case-control design (Fig.1). For this analysis, the annual moving average KalfOK scores of four time points (the average up to the fourth quarter of 2019, 2020, 2021 and up to 2022) were selected with 'annual moving average score' being the average KalfOK score a farm has obtained over the past four quarters. These annual scores were used instead of the quarterly KalfOK scores to account for seasonality. A case farm was defined as a farm with a continuously high KalfOK year score, with ‘continuously high’ being defined as an annual moving average KalfOK score equal to or above the 75th percentile of the study population in at least three out of four years (2019, 2020, 2021 and 2022). This yielded a total of 2,247 case farms. A control farm was defined as a farm with a continuously low KalfOK score, with ‘continuously low’ being an annual moving average KalfOK score equal to or below the 25th percentile of the study population in at least three out of four selected years (2019, 2020, 2021 and 2022). A total of 2,099 control farms were selected.

To control for potential confounders, farms in the case and control groups were matched according to their herd size in 2019, since it is known that herd size is associated with the KalfOK score and this factor was not of interest. Farms were divided into four categories (Table 1), using the herd size distribution of the study population (based on quartile values). A case to control ratio of 1:1 was used in which case farms were matched to a control farm in the same herd size category. One case was randomly sampled per control within each herd size category resulting in 1,234 case farms and 1,234 control farms.

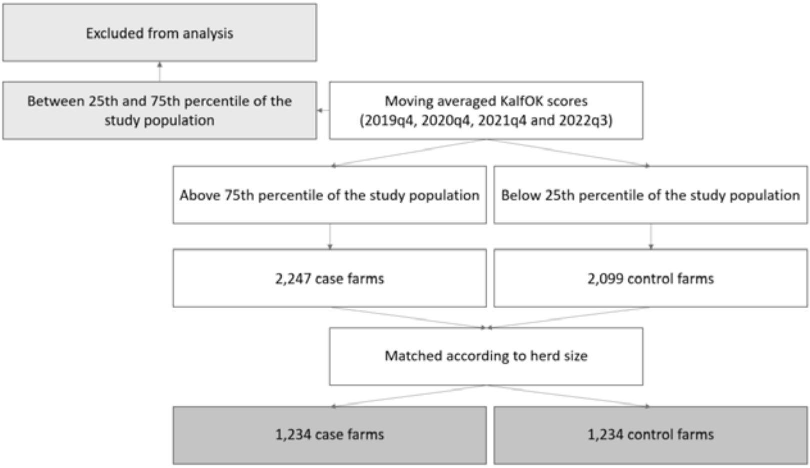

Figure 1. Selection of case and control herds for the multivariable analysis assessing which farm characteristics can explain the difference between a farm with a continuously high or a continuously low KalfOK score

Table 1. Frequency of farms per farm size category, according to the farm size in 2019

Category	Farm size	Frequency
1 (<25th percentile)	<63 cows	230
2 (>=25th percentile & <50th percentile)	>=63 cows & <96 cows	926
3 (>=50th percentile & <75th percentile)	>=96 cows & < 136 cows	846
4 (>=75th percentile)	>=136 cows	466
Total		2468

Farm characteristics obtained via dataset A were used as independent variables for analysis. For the variables ‘incentive’ (financial reward for farmers participating in KalfOK), ‘multiple locations’, ‘import’ and ‘farming system’, data of the last quarter of 2019, 2020, 2021 and 2022 was used to determine a status for each of these variables. To qualify for a specific status, a variable had to fall within a particular category, such as "closed" or “open” for farming system, for at least three out of the four selected years. For the continuous variables ‘replacement rate’, ‘percentage of growth in herd size’, ‘average age of the cows’ and ‘average age of the total number of cattle’, the average of the whole study period was calculated. Based on the result, herds were classified in one of four categories based on quartile values of the study population.

Farm characteristics explaining the difference between a continuously high and a continuously low KalfOK score were then analysed using a conditional multivariable logistic regression model (clogit). The model can be described by Eq.(1):

$$\begin{aligned} & logit\,[P(\,Z_j = 1)] \\ & = \mu_j + \mu_1 Prov_j + \mu_2 Incentive_j + \mu_3 MultLoc_j \\ & + \mu_4 ReplaceRate_j + \mu_5 GrowthRate_j + \mu_6 Import_j \\ & + \mu_6 Agecows_j + \mu_7 Agecattle_j + \mu_8 CalvPat_j \\ & + \mu_9 Farm\ system_j + \varepsilon_j \end{aligned} \tag{1}$$

where Zj is the dichotomous dependent variable indicating whether farm j is a case or control farm; μj the intercept for farm j; $Prov_j$ the province where farm j is located; $Incentive_j$ whether farm j receives a financial reward for participation in KalfOK (yes/no); $MultLoc_j$ whether farm j outsources (yes/no); $ReplaceRatej$ the replacement rate (<25th percentile/ 25th-50th percentile/ 50th-75th percentile / >75th percentile) of farm j; $GrowthRatej$ the growth rate (<25th percentile/ 25th-50th percentile/ 50th-75th percentile / >75th percentile) of farm j; $Importj$ whether farm j imports cattle (yes/no); $Agecowsj$ the average age category of cows (<25th percentile/ 25th-50th percentile/ 50th-75th percentile / >75th percentile) at farm j; $Agecattlej$ the average age category of all cattle (<25th percentile/ 25th-50th percentile/ 50th-75th percentile / >75th percentile) at farm j; $CalvPatj$ the calving pattern (seasonal/not seasonal) of cows at farm j; $Farm\ systemj$ the farming system (open/closed/variable over time) of farm j; and εj the random error for farm j. In the regression analysis, the sample mean was established as reference category for all continuous variables that were categorized, as well as for the variable ‘province’.

All independent variables were subjected to univariable pre-screening of having potential to be included in the subsequent multivariable analysis. Variables with a p-value ≤0.20 in the univariable screening were selected for inclusion in the multivariable model. The final multivariable model was obtained using a forward stepwise selection procedure, adding each variable with a univariable *p*-value smaller than 0.05. Confounding of variables was monitored during this procedure by the change in coefficient values. If the change exceeded at least 25%, or > 0.1 when the value of the coefficient was between -0.4 and 0.4, the added value was considered a potential confounder and re-entered in the model. For all model parameters, a check for collinearity was performed using a correlation matrix. If two variables had a high correlation (>0.50), they were re-entered in the model separately. De variable resulting in the best model fit was included in the final model. In the final model, biologically relevant two-way interactions were tested, and the model fit was monitored using Akaike's information criterion (AIC).

RESULTS

Farm characteristics of herds with a continuously high KalfOK score

Descriptive results: the number of farms participating in KalfOK increased up to >90% of the dairy population in 2020 and stabilized thereafter. The study population consisted of 13,343 Dutch dairy herds in 2022. The average size of all dairy herds that were included in this study was 108 (median: 95) cows (≥2 years old) and 25 (median: 21) cattle (1-2 years old) in 2023q3. More descriptive results of these herds can be found in Table 2.

Table 2. Description of herd characteristics of the study population of 13,343 Dutch dairy herds in 2022

Herd characteristic	Mean (median)	10th & 90th percentile
Cows ≥ 2 years old	108 (95)	46 – 184
Youngstock (1-2 years old)	25 (21)	4 – 47
Ear tagged calves <1 year old	36 (30)	11 – 64
Replacement percentage [a]	23,6 % (22,6 %)	15,2 % - 32,5 %

[a] Percentage of adult cows that have been replaced compared to one year ago

The median KalfOK score of the study population showed a seasonal trend, with the highest scores in spring and the lowest scores in winter months. This seasonal trend is most noticeable in farms with lower KalfOK scores and appears to be less prominent in the higher scoring farms (Fig.2). An overview of the results of some of the underlying KalfOK indicators, average percentage of live births and successful rearing, is provided in Figure 3. Over time, part of the indicators show an improvement which was also concluded by Santman-Berends et al. (2021).

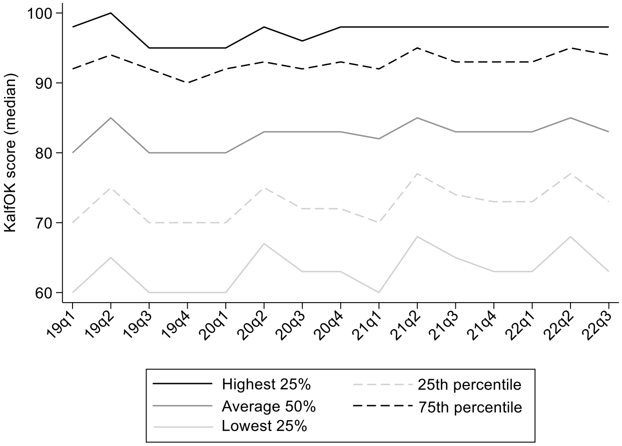

Figure 2. Median KalfOK score per quarter for the highest scoring 25%, lowest scoring 25% and average 50% of participating herds. The 25th and 75th percentile, determining in which group herds are categorized, are also displayed. Herds can alternate between groups over time

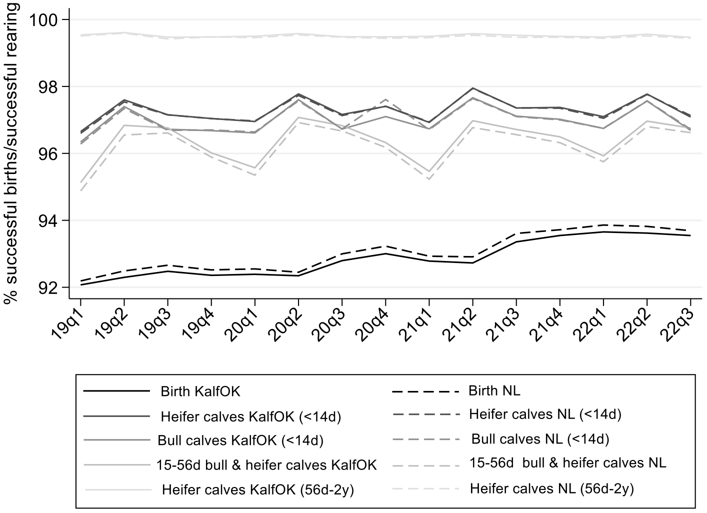

Figure 3. Average percentage of live births and successful rearing (bull calves <14d, heifer calves <14d, bull & heifer calves 15-56d, heifer calves 56d-2y) per quarter (solid line). The average percentage of live births and successful rearing in Dutch dairy farms are also displayed as a reference (dashed line)

Multivariable results: variables that were excluded based on the univariable results, include ‘farm size’, ‘multiple locations’ and ‘calving pattern’. Following a forward selection process the variables “stimulant”, “farm growth percentage”, “replacement rate”, “farming system” and “province” were identified as significant (Fig.4). Replacement rate, farming system and province were considered as potential confounders and forced in the model. Farms not provided with a incentive to participate or reach a certain score in KalfOK were 1.7 times less likely to have a continuously high KalfOK score compared to farms that were provided with a stimulant. Farms with a closed farming system were more likely to have a continuously high KalfOK score (OR=2.5, 95% CI: 1.5–3.0) compared to farms with an open farming system. Participating farms with a replacement rate <19.4% and farms with a replacement rate between 19.4% and 22.4% obtained a continuously high KalfOK score more often (OR=2.9, 95% CI: 2.4–3.6 and OR=1.3, 95% CI: 1.1–1.6 respectively) than farms with a replacement rate between 22.4 and 26.0 percent and farms with a replacement rate >26.0% (OR=0.7, 95% CI: 0.6–0.8; OR=0.4, 95% CI: 0.3–0.5). Farms with a growth percentage >3.6% were less likely to have a continuously high KalfOK score (OR=0.8, 95% CI: 0.7–1.0), while farms with a growth percentage between -0.8% and 1.3% were more likely to have a continuously high KalfOK score (OR=1.34, 95% CI: 1.1–1.6). Furthermore, the results showed statistically significant regional differences.

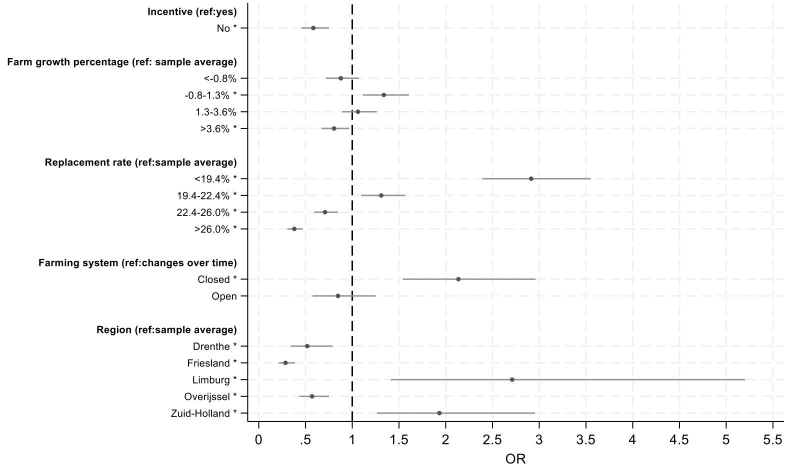

Figure 4. Odds ratios of farm characteristics associated with the difference between a continuously high and a continuously low KalfOK score. Significant odds ratios are indicated by an asterisk. For the farm characteristic region only the significant regions are displayed

Farm characteristics for herds included in the survey on the use of KalfOK in their herd management

Descriptive results: In total, 324 out of the 1200 farmers that were contacted replied (response rate of 27%) and were included in this part of the study. The majority of the participating farmers had been farming for more than 20 years. Of the farmers, 63% used a conventional milking system and 37% used a milking robot. On average, there were 107 (median: 95) calves born per farm in 2022. This was comparable to an average of 112 (median: 97) births in 2022 per farm for all KalfOK participants. Ninety-two percent (92%) of the farmers reared their own youngstock in comparison to 5% who outsourced the young stock rearing and 3% who did not keep any youngstock. The KalfOK scores of the respondents had an annual moving average score of 83 (median: 84, min-max: 37-100) points in 2022q4. This was comparable to the moving average year score of 82 (median: 84, min-max: 28-100) of all KalfOK participants.

Of the respondents, 53% (n=162) indicated to actively use their KalfOK report. Of the active users, 57% used KalfOK to obtain more insight in calf mortality on their farm, 46% used the report to see how their calves were doing compared to calves on other farms. The farmers who indicated that they did not actively use KalfOK while they did receive the quarterly report and were officially participating in the system (n=162), were asked which modification could be made to the system, so that they would start using the reports in thei day-to-day management. Part of these farmers (38%) did not have an answer to this question, or did not see added value in using KalfOK, while 14% stated that they did not have any problems with their calves and youngstock and were therefore not interested in detailed figures. Four percent (4%) of the farmers stated that the availability of the results was not timely enough and 7% stated only to have a look at the data because it is required by their dairy company.

The majority of the participating farmers discuss their KalfOK report sometimes (48%) or regularly (16%) with their veterinarian. Additionally, 71% of participating farmers has authorized their veterinarian to access KalfOK, while 23% does not know whether their veterinarian has access to their report or not (while they are the ones that need to give acces to the veterinarians). According to 82% of the respondents, the health of their calves/youngstock did not improve since they started participating in KalfOK, while 18% of farmers indicated that they did see improving health. Of the participating farmers, 34% indicated to have adjusted their youngstock rearing management since the start of participation, such as improvements in feeding (e.g. colostrum), housing (e.g. ventilation) and/ or vaccinations (of cows and/or youngstock).

DISCUSSION

In this study, farms with a continuously high and a continuously low KalfOK score, indicative for their calf rearing quality, were compared to assess associated farm characteristics. The results showed multiple factors associated with having a low or high KalfOK score. First, a statistically significant difference in KalfOK group between farms with a low and high replacement rate was found. Several studies have reported the association between healthy calves, maximized cow health and long-term productivity (Hultgren & Svensson, 2009; De Vries et al., 2011; Soberon et al., 2012), all potentially leading to a lower replacement rate. Also, a closed farming system was associated with a higher odds of belonging to the group of herds with a continuously high KalfOK score. A closed farming system prevents introductions of infectious diseases, such as BVD and IBR (Van Schaik et al., 2001, Sarrazin et al., 2014, Shortall et al., 2017). Furthermore, the results showed statistically significant regional differences. One explanation for these regional differences could be the influence of veterinarians. Veterinarians are often perceived as a primary source of information on animal health and welfare by farmers (Gunn et al., 2008; Heffernan et al., 2008; Ellis-Iversen et al., 2010; Vande Velde et al., 2015; Biesheuvel et al., 2021). This is demonstrated by the surveyed farmers where the majority discusses their KalfOK report with their veterinarian.

Sociological factors might partly explain the difference between high and low scoring farms. Santman-Berends et al. (2014), for instance, showed that the mindset of farmers was associated with calf mortality and thus rearing practices. Additionally, in recent years increasing evidence became available on the influence of factors such as farmers 'perceived risk, perceived knowledge, perceived control, incentivization, emotions and normative beliefs on farmers decision making (Valeeva et al., 2007; Jansen et al., 2009; Shortall et al., 2016; O'Kane et al., 2017; Doidge et al., 2021). The regression analysis in this study did not focus on inclusion of the sociological aspects as it evaluated routinely collected data. It is commendable to evaluate the effect of social aspects on calf rearing in future research. Awareness for calf health and welfare for example. In the survey, even though only 18% of the respondents indicated that the health of their calves/youngstock did improve since the start of KalfOK, 34% of the farmers stated that they adjusted their youngstock rearing management since the start of participation. It cannot be concluded whether an improvement in KalfOK score is due to participation in the program, but KalfOK could bring youngstock rearing practices under the attention of farmers.

To conclude, this study shows differences in farm characteristics between herds with a continuously high and continuously low KalfOK score. The findings emphazise the importance of maintaining a low replacement rate and adopting a closed farming system to improve in KalfOK score. Furthermore, future research incorporating sociological aspects can provide a more comprehensive understanding of other dynamics influencing calf rearing and contribute valuable insights for enhancing the effectiveness of programs like KalfOK in promoting calf health and welfare.

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ADAPTIVE FARMING STRATEGIES AFTER CHEMICAL CONTROL FAILURE: A CROSS-SECTIONAL STUDY IN SUBTROPICAL ECUADORIAN DAIRY FARMS

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SUMMARY

Tick infestation is a concern in tropical livestock management. However, overdependence on chemical treatment and the improper application of these drugs has contributed to increased acaricide resistance among tick populations. This study aimed to identify common and uncommon tick-control practices employed by dairy farmers in subtropical areas of Ecuador and associate them with infestation levels and acaricide resistance. One hundred thirty-eight farms were visited, and a cross-sectional survey was conducted. Multiple correspondence analysis (MCA) was used to summarize associations between tick-control practices, farmers' knowledge, level of tick infestation, and acaricide resistance. The results of the MCA analysis showed that low levels of infestation were related to the use of intensive grazing and acaricide dips every three to four weeks. High infestation was related to the wipe cloth application, the application of organophosphate acaricides, and tick-borne disease reports.

INTRODUCTION

The predominant zoosanitary challenge in global cattle breeding within tropical and subtropical regions is presented by the cattle tick *Rhipicephalus microplus* and its control (Reck et al., 2014). With demonstrated and remarkable capacity for adaptation and propagation, *Rhipicephalus microplus* has successfully expanded its presence across various geographical areas in the world.

In Ecuador, this tick species exhibits a habitat range from sea level to 2600 meters above sea level (m.a.s.l.), and given its equadorial position, most of the country, excluding parts of the Andean highlands, experiment a tropical climate (Pourrut et al., 1983; Rodríguez-Hidalgo et al., 2017). In this context, approximately 75% of cattle herds are concentrated in tropical and subtropical areas (Bustillos & Rodríguez, 2014).

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For chemical control acaricides with diverse, active compounds, modes of action, and application forms are available in the country (Orozco, 2018; Paucar et al., 2022). Six families of chemical acaricides are in the market, encompassing organophosphates, amides, synthetic pyrethroids, macrocyclic lactones, phenylpyrazolones, and benzoylphenyl ureas (Agrocalidad, 2023). Inappropriate application practices of acaricides such as underdosing, insufficient preparations, and misapplications, contributes significantly to treatment failures (Betancur et al., 2019; Jongejan & Uilenberg, 2004). This not only exacerbates the prevalence of tick infestations but also leads to acaricide resistance against ticks. In Ecuador, numerous studies have highlighted the prevalence of *Rhipicephalus microplus* infestations in cattle (Paucar et al., 2022), and the development of acaricide resistance to amitraz, alpha-cypermethrin, and ivermectin (Maya-Delgado et al., 2020; Paucar et al., 2022; Rodríguez-Hidalgo et al., 2017).

Understanding the reasons behind farmers' misuse of acaricides is crucial for developing targeted interventions and educational programs. Factors such as limited awareness, insufficient training, economic constraints, and the perceived complexity of acaricide application may contribute to these mismanagement practices (Jack et al., 2022). Addressing these underlying issues is pivotal for promoting effective tick control strategies, minimizing acaricide resistance, and enhancing cattle herds' overall health and productivity (Van Huis, 1981). This research aimed to qualitatively assess the perspectives, understanding, and prevailing practices for tick control employed by dairy farmers in subtropical regions of continental Ecuador.

MATERIALS AND METHODS

Study setting

This study was part of the project entitled “Socio-eco-epidemiology of ticks, tick-borne parasites, acaricide resistance and residual effects of acaricides in tropical Ecuadorian livestock: environmental, animal and public health impacts”. The study was conducted in two subtropical dairy production areas of Ecuador: Area 1, located in the Northwest of Pichincha Province (Western Andean foothills), and Area 2, situated in the Quijos river valley (Eastern Andean foothills) in Napo province. The two areas were intentionally selected on the basis that they are subtropical areas with very low productive efficiency, located in ecologically vulnerable areas, and where farmers have introduced short-term improved tropical pastures and improved dairy breeds (*Bos taurus*) susceptible to ticks to increase farm productivity (Acebo, 2016; Hüe & Fontfreyde, 2019; Kerario et al., 2018). The Northwestern region of Pichincha province comprises six localities: Nanegal, Nanegalito, Pacto, Gualea, San Miguel de los Bancos, and Pedro Vicente Maldonado (Echeverría, 2017). This area, positioned on the western slopes of the Andes Mountains and intersected by the Chocó Andino of Pichincha Biosphere Reserve (RBCAP, 2019), exhibits various altitudinal floors and microclimates. The average altitude and mean annual maximum temperature in this region range from 1150 m.a.s.l. and 21°C in Pacto (highest locality) to 600 m.a.s.l. and 25°C in Pedro Vicente Maldonado (lowest locality) (Consejo Municial PVM, 2011; HCPP, 2000). The Quijos River valley comprises five localities: Baeza, San Francisco de Borja, Sumaco, Linares, and Sardinas (Guamán et al., 2019). This area is positioned between the eastern Andes Mountains' foothills and the Amazon region's high jungle, surrounded by the Cayambe Coca National Park and Sumaco Napo Galeras National Park (Cárdenas, 2010). The temperature in the central zones (Baeza, Borja, and Sumaco) ranges from 16 to 19°C at 1800 to 2000 m.a.s.l., while the lower parts like

Sardinas and Linares experience temperatures of 18 to 24°C at 1800 to 1500 m.a.s.l. (Grijalva et al., 2004; PDOT El Chaco, 2019; PDOT Quijos, 2015).

Sampling and data collection

Snowball sampling, as outlined by Lupo et al. (2016) was used for the participant selection. Participants were chosen without considering age, gender, or educational background. The sole criterion for inclusion was being the person with the most knowledge about farm management on their respective farms. A deliberate effort was made to incorporate farmers from small and medium-sized cattle ranches. Verbal informed consent was sought and obtained from all participants.

A face-to-face survey was conducted on 126 farmers, 65 from the Quijos River valley and 61 from the Northwest of Pichincha. The data were collected using the Epicollect-5 mobile application (Epicollect5, 2021). A pre-tested questionnaire containing both closed and open-ended questions was applied. The survey encompassed inquiries about herd management, livestock diseases, ticks, and acaricide-related aspects, including perceptions, knowledge, and methods of acaricide control. Efficacy evaluation was quantified as percentages based on farmers' perceptions, ranging from 0 to 100%. Visual aids featuring figures of cattle ticks were employed to facilitate the identification of tick species on the farm's cattle.

The assessment of farmers' knowledge about ticks and ticks’ management comprised six key elements: understanding the biology of ticks, breed predisposition, tick-borne diseases, awareness of economic losses caused by ticks, and familiarity with appropriate acaricide treatment. Biological knowledge involved accurately identifying the tick species on the farm and understanding its life cycle, including the presence of larvae in paddocks. Farmers were presented with illustrations of *Rhipicephalus microplus*, *Amblyomma cajennense* sensu lato, and *Ixodes boliviensis*, and they identified the most prevalent ticks on their hosts (Enríquez et al., 2020). Morphological identification of the ticks collected in the farms was conducted in the entomology laboratory and contrasted with the identification made by the farmers. Evaluation of knowledge regarding tick-borne diseases (TBDs) was considered correct if farmers mentioned anaplasmosis, babesiosis, or tick fever (the colloquial term for TBDs). The rotation of acaricides was deemed incorrect if different acaricide brands within the same acaricide group were used or if the farmer needed clarification about the brand name of the previously used acaricide.

Tick infestation and acaricide resistance

The level of infestation at the farm level was determined following the methodology outlined by Paucar et al. (2022). This variable was assessed at the farm level, enabling the categorization of farms into low or high levels of tick infestation. Subsequent resistance testing for three acaricides (amitraz, alpha-cypermethrin, and ivermectin) was conducted with the engorged tick females, using the larval package test. Farms were then classified based on the presence or absence of acaricide resistance taking into account the above categorization (Paucar et al., 2023).

Data analysis

All data collected in the cross-sectional survey were incorporated into a Microsoft Excel® database. To safeguard the anonymity of study participants, surveys were coded using a combination of numbers (Farm ID, 01) and letters (study areas, B = Northwest of Pichincha Province, L = Quijos River valley).

For the analysis of associations between practices of tick control, knowledge (about ticks and TBDs), tick infestation levels, and acaricide resistance, Multiple Correspondence Analysis (MCA) was employed. The FactoMineR package (Lê et al., 2008) in an R environment was utilized for this analysis. Twenty variables (Table 1) were used to establish relationships and to group farmers based on control practices and perceptions. Covariates with minimal or no variability were excluded from the analysis.

Knowledge levels were categorized as poor, fair, or good based on the number of correct answers. Poor knowledge was defined as 0% to <35% correct answers, fair knowledge as 35% to 65% correct answers, and good knowledge as >65% of correct answers (Coffin et al., 2015). The reported efficacy of acaricide treatments was grouped into three categories: low (0% to 50%), medium (51% to 80%), and high (81% or above). Alternative control methods encompassed acaricide control with paddock control (equalization cuts), medicinal plants and/or the use entomopathogenic fungi.

RESULTS

According to the evaluated items, most participants (70%) demonstrated a fair level of knowledge, while 27% of farmers exhibited good knowledge. Three percent (3%) of the participants needed better knowledge of the items evaluated (poor knowledge). Farmers categorized under “good knowledge” possessed a comprehensive understanding of tick biology, TBDs, economic losses associated with ticks, and breed predisposition, and they have relatively good acaricide management. On the other hand, the “fair knowledge” group differed from those with “good knowledge” primarily due to their limited comprehension of TBDs and the correct use of acaricides. Our results indicate the most farmers need to develop a better understanding about using the correct acaricide dosage and rotation.

The results from the MCA yielded 23 dimensions. However, only the first two dimensions were retained for clarity and succinct representation of the results.

Table 1. Variables used for the MCA

Variable	Categories	Code
Presence of high level of tick infestation	No	low infestation
	Yes	high infestation
Am[a] resistance	No	am res no
	Yes	am res yes
Cy[b] resistance	No	cy res no
	Yes	cy res yes
Iv[c] resistance	No	iv res no
	Yes	iv res yes
Multiresistance: Am[a] and Iv[c]	No	am iv res no
	Yes	am iv res yes
Multiresistance: Am[a] and Cy[b]	No	am cy res no
	Yes	am cy res yes
Multiresistance: Cy[b] and Iv[c]	No	cy iv res no
	Yes	cy iv res yes
Multiresistance: Am[a], Cy[b] and Iv[c]	No	X3res no
	Yes	X3res yes
Who preparated the acaricide treatment	Employed	employed
	Owner	Owner
Mixture of different acaricides	No	acaricide mix no
	Yes	acaricide mix yes
Addition of additives	No	additives no
	Yes	additives yes
Acaricide application with a wipe cloth	No	appl wipe no
	Yes	appl wipe yes
Use of organophosphate acaricides	No	org use no
	Yes	org use yes
Alternative acaricide control	No	alt no
	Yes	alt yes
Manual removal of ticks	No	m removal no
	Yes	m removal yes
Frequency of bath sprays	1 or 2 weeks	bath 1-2
	3 or 4 weeks	bath 3-4
	5 weeks or more	bath >5
Reported efficacy: bath spray	high efficacy bath	high eff bath
	low efficacy bath	low eff bath
	medium efficacy bath	med eff bath
Reported efficacy: injection	high efficacy bath	high eff injection
	low efficacy bath	low eff injection
	medium efficacy bath	med eff injection
Tick-Borne Diseases Report	No	TBDs no
	Yes	TBDs yes
Knowledge level	fair knowledge[d]	fair knowledge
	good knowledge	good knowledge
Grazing system	extensive grazing	ext graz
	intensive grazing	int graz

[a] Amitraz; [b] Alpha-cypermethrin; [c] Ivermectin; [d] Due to the small amount of data from farmers with poor knowledge, they were grouped into the group with fair knowledge.

These two dimensions effectively capture and account for nearly 30% of the variance, as illustrated in Fig.1. This decision ensures a concise and accessible depiction of the primary patterns and relationships in the dataset. The first and second dimensions accounted for 19.1%

and 8.9% of the variance, respectively. The first dimension was designated as the "gradient of acaricide resistance" because the variables that exerted the most influence on this dimension were related to the presence or absence of both acaricide resistance. In contrast, the second dimension was termed a "gradient of good to bad acaricide control practices". The variables contributing significantly to this dimension encompassed the perception of the efficacy of acaricide control, the level of knowledge about ticks and common control practices (chemical control), uncommon control practices (acaricide application with a wipe cloth; use of injectable or pour on acaricides as bath acaricides), and/or alternatives to acaricide control. The first quadrant shows the relationship between good acaricide control practices with the presence of acaricide resistance and high infestation. The second quadrant shows the relationship between good acaricide control practices with the absence of acaricide resistance and high infestation with. In the third quadrant, the absence of acaricide resistance and high tick infestation are associated with poor acaricide control practices. Finally, the fourth quadrant correlates acaricide resistance and high infestation levels with inadequate acaricide control practices. This quadrant provides a comprehensive understanding of the intricate relationships between acaricide resistance, tick infestation levels, and the effectiveness of acaricide control practices.

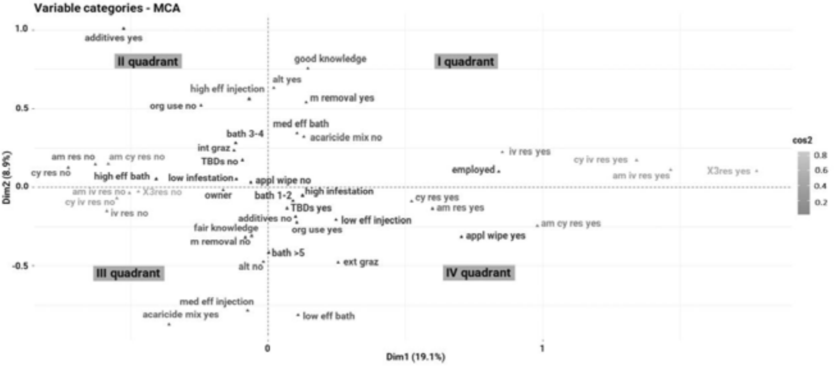

Figure 1. Multiple correspondence analysis map of risk and perceptions associated with the presence of infestation and acaricide resistance

As depicted in Fig.1, the outcome of the MCA reveals notable associations within the dataset. The presence of acaricide resistance was notably linked with instances where the employee assumes the responsibility for preparing and applying the acaricide treatment and using a wipe cloth for acaricide application. The presence of a low tick infestation was correlated with specific practices, including the non-use of organophosphate acaricides, spray baths administered every three to four weeks, the absence of a wipe cloth for acaricide application, intensive grazing practices, and the absence of reported cases of TBDs on the farm. In contrast, a high level of tick infestation demonstrated associations with practices, such as the use of organophosphate acaricides, frequent acaricide treatments (every one or two weeks) or irregular treatments (five or more weeks), fair knowledge levels, low perception of the efficacy of injectable acaricide treatments, the presence of TBDs cases on the farm, and the implementation of extensive grazing practices.

Fair and poor knowledge were associated with the non-implementation of alternative control methods and the non-practice of manual tick removal. The perceived high efficacy of acaricide treatment administered through spray baths demonstrated an association with the absence of acaricide resistance. Conversely, the perception of low efficacy in acaricide bath spray was linked to the practice of mixing acaricides and a relatively medium efficacy of injectable treatment. In contrast, fair and poor knowledge levels were associated with the non-implementation of alternative control methods and the absence of manual tick removal practices. These associations highlight the significance of both perception and knowledge levels in influencing the choice and effectiveness of tick control measures on the surveyed farms.

Farmer characteristics were gruped into three main clusters. The first cluster grouped 35%, the second cluster 44%, and the third cluster grouped 21% of farmers (Fig.2). The first cluster exhibited distinct characteristics, with farms showcasing mono-resistance (Mean=33%; SD=7%), resistance to two acaricides (Mean=10%; SD=3%), and a high infestation rate in 39% of cases. Typically, within this group, acaricides were not applied using a wipe cloth, the utilization of organophosphate acaridides in 47% of the cases, and a minority of farms mixed different acaricides (13%). The second cluster was marked by farms primarily demonstrating mono-acaricide resistance (Mean=41%; SD=17%), resistance to two acaricides (Mean=16%; SD=13%), and a high infestation rate observed in 57% of cases. Within this cluster, the majority of farms adopted the use of organophosphate acaricides (93%) and engaged in the mixing of acaricides (53%). The third cluster was characterized by farms exhibiting mono-resistance and multi-resistance, accounting for 100% in both cases and a high infestation rate in 50% of cases. Generally, this group refrained from mixing different acaricides but frequently employed organophosphate acaricides (78%).

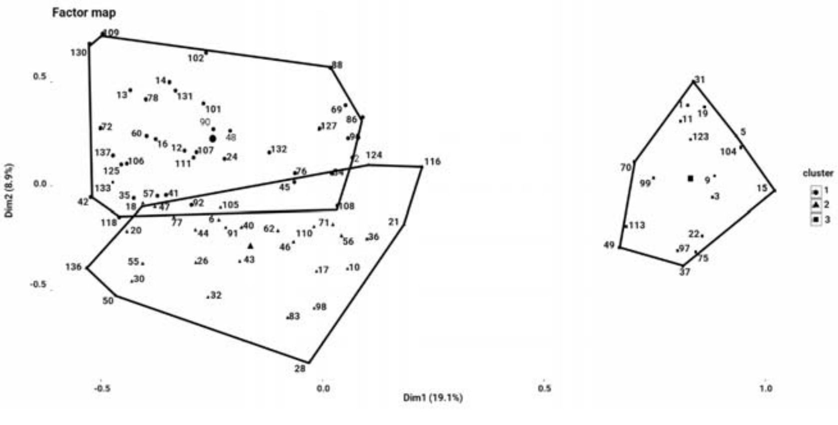

Figure 2. Graphical representation of farmers

While only a few farms demonstrated good knowledge (27%), the majority were classified in Group 1. Likewise, in terms of employing alternative methods for acaricide control, the majority of farms using these methods were concentrated in Group 1. Among farms implementing alternative control practices, 58% were categorized in Group 1, 18% in Group 2, and 24% in Group 3. Farms utilizing a wipe cloth for acaricide application were distributed between Groups 2 and 3. In these groups, the frequency of acaricide treatment through spray baths occurred every two weeks or less, with reported cases of TBDs standing at 60% and 56%, respectively. Additionally, extensive grazing practices were observed in 50% of the cases. Conversely, in Group 1, the frequency of spray baths occurred every three or four weeks, intensive grazing was predominant (84%), and there were fewer reported cases of TBDs (34%). Notably, in Groups 1 and 2, the person responsible for preparing the acaricide solution and administering the treatment was typically the owner. In contrast, in Group 3 the owner's involvement occurred in only 50% of the cases.

Regarding the perception of efficacy, most farmers in all three groups rated the efficacy of acaricide baths as "medium efficacy." In Group 1, farmers rated the efficacy of injectable treatments as "high efficacy." These distinct patterns highlight variations in practices and perceptions across different farm groups, providing valuable insights for targeted interventions in tick management strategies.

DISCUSSION

The results of this study reveal several key findings that warrant discussion. Firstly, a noteworthy 93% of the respondents demonstrated a proficient understanding of tick species prevalent in their respective areas and the associated economic losses. However, this practical knowledge basis is juxtaposed with a conspicuous lack of awareness regarding TBDs and the intricacies of acaricide control, specifically concerning the accurate dosage and rotation of acaricides. This knowledge gap aligns with the findings of Sungirai et al. (2016), which emphasized a direct correlation between TBD awareness and effective tick control, highlighting the pivotal role of education and training in farmers' proficiency. This correlation aligns with the contextual background of the study area, where farmers boast a substantial 20 years of farming experience, yet predominantly possess basic or secondary education levels (Paucar et al., 2022).

The investigation also unveiled farmers' engagement in risky practices, notably blending various acaricides and adopting more extreme measures. These extreme practices encompassed the excessive and overdosing of acaricides, typically involving organophosphate acaricides, along with the utilization of other irritants, such as motor oil. Previous studies have consistently reported that these practices pose several health problems for individuals, emphasizing the critical need for awareness and regulatory measures in mitigating potential health risks associated with such practices (Pajurek et al., 2023; Saegerman et al., 2006).

A discernible relationship has been established between the frequency of acaricide treatments, extensive grazing practices, and a heightened level of tick infestation. This association may stem from the inadequate control of larval stages of ticks, causing rapid infestation in animals. Consequently, farmers may resort to increased acaricide treatments (at one or two weeks) as a temporary measure to diminish resistant tick populations (Miyama et al., 2020; Vudriko et al., 2016). Intensive grazing emerges as a valuable strategy in reducing infestation in animals, as supported by studies (Cruz-González et al., 2023; Muhammad et al., 2008). However, its effectiveness requires a meticulous implementation every 45 days, accompanied by acaricide applications to achieve a substantial reduction in tick burden (Abbas et al., 2014). Conversely, high infestation levels correlate with the application of long-term acaricide baths (five weeks or more), which is indicative of potential animal care negligence on the part of farmers. This variable is further linked to extensive grazing, suggesting that livestock activities may not constitute the primary income source, leading to insufficient attention. Paddock rotation and acaricide spray baths administered every three or four weeks have shown an association with low tick infestations. The frequency of bathing aligns with the recommended interval for *Rhipicephalus microplus* treatments, crucial for disrupting the parasite's life cycle every 21 days (Muhammad et al., 2008).

The study underscored a significant correlation between farmers' adoption of alternative control practices and their level of knowledge about ticks. Individuals with a higher knowledge tend to explore alternatives to chemical control. While these alternative methods present viable options for farmers, their feasibility, efficacy, and the establishment of standardized protocols necessitate further exploration. In the cluster formation, it was noteworthy that although most farmers employing alternative control methods were concentrated in Group 1, a few were also observed in Groups 2 and 3, characterized by acaricide resistance and high infestation levels. This observation indicates that there is a concurrent need for the judicious use of chemical acaricides and for alternative control measures to be effective. As highlighted by Jack et al. (2022), integrated cattle-tick management necessitates a synergistic approach, combining various tools and strategies to manage tick infestations effectively. Implementing these strategies requires the proper and strategic use of acaricides. This information is pivotal for enhancing tick control management, especially practices that address acaricide resistance, ensuring sustainable, long-term solutions that contribute to the enduring efficacy of tick control measures.

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DEEP MECHANISTIC MODEL: INTEGRATING DEEP LEARNING AND STOCHASTIC MECHANISTIC APPROACHES FOR BOVINE RESPIRATORY DISEASES DIAGNOSIS AND EPIDEMIOLOGICAL FORECASTING

T.G.L. EYANGO TABI*, M. ROUAULT, V. POTDEVIN, X. L'HOSTIS, S. ASSIE, S. PICAULT AND N. PARISEY

SUMMARY

Bovine Respiratory Disease (BRD) poses a significant challenge in beef fattening due to its complex causes. Relying solely on data-driven sensor methods for early detection may yield false alarms. This paper introduces an innovative approach that integrates a deep learning model with a BRD mechanistic model, utilizing pulmonary ultrasounds and clinical exams as sensor data and ground truth, respectively. By employing reliable clinical diagnostics, three crucial biological parameters were inferred, enabling the forecast of the number of asymptomatic animals up to 30 days. The deep learning model achieves 70% accuracy in diagnosis, and the BRD mechanistic model forecasts disease dynamics with less than 5% error. However, the hybrid method's weakness lies in clinical exams' uncertainty for some animal diagnosis, and improvements to the BRD model have been addressed in existing literature. Future work could explore incorporating biological exams or utilizing a pathogen-specific model for enhanced accuracy.

INTRODUCTION

The Bovine Respiratory Disease (BRD) poses significant challenges to farmers, as it results in substantial economic losses, accounting for as much as 20% of farmers' incomes (Bareille et al., 2009). This disease raises critical concerns for animal welfare, as it can lead to fatal pneumonia in calves (Engler et al., 2014; Delabouglise et al., 2017). The predominant treatment for BRD relies on antimicrobials, however, practices like systematic collective treatments and misdiagnosed BRD, including false detection, contribute to antimicrobial misuse. It is crucial to ensure proper and judicious administration of these antimicrobials to prevent the emergence of antibiotic resistance. The complexity of diagnosing BRD stems from numerous factors, including the involvement of multiple pathogens such as bacteria and viruses, as well as susceptibility to external and environmental influences like weaning, stress, breed, immunity, and farming conditions (Hay et al., 2016; Kudirkiene et al., 2021). On a more positive note, farms are generating a wealth of valuable data, providing significant potential for insights. Farms are also increasingly incorporating sensor technologies to enhance and automate data

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collection. Consequently, precision livestock farming emerges as a promising tool for real-time monitoring and farm management, with the potential to improve animal health and welfare (Berckmans, 2014).

Various approaches have been explored to leverage farm data and study the spread of diseases. One type uses data-driven methods in sensors, excelling at detecting straightforward symptoms or events, such as heat (Kosanovic et al., 2022), heat stress (Hoffman et al., 2022), calving and hyperthermia. However, these sensors have limitations when it comes to complex diseases like BRD, which involve intricate underlying epidemiological processes (Concordet et al., 2022; Pfeiffer et al., 2022). For instance, hyperthermia, a symptom of BRD, can result from causes other than BRD, such as overexertion. Thus, relying solely on early detection of hyperthermia infectious episodes may also lead to false alarms. Additionally, cattle tend to hide early symptoms as a survival behaviour (Griffin, 2010), reducing the quantity of observations needed to adjust data-driven methods. Alternatively, knowledge-driven methods, like mechanistic models, are widely used to understand how pathogens spread across different scales and according to contrasted scenarios, from individual animals to entire regions (Ezanno et al., 2020). As such, mechanistic models have contributed a lot to modelling and understanding the spread of pathogens involved in BRD (Picault et al., 2022; Sorin-Dupont et al., 2023), however their calibration remains a substantial challenge.

The hypothesis of this paper suggests that by combining data-driven and knowledge-driven methods, an integrated and innovative approach can be developed. This approach is applied to automatically diagnose male beef cattle and forecast the dynamics of BRD. The designed workflow integrates a spatiotemporal convolutional neural network with a stochastic mechanistic models and pulmonary ultrasound videos used as sensor data.

MATERIALS AND METHODS

Figure 1 illustrates the overall workflow and unfolds as follows: in the first section (Fig.1a), pulmonary ultrasound videos are employed as sensor data. These videos were selected to test the pipeline because they provide an internal view of the lungs, potentially serving as a reliable sensor for detecting respiratory symptoms. Moving to the second section (Fig.1b), clinical observations (categorized as either healthy or diseased) are considered as ground truth. These states are determined by a veterinarian through clinical assessments, including physiological parameters like rectal temperatures and other clinical signs (cough, nasal or ocular discharge, depression, etc.). The underlying hypothesis is that animals exhibiting a certain number of clinical signs (symptomatic) are considered diseased. In the third section (Fig.1c), sensor data and ground truth are utilized to train and compare multiple deep learning models, specifically spatiotemporal convolutional neural networks. Their objective is to accurately predict the clinical health state of each animal. During training, the predictions are statistically evaluated for potential use in real farm conditions. However, in operational conditions, clinical assessments will not be necessary as the best-trained deep learning model would automatically predict clinical health states using pulmonary ultrasound videos. In the last section (Fig.1d), reliable clinical health states serve as input to calibrate parameters of a mechanistic model. The performance of various parameter inference methods was compared for three crucial parameters of an average pathogen stochastic mechanistic model (Picault et al., 2022). This calibration enables a 30-day forecast of the number of asymptomatic animals within specific batches across two farms with contrasting configurations.

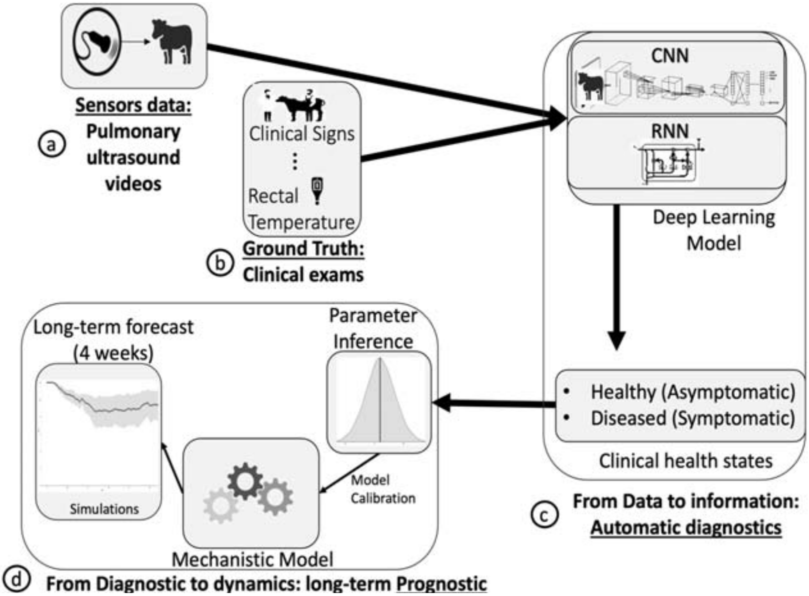

Figure 1. Workflow of the "deep mechanistic model" approach. This hybrid approach integrates a deep learning model (data-driven) with a mechanistic model (knowledge-driven)

Data acquisition, sensor data and ground truth

The experiment encompassed nine fattening farms, each simultaneously managing up to three batches of male beef cattle, with each batch comprising eight to twelve calves. In terms of breed distribution, 78% of the cattle were Charolais, 12% Limousin, and the remaining 10% were of mixed races. The Charolais breed was predominantly chosen due to their clearer display of clinical symptoms. Data collection started on the day the cattle arrived at the farm (Day 0) and extended for one month, considering it as the period when cattle are most susceptible to BRD (Babcock et al., 2009). Data collection spanned from January 2023 to June 2023, encompassing a total of 480 beef cattle in the experimentation.

Sensor data (Fig.1a): portable ultrasound scanners were used to assess the animals' lungs on multiple days: Day 0, Day 2, Day 5, Day 14, Day 21, and Day 28. The selection of dates varied across farms, based on the availability of farmers and a veterinarian. The ultrasound scanner captured lung images in video format, with 28 frames per second in black and white, lasting up to 20 seconds each, and 480x560px resolution. The animals' lungs were divided into eight intercostal zones, following a standardized scanning protocol from the shoulder to the stomach. A veterinarian validated the dataset to ensure accuracy. Recording a video with an ultrasound scanner is time-consuming, tedious, and challenging, requiring animals to be kept in a fixed position, which is not an easy task. This led to prioritizing cases for study, considering only lesions larger than 1 cm^2 as meaningful (Masset et al., 2022). For each intercostal zone, a video of only the largest lesions is saved. On Day 0, one-third of each batch was examined, while from Day 2 to Day 28, all animals in a selected batch were examined. To maintain balance in the dataset, videos of clinically healthy lungs (without lesions) were also recorded, resulting in a total of 255 lung ultrasound videos.

Ground truth (Fig.1b): several veterinarians participated in annotating the ground truth data, having undergone the same training to minimize annotation bias. The determination of ground truth for identifying diseased animals relied on clinical assessments. The decision rule utilized was established in various publications (Timsit et al., 2019, 2011). An animal was considered diseased if it had a rectal temperature exceeding 39.7°C and displayed at least one clinical symptom. These clinical symptoms were defined based on a clinical assessment table (Table 1) established by veterinarians. This method of diagnosing diseased animals is widespread in France, with three out of nine farms in this experiment already using it. Every animal in each batch underwent clinical examinations, following the same frequency as the collection of sensor data.

Table 1. Data dictionary of the clinical assessments

Observable Symptoms	Tiredness	Shape of flank	Nasal discharge	Cough	Ocular discharge	Breathing amplitude	Breathing rate
Levels	Absent, Mild, Severe	Hollow, Flat, Rounded	Absent, Mucous, Purulent, Serous	Absent, Weak, Strong	Absent, Mucous, Purulent, Serous	Normal, Augmented	Regular, Irregular

From Data to information: automatic diagnostics (Fig.1c)

Data Preprocessing: four steps were taken in data preparation process. Initially, the distribution of the entire dataset of pulmonary ultrasound videos was adjusted to address a significant class imbalance. Only 23.2% of videos belonged to diseased animals, while 76.8% belonged to healthy animals. To rectify this, a downsampling strategy was employed, using stratified random sampling considering factors like the intercostal zone, lesion size, lesion count, and the day of clinical assessment. In the second step, the dataset was split with a random shuffle: 60% for training (80 videos), 19% for validation (22 videos), and 13% for testing the model's performance (16 videos). The validation dataset served to adjust the model’s weights during training, while the test set was exclusively used for accuracy evaluation after training. In the final step, ultrasound videos were cropped to eliminate areas containing text or watermarks.

Handling ultrasound videos is challenging due to varying frame counts and noisy images. Some videos were shorter than expected due to technical issues related to the ultrasound scanner. A straightforward solution involved extracting images from the videos until reaching a maximum count. If a video had fewer images, the missing frames would be filled with zeros, which is a method similar to handling text sequences.

Deep learning model: a video contains both spatial information within individual frames and temporal information across the entire sequence. To effectively address both aspects in video analysis, a hybrid architecture, specifically a spatiotemporal convolutional neural network was chosen. In our approach, we combined convolutional layers (CNN) with recurrent layers (RNN). The convolutional layers focus on extracting spatial features, such as lesions, pleura lines, or any other relevant anatomical details. Meanwhile the recurrent layers capture temporal information, which pertains to the sequence or frequency of appearance of spatial features.

For the training phase (Fig.2), various convolutional layer architectures (spatial feature extractor) were compared. Depending on the number of layers, the depth, the structure, different architectures will extract different spatial features. The tested architectures include five classical networks pre-trained on imagenet, such as efficientNetB7, inceptionResnetV2, inceptionV3, VGG16, and a late fusion ensemble model of inceptionV3 with inceptionResnetV2. The temporal layer architecture is composed of eight layers: the first with sixteen neurons, the second with eight neurons, followed by a dropout layer that suppressed 40% of the neurons, and a final dense layer with eight neurons using a ReLU activation function. The classification layer employes a softmax function with two output neurons. The loss function used is a sparse categorical cross-entropy with an Adam optimizer. In conclusion, this model analyses an entire pulmonary video and predicts the clinical health state (diseased or healthy).

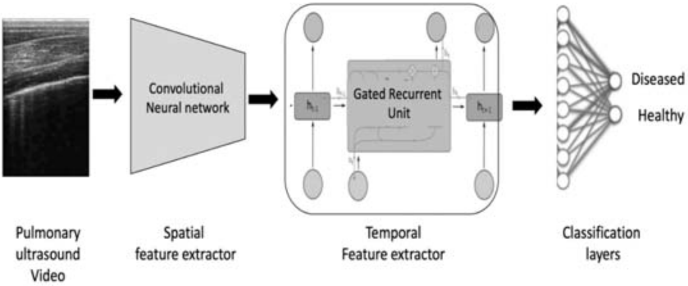

Figure 2. Deep learning Architecture Trained

Evaluation: to evaluate the model's performance, four essential metrics were considered. The *weighted precision* measures the proportion of correctly identified positive cases among all cases predicted as positive. The *weighted recall* reflects the ability of the model to identify many actual positive cases, providing insights into the model's capacity to capture relevant instances. The *weighted F1-score*, as the harmonic mean of precision and recall, offers a balanced assessment, considering both false positives and false negatives. Lastly, the *accuracy* indicates the overall proportion of videos that were correctly classified, serving as a general measure of the model's predictive power and overall performance. These metrics together provide a comprehensive understanding of the model's effectiveness in distinguishing between healthy and diseased cases in the pulmonary ultrasound videos.

From diagnostics to disease dynamic: prognostic (Fig.1d)

Mechanistic model: to date, only two mechanistic models for BRD have been published. The model introduced in (Picault et al., 2019a) was mechanistic (to explicitly represent processes), stochastic (to account for intrinsic variability in biological processes), and individual-based (to ensure a fine-grained detail level). This model aimed to investigate the spread of BRD in French fattening pens by capturing the evolution of infection, emergence of clinical signs, detection, and subsequent treatment. To tackle the limited knowledge about interactions between multiple BRD pathogens, model parameters were calibrated assuming an average pathogen infection (Picault et al., 2022). A sensitivity analysis was also carried out to understand its behaviour and the impact of parameter uncertainty. Results emphasized the significance of parameters such as the pathogen transmission rate, the average duration in the infectious state, and the average duration in the pre-infectious state, crucial for controlling antimicrobial usage and mortality rates.

This study employed this average pathogen BRD model (Fig.3), utilizing the three biological parameters as essential input, with the output focusing on the count of symptomatic animals, encompassing those exhibiting both mild and severe clinical signs. Model predictions were given with a 12-hour time grain, aligning with the interval between successive visual assessments of beef cattle during feeding. Implementation was facilitated by the EMULSION platform (Picault et al., 2019b), allowing the depiction of all model components in a human-readable, flexible structured text file processed by a generic simulation engine. This facilitates collaboration and model refinement by scientists with diverse backgrounds, including veterinarians and epidemiologists.

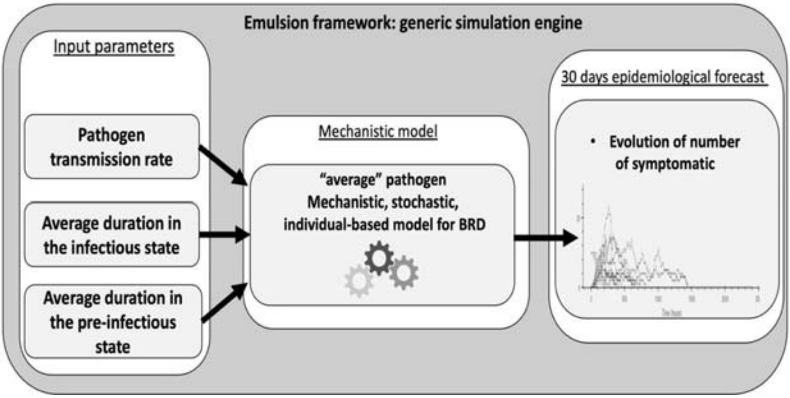

Figure 3. Simplified process of how the average pathogen model was used

<u>Integrating a deep learning model with a mechanistic model:</u> involves employing parameter inference, a computational approach used in various scientific disciplines to estimate the unknown parameters of a statistical model so that its predictions match, at best, observed data. The deep learning model predicts the clinical health state of an animal, distinguishing between symptomatic and asymptomatic states (Fig.4a). The total count of predicted diseased animals in a pen corresponds to the number of symptomatic animals. The average pathogen mechanistic model generates various outputs, only the count of symptomatic animals is considered (Fig.4b). Both the outputs of the deep learning model and the mechanistic model align, making parameter inference an ideal method to link two models, using deep learning predictions to estimate three parameters of the mechanistic model (Fig.4d), namely the pathogen transmission rate, the duration in infectious state and the duration in pre-infectious state.

The average pathogen model is categorized as an implicit generative model, capable of simulating samples however its likelihood is hardly obtainable. Hence, to estimate its parameters, a likelihood-free inference method, namely Approximate Bayesian Computation

(ABC) (Beaumont, 2019) was employed. This involved sampling 10,000 parameter values within a biologically acceptable domain and using them to generate simulated datasets through the average pathogen model. Chosen summary statistics, in this case, the count of symptomatic animals, captured essential features of the observed data. The similarity between simulated and observed data was assessed using distances in their summary statistics the closest 1% of sampled simulated parameters were accepted. This process allowed for the estimation of the distribution of potential values for the chosen parameters. One extension of the ABC method was selected for this study, the ABC-NN (neural network), as it gave the most consistent results in our use cases.

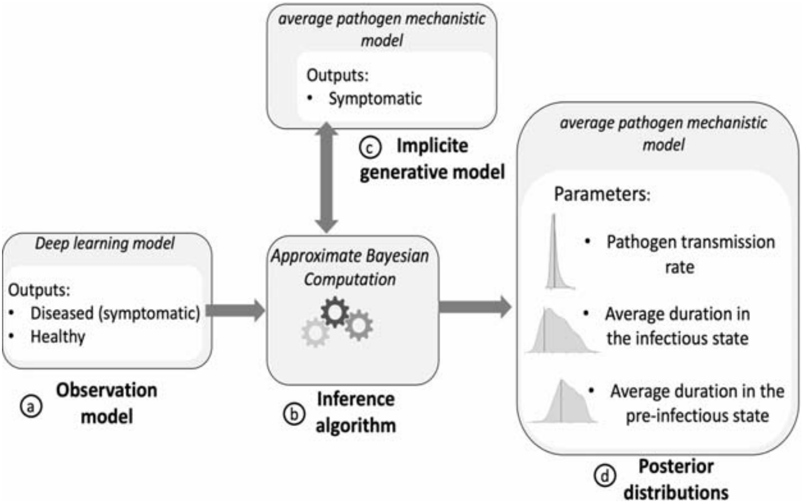

Figure 4. Method of coupling a deep learning model with a mechanistic model. Inference of the three biological parameters of an average pathogen BRD model

Evaluating the effectiveness of the inference method involved sampling values from the joint posterior distributions (Fig.4) and utilizing them to predict the number of symptomatic animals (also considered clinically diseased). This prediction was then compared to the actual number of detected diseased animals, using the mean absolute percentage error as the metric. The assessment of the inference method was carried out on two farms with different breeding practices, and the forecasting period was set at 30 days.

RESULTS

From Data to information: automatic diagnostics

Training the hybrid (CNN-RNN) deep learning architecture, using various spatial feature extractors demonstrated varying performance (Table 2.) due to their distinct architectural characteristics, including differences in structures and depth. VGG16 exhibited the poorest performance with a weighted F1-score of 14%, while InceptionV3 outperformed the rest with a weighted F1-score of 70%.

Table 2. Deep learning performance

Feature Extractor	Weighted Precision	Weighted Recall	Weighted F1-score	Accuracy
EfficientNetB7	0.67	0.62	0.63	0.62
InceptionResnetV2	0.71	0.50	0.49	0.50
InceptionV3	**0.72**	**0.69**	**0.70**	**0.69**
VGG16	0.09	0.31	0.14	0.31
InceptionV3 + InceptionResnetV2	0.71	0.62	0.63	0.62

In summary of the diagnostic phase, it is feasible to train a deep learning model using sensor data, particularly pulmonary ultrasound videos, to estimate the clinical health status of animals. However, it is important to note that the margin of error for the best model, when making predictions for 12 animals, is approximately ± 0.259 (or 25.9%) at a 95% confidence level. While the accuracy of the best model is reasonable considering it has been trained exclusively on ultrasound videos, using this model in real-life scenarios would result in an unacceptable margin of error.

From diagnostics to disease dynamic: prognostic

Due to the margin of error being too large for practical use on a farm with 12 animals, reliable data was used for the inference, specifically the ground truth. In Table 3 illustrates the estimated values of the three most critical biological parameters for two farms. The estimations are presented as the median, Q1, and Q3. Additionally, Table 3 includes the nominal values for these three parameters for comparison. In both farms, the estimated parameter values appear acceptable and closely align with the nominal values.

Table 3. Inferred value of parameters vs nominal value of parameters

Parameter name	**Farm 1**			**Farm 2**			**Default model values**
	Median	**Q1**	**Q3**	**Median**	**Q1**	**Q3**	**calibrated**
Pathogen Transmision rate	0.009	0.006	0.012	0.019	0.014	0.023	0.008
Mean duration in infectious	150	118	193	123	100	156	120
Mean duration in pre-infectious	87	68	115	76	58	100	72

Utilizing these estimated values, the number of animals considered asymptomatic over a 30-day period in both farms (Fig.5) was projected. The mechanistic model was run at discrete time steps, with each step occurring every 12 hours. For farm 1, the forecasted trajectory demonstrates an average error below 5%. However, for farm 2, the projected trajectory indicates an average error close to 23%.

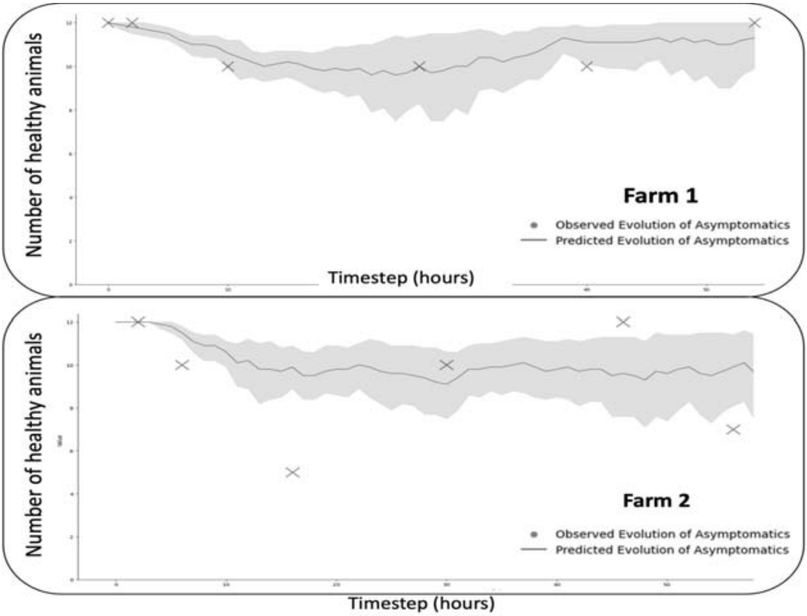

Figure 5. Asymptomatic forecast, ground truth vs calibrated average pathogen mechanistic model

In summary of the forecast phase, using clinical health status gives enough information to estimate the values of three crucial biological parameters: the pathogen transmission rate, the average duration in the infection state, and the average duration in the pre-infectious state. However, it is important to note that the average pathogen model is not suitable for every scenario.

DISCUSSION

This study demonstrated the feasibility of creating a hybrid approach that combines a deep learning model with a mechanistic model for diagnosing and predicting the dynamics of BRD. This adaptable approach can be implemented across various farms and scenarios, providing personalized diagnostics and predictions tailored to each farm's unique conditions. This has the potential to support the development of individualized control strategies and animal management practices based on specific farm circumstances.

However, it is important to acknowledge certain limitations in the proposed pipeline. Firstly, relying solely on pulmonary ultrasound videos as sensor data may not be sufficient to accurately estimate the clinical health status of each animal. This limitation arises because some symptoms caused by BRD, especially those affecting the upper respiratory tract, may not be visible in the lungs. Additionally, lung lesions become apparent only in the advanced stages of the disease. To address this issue, incorporating diverse sensor data, such as audio data already at our disposal, could be beneficial.

Secondly, the ground truth based on clinical symptoms may be more uncertain in detecting animals in the pre-infectious state. To tackle the challenge of a lack of a clear gold standard (Timsit et al., 2016) future research could explore the inclusion of biological exams, such as PCR and serological tests, which are presumed to provide more informative insights, especially regarding the type of pathogen infection.

Lastly, the average pathogen model may not be universally applicable, particularly in scenarios involving viral infections. In such cases, depending on the type of infectious agent, employing a pathogen-specific model (Sorin-Dupont et al., 2023) could enhance the accuracy of forecasting.

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BIOSECURITY

DO ANTIMICROBIAL USE LEVELS IMPACT HEALTH AS SIGNIFIED BY MEAT INSPECTION FINDINGS IN DANISH FINISHERS?

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SUMMARY

To ensure prudent antimicrobial use (AMU), Denmark has introduced mitigating measures, including the Yellow Card scheme, targeting pig herds with high AMU. We wanted to see whether pig health was poorer in herds with high AMU compared to low AMU. Mixed effects logistic regression models were used to examine meat inspection lesions from 10,511,056 finishers from herds with high, medium, or low AMU. Despite differences in AMU, only marginal differences in meat inspection lesions were present. Herds with high AMU were more likely to deliver pigs with arthritis or abscesses in extremities, while herds with low AMU were more likely to deliver pigs with hernia, osteomyelitis, tail lesions, or abscesses in the trunk. Hence, both high and low AMU can be linked with health and welfare issues. This demonstrates that the advisory role of the herd veterinarian is pivotal in preventing adverse effects of antimicrobial reduction measures on animal health and welfare.

INTRODUCTION

The debate on prudent antimicrobial use (AMU) addresses usage patterns in livestock production and humans. The main concern is the emergence of antimicrobial resistance (AMR) with its potentially far-reaching implications for human and animal health. To mitigate this risk, several initiatives focusing on prudent use of antimicrobials (AMs) in humans and animals are in place.

In Denmark, efforts towards AMU-reducing measures in pig production have gained considerable attention since the early 1990s. These measures were enabled by close collaboration with the industry. Following the emergence of vancomycin resistance in Danish livestock, the use of avoparcin as a growth promoter was banned in 1995. The ban was implemented to reduce cross-resistance to vancomycin, a critically important AM in human medicine. It also marked the beginning of systematic monitoring of AMR in animals and humans, published in the annual DANMAP reports (DANMAP, 2021). The industry followed with a voluntary phase-out of growth promoters in finishers and weaners in 1998 and 1999, respectively (Moura et al., 2023). A restriction on the use of fluoroquinolones and polymyxin was introduced in 2002 and 2016, as was a voluntary ban on 3rd and 4th generation cephalosporins in 2010 (DANMAP, 2022). As a result, critically important AMs are mostly not used in Danish pig production (DANMAP, 2022).

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The use of veterinary AMs in Denmark is limited to prescription only, and all sales of veterinary medicinal products have been recorded in the Danish Veterinary Statistics database (VetStat) since 2000 (Stege et al., 2003). The database has a high level of granularity, as each record specifies animal species, age group, treatment indication, and route of administration, as well as product information, like active ingredient, ATC code, product name, amount, and concentration (Stege et al., 2003). The VetStat database has enabled risk-mitigating initiatives, such as the Yellow Card scheme, introduced by the Danish Veterinary and Food Administration (DVFA) in 2010. The Yellow Card scheme is a legal intervention promoting the prudent use of AMs in Danish pig production (Anonymous, 2018a). In the Yellow Card scheme, data from VetStat are used as a proxy for AMU, presented as a 9-month rolling average. The AMU is measured in Animal Daily Doses (ADD) per 100 animals per day (ADD/100 animals/day), denoting the percentage of animals treated per day in each of the three age groups (sows with their piglets, weaners, and finishers). The scheme allows the DVFA to identify herds where AMU exceeds the permitted limits, which results in a Yellow Card. This entails a mandatory reduction of AMU within the specific age group, accompanied by advisory visits from the authorities at the cost of the producer. Moreover, the option to keep and refill prescriptions for previously prescribed AMs for group treatment is abolished, which is costly for the producer (Anonymous, 2018b). Initially, the permitted limits for each age group were set as twice the mean of the AMU of all Danish pig farms. The permitted limit has been lowered several times, most recently in 2019, and it now stands at 4.4 ADD/100 animals/day for finishers (Anonymous, 2018a). In 2016, the differentiated Yellow Card was introduced, which has a dynamic weighting for specific, critically important AMs, currently giving colistin, 3rd and 4th generation cephalosporins, and fluoroquinolones an additive factor of 10 and tetracyclines a factor of 1.5 (Anonymous, 2018a).

Since the Yellow Card was implemented in 2010, the total annual AMU in pig production decreased from 100.5 tons (DANMAP, 2010) to 71.3 tons in 2022 (DANMAP, 2023). In the meantime, the number of pigs produced increased from 28.6 million head to 32.6 million (Danish Agriculture & Food Council, 2022). In 2021, weaners for export accounted for almost 44% of the total production, with around 14 million animals exported annually (Danish Agriculture & Food Council, 2022). Denmark is ranked fourth among EU Member States (MS) with regard to pig population size, only surpassed by Spain, France, and Germany (Danish Agriculture & Food Council, 2022). The total AMU in Danish livestock production is low per animal unit compared to other MS, especially those with similar pig production (European Medicines Agency, 2022). In Denmark, sales of AMs for use in pig production account for 75% of the total sales of veterinary AMs (DANMAP, 2022). For more details about the AMU in Danish pigs, please see Moura et al. (2023).

The profit margin for veterinary medicine in Denmark is restricted to 5% above the market value (Anonymous, 2023). Instead, Danish veterinarians rely on health advisory contracts with farmers. These contracts are a prerequisite for allowing the producers to initiate AM treatment independently of a veterinarian (Anonymous, 2021). Veterinarians commit to making regular visits to the farm. Typically, there is a minimum of nine annual visits, but this can be reduced to four if there are only finishers in the herd. During the visits, veterinarians document recurring diseases, perform autopsies, and conduct diagnostic sampling to identify infectious agents and test for AMR. These activities help diagnose herd problems, which guide AM prescriptions. Additionally, during the advisory visits, action plans are created to prevent disease and reduce the future need for treatment. The main indications for AM treatment in commercially raised finishers are 1) gastrointestinal diseases, 2) respiratory disorders, and 3) conditions related to the skin, central nervous system (CNS), and joints (Moura et al., 2023). Gastrointestinal and respiratory infections influence herd-level AMU, as they often require oral preparations (Moura et al., 2023). Pigs can recover fully from such infections when they occur early in the animal's life, whereas infections occurring closer to the time of slaughter can result in lesions that will be detected at post-mortem meat inspection. Lesions related to respiratory disorders are frequent findings at meat inspection (Enøe et al., 2002).

Tail-biting behaviours are widespread in alternative and intensive pig production (Alban et al., 2015). The bite site provides an entry point through the skin and tail vertebrae, and the spread of pyogenic bacteria can result in several sequelae, including osteomyelitis in the pelvic area, abscesses in the hindquarters, and pyaemia (Schrøder-Petersen and Simonsen, 2001). In addition to increased AMU (Moura et al., 2023) and reduced animal welfare, production-related diseases are also responsible for financial losses due to partial or total condemnation at the abattoir (Schrøder-Petersen and Simonsen, 2001; Harley et al., 2012).

Meat inspection is highly important for food safety, animal welfare, and animal health. Foremost, meat inspection ensures that only meat fit for human consumption enters the food chain (Anonymous, 2022). Moreover, meat inspection has the potential to be used as part of animal welfare assessment in livestock production (Stärk et al., 2014), as it is capable of documenting breaches in animal welfare requirements. Pigs delivered to the abattoir are subjected first to ante-mortem inspection, and if accepted, the resultant carcass and organs also undergo post-mortem inspection. In Denmark, the inspections are undertaken by official veterinarians and official auxiliaries, and various findings detected during inspection are logged using a comprehensive coding system (Anonymous, 2022). Meat inspection findings determine whether parts of or the whole carcass need to be condemned, and this is based on pre-defined condemnation criteria (Vieira-Pinto et al., 2022). Overall, meat inspection acts as an extensive surveillance system, thus also providing a valuable source of information on animal health at the time of slaughter, potentially serving as an indicator of the on-farm environment and of AMU treatment regimes.

It might be hypothesised that herds with a high AMU are linked to a high need for treatment due to compromised animal health. However, it is important to consider that AM treatment of a herd can be instigated for various underlying reasons. Moreover, the AM administered could effectively treat disease outbreaks in the herd other than that being targeted, thus actually reducing the herd's overall disease burden. Conversely, some herd owners could intentionally delay or avoid treating animals needing AMs to maintain a low herd-level AMU. To better understand the possible outcomes of future AMU restrictions, it is important to assess the current health status of Danish finishers across different AMU classes and verify whether high AMU is associated with reduced finisher pig health. More specifically, the objectives of the study were to 1) explore the variations in meat inspection findings among finishers from herds with either a high, medium, or low AMU and 2) examine the association between meat inspection findings and AMU class, considering herd size, regional location within Denmark, and production type.

MATERIALS AND METHODS

Herd selection

Information on herds assigned a Yellow Card between 2016 and 2020 was obtained from the DVFA, and AM prescription data for Danish finishers were obtained from VetStat. Herds that had not previously received a Yellow Card were assigned a random date between 2016 and 2020, and the AMU data for the nine months before this specified date were included in the study. The level of AMU in those nine months was applied to categorise the herd as having either medium or low AMU. An AMU higher than 2.5 ADD/100 animals/day in at least one month out of the nine months was categorised as a medium AMU, and an AMU equal to or lower than 2.5 ADD/100 animals/day in all nine months was categorised as a low AMU. Herds with a Yellow Card were categorised as having a high AMU. Information on herd type and geographical location were obtained from the Central Husbandry Register (CHR). The herds were divided into two production groups, indoor or outdoor production, where the latter included organic and conventional free-range herds. The geographical location of the herd was divided into two regions, where herds in Jutland and Funen were categorised as Region West and herds in Zealand and the other islands, including Bornholm, were categorised as Region East.

Meat inspection data

The meat inspection data originated from 10 large Danish abattoirs in Jutland, Zealand, and Bornholm. Data covered monthly recordings of selected meat inspection codes and the number of pigs slaughtered for each herd. A total of 17 meat inspection codes were included in the study as indicators of pig health, covering: abscesses (in the head, front, mid and hind part, and leg/toe), arthritis, hernia, osteomyelitis, pericarditis, peritonitis, pleuritis, pneumonia, pyaemia, scar/contusion/bursitis, tail bite, and tail infection. The selected codes primarily represent lesions associated with a need for AM treatment at herd level. The code “totally condemned” was also included, as it is used in cases of generalised or extensive disorders affecting the carcass. Lesion codes related to each of lungs, tail, or abscesses were combined into three respective groups. Specifically, localised tail bites (lesions only found locally in the pelvis) and tail infections (lesions found beyond the pelvis as an indication of septicaemic spread) were grouped as “tail lesions”. Chronic pneumonia and chronic pleurisy were grouped as “lung lesions”. Abscesses detected in the front, middle, or hindquarters were combined into “abscesses, trunk”, while abscesses in the head or legs were grouped as “abscesses, extremities”. The median number of pigs delivered to the abattoir from each herd during the study period was used as a proxy for herd size. Herds delivering more than the median number of pigs were categorised as large herds, while herds delivering fewer than the median were characterised as small herds.

The study analysed meat inspection data for nine consecutive months, which is the same period used in the Yellow Card Scheme. However, this period was adjusted to cover six months before and three months after the specified dates for each herd. This was done because Danish finishing pigs usually reach their final slaughter weight within 10-12 weeks after entering the fattening unit. So, a herd with a Yellow Card could potentially have an increasing AMU from up to nine months before receiving the Yellow Card. But pigs introduced to the fattening unit at the beginning of this period will not reach slaughter weight until 10-12 weeks. The same applies to the period following the Yellow Card, where pigs will reach slaughter weight during the following three months.

Statistical analysis

Data were managed using the statistical software program R (version 4.3.0). Statistical analyses were carried out using the glmer function in the lme4 package. The prevalence of the meat inspection lesions was calculated for each AMU class. Chi-square tests were used to test the statistical significance between each lesion and the AMU class. Fisher’s exact test was used for lesions with a very low prevalence (i.e., an expected cell frequency lower than 5). Only lesions with a prevalence > 0.2% and P-value < 0.1 were considered for further analysis.

The potential associations between AMU class and meat inspection lesions were investigated in mixed-effects logistic regression models. A model was fitted for each lesion, with AMU class, region, production type, and herd size as covariates. Because meat inspection data are reported as the number of animals with a given lesion out of the total number of animals delivered for slaughter, the outcome was set as the number of pigs with a given lesion (y) detected by post-mortem inspection divided by the overall number (n) of finishers delivered from the herd during the selected nine-month period.

To account for any differences in the use of the meat inspection code system by the 10 abattoirs, herd and abattoir were included as random effects in all models.

Model reduction was performed using stepwise backward elimination, retaining only statistically significant covariates. Because of the large dataset size, P < 0.01 was used to determine statistical significance. Confounding effects on AMU and modifying effects were assessed by re-entering covariates into the final models and adding interaction terms between significant covariates, respectively. The full model was as follows (Eq.1):

$$GLMM\left(\frac{y}{n}\right) = AMU\ class + region + herd\ size + production\ type + (1|herd) + (1|abattoir) \tag{1}$$

RESULTS

<u>Descriptive statistics</u>

In total, 84 herds (348,124 pigs) with a high AMU (herds assigned a Yellow Card), 1332 herds with a medium AMU (5,976,589 pigs), and 1305 herds with a low AMU (4,186,343 pigs) were included in the analyses. Figure 1 illustrates the proportion of each lesion in each of the three AMU classes. Overall, lung lesions were the most common (16-17%) lesion type. The other lesions were recorded less frequently (each at < 3%). The difference between the three AMU classes with respect to lesions was minimal, with less than 0.5% variation. However, lung lesions, abscesses in the extremities, tail lesions, and arthritis were more common in herds with a high AMU. Scar/contusion/bursitis were more prevalent in herds with a medium AMU. Conversely, abscesses in the trunk, pericarditis, hernia, peritonitis, and osteomyelitis were more prevalent in herds with a low AMU. There was no association between AMU class and pyaemia (P-value = 0.21) or between AMU class and scar/contusion/bursitis (P-value = 0.28) in the univariable analysis. For the remaining nine lesions, their correlations with AMU class were statistically significant. Therefore, the subsequent models did not include pyaemia or scar/bursitis/contusion. Hence, nine meat inspection lesion codes were included in the multivariable analyses.

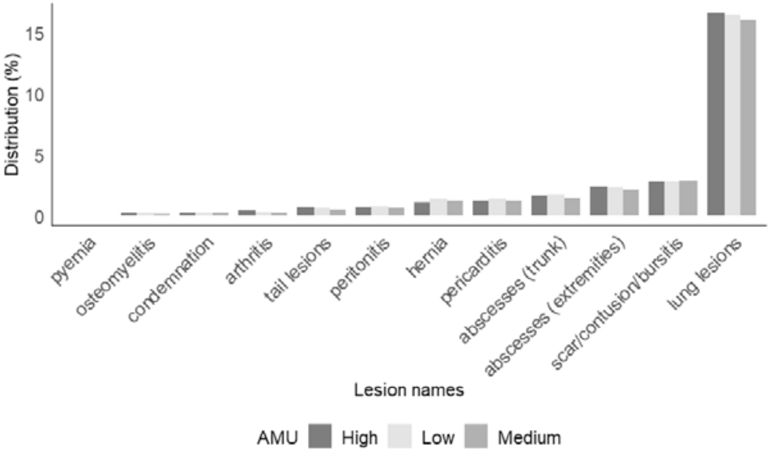

Figure 1. Prevalence of selected lesions recorded during meat inspection in Danish finishers from herds with a Yellow Card (=high AMU) compared to finishers from herds with a medium or low antimicrobial use. Data were collected over 5 years, from 2016 to 2020, from 10 Danish abattoirs

Mixed-effects logistic regression models

The results of the nine mixed-effects logistic regression models are presented in Table 1. Herds with a high AMU were more likely to deliver pigs with arthritis or abscesses in the extremities than herds with a medium or a low AMU. Conversely, herds with a low AMU were more likely to deliver pigs with hernia, osteomyelitis, abscesses in the trunk, or tail lesions. The prevalence of pigs with peritonitis was equal for all three AMU classes. Outdoor production, compared with indoor production, was associated with a higher prevalence of osteomyelitis, arthritis, and tail lesions, whereas hernia was less prevalent in outdoor production compared to indoor production. A large herd size, compared to a small herd size, was associated with a lower prevalence of peritonitis, hernia, osteomyelitis, arthritis, tail lesions and abscesses in the trunk and extremities (Table 1).

The prevalence of lung lesions was higher for herds in western regions than in eastern regions of Denmark. The variance introduced to the models due to animals delivered from the same herds was greater than that due to the different abattoirs (Table 1). No multicollinearity was present in any of the full models.

DISCUSSION

The first objective of this study was to assess variations in meat inspection lesions among herds with a high, medium, or low AMU. This was done to investigate potential associations between a high AMU and poor health in finishers. Despite the large differences in AMU, the prevalence of each lesion differed by less than 1% between the three AMU classes, making the lesion prevalences very uniform across classes (Fig.1). There are several possible reasons for this finding. AMU in the Danish livestock sector is in the lowest third among all reporting EU MS (European Medicines Agency, 2022), which could reflect the very uniform production methods in the Danish pig industry. The relatively low AMU can be explained by the extensive efforts undertaken by Danish policymakers, the livestock industry, and other stakeholders in working toward prudent AMU, including establishing the VetStat database and implementing the Yellow Card scheme. Therefore, herds with high AMU in Denmark may not necessarily reflect herds with high AMU in other MS.

In Denmark, ongoing efforts are dedicated to reducing AMU in pig production, with a significant achievement of a 16% reduction in 2019 compared to 2014 (DANMAP, 2023). This highlights the second objective of this study, which aimed to investigate the potential relationship between AMU classes and meat inspection lesions. The findings showed that herds with a high AMU were more likely to deliver pigs with arthritis or abscesses in the extremities than those with a medium or with a low AMU (Table 1). Conversely, herds with a low AMU were more likely than herds in the other AMU classes to deliver pigs with hernia, osteomyelitis, abscesses in the trunk, or tail lesions. A medium AMU was not associated with any of the meat inspection lesions. It is plausible that herds with a medium AMU do not experience severe health challenges, as the medium AMU class likely mirrors what is expected in Danish pig herds with appropriate treatment protocols and a manageable disease burden in the finishers. In contrast, herds with a low AMU could encompass labelled production systems such as organic and RWA (raised without antimicrobials), where there is a financial incentive to maintain a low AMU.

Table 1. Results of the multivariable analyses of the associations between nine selected meat inspection lesions and AMU class, herd size, region, and production type in 10.5 million finishing pigs delivered to 10 Danish abattoirs between 2016 and 2020

Model with lesion as outcome	Fixed effects					Random effects		
	Covariates[a]	Categories	Estimate	Odds ratio (CI)	P-value	Levels	Variance	Standard deviation
Pericarditis	Herd size	Intercept	-6.72		<0.001	Herd	0.59	0.77
		Small		1		Abattoir	7.74	2.78
		Large	-0.11	0.89 (0.83 – 96)	0.004			
Lung lesions[b]	Region	Intercept	-2.74		<0.001	Herd	1.27	1.13
		East		1		Abattoir	0.06	0.24
		West	0.80	2.22 (1.99 – 2.47)	<0.001			
Peritonitis	AMU class	Intercept	-5.06		<0.001	Herd	0.25	0.50
		High		1		Abattoir	0.39	0.62
		Medium	-0.11	1.00 (0.79 – 1.02)	0.10			
		Low	-0.001	1.00 (0.87 – 1.13)	0.93			
	Herd size	Small		1				
		Large	-0.22	0.80 (0.77 – 0.84)	<0.001			
Hernia		Intercept	-4.30		<0.001	Herd	0.65	0.81
	AMU class	High		1		Abattoir	0.24	0.49
		Medium	-0.03	1.00 (0.80 – 1.16)	0.67			
		Low	0.16	1.17 (0.97 – 1.41)	0.12			
	Herd size	Small		1				
		Large	-0.49	0.61 (0.57 – 0.65)	<0.001			
	Production type	Indoor		1				
		Outdoor	-0.28	0.76 (0.64 – 0.90)	0.002			
Osteomyelitis		Intercept	-6.62		<0.001	Herd	0.56	0.75
	AMU class	High		1		Abattoir	0.12	0.34
		Medium	0.002	1.00 (0.81 – 1.23)	0.98			
		Low	0.29	1.34 (1.10 – 1.65)	0.006			
	Herd size	Small		1				
		Large	-0.33	0.72 (0.67 – 0.77)	<0.001			
	Production type	Indoor		1				
		Outdoor	0.59	1.80 (1.40 – 2.32)	<0.001			

Table 1 (continued). Results of the multivariable analyses of the associations between nine selected meat inspection lesions and AMU class, herd size, region, and production type in 10.5 million finishing pigs delivered to 10 Danish abattoirs between 2016 and 2020

Model with lesion as outcome	Fixed effects					Random effects		
	Covariates[a]	Categories	Estimate	Odds ratio (CI)	P-value	Levels	Variance	Standard deviation
Arthritis		Intercept	-5.91		<0.001	Herd	0.38	0.62
	AMU class	High		1		Abattoir	0.16	0.41
		Medium	-0.16	0.85 (0.73 – 1.01)	0.06			
		Low	-0.04	0.96 (0.81 – 1.14)	0.65			
	Herd size	Small		1				
		Large	-0.29	0.75 (0.71 – 0.79)	<0.001			
	Production type	Indoor		1				
		Outdoor	0.92	2.51 (2.10 – 3.01)	<0.001			
Abscesses, trunk[c]		Intercept	-4.24		<0.001	Herd	0.26	0.51
	AMU class	High		1		Abattoir	0.07	0.27
		Medium	-0.04	0.96 (0.85 – 1.09)	0.53			
		Low	0.16	1.17 (1.04 – 1.33)	0.01			
	Herd size	Small		1				
		Large	-0.19	0.82 (0.79 – 0.86)	<0.001			
Abscesses, extremities[d]		Intercept	-3.71		<0.001	Herd	0.30	0.55
	AMU class	High		1		Abattoir	0.10	0.32
		Medium	-0.14	0.87 (0.77 – 0.99)	0.03			
		Low	-0.04	0.96 (0.85 – 1.09)	0.56			
	Herd size	Small		1				
		Large	-0.23	0.79 (0.76 – 0.83)	<0.001			
Tail lesions[e]		Intercept	-5.7		<0.001	Herd	1.10	1.05
	AMU class	High		1		Abattoir	0.13	0.35
		Medium	-0.09	0.94 (0.73 – 1.21)	0.47			
		Low	0.08	1.09 (0.85 – 1.40)	0.69			
	Herd size	Small		1				
		Large	-0.39	0.68 (0.62 – 0.74)	<0.001			
	Production type	Indoor		1				
		Outdoor	0.56	1.75 (1.38 – 2.23)	<0.001			
	Region	East		1				
		West	0.21	1.23 (1.10 – 1.38)	<0.001			

Herds with a high AMU were more likely to deliver pigs with arthritis than herds with a medium or low AMU. In Danish pig production, about one-third of the AMU is to treat conditions of the skin, CNS, and joints (Moura et al., 2023). Treatments typically require single-animal treatment, which is considerably more time-consuming than group medication. In addition to compromising animal welfare, arthritis is thus costly and time-consuming for the producer. It is unknown if increased arthritis caused the high AMU. Arthritis may have gone undetected, or treatment could have been delayed to avoid a greater increase in AMU.

This study found that herds with a low AMU were more likely to deliver pigs with tail lesions than herds with a high or a medium AMU. The same was true for osteomyelitis, a common sequela of tail lesions (Schrøder-Petersen and Simonsen, 2001). For both tail lesions and osteomyelitis, the likelihood of pigs being delivered with these conditions was higher in outdoor production than in indoor production. Other studies have also reported greater odds for tail lesions in herds with outdoor production and in herds where pigs have undocked tails (Alban et al., 2015; Kongsted and Sørensen, 2017). Most herds with low AMU in this study were from indoor production where tails were docked, meaning tail lesions were also detected in pigs with docked tails. These findings suggest that tail docking is insufficient and that additional measures must be taken, not only to comply with the EU Commission's directive on undocked tails in pig production (Council of European Union, 2008), but also to ensure that animal welfare is not compromised in pursuing a low AMU. The close correlation between tail lesions, pyaemia, and abscessation (Huey, 1996) provides another important economic justification for minimising disease prevalence at the herd level and thus reducing the number of condemned carcasses at the abattoir.

The prevalence of delivering pigs with hernias was also higher in herds with a low AMU compared to those with a high or a medium AMU. Production type also affected the likelihood of hernias, with outdoor production having lower odds than indoor production. Previous Danish studies have also reported a lower prevalence of hernias in pigs raised outdoors compared to pigs from conventional indoor housing (Alban et al., 2015; Kongsted and Sørensen, 2017). The pathogenesis behind hernias is not fully understood, but predisposing factors of anatomical and environmental origin are considered (Hovmand-Hansen et al., 2021). This study does not focus on the prevalence of hernias in young animals but on the prevalence of hernias in finishers delivered to the abattoirs. Therefore, the results from this study are likely a reflection of management routines for the individual herds, which could vary according to size and production type.

In all three AMU classes, lung lesions were the most prevalent (16-17%). However, the prevalence of lung lesions in this study was slightly below previous findings from Denmark (Alban et al., 2015; Cleveland-Nielsen et al., 2002). Since the introduction of the Yellow Card scheme, the use of vaccines against pulmonary infections has increased (Kruse et al., 2017), which is likely to have had an effect on reducing lung lesions. Additionally, non-infectious risk factors are considered significant in spreading lung diseases, including proximity to neighbouring farms and pig density (Cleveland-Nielsen et al., 2002), enhancing the airborne spread between herds. The density of pigs could have had a decisive impact on the results of our study. Only the regional location of the herds was statistically significantly associated with the probability of delivering pigs with lung lesions, with the highest odds for herds in the west of the country compared to herds in the east. This is consistent with the current pig density in Denmark, which is higher in the west (Danish Agriculture & Food Council, 2022).

The potential for meat inspection data to be included in national welfare indices has previously been explored by Nielsen et al. (2017). The present study also emphasises that meat inspection data could be a valuable source of information for the consulting veterinarian if presented in a way that makes the data easy to use. Since the Yellow Card was introduced, the permitted limits have been lowered several times as a part of national action plans to reduce AMU (DANMAP, 2023). If the permitted limits are to be lowered again, the role of the veterinarian with regular advisory visits will become even more important to ensure that less AMU does not inadvertently contribute to a decline in animal welfare.

CONCLUSION

This study investigated the association between AMU and meat inspection lesions in finisher pigs as a proxy for animal health. Despite notable differences in AMU among finisher pig herds, there were only marginal differences in the overall prevalences of meat inspection lesions among herds with a high, medium, or low AMU. Herds with a high AMU were more likely to deliver pigs with arthritis or abscesses in the extremities, while herds with a low AMU were more likely to deliver pigs with hernias, osteomyelitis, abscesses in the trunk, or tail lesions. A medium AMU was not associated with a greater likelihood of any of the meat inspection lesions studied occurring in finisher pigs on delivery to the abattoir. Hence, neither a high nor a low AMU resulted in optimal finisher pig health. Furthermore, each of the nine lesions studied was associated with at least one of region, herd size or production type. If the permitted limits for AMU are further reduced, the veterinary advisory role should become even more vital in preventing adverse effects on animal health and welfare.

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IMPLEMENTATION OF BIO…WHAT?’: SUBJECTIVITIES OF FARM WORKERS BETWEEN CONFLICTS WITH AND INSTRUCTIONS FROM FARMERS ON DAIRY CATTLE FARMS

S. MOYA*, G. ENTICOTT AND J. ESPLUGA

SUMMARY

The implementation of biosecurity measures on dairy farms is mainly decided, planned, and carried out by farmers and veterinarians, but it is the farm workers who carry out the management tasks. The aim of this study was to analyse the implementation of biosecurity measures by farm workers in order to understand their dynamics and contribute to the improvement of biosecurity standards on dairy farms in Spain. Four ethnographies were carried out in two regions of Spain: two in Catalonia and two in Galicia. The results showed that there were communication and hierarchical differences between farm workers and farmers that affected the implementation of biosecurity measures. Farm workers did not always understand or follow the farmer’s instructions, sometimes because they relied on a practical anthropological approach to implementing biosecurity measures. In order to improve the implementation of biosecurity measures on dairy farms, farm workers need to be considered as one of the key stakeholders. This is usually neglected in the literature and policy making.

INTRODUCTION

Biosecurity measures, understood as isolation, hygiene or management practices specifically designed to reduce the likelihood of introduction, establishment, survival or spread of a potential pathogen on a farm (Huber et al., 2022), are mainly decided, planned, and carried out by farmers and veterinarians. On dairy cattle farms, however, it is mainly farm workers who carry out management tasks on behalf of the farmer. These workers have close contact with the animals and spend most of their time on the farms. Therefore, they may also be responsible for the implementation of biosecurity measures on these farms.

Farm workers perform a variety of roles, such as managers, supervisors (Diniso and Jaja, 2021; Merenda et al., 2023), general workers, temporary workers (Diniso and Jaja, 2021), caretakers and even veterinary care (Merenda et al., 2023). In general, these positions involve interaction with other stakeholders and responsibility for certain management tasks, although

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these responsibilities are broad and unspecific and vary from farm to farm. In addition, a significant number of these workers are migrants or non-technical staff, such as Latin Americans working on dairy farms in California (United States of America) (Merenda et al., 2023).

Research on the influence of psychosocial factors on the implementation of biosecurity measures by farm workers on dairy farms has been largely neglected. Existing studies have primarily focused on farmers (Roche et al., 2019; Wolff et al., 2019; Singh et al., 2020; Moya et al., 2021; Imada et al., 2023; Zhu et al., 2023) and veterinarians (Roche et al., 2019; Singh et al., 2020; Moya et al., 2021). Other stakeholders such as paraveterinarians (Singh et al., 2020), veterinary academics (Singh et al., 2020), other animal health professionals (Zhu et al., 2023) and government veterinarians (Moya et al., 2023) have received little attention. Conversely, most studies on dairy farm workers have focused on infectious disease hazards associated with work practices (Torres-Gonzalez et al., 2013; Benschop et al., 2017; Elsohaby et al., 2020; Dobos and Balla, 2021).

Despite efforts to implement biosecurity measures according to legislation and recommendations, there are still gaps that favour the introduction, establishment, survival or spread of infectious diseases on dairy farms, where farm workers could also play an important role. The aim of this study was to analyse the implementation of biosecurity measures by farm workers in order to understand their dynamics and contribute to the improvement of biosecurity standards on dairy cattle farms in Spain.

MATERIALS AND METHODS

The present study was carried out on four dairy farms in Spain, two in Galicia (north-east) and two in Catalonia (north-west), using an ethnographic approach. These farms were named Galicia-I, Galicia-II, Catalonia-I and Catalonia-II. Farms and participants were selected using convenience sampling, coordinated by JD and EY in Galicia and AA and JC in Catalonia. Participants were informed in detail about the objectives and conditions of the study, guaranteeing that their participation was completely voluntary and without financial compensation, and subsequently gave their consent by signing an informed consent form.

The participants represented different roles within the farms: farmer-worker (4), farmer-family worker (7), internal worker-employee (3) and external worker-employee (4). The internal and external workers came from a variety of backgrounds, including countries outside Europe and non-livestock technical sectors. Participants interacted with a variety of stakeholders, including veterinarians, transporters, sellers and maintenance staff.

Data were collected through participant observation and conversational interviews conducted by SM, a veterinarian trained in qualitative methods and a PhD student at the time of the study. SM interacted with participants on a daily basis, asking spontaneous and informal questions focused on biosecurity practices, and recorded both observations and interviews using a field diary and voice recordings. The average duration of the ethnographies on each farm was two weeks. It should be noted that the participants and SM had no prior relationship. A pilot study was carried out on the Catalonia I farm.

After data collection, the data were reviewed with the participants after one week to allow them to give feedback and clarify certain biosecurity practices.

The data collected was coded in two stages: using pre-established codes and codes that emerged from the data. These codes were then organised into two main themes: implementation of biosecurity measures (descriptive observations) and subjectivities of implementation (explanatory conversations). ATLAS.ti software was used for thematic analysis.

Vignettes drawn from the observations and interviews were used to illustrate the findings in the results section.

RESULTS

The field ethnographies revealed that there were communication and hierarchical differences between farm workers and farmers, which in some cases led farm workers to implement biosecurity measures that were incomplete or contrary to the instructions of their superiors. Such implementation, in turn, led to conflicts between the two stakeholders. It was also observed that the behaviour of the farm workers was shaped by what the authors described as a practical anthropological approach (i.e. managing animals in a useful, efficient and effective way, taking into account that animals experience their environment in a similar way to humans), which also led these farm workers to implement biosecurity measures in a different way from the farm guidelines.

Communication differences

Differences in communication between farm workers and farmers were found to be related to differences between mother tongues and between technical and non-technical language. On the Catalonia I farm, the farm worker was from Gambia and his mother tongue was Mandinka, although he had a basic knowledge of Catalan-Spanish, the farmer's mother tongue. However, this basic knowledge was not sufficient for the farm worker to fully understand the farmer's instructions on how to feed the calves. He misunderstood and partially carried out these instructions, causing tension between the two.

> "It was winter after milking. The farmer instructed the farm worker to feed the calves with a bucket of warm colostrum that he had taken out and placed outside the milking parlour. However, the labourer used another bucket of cold milk from a cow with mastitis. The farmer got angry, argued with the worker and immediately sent him to clean the parlour" (Excerpt of the field notes of Catalonia-I)

On the Galicia I farm, the three farm workers came from the administration and transport sector, although they had a superficial knowledge of the technical language of dairy farming. However, this superficial knowledge did not help them to understand the basics of cleaning the calves' bedding. They carried out this task in their own way and contrary to the basics, which also led to conflicts between them and the farmer.

> "Each area of the farm has its own tools to prevent the spread of infectious diseases, is one of the explanations given to farm workers when they join the farm. But to clean the beds in the calf area, the labourers used the garden shovels and rakes from the lactation area. 'Ideally I shouldn't be doing this, but it's not so bad if I do it once in a while', justified one of the workers, who, when was asked about the basics of biosecurity,

> replied ‘Implementation of bio...what?’. At this point, the farmer argued with this farm worker, who abandoned his task” (Excerpt of the field notes of Galicia-I)

Hierarchical differences

The hierarchical difference between farm workers and farmers was mainly observed in their treatment, with farmers attacking farm workers in a vexatious manner and farm workers defending themselves in the same way. On the Catalonia I farm, the farmer saw the farm worker as an incompetent person who was carrying out his daily tasks in the wrong way. This treatment was characterised by an argument in which the farmer vehemently shouted at the farm worker without first trying to establish a dialogue with him. The farm worker never tried to change his wrong practices, according to the farmer, who never gave the farm worker feedback to correct them.

> “The farm worker had to move male calves and young bulls as a block from one area to another because new male calves had to be brought in. However, the labourer moved all these animals together in one area, not as a block. The farmer was fed up with this situation and treated the worker badly, insulting him in front of his customers. Previously, the farmer had never explained to the farm worker, who had only done this once before with the farmer, all the steps involved in the correct handling of these animals” (Excerpt of the field notes of Catalonia-I)

On the Galicia I farm, one of the three farm workers saw the farmer as a person who only gave orders and controlled him, making him feel intimidated. This treatment led to the farm worker deliberately doing his daily tasks incorrectly in order to annoy the farmer. This situation ended with the farm worker being fired from the farm for being confrontational, according to the farmer, who did not recognise his harassment.

> “The three farm workers had been stockpiling straw for the animals’ bedding all day, and the farmer had coordinated their organisation and layout. However, one of the labourers reprimanded the farmer, saying: ‘I can’t stand it anymore when you wait all day for us to make a mistake so you can throw it in our faces’. The farmer simply pointed out that clean straw had been mixed with dirty straw (containing animal waste). At the time, the worker admitted that he had done this on purpose because he was tired of being watched for no reason” (Excerpt of the field notes of Galicia-I)

Practical anthropological approach

Farm workers were found to use a practical anthropological approach to their daily tasks. On one hand, this approach involved viewing animals as similar, also with inherent organic processes, and on the other hand, simplifying their practices in order to conserve resources in their development. On the Galicia I farm, workers had the option of using the workers’ toilet. However, while cleaning the lactation area, two of the three farm workers urinated with the animals. The workers argued that the cows were an animal with organic processes, like themselves, and that they should be exposed to infectious diseases in order to have a better immune system. They also pointed out that they were saving time going to the toilet, even though the toilet was less than five minutes away.

> “While the farm workers were cleaning the lactation area, two of them started to urinate. One of them commented ‘Urine is urine’, while the other added ‘The cows also boost

> their immune systems this way'. When I asked about management protocols, the first replied 'Cows piss, shit and eat just like us, and they are here just like us, so there is no problem'" (Excerpt of the field notes of Galicia-I)

On the Catalonia I farm, the farm worker had the opportunity to wash his clothes in a washing machine and also to clean his boots at water points. However, the farm worker used the same clothes and boots in all areas without cleaning them before entering each area. The farm worker implied that all waste was the same, both animal and human, and that the clothes would get dirty again. In addition, he shared these boots with another farm where he also worked, as he did not have his own equipment on each farm.

> "The farm worker moved from the carcass area to the lactation area without cleaning his boots before entering. He only cleaned his boots when they got too dirty. He tried to explain to me that he did not clean his boots because he had to go to the water points, which were not at the entrance to each area, and that they would end up dirty again" (Excerpt of the field notes of Catalonia-I)

In addition to the communication and hierarchical differences and the practical anthropological approach, it is important to note that the farms did not provide any training or feedback on biosecurity and other management procedures to their farm workers. According to the farmers, this was mainly due to the fact that there was a high turnover of these staff and they lost resources in the process, so they no longer offered it, nor did they use the services of any other institution or organisation for this purpose.

DISCUSSION

The results of this study show that the subjectivities of farm workers in relation to biosecurity measures are influenced at three different levels. Firstly, the dominant social structures, characterised by the Galician-Catalan-Spanish language and the technical language. Secondly, the existing power relations that limit the correction of faulty practices or encourage the performance of deliberately incorrect tasks. Finally, representations of animals or everyday practices that generate a practical anthropological approach. These factors interact in a complex way and influence the subjectivities of the farm workers, which in turn have a direct or indirect impact on the implementation of biosecurity measures on dairy cattle farms in Spain.

In this study, the different mother tongues of farm workers and farmers resulted in workers implementing biosecurity measures differently from the farmers' instructions. This is in line with the study by Viveros-Guzmán and Gertler (2015), where this difference disrupts sustained two-way communication between these stakeholders, which in turn contributes to the spread of this disruption. Thus, this difference in mother tongues is a communicative barrier that requires the efforts of both the farm workers to learn Galician-Catalan-Spanish and the farmers to try to better understand their workers in their language. In addition, this barrier can pose a particular risk to the mental well-being of the farm workers (Viveros-Guzmán and Gertler, 2015), who may even experience discrimination for speaking their mother tongue at work (Farquhar et al., 2008).

The difference between the technical and non-technical language of farm workers and farmers in this study also leads to a distant biosecurity implementation of farmers' instructions by farm workers. This can be explained by the fact that farm workers enter the farms with a superficial knowledge of the sector, without any training or feedback on their practices. In fact, this lack of technical knowledge can also be found in other areas, such as pesticide use (Farquhar et al., 2008). In this sense, farm workers acquire this knowledge only over time, and those who become experienced are considered valuable for their contribution to productivity (Klocker et al., 2020).

In this study, hierarchical differences lead farm workers to make mistakes, accidentally or deliberately, in the implementation of biosecurity measures. These hierarchical differences arise in the relationship between farm workers and farmers through tensions and conflicts. However, these differences are marginalised, which may be due to the invisibility of farm workers themselves within the primary sector (Saldanha, 2020) and also in the rest of society. One way to address these differences is to establish open channels of communication between all stakeholders, where farm workers' perspectives can be taken into account by farmers (Klocker et al., 2020).

Farm workers have their own subjectivities that may influence the implementation of biosecurity measures and should therefore be considered as an important stakeholder, especially as they have close contact with the animals and spend most of their time on the farm. Moreover, although dairy cattle farms in Spain are heterogeneous in terms of their social structure (i.e. a variety of established frameworks of social relations between farms), they are homogeneous in terms of their social organisation (i.e. a uniform dynamic of interaction between farm workers within these frameworks), which makes it possible to extrapolate farm workers' subjectivities to the sector as a whole. To some extent, the poor working conditions in the sector, resulting in high levels of temporary employment, could be seen as an unfavourable context for biosecurity measures. In this sense, this stakeholder should be considered in intervention programmes such as those on communication and treatment between farm workers and farmers, including biosecurity training. Farm workers can not only professionalise their role, but also be made visible as a key stakeholder within dairy farms in Spain. And as a consequence, this can motivate farm workers (Kolstrup, 2012) by reducing their turnover and increasing their permanence within these production systems.

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ECONOMICS

AN EPIDEMIOLOGICAL-ECONOMIC MODELLING FRAMEWORK TO ESTIMATE THE PRODUCTION LOSSES ATTRIBUTABLE TO LIVESTOCK BRUCELLOSIS AND THE POTENTIAL BENEFITS OF DISEASE CONTROL

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SUMMARY

Brucellosis is a globally widespread zoonosis. This study aimed to estimate the production losses attributable to livestock brucellosis and the potential benefits of livestock vaccination for brucellosis control in pastoral communities of northern and central Tanzania. Data from household surveys were used to determine the prevalence of livestock brucellosis. A clustering algorithm was applied to classify households into pastoral and non-pastoral systems. A herd-growth model was applied to estimate production losses attributable to brucellosis. A disease transmission model was applied to estimate the costs and benefits of livestock vaccination. The median (95% uncertainty interval) annual losses attributable to brucellosis per infected animal were 74.4 (26.2-211.7) international dollars in cattle, 9.7 (3.4-23.1) in sheep, and 10.6 (3.7-25.0) in goats. The estimated production losses averted by vaccination were 3.4 (1.3-9.7) times higher than vaccination costs. This study quantifies the considerable impacts of brucellosis and the potential benefits of disease control in Tanzania.

INTRODUCTION

Livestock production is critical to the economies of low- and middle-income countries, representing a source of protein-rich foods, fertiliser, draught power, and financial and social capital (e.g. insurance, credit, reputation) (Thornton, 2010). Many factors, including infectious diseases, can affect livestock productivity and have impacts on household income, food security, and human health and well-being (Randolph et al., 2007).

Brucellosis is an infectious disease that affects livestock productivity in many low-resource settings (Franc et al., 2018). Brucellosis is caused by bacteria of the genus *Brucella*, which can infect a wide range of hosts, including humans, livestock, and wildlife (Whatmore and Foster, 2021). In livestock, brucellosis is characterised by low mortality risk but high reproductive morbidity (e.g., abortion, infertility, and sterility) (Corbel et al., 2006). Symptoms of livestock brucellosis are often non-specific and insidious, which can lead to poor recognition of disease impacts by households, particularly those facing numerous other sources of risk (e.g. climate

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change, food insecurity, political conflicts, and highly fatal livestock diseases) (Cleaveland et al., 2017; Moreno et al., 2022; Moriyón et al., 2023).

Brucellosis is endemic in Tanzania, a country with one of the largest livestock populations on the African continent (Mtui-Malamsha et al., 2022; Mengele et al., 2023). The prevalence of brucellosis is higher in pastoral communities of northeastern Tanzania compared to other parts of the country. In these pastoral communities, the disease is one of the most common bloodstream infections in humans and over 6% of cattle are seropositive (Bodenham et al., 2020; Bodenham et al., 2021). Pastoral households raise livestock on a subsistence basis, mostly in large herds, following traditional cultural practices (de Glanville et al., 2020).

Mass livestock vaccination is often recommended to control brucellosis in settings such as northeastern Tanzania, where the prevalence is high and veterinary services have limited resources (Ducrotoy et al., 2017; Mtui-Malamsha et al., 2022). The safest and most effective brucellosis vaccines currently available are live-attenuated and host-specific. These include *B. abortus* strain 19 (S19) for cattle and *B. melitensis* strain Rev-1 for sheep and goats (Ducrotoy et al., 2017).

Policymakers considering allocation of public resources to livestock vaccination for brucellosis control need information about the production losses attributable to the disease and the potential intervention costs and benefits (Zinsstag et al., 2016). Producing this evidence in endemic settings is challenging for several reasons. First, there are significant data gaps on disease prevalence, transmission pathways, and direct impacts on livestock productivity (Godfroid, 2017; Franc et al., 2018). Second, there is a lack of methodological standardisation in livestock disease impact estimation literature and benefit-cost analysis of livestock vaccination for brucellosis control. Third, multiple factors must be considered to design and deliver a vaccination programme for brucellosis control, including the challenges of differentiating vaccinated from infected animals (WOAH, 2019) and the risks posed by vaccination during pregnancy. Vaccination may cause abortion and infection of the mammary gland with excretion of the vaccine strain in the milk, which poses a risk to humans (WOAH, 2019).

This study aimed to develop and apply an integrated epidemiological-economic modelling framework to estimate the production losses attributable to livestock brucellosis and the potential benefits of livestock vaccination for brucellosis control in endemic settings. This framework was applied in the pastoral context of northern and central Tanzania.

MATERIALS AND METHODS

Study area

The estimation of production losses attributable to livestock brucellosis focused on eight regions of Tanzania: Arusha, Manyara, Kilimanjaro, Tanga, Dodoma, Singida, Simiyu, and Mara. These regions cover an area equivalent to approximately 29% of mainland Tanzania and include around half of the livestock in the country (Government of The United Republic of Tanzania, 2013, 2017).

The estimation of the costs and benefits of livestock vaccination for brucellosis control focused specifically on the Ngorongoro Conservation Area (NCA). The NCA is a protected area in the Arusha region with an estimated population of about 100,000 people, 80% of which are Maasai pastoralists who keep around 115,000 cattle and 180,000 sheep and goats (Tanzania Wildlife Research Institute, 2016; Manzano and Yamat, 2018; Government of The United Republic of Tanzania, 2019).

Data

A protocol based on the Maelstrom Research guidelines for retrospective data harmonisation was followed (Fortier et al., 2017). Informal reviews of the literature on household surveys in northern and central Tanzania and classification of livestock production systems were conducted. The following surveys were identified for inclusion in this study: i) The 'Social, Environmental, and Economic Drivers of Zoonotic disease' (SEEDZ) project; ii) the 'Living Standards Measurement Study' (LSMS); and, iii) the 'Rural Household Multiple Indicator Survey' (RHoMIS).

The SEEDZ project delivered a large-scale, cross-sectional survey, dedicated to the analysis of risk factors of human and animal zoonoses, between February and November 2016, in two regions of northern Tanzania: Arusha and Manyara. The study design and the methods used have been described in detail elsewhere (de Glanville et al., 2020). The SEEDZ survey was used as a contextual, spatial, and temporal reference for gathering data on livestock production from additional surveys.

The LSMS is a multi-country, nationally representative household survey programme led by the World Bank (World Bank, 2021). Data from the latest two waves (October 2012 to November 2013, and October 2014 to November 2015) were used due to their temporal proximity to the SEEDZ survey.

RHoMIS is a standard survey tool focused on household socioeconomic characteristics, which has been applied in more than 30 countries since 2015 (van Wijk et al., 2020). Two applications of the RHoMIS tool were used: i) RHoMIS1 - a survey led by the International Livestock Research Institute (ILRI) of livestock keepers randomly selected from community animal health service records in 2017; and, ii) RHoMIS2 - a survey led by the International Centre for Research in Agroforestry (ICRAF) of randomly selected households based on village lists obtained from the village elders in 2018.

Modelling framework

The modelling framework developed and applied in this study had three parts: Part I – classification of households by production system; Part II – estimation of production losses due to livestock brucellosis; and, Part III – estimation of the costs and benefits of livestock vaccination for brucellosis control.

Part I – classification of households by production system: de Glanville et al. (2020) applied multiple factor and hierarchical clustering analyses to classify SEEDZ households as pastoral, agro-pastoral, or smallholder. The goal was to apply the same methodology to classify LSMS and RHoMIS households. However, many of the variables in SEEDZ were not available in LSMS and RHoMIS. The SEEDZ dataset included in de Glanville et al. (2020) had 76 variables. Out of these 76 variables, 12 (15.8%) were absent in LSMS, and 28 (36.8%) were absent in RHoMIS. All the variables that were absent in LSMS were also absent in RHoMIS.

Given differences in availability of variables between datasets, several household classification models were run with different combinations of datasets and variables. A number of integration procedures were also tested, as follows: i) adding one non-SEEDZ household at a time to the original model (M0), which initially contained SEEDZ data only, as per de Glanville et al. (2020); ii) adding one non-SEEDZ dataset at a time to M0; or, iii) adding both non-SEEDZ datasets to M0.

The performance of each model was evaluated with reference to the classification of SEEDZ households derived with M0. The performance metric used was the probability of a model classifying a SEEDZ household correctly as pastoral or non-pastoral. The best-performing model was selected for classifying LSMS and RHoMIS households by production system. Data from pastoral households were used to estimate losses due to livestock brucellosis (Part II) and the costs and benefits of livestock vaccination for brucellosis control (Part III).

Part II – estimation of losses due to livestock brucellosis: A Bayesian latent-class analysis (LCA) model was applied to derive livestock brucellosis prevalence estimates for pastoral households in the SEEDZ dataset, as described by Bodenham et al. (2021).

A herd-growth model was developed to estimate the losses attributable to livestock brucellosis. This herd-growth model used prevalence estimates from the LCA model, demographic and production data from the three survey datasets, and additional literature-based information on disease impacts.

The herd-growth model was set up with differential equations Eq.(1-3) that enabled estimation of the number of animals in each age and sex group (juveniles - J, adult males - M, and adult females - F), and the number of events (births, deaths, abortions, etc.) at any point in time for the duration of the simulation (10 years). Animals could (i) leave the system through death (at rate μ), or offtake (at rate θ), and (ii) enter the system through birth (at rate α), as follows (rates are annual):

$$\begin{aligned}\frac{dJ}{dt} = \big((1-\pi) + \pi(1-\delta-\eta-\gamma)\big)\alpha zF - \mu_J(J + \nu\pi(1-\delta-\eta-\gamma)\alpha zF) \\ - (\theta + \sigma_M + \sigma_F))J\end{aligned} \tag{1}$$

$$\frac{dM}{dt} = \sigma_M J - (\mu_M + \theta)M \tag{2}$$

$$\frac{dF}{dt} = \sigma_F J - \big(\mu_F\big(1 + \psi\pi\alpha\delta(1-\eta-\gamma)\big) + \theta\big)F \tag{3}$$

where π is the prevalence of brucellosis, δ is the probability of abortion in infected females that became pregnant, η is the probability of infected females being temporarily infertile, γ is the proportion of infected females that are sterile due to a previous abortion event, z is the average number of offspring per term pregnancy, ν is the proportional increase in perinatal mortality rate in offspring from infected females, ψ is the proportional increase in mortality rate of infected females that aborted, and σ_F and σ_M are the transition rates from juveniles to adult females and adult males, respectively.

Losses were calculated as the difference between the estimated value of livestock production simulated in the presence and in the hypothetical absence of brucellosis. The same workflow was used to estimate losses in cattle, sheep, and goats. Offtake (wholesale, home slaughter, or gifts given away) and milk contributed to production or income from all species. For cattle, offtake values also included draught power and dung. Brucellosis impacts included changes in the number of animals in a herd, reduction in daily milk yield, reduction in meat production, and reduction in draught power. A total of 50,000 Monte Carlo iterations of the herd-growth model were run. Estimated annual losses are reported per infected animal, as a percentage of livestock-derived income per household, and as a total for the pastoral community in the study area. All monetary values were discounted over time at a rate of 3% per year.

Part III – estimation of the costs and benefits of livestock vaccination for brucellosis control: A livestock vaccination strategy was designed for brucellosis control in the pastoral community of the Ngorongoro Conservation Area. This strategy consisted of mass vaccination, through conjunctival instillation, of cattle, sheep, and goats, every two years over a period of ten years. The benefits and costs of vaccination were estimated over this period and the subsequent ten years. In each vaccination year, the vaccine would be administered at a time of the year immediately after a calving, lambing, and kidding season, or before mating. The vaccines S19 (supplied by the Tanzania Vaccine Institute) and Rev-1 (Ocurev, supplied by CZ Vaccines, Spain) were assumed to be used in cattle (5×10^9 viable organisms; reduced dose) and sheep and goats ($1\text{-}2 \times 10^9$ viable organisms; standard dose), respectively (Blasco, 2010; WOAH, 2019). The reduced dose (in cattle), route, and time windows for vaccination aimed to (i) minimise the possible side effects of the vaccine, including abortion, mammary infection, and excretion of the vaccine strain in the milk, and (ii) avoid a persistent antibody response that could potentially affect the ability to distinguish vaccinated from infected animals in the future.

The cost of the livestock vaccination programme included two cost types: i) information campaign expenditure to communicate the programme's objectives to the community, promote engagement of livestock keepers, and increase adherence to the programme; and, ii) vaccination costs, which included consumables, transport, labour, and cold chain costs.

A compartmental, discrete-time, susceptible-infected (SI) model was designed to represent brucellosis transmission dynamics in livestock. In addition to susceptible and infected compartments for each host species, a vaccinated compartment was used to represent the immunisation of livestock against brucellosis. The difference equations used were as follows:

$$S_{i,t+1} = S_{i,t} + \alpha_i N_{i,t} - \beta_{ii} S_{i,t} \frac{I_{i,t}}{N_{i,t}} - \sum_{\substack{j=1 \\ j \neq i}}^{n} \beta_{ij} S_{i,t} \frac{I_{j,t}}{N_{j,t}} - \mu_i S_{i,t} - \varepsilon_i \lambda_{i,t} \rho_i S_{i,t} \tag{4}$$

$$I_{i,t+1} = I_{i,t} + \beta_{ii} S_{i,t} \frac{I_{i,t}}{N_{i,t}} + \sum_{\substack{j=1 \\ j \neq i}}^{n} \beta_{ij} S_{i,t} \frac{I_{j,t}}{N_{j,t}} - \mu_i I_{i,t} \tag{5}$$

$$V_{i,t+1} = V_{i,t} + \varepsilon_i \lambda_{i,t} \rho_i S_{i,t} - \mu_i V_{i,t} \tag{6}$$

where t is the time (simulation year, from zero to 19), the subscripts i and j indicate livestock population (cattle, or sheep and goats; sheep and goats were considered together as a population), N is the total number of individuals, V is the number of vaccinated individuals, S is the number of susceptible individuals, I is the number of infected individuals, ρ is the proportion of animals that are eligible for vaccination, ε is the vaccine efficacy, λ is the proportion of eligible animals that are administered a vaccine, β_{ii} is the transmission rate between animals of population i, and, β_{ij} is the transmission rate from animals of population j to animals of population i.

Reference and alternative scenarios of vaccination were considered to assess the influence of key variables and parameters on the cost-benefit analyses outcomes. For the reference scenario, the value of ρ was obtained from the SEEDZ dataset: 67% in cattle and 83% in sheep and goats. The values of ε and λ were assumed to be 70% and 75%, respectively. The transmission rates (β_{ii} and β_{ij}) were calculated assuming that brucellosis is in endemic equilibrium, i.e., the incidence in livestock remains steady and the effective reproductive number (R_e), is 1.00.

Alternative scenarios consisted of different combinations of (i) population to be vaccinated, (ii) frequency of vaccination, (iii) efficacy of the vaccine used, and (iv) proportion of eligible animals to be vaccinated.

The disease transmission model was run for 20 years, under the assumption of no vaccination (*status quo*), and under the reference and alternative scenarios of livestock vaccination. The 20-year time horizon encompassed ten years dedicated to the vaccination program, except in the *status quo* scenario of no vaccination, and an additional ten years for follow-up. The monetary benefits of livestock vaccination (averted production losses in livestock) were estimated as the difference between the outputs of (i) the model run without vaccination (*status quo*) and (ii) the model run with vaccination (reference and alternative scenarios). A total of 50,000 Monte Carlo iterations of the disease transmission model were run for each of the reference and alternative scenarios. All monetary values were discounted over time at a rate of 3% per year.

To determine if and how cost-beneficial the reference and alternative vaccination strategies might be, the benefit-cost ratio (BCR) was used. The BCR was calculated as the production losses averted (benefits) divided by the cost of the vaccination programme.

RESULTS

Classification of households by production system

A total of 384 (24.9%) out of 1,541 households in SEEDZ, LSMS, and RHoMIS were classified as pastoral with the best-performing model. Key demographic and production characteristics of pastoral households are shown in Table 1.

Production losses attributable to livestock brucellosis

The median estimated individual-level prevalence values for brucellosis in cattle, sheep, and goats were 5.1% (95% uncertainty interval, UI: 3.4-6.9), 1.3% (95% UI: 0.1-3.0), and 2.5% (95% UI: 0.3-4.8), respectively. The median annual losses attributable to brucellosis in cattle, sheep, and goats, per infected animal, were 74.4 (95% UI: 26.2-211.7), 9.7 (95% UI: 3.4-23.1), and 10.6 (95% UI: 3.7-25.0) international dollars (int. $). At the household level, losses attributable to livestock brucellosis were equivalent to 4.4% (95% UI: 2.1-8.8) of the livestock-derived income per year. The total annual losses attributable to livestock brucellosis in the pastoral community of the study area were estimated at 14.0 million int. $.

Table 1. Key demographic and production characteristics of pastoral households in SEEDZ, LSMS, and RHoMIS

Parameter	Livestock species Cattle	Sheep	Goats	Data source
Total number of animals kept	10,108	8,697	9,934	SEEDZ
Birth rate[a]	0.23	0.33	0.38	SEEDZ
Death rate[a]	0.15	0.31	0.32	SEEDZ
Offtake rate[b]	0.08	0.02	0.06	SEEDZ
Wholesale price (int. $)[c]	467	57	57	LSMS and RHoMIS
Milk price (int. $/L)[d]	0.81	0.55[h]	0.55[h]	LSMS and RHoMIS
Milk harvest (%)[e]	82.3	22[h]	22[h]	LSMS and RHoMIS
Milk yield (L/day)[f]	1.3	1[h]	1[h]	LSMS and RHoMIS
Lactation length (days)[g]	180	120[h]	120[h]	LSMS

Int. $: international dollars. [a]The birth and death rates were obtained by dividing the total number of animals that were born and died in the 12 months preceding the interview, respectively, by the total number of animals kept at the time of the interview. [b]The offtake rate is the difference between the birth and death rates. [c]The wholesale price is the median income generated from the sale of each live animal during the 12 months preceding the interview. [d]The milk price is the average income from sale of milk per litre. [e]The milk harvest is the proportion of households that reported harvesting milk from livestock at least once in the 12 months preceding the interview. [f]The milk yield is the median volume of milk harvested per lactation day. [g]The lactation length is the average number of days lactating animals are milked for. [h]Sheep and goats combined.

Costs and benefits of livestock vaccination for brucellosis control

The total cost of the 10-year vaccination programme (reference scenario) in the Ngorongoro Conservation Area was estimated at 1,368,390 (95% UI: 1,186,279-1,710,905) int. $. The information campaigns were estimated to cost approximately 7.5% (95% UI: 6.0-8.7) of the total cost of the vaccination programme.

The expected prevalence of brucellosis under the reference vaccination scenario is shown in Fig.1. Over the 20-year time horizon, the prevalence of brucellosis in cattle would decrease from 5.1% (95% UI: 3.4-6.9) to 1.3% (95% UI: 0.9-1.9). In sheep and goats, the prevalence would decrease from 2.2% (95% UI: 0.2-4.6) to 0.6% (95% UI: 0.1-1.2).

Over the 20-year time horizon, the livestock production losses averted due to the vaccination programme (reference scenario) were estimated at 4,722,023 (95% UI: 1,774,978 - 13,159,388) int. $, 88.5% of which were in cattle. The benefit-cost ratio (BCR) was estimated at 3.4 (95% UI: 1.3-9.7).

All alternative vaccination scenarios had a median BCR greater than 1.0. The vaccination scenarios that were most cost-beneficial were those that consisted of cattle-only vaccination every two years, using a vaccine with high efficacy.

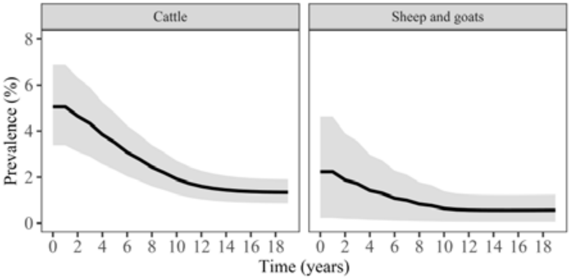

Figure 1. Estimated prevalence of brucellosis in cattle, and sheep and goats, over the 20-year time horizon, in the Ngorongoro Conservation Area (reference vaccination scenario). The solid lines indicate the median. The bands indicate the 95% uncertainty interval obtained from Monte Carlo simulation (50,000 iterations). The 20-year time horizon encompasses ten years dedicated to the vaccination program (vaccination every two years) and an additional ten years for follow-up

DISCUSSION

This study developed an integrated epidemiological-economic modelling framework to estimate the production losses attributable to livestock brucellosis and the potential costs and benefits of disease control. This framework was applied in the pastoral context of northern and central Tanzania where brucellosis is endemic in livestock and people.

The median household-level losses attributable to brucellosis were equivalent to 4.4% of the median annual, livestock-derived, household income expected in the absence of the disease. The endemic circulation of brucellosis and households' significant dependency on livestock and livestock products in this pastoral setting result in considerable losses.

The livestock production losses per infected animal estimated in this study were lower than those reported elsewhere. The median losses per infected animal were 74.4 int. $ per year in cattle, 9.7 int. $ in sheep, and 10.6 int. $ in goats. Elsewhere, the losses per infected animal have been estimated at over 100 int. $ in cattle (India, Brazil, Colombia, Uganda, Turkey, and Sudan) and over 20 int. $ in sheep and goats (India, Iraq, and Malaysia) (Santos et al., 2013; Patel et al., 2014; Bamaiyi et al., 2015; Singh et al., 2015; Angara et al., 2016; Homem et al., 2016; Alshwany, 2019; Bardhan et al., 2020; Guma et al., 2020; Al Hamada et al., 2021; Eşki et al., 2021).

Significant heterogeneity in methods used to estimate the losses attributable to livestock brucellosis hampers comparisons between study systems (Deka et al., 2018). This study aimed to estimate losses attributable to brucellosis, as defined by McInerney et al. (1992), i.e., the production potential that is not realised over time due to the presence of the disease. This definition does not include treatment expenditure, often considered in other studies (Santos et al., 2013; Angara et al., 2016). Also, specific impacts of the disease (e.g., temporary infertility or sterility) are often valued at the cost, at least partially, of the replacement animal (e.g., the price of a fertile adult animal), which overestimates the loss caused by brucellosis because the offtake value of an infected animal is, in many cases, not depreciated by the occurrence of sterility (Santos et al., 2013; Singh et al., 2015). Despite conservative assumptions and costings for impacts, the modelled vaccination intervention was highly cost-beneficial.

The monetary benefits of the livestock vaccination programme (reference scenario) outweighed its costs. An investment of approximately 1.4 million int. $ in the 10-year vaccination programme would result in a median of 4.7 million int. $ in production losses averted, representing a significant return on investment. The benefits of brucellosis control in this context would be over three times greater than its costs. The monetary benefits would exceed the costs in most alternative scenarios of livestock vaccination for brucellosis control in the NCA.

This study did not consider the potential benefits of reduced human brucellosis incidence that would be expected due to livestock vaccination. Possible benefits of reduced human brucellosis incidence include increased human productivity and reduced health-related out-of-pocket expenditure. Downstream effects on the household economy and health services would also be expected. Livestock disease control through vaccination has been associated with increased school attendance by children, particularly girls, in pastoral communities (Marsh et al., 2016). Potential impacts of livestock vaccination on the human health service include reduced resource use (e.g., beds) in local hospitals and health centres.

In conclusion, our study shows that brucellosis causes considerable monetary losses in pastoral communities of northern and central Tanzania. Livestock vaccination for brucellosis control, as modelled in this study, would be cost-beneficial. The results of this study may help inform the development and implementation of brucellosis control efforts in Tanzania. The methods applied to estimate the economic impact of brucellosis and the benefits of control actions are likely applicable in other endemic settings.

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COST EVALUATION OF THE IMPLEMENTATION OF BIOSECURITY MEASURES IN BOVINE DAIRY FARMS

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SUMMARY

An efficient biosecurity program is crucial for maintaining animal health, enhancing farm productivity, and ensuring profitability. This study focused on evaluating the costs associated with implementing and maintaining specific biosecurity measures (BSMs) in dairy cattle farms. An Excel-based tool was developed to calculate total expenses (with and without amortization) linked to BSMs, considering farm characteristics and the chosen set of measures. Costs varied based on farm size and the comprehensiveness of their biosecurity program. Across all farms, the primary contributors to total cost were BSMs related to introducing new animals and managing water and feed. The tool, with its itemized breakdown of costs, serves as a valuable resource for farmers, assessors, and veterinarians, aiding decision-making in implementing biosecurity programs to prevent the introduction of infectious pathogens.

INTRODUCTION

Over the past five decades, there has been a notable intensification of the dairy industry, predominantly observed in high-income countries. This intensification is motivated by the goal to enhance the economic efficiency of production, leading to a consolidation of farms into fewer, larger entities housing a greater number of animals, thereby achieving significant increases in milk production (Clay et al., 2020).

A farm environment constitutes an intricate mix of production activities. Realizing optimal returns from these activities requires a profound understanding of technical practices and an appropriate resource allocation. The rationale of production economics provides a structured framework for decision-making within the livestock unit (Rushton, 2008).

Several infectious pathogens have the potential to impact bovine cattle, thereby creating an economic burden on the farm's finances. Neosporosis (Reichel et al., 2013), paratuberculosis (Garcia and Shalloo, 2015), bovine viral diarrhoea (Stott et al., 2003), and infectious bovine rhinotracheitis (Iscaro et al., 2021) are instances of diseases that significantly affect farms. These agents can be introduced into farms in several ways: through the entry of animals, through contaminated water and feed, through contact with wildlife or transported by employees or vehicles entering the facilities (Dewulf and Immerseel, 2019). The implications of a disease establishing itself on a farm differ based on the pathogen, the extent of its spread

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within the herd, and the specific country or zone where the farm is situated. These consequences can be detrimental to various stakeholders. The most immediate impact involves economic losses stemming from disease-related mortality, culling of animals, veterinary services, a decline in milk production, delayed age at first calving, herd replacement, among others (Maunsell and Donovan, 2008).

The optimal method for addressing this concern involves adopting a preventive strategy to minimize exposure to pathogens, thereby safeguarding animal health and sustaining elevated production yields. Biosecurity entails implementing a variety of measures within a production system to diminish the risk of introducing and spreading disease agents (Kristensen and Jakobsen, 2011; Sayers et al., 2013; Dewulf and Immerseel, 2019; Alarcón et al., 2021). A sound biosecurity program's implementation should be tailored to each farm, considering the risks in the region, the production system, herd size, and the farm's premises and location. This customization is essential to prevent farmers from resisting the adoption of biosecurity programs (Brennan and Christley, 2012).

Biosecurity is generally divided in two components. External biosecurity refers to preventive measures designed to avoid the introduction of pathogens, whereas internal biosecurity relates to measures to limit within-farm transmission of infectious agents (Mee et al., 2012).

In the case of external biosecurity, aspects related to the purchase and introduction of replacement animals, contamination of water and feed, visitors, vehicles and contact with animals from neighbouring farms and wildlife are considered. It is important to consider the health status of the farms of origin for new entries, appropriate quarantine, testing for relevant pathogens on arrival, and good hygiene practices. Purchasing feed from a supplier with regular quality controls and having separate storage areas where feed is kept is recommended. Similarly, the hygiene of drinking water should be guarantee through adequate storage, treatment (e.g., peroxides, chlorine dioxide, etc) and tested regularly to control the presence of pathogens. Access for visitors and vehicles should be limited. It is essential that farm workers and visitors change clothes and boots before having contact with animals from the farm. The adoption of an effective pest control strategy can reduce the likelihood of encountering rodents or insects in the farm (Brennan and Christley, 2012; Dewulf and Immerseel, 2019; Benavides et al., 2021).

Hence, a biosecurity programme entails a substantial investment demanded from the farmer, along with monthly expenses arising from the implementation of these measures and the additional time spent on training employees and executing essential tasks. Among the different factors that can influence attitude and behaviour, the costs associated with biosecurity measures as well as the expected benefits resulting from implementing biosecurity measures are among the most important elements and have been linked to the decision to implement biosecurity measures on livestock farms (Gunn et al., 2008; Sayers et al., 2013, 2014; Toma et al., 2015; Broughan et al., 2016; Renault et al., 2018; Moya et al., 2020). Based on this knowledge, some studies have tried to relate the benefits of applying biosecurity measures on cattle farms to the improvement of farm productivity and profitability (Rojo-Gimeno et al., 2016; Renault et al., 2018; Damiaans et al., 2020).

Given the above, the aim of this study was to estimate the economic cost of the different external biosecurity measures applied in Spanish dairy cattle farms using a cost accounting analysis (Rushton, 2008; Rojo-Gimeno et al., 2016).

MATERIALS AND METHODS

Constructing a price database for biosecurity measures.

Four main pathways of pathogen introduction into the farm were identified, including through the introduction of new animals into the herd, people in close contact with the animals and vehicles entering the farm premises, water and feed contamination, and direct or indirect contact with pests or cattle from neighbouring farms.

For each of the above-mentioned pathways, the specific measures were extracted from the literature, listed and broken down into individual “items” to which a market price could be allocated. An excel spreadsheet was constructed in an Excel spreadsheet.

Once the "items" for each biosecurity measure had been selected, a further review was carried out to establish a market price for each of them, checking this information on the websites of livestock or veterinary suppliers. The criteria for the inclusion of these suppliers were that they were based in or distributed to Spain so that the price could be adjusted and comparable. At least one and maximum three different prices were sought from different sources for each item to calculate an average price per unit.

Creating the tool to calculate costs.

An Excel sheet was to be filled in with the following data concerning the assessed farm, separated into five sections. These sections included general information, such as the farm's name, animal census, number of employees per day, and standard salaries. Additionally, there was information about the introduction of new animals, covering aspects such as the annual frequency, total number, and quarantine details, such as duration of the quarantine period, perimeter of the area designated for quarantine, and daily frequency of entrance (by employees) and time spent in this area. Information on access to the farm, such as fencing or wall perimeter, monthly non-employee visits, and vehicle frequency. The data also encompassed information about water and feed, specifically the number of feeding points. Finally, details about pest and animal control were included, specifying whether the farm engaged an external company for pest extermination and control.

Under each section, the corresponding external biosecurity measures were listed to indicate whether each measure was implemented in the farm or not. For each section there was a “COST” column linking the selected biosecurity measures and the farm inputs with the prices database, computing the annual total cost of each measure, and the annual total in relation to the amortization of the assets which can be used for more than one year (e.g., building the perimeter fence involves a high total cost, but it can last up to 30 years, so the total cost is divided by its years of depreciation). The result was the total annual cost of all the external biosecurity measures selected, and a second total annual cost considering amortization.

To test the tool, a survey was created to collect the necessary data on the background and biosecurity measures of existing dairy cattle farms and was given to farmers or veterinarians. In addition, two "prototype" farms were designed with a full external biosecurity protocol applied to assess what their hypothetical cost would be.

RESULTS

The tool was used in seven farms. A brief description of each farm is given below:

Farm 1: A prototype intensive dairy farm, designed with a census of 600 animals, 800 meters (m) of fenced perimeter and a 100 m fenced perimeter for an area designated for quarantine. A total of 200 new animals are introduced to the herd each year divided in 4 entries, where they spend 15 days in quarantine in which they are tested for BVD and IBR. The access to the premises has a drive-through disinfection bath for vehicles and footbaths for the employees and external visits. The farm also applies periodical insect and rodent control measures and performs chemical treatment of the drinking water for the herd.

Farm 2: A second prototype of a smaller extensive grazing farm, with a census of 60 animals, with a total of 150 m fenced (including fencing of a quarantine area to separate animals), and some of the fences are electrified. Only 20 new animals are introduced each year at one time, spending 32 days in quarantine where they are tested for BVD, IBR and Neosporosis. Water is chemically treated and some measures to avoid indirect contact with wildlife are implemented.

Farm 3: An intensive system farm in Catalonia, with 1150 animals and a fenced perimeter of 950 m. 140 new animals are introduced each year and tested for IBR and Neosporosis, but not placed in quarantine when arriving to the farm. The drinking water is chlorinated, and they perform rodent and insect control.

Farm 4: An intensive dairy farm located in Catalonia with a census of 1100 animals and a fenced perimeter of 740 m. There is no entry of new animals as they have internal replacement, therefore no quarantine is carried out; the water is chlorinated, and there is periodic control of rodents and insects.

Farm 5: A farm in Catalonia with 924 animals and an unfenced perimeter of 1024 m has a quarantine area for the 463 new animals they introduce each year (in separate batches twice a month), which are tested for BVD and IBR. Drinking water is chemically treated and there is no pest control programme.

Farm 6: An intensive dairy farm in Catalonia, with 700 animals and no perimeter fencing. A total of 240 animals are introduced per year (in separate batches every 15 days), tested for BVD, IBR and Neosporosis on the farm of origin and not quarantined on arrival. Drinking water is chlorinated and regular rodent and insect control is carried out.

Farm 7: The farm located in Catalonia has a census of 83 animals, the perimeter of 183 m is not fenced, and 42 new animals are introduced per year, tested for BVD and IBR, but not quarantined. Drinking water is not chemically treated and no rodent control measures are applied.

The following tables show the total annual external biosecurity costs for each farm in euros, separated in four sections corresponding to the four pathways of pathogen introduction mentioned previously (introduction of new animals, access to the farm, contamination of water and feed, and contact with pests and other animals) and the total sum. Table 1 shows the total costs while Table 2 shows the costs considering the amortization of certain measures.

Table 1. Total external biosecurity costs for each farm divided into the four pathways of pathogen introduction considered

Farm	New animals	Access to farm	Water and feed	Pests & neighbors	Total
1	11.107,54 €	138.904,74 €	4.681,12 €	3.740,17 €	158.460,57 €
2	2.292,49 €	3.129,99 €	3.200,94 €	877,4 €	9.500,82€
3	2.403,33 €	29.711,97 €	8.435,08 €	2.634,87 €	40.814,25 €
4	0 €	43.407,27 €	8.203,08 €	2.337,93 €	53.949,28 €
5	13.476,67 €	0 €	6.211,42 €	0 €	19.688,09 €
6	5792 €	1.712,88 €	5.172,12 €	2.837,64 €	15.514,64 €
7	700 €	0 €	1.924,12 €	722,69 €	3.346,81 €

Table 2. Total external biosecurity costs (with amortization) for each farm divided into the four pathways of pathogen introduction considered

Farm	New animals	Access to farm	Water and feed	Pests & neighbors	Total
1	4.385,34 €	8.573,74 €	2.890,9 €	3.740,17 €	19.590,15 €
2	808,59 €	162,19 €	548,9 €	95,62 €	1.615,3 €
3	2.403,33 €	1.517,86 €	5.540,81 €	2.634,87 €	12.096,87 €
4	0 €	2.279,8 €	5.288,94 €	2.337,93 €	9.927,54 €
5	13.476,67 €	0 €	4.394,25 €	0 €	17.870,92 €
6	5.792 €	1.650,1 €	3.354,89 €	2.837,64 €	13.634,63 €
7	700 €	0 €	106,9 €	722,69 €	1.529,59 €

DISCUSSION

Comparing Table 1 and Table 2, it is evident that the amortized costs are significantly lower than the total costs. This observation is particularly pronounced in the prototype farms (farms 1 and 2), where amortized costs decrease by over 80% in comparison to total costs. In the case of real farms (farms 3-7), this reduction ranges from 9.2% to 70.3%. The variance arises due to the enduring nature of certain substantial costs. For instance, constructing a perimeter wall for the farm incurs a high initial investment, but this cost is distributed over the total years of amortization, and maintenance costs are relatively low. Similar considerations apply to the procurement of equipment for feed storage and feeding and drinking troughs (Rushton, 2008). Farms 1 and 2 exhibit a comprehensive external biosecurity protocol, with measures covering all four paths of pathogen entry, leading to a higher initial cost but a significant difference with amortized costs. Conversely, farms 3-7, being operational farms, implement lighter biosecurity programs, with variations in fencing and quarantine areas, resulting in less disparity between total and amortized costs.

Focusing on farms 1 and 2, the cost disparity arises from farm 1 having a larger animal census and introducing more animals annually. Moreover, farm 2's extensive grazing system limits the implementation of biosecurity protocols compared to an intensive system. In both farms, measures related to introducing new animals and water and feed contamination contribute most to total costs.

Similarly, real farms (farms 3-7) differ in animal census and biosecurity measures but share a proportional distribution of costs. On average, measures related to introducing new animals constitute 40% of total amortized costs, followed by water and feed measures at 34%, pests and wildlife at 16%, and farm access at 10%. However, direct comparison is challenging due to variations in costs, such as farms lacking certain fencing or exchange areas.

Analysing the biosecurity measures in real farms reveals areas for potential enhancement. In the case of farm 3, despite testing introduced animals for BVD and neosporosis, it would be prudent to establish an isolated area for their observation during a quarantine period to safeguard against the potential introduction of other diseases. Additionally, allocating a designated area within the enclosure as a changing room for employees could prevent contamination outside the farm by ensuring that their daily work clothes and boots remain within the premises. This measure could also enhance the biosecurity of farm 4, which, despite having a more comprehensive program, could benefit from adopting a similar practice. Notably, farm 4 does not require a quarantine area for new animals, as they manage replacements internally.

In the cases of farms 5, 6, and 7, a potential method for enhancing biosecurity would be to enclose the area or install fencing to segregate areas that present a higher risk of trespassing or contact with other animals. Additionally, farm 5 could consider introducing pest control measures to assess and eradicate potential infestations. Farm 6 exhibits a vulnerability in its practice of placing new animals in a separate area, which, despite being distinct from the herd, involves sharing feeding troughs, thus lacking genuine isolation. Similarly, farm 7 faces shortcomings in lacking a quarantine protocol, pest control, and stands as the sole farm without any form of drinking water treatment.

However, it is essential to emphasise that the number and effectiveness of biosecurity measures that can be implemented on each farm depends on several factors, such as financial resources, the topography of the site and the specific risks faced. Furthermore, even with the introduction of new measures, there is a continuous need to assess employee compliance and effectiveness and to identify limitations and weaknesses to develop a tailored and efficient programme.

This tool could be a valuable for a cost-benefit analysis of implementing a biosecurity programme on dairy cattle farms (Mcleod, 1995). However, a comprehensive analysis would require data on the costs of internal biosecurity measures, the costs of diseases and the effectiveness of biosecurity measures to prevent these diseases. If all the necessary information were available, it could be of great benefit to farmers in determining the optimal allocation of resources and investments needed to improve animal health and productivity.

Developing a tool to accurately calculate biosecurity costs for each farm is challenging due to the various limitations and assumptions of the process. Firstly, the selection of measures and the "items" required may not be universally applicable, as farms may have implemented different versions of the same measure. For example, when calculating the cost of perimeter fencing, the tool offers four options: brick wall, concrete wall, concrete block wall and wire fence. The cost of each of these is calculated based on a specific height per linear metre. Therefore, if a farm were to use a different material or height, the price would vary, making the tool's cost calculation inaccurate. Similarly, some costs require an on-site assessment, such as when farms contract an external company for pest control. To obtain a quotation for these services, the company must visit and assess the actual premises, as the cost depends on the size and degree of infestation the farm may have.

To calculate specific costs, it was necessary to make certain assumptions, regarding additional time spent on specific tasks, such as time spent by employees inspecting the perimeter fence/wall, checking and replacing rodent or insect traps, as well as daily time spent in the quarantine area. In addition, to standardise the cost of the disinfectant used in the footbaths and for vehicle tyres when entering the farm, a specific standard volume of water was proposed. After that, the amount of disinfectant was calculated according to the manufacturer's specifications. On the other hand, regarding the diagnosis of diseases in replacement animals introduced into the herd (e.g., BVD, IBR, neosporosis), the cost was calculated assuming that all incoming animals are tested. However, it is important to note that farms with a high replacement rate may choose to test only a fraction of these animals. A factor that also limits the accuracy of the tool is that economies of scale are not considered. The price per unit of each item decreases when purchased in bulk, thus the bigger farms with a higher census could have lower costs than the ones procured by the tool.

To assess the accuracy of the tool in determining the annual cost of external biosecurity measures, it would be necessary to examine the financial records of the farms and compare the actual expenditure to the values generated by the tool. The latter, coupled with the fact that farmers may be reluctant to publicly disclose such information (Gilmour et al., 2011), can make it challenging to have fully reliable information.

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INTERVENTION EVALUATION

THE IMPACT OF FLOCK DEMOGRAPHICS ON POST-VACCINATION IMMUNITY LEVELS AGAINST PESTE DES PETITS RUMINANTS VIRUS IN HETEROGENEOUS SMALL RUMINANT POPULATIONS

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SUMMARY

Peste des petits ruminants (PPR) is an acute infectious disease of small ruminants affecting flocks throughout large parts of Africa, Asia and the Middle East. The Global Strategy for Control and Eradication (GSCE), targeting global eradication by 2030, recommends vaccination of small ruminants in endemic regions to attain 70% post-vaccination immunity. We develop a deterministic, age- and sex-structured, demographic model of flock dynamics and conduct a global sensitivity analysis to identify the most important demographic parameters influencing post-vaccination immunity in pastoral small ruminant populations in sub-Saharan Africa. The model is parameterised using empirical demographic data extracted from the literature to explore post-vaccination immunity levels in different flock profiles. We show that under the GSCE recommendation, immunity decays below 70% within 12 months for all profiles. We highlight the importance of tailoring vaccination to local demographic contexts and the need for widely available, standardised demographic data to characterise small ruminant populations.

INTRODUCTION

Peste des petits ruminants (PPR) is an acute infectious disease of small ruminants, caused by peste des petits ruminants virus (PPRV), which is endemic throughout large parts of Africa, Asia and the Middle East. PPRV endemicity disproportionately impacts subsistence farmers in affected regions, with outbreaks causing high morbidity and mortality in susceptible flocks and estimated global economic losses of up to US$2billion per year (Jones et al., 2016). The World Organisation for Animal Health (WOAH) and the Food and Agriculture Organisation of the United Nations (FAO) launched the global strategy for the control and eradication of PPRV (GSCE) in 2015 with the target of global PPRV eradication by 2030 (FAO and OIE, 2015). Central to the GSCE is mass vaccination of endemic small ruminant populations aiming for a post-vaccination immunity level of at least 70% to prevent viral circulation (FAO & OIE, 2015).

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Progress towards PPRV eradication is challenged by the diversity of small ruminant populations affected. In endemic regions, livestock are managed under three main production systems: pastoral, agro-pastoral and mixed crop-livestock. Variations in the geographic and climatic conditions, and religious and cultural contexts in which small ruminants are reared results in adoption of diverse husbandry practices. These differences generate heterogeneity in the demographic characteristics of small ruminant flocks impacting flock turnover rates. The effectiveness of vaccination depends on flock turnover as immune animals exit the flock through deaths (e.g. predation, disease) or offtake (e.g. slaughter, sales, gifts given, loans) and new, susceptible animals enter the flock through births, and intake (e.g. purchases, gifts received) resulting in a decay in flock immunity levels. Demographic processes which are linked to high turnover rates may therefore drive rapid decay in post-vaccination immunity. Through improved understanding of how demographics affect flock turnover and immunity dynamics, it may be possible to characterise types of flock or production systems – associated with certain demographic characteristics – where current vaccination strategies fail to maintain immunity levels, and to inform the design of tailored vaccination campaigns.

The objective of this study was to explore post-vaccination immunity dynamics in different small ruminant populations ("flock profiles") using a deterministic, age- and sex-structured, demographic model of flock dynamics. Through a sensitivity analysis, the most important demographic parameters influencing flock immunity levels were identified, providing targets for data-collection activities. The model was then used to simulate post-vaccination immunity dynamics in different small ruminant flock profiles to assess the effectiveness of the GSCE vaccination programme for small ruminant flocks in PPRV-endemic regions of sub-Saharan Africa.

MATERIALS AND METHODS

Overview

A deterministic, age- and sex-structured flock dynamics model was developed to explore the impact of demographic processes on small ruminant flock immunity levels following vaccination. A global sensitivity analysis was conducted to assess the sensitivity of post-vaccination immunity levels to uncertainty in different demographic parameters. The model was parameterised using empirical demographic data, extracted from the literature, to represent different small ruminant flock profiles in PPRV endemic regions of sub-Saharan Africa (primarily East and West Africa). The model was used evaluate the effectiveness of the GSCE vaccination-programme.

Demographic Model Structure

The model considered a single small ruminant flock, structured into age classes and differentiated by sex (denoted m for males and f for females). The model was in discrete time (t), with a timestep of 1 week, such that animals within each age group (denoted i) could move into the next age-class (i+1), or leave the flock, within a timestep.

Health State Transitions

The model comprised two mutually exclusive health states: susceptible (S, animals with no history of infection or vaccination) and immune (R, animals with lifelong immunity from vaccination). At time T_v a proportion , p_v, of susceptible animals (S) were vaccinated and moved into the immune state (R). For animals under 4 months ($i \leq$ 17 weeks) only, an additional compartment, *born-immune* (B), was included to account for offspring which gain PPRV immunity due to maternal antibodies. Newborn offspring born to immune females (R_f) entered the *born-immune* state ($B_{f,1}, B_{m,1}$) with probability p_B or the susceptible state ($S_{f,1}, S_{m,1}$) with probability $1 - p_B$. In each timestep, *born-immune* animals (B_i) could transition to susceptible (S_i) with rate τ_i. The decay in maternal immunity was informed by empirical data on the waning of maternal antibodies in lambs born to vaccinated mothers (Bodjo et al., 2006).

Demographic Transitions

Within a timestep animals exited the flock through mortality and offtake, and entered the flock through births and intake. Females above the minimum age of reproduction (A_R) reproduced with rate α, defined as the mean number of (live) offspring produced per female within a timestep (one week). α was fixed and equal for all reproductive females for the duration of the simulation and the ratio of female to male offspring was 1:1. Hence, female births per timestep could be computed from equations 1 and 2:

$$B_{f,1}(t) = \frac{\alpha}{2} \cdot p_B \cdot \sum_{i=A_R}^{A_F} R_{f,i}(t-1) \tag{1}$$

$$S_{f,1}(t) = \frac{\alpha}{2} \cdot (1 - p_B) \cdot \sum_{i=A_R}^{A_F} R_{f,i,}(t-1) + \frac{\alpha}{2} \cdot \sum_{i=A_R}^{A_F} S_{f,i}(t-1) \tag{2}$$

The weekly mortality risk (μ_i) varied with age but was equal between sexes, with μ_1 for animals younger than 6 months ($i \leq$ 26 weeks), and μ_2 for animals older than 6 months ($i >$ 26 weeks) such that $\mu_1 \gg \mu_2$ accounting for the high reported mortality in young stock (Otte & Chilonda, 2002; Hassen & Tesfaye, 2014). A maximum age for males (A_M) and females (A_F) was defined as the final age category ($\mu_{i,f} = 1$ for $i = A_F$, and $\mu_{i,m} = 1$ for $i = A_M$). The weekly net offtake risk (θ) was the net movement of animals out of the flock (θ = weekly intake – weekly offtake). A minimum age of exchange, A_X, was defined such that male or female animals could be exchanged if $i \geq A_X$. θ was age and sex stratified resulting in 4 offtake parameters: θ_y for animals (male or female) younger than the minimum age of exchange ($i < A_X$), here we assumed θ_y = 0; θ_f for females older than the minimum age of exchange ($i \geq A_X$); θ_{m1} for young adult male animals, older than the minimum age of exchange and younger than 24 months ($i \geq A_X$ and $i \leq 104$ weeks) and θ_{m2} for adult male animals older than 24 months ($i > 104$ and $i < A_M$). The young adult male offtake risk (θ_{m1}) accounted for the high reported offtake risks of males from puberty to 2 years such that $\theta_{m1} \geq \theta_{m2}$ and $\theta_{m1} \gg \theta_f$ (Hassen & Tesfaye, 2014; Lesnoff, 1999; Tadesse et al., 2014). For each group, θ was fixed and constant throughout the simulation (although see Hammami et al., 2016 for an evaluation of the impact of temporal offtake dynamics on post-vaccination immunity dynamics). The demographic equations for female animals were computed as follows:

$$B_{f,i}(t) = B_{f,i-1}(t) \cdot \tau_{i-1} \cdot \left(1 - \theta_{f,i-1}\right) \cdot \left(1 - \mu_{f,i-1}\right) \qquad if\ i \leq 17 \tag{3}$$

$$S_{f,i}(t) = \begin{cases} S_{f,i-1}(t-1) \cdot \left(1 - \theta_{f,i-1}\right) \cdot \left(1 - \mu_{f,i-1}\right) + B_{f,i-1}(t-1) \cdot (1 - \tau_{i-1}) & if\ i \leq 17 \\ S_{f,i-1}(t-1) \cdot \left(1 - \theta_{f,i-1}\right) \cdot \left(1 - \mu_{f,i-1}\right) & if\ i > 17 \end{cases} \tag{4}$$

$$R_{f,i}(t) = R_{f,i-1}(t-1) \cdot \left(1 - \theta_{f,i-1}\right) \cdot \left(1 - \mu_{f,i-1}\right) \qquad if\ i \leq 17 \qquad (5)$$

Global Sensitivity Analysis

A global sensitivity analysis was conducted to determine the most important demographic parameters influencing post-vaccination immunity levels. Demographic parameter ranges were extracted from the literature, and supplemented by expert opinion where data were lacking, to represent pastoral and agro-pastoral small ruminant flocks in PPRV endemic regions of sub-Saharan Africa (primarily East & West Africa) (Table 1). A latin hypercube sampling procedure was used to generate 200,000 parameter sets with 10 variable demographic parameters included using the R sensitivity package (Iooss et al., 2023). The simulation period was 25 years and all animals were initially susceptible. Vaccination was implemented at the mid-point of the simulation (12.5 years, $T_v = 652$) allowing time for flock growth rates and age-sex structures to stabilise before vaccination. At T_v, 100% animals were vaccinated within one timestep, assuming a post-vaccination immunity level of 100% (i.e. 100% efficacy). Three immunity metrics were computed to analyse post-vaccination immunity dynamics: (i) immunity at 6 months post-vaccination, (ii) immunity at 12 months post-vaccination and (iii) weeks post-vaccination with immunity ≥70%. Population growth in the final 10 years of the simulation was also computed. Model outputs were validated against the expected growth rates and age-sex structures of pastoral and agro-pastoral small ruminant populations (Table 2). Parameter sets failing to meet these criteria were discarded. Partial rank correlation coefficients (PRCCs) were computed using the epiR package in R (Stevenson et al. 2023). PRCCs are commonly used in sensitivity analyses of infectious disease models (Sanchez and Blower, 1997; Wu et al., 2013; Sumner et al., 2019; Sumner and White, 2022), providing a measure of the monotonicity between model inputs and outputs to indicate the extent to which uncertainty in input parameters accounts for variation in output metrics while considering possible correlations between input parameters.

Table 1. Demographic parameter ranges extracted from the literature for global sensitivity analysis, representing pastoral and agropastoral small ruminant flocks in sub-Saharan Africa

Parameter	Symbol	Minimum	Maximum	References
Net offtake risk, young[a]	θ_y	0	0	Hassen & Tesfaye, 2014
Net offtake risk, young male[a]	θ_{m1}	0.4	0.99	Lesnoff, 1999; Hassen & Tesfaye, 2014; Tadesse et al., 2014
Net offtake risk, adult male[a]	θ_{m2}	0	0.3	Otte & Chilonda, 2002; Tadesse et al., 2014; Yirga et al., 2020
Net offtake risk, adult female[a]	θ_f	0	0.3	Otte & Chilonda, 2002; Tadesse et al.,; Yirga et al., 2020
Mortality risk young[a]	μ_y	0.15	0.55	Otte & Chilonda, 2002; Hassen & Tesfaye, 2014
Mortality risk adult[a]	μ_a	0.05	0.33	Otte & Chilonda, 2002; Hassen & Tesfaye, 2014; Tadesse et al., 2014; Yirga et al., 2020
Birth rate[a]	α	1	2.5	Otte & Chilonda, 2002; Hassen & Tesfaye, 2014; Tadesse et al., 2014; Yirga et al., 2020
Minimum age of exchange (months)	A_X	9	14	Hassen & Tesfaye, 2014; Tadesse et al., 2014
Age of first parturition (months)	A_R	10	18	Otte & Chilonda, 2002; Tadesse et al., 2014; Yirga et al., 2020
Maximum age of males (years)	A_M	3	5	Kosgey et al., 2008
Maximum age of females (years)	A_F	7	12	Kosgey et al., 2008

[a] Annual risk/rate.

Table 2. Flock growth and age-sex structure conditions extracted from the literature representing pastoral and agro-pastoral small ruminant flocks in sub-Saharan Africa

Parameter	Minimum	Maximum	References
10-year growth	0.85	1.15	Tadesse et al., 2014; Yirga et al., 2020
Female <6months	0.05	0.19	Gebre Mariam, 1991; Tadesse et al., 2014; Tesfahun et al., 2017; Yirga et al., 2020
Female 6-12months	0.06	0.15	Gebre Mariam, 1991; Tadesse et al., 2014; Tesfahun et al., 2017; Yirga et al., 2020
Female >12months	0.21	0.62	Gebre Mariam, 1991; Tadesse et al., 2014; Tesfahun et al., 2017; Yirga et al., 2020
Male <6months	0.05	0.16	Gebre Mariam, 1991; Tadesse et al., 2014; Tesfahun et al., 2017; Yirga et al., 2020
Male 6-12months	0.04	0.15	Gebre Mariam, 1991; Tadesse et al., 2014; Tesfahun et al., 2017; Yirga et al., 2020
Male >12months	0.01	0.15	Gebre Mariam, 1991; Tadesse et al., 2014; Tesfahun et al., 2017; Yirga et al., 2020

Applied Analysis

The effectiveness of the PPRV GSCE vaccination programme was assessed by analysing post-vaccination immunity dynamics in different small ruminant populations. Flock profiles were extracted from the literature to represent populations found in PPRV-endemic regions, including sheep and goat flocks under pastoral and mixed crop-livestock systems in different agro-ecological zones across East and West Africa (Otte and Chilonda, 2002). For each flock profile, 10,000 parameters sets were generated through latin hypercube sampling of the parameter ranges reported, if no range was provided $\pm 10\%$ variation was added to the reported value. Net offtake of young males (θ_{m1}) was rarely specified, we assumed that the lower boundary was equal to the minimum net offtake risk of the flock and the upper boundary was equal to the maximum value which produced valid population growth and age-sex structures in the global sensitivity analysis (Methods; *Global Sensitivity Analysis*). The simulation period was 20 years (1040 weeks), and all animals were initially susceptible. The GSCE vaccination programme was simulated including 4 consecutive annual campaigns, beginning at the mid-point of a simulation (T_v = 520 weeks). In rounds 1 and 2, animals aged over 4 months were vaccinated and in rounds 3 and 4 animals aged 4 to 12 months were vaccinated, as specified in the GSCE (FAO and OIE, 2015). Each vaccination round was implemented within one timestep, and all eligible animals gained immunity (i.e. pV = 100% for eligible animals with 100% vaccine efficacy assumed). Flock growth over the ten-year simulation period, and the stable age-sex structure for each parameter set were initially validated against the conditions used for the global sensitivity analysis (Table 2). Parameter sets failing to meet these criteria were discarded and, for the remaining parameter sets, immunity at 6 months post-vaccination, immunity at 12 months post-vaccination, and the number of weeks with immunity ≥70%, were computed. The proportion of immune animals at the first timestep following vaccination, was also computed. Model development and all analyses were carried out using R statistical computing environment (R Core Team, 2023).

RESULTS

Global Sensitivity Analysis: the most influential demographic parameters on flock immunity

When validated against flock growth and age-sex structure conditions (Table 2), 4140 of the 200,000 parameter sets generated through latin-hypercube-sampling produced valid outputs. Invalid parameter sets were discarded from the sensitivity analysis. The PRCCs for the flock immunity metrics and each of the 10 demographic parameters are given in Table 3. All PRCCs were statistically significant ($p<0.05$) except the PRCCs for the maximum age of males, the maximum age of females, and the net offtake risk of adult females. The order of the PRCC indicates the degree to which uncertainty in a given demographic parameter influences variability in post-vaccination immunity. The PRCCs indicated that the demographic parameters which were most influential on post-vaccination immunity levels (i.e. PRCC $> \pm 0.5$), in pastoral and agro-pastoral small-ruminant flocks were young male offtake risk, birth rate, minimum age of offtake, minimum age of reproduction, and youth mortality risk (based on PRCCs for weeks with >70% immunity, in descending order of magnitude). Demographic parameters which were positively correlated with flock immunity levels, an increase in the parameter value resulted in higher flock immunity levels, included: the minimum age of offtake, the minimum age of reproduction, and the mortality rate of young animals. Demographic parameters which were negatively correlated with flock immunity levels, i.e. an increase in the parameter value resulted in lower flock immunity levels, included: birth rate, the mortality risk of adults and net offtake risks (θ_{m1}, θ_{m2}, θ_f).

Applied analysis: the effectiveness of GSCE vaccination in different small ruminant populations

This analysis of flock immunity following GSCE vaccination in different small ruminant flock profiles focuses on immunity levels following the first annual vaccination campaign (at $T_v = 520$). Following vaccination of all eligible animals the median flock immunity level was 0.849 over all flock profiles (range 0.78-0.90). The proportion of animals which failed to gain immunity is accounted for by the proportion of animals younger than 4 months which are not eligible at the time of vaccination. Post-vaccination immunity levels were highly variable within and between different flock profiles, however, at 12 months post-vaccination, immunity was not maintained above 70% in any flock profile. The median duration of immunity above the 70% threshold was 32 weeks post-vaccination, approximately 7 months (range 20-50 weeks) (Table 4, Fig.1).

Table 3. Partial Rank Correlation Coefficient (PRCC) values for flock immunity metrics and 10 demographic parameters, ranked in descending order of magnitude for weeks with immunity >70%

Parameter	Symbol	Weeks with immunity >70%		Immunity at 6 months		Immunity at 12 months	
		PRCC	p	PRCC	p	PRCC	p
Net offtake risk, young male[a]	θ_{m1}	-0.865	<0.001	-0.903	<0.001	-0.860	<0.001
Birth rate[a]	α	-0.802	<0.001	-0.859	<0.001	-0.813	<0.001
Minimum age of exchange[a]	A_X	0.759	<0.001	0.825	<0.001	0.612	<0.001
Age of first parturition[a]	A_R	0.722	<0.001	0.791	<0.001	0.746	<0.001
Mortality risk, young[a]	μ_y	0.640	<0.001	0.469	<0.001	0.695	<0.001
Net offtake risk, adult male	θ_{m2}	-0.285	<0.001	-0.331	<0.001	-0.287	<0.001
Mortality risk, adult	μ_a	-0.250	<0.001	-0.336	<0.001	-0.222	<0.001
Net offtake risk, female	θ_f	-0.085	<0.001	-0.022	0.167	-0.125	<0.001
Maximum age of males	A_M	0.016	0.323	0.013	0.424	-0.008	0.590
Maximum age of females	A_F	0.008	0.622	-0.052	0.001	0.022	0.164

[a] Parameters with PRCC>±0.5 for weeks with immunity >70%.

Table 4. Summary statistics for post-vaccination immunity levels after 1 round of vaccination in small ruminant populations in sub-Saharan Africa. Vaccination was implemented with 100% coverage of animals older than 4 months

Immunity metric	Mean	Median	Minimum	Maximum
Flock proportion immune at first timestep post-vaccination.	0.847	0.849	0.780	0.901
Flock proportion immune 6-months post-vaccination.	0.734	0.738	0.642	0.824
Flock proportion immune 12-months post-vaccination.	0.580	0.585	0.432	0.705
Weeks with immunity >70%	31.8	32	20	50

DISCUSSION

The global sensitivity analysis indicated that young male offtake, births, the minimum age of exchange, age of first parturition, and youth mortality were the most important influences on post-vaccination immunity levels in pastoral and agro-pastoral small ruminant flocks. A small number of studies have explored the impact of different demographic features on post-vaccination flock immunity dynamics in specific small ruminant populations (Hammami et al., 2016, 2018; El Arbi et al., 2019), however, this sensitivity analysis offers more general conclusions about the relative importance of different demographic parameters for diverse PPRV-affected small ruminant populations. Understanding the role of different demographic parameters in driving post-vaccination immunity dynamics can provide insights to inform data-collection activities, enabling the prioritisation of important parameters and optimised resource allocation (Wu et al., 2013). This is particularly relevant in PPRV endemic regions where data collection activities can be limited due to lack of resource (economic, logistic) and practically challenging due to the characteristics of extensively managed, mobile small ruminant populations.

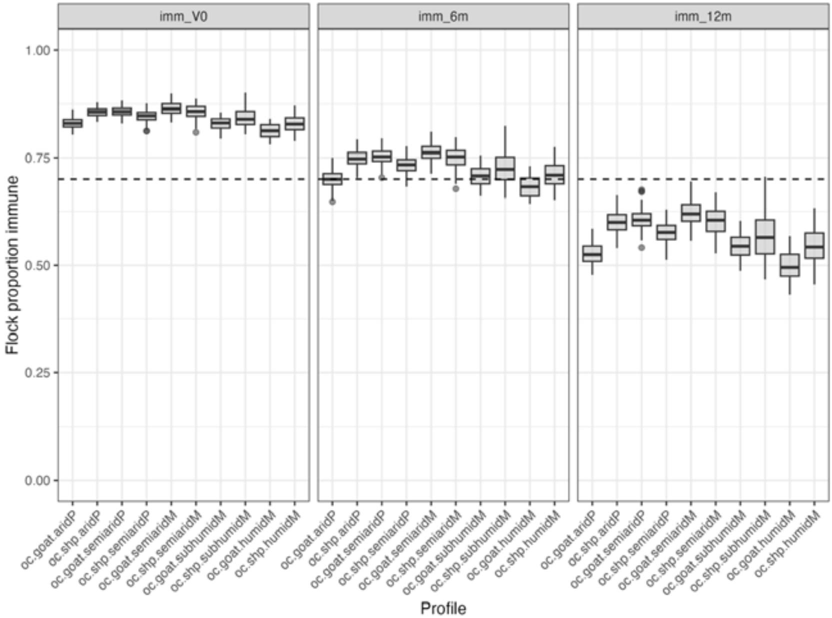

Figure 1. Box-and-whisker plots of post-vaccination flock immunity metrics for different small ruminant flock profiles in sub-Saharan Africa. Dashed line indicates flock immunity level = 70%. Immunity abbreviations: flock proportion immune at first timestep (imm_V0), at 6 months (imm_6m) and at 12 months post-vaccination (imm_12m). Flock profile abbreviations: goat flocks (oc.goat), sheep flocks (oc.shp), pastoral (P), mixed crop-livestock (M)

The results of the applied analysis demonstrated that the current GSCE vaccination programme fails to maintain flock immunity levels above the targeted 70% threshold up to 12 months post-vaccination in populations with constant reproduction. This finding supports previous research which showed a decay of flock immunity to below 70% within a year of vaccination (Hammami et al. 2018). In this study, flock immunity was maintained above 70% for a median of 31 weeks (or 7-months), suggesting that implementing vaccination campaigns twice per year may be more appropriate. The highest immunity level attained immediately following vaccination was 80%, with the non-immune proportion comprised of animals younger than 4 months which were ineligible for vaccination. In small ruminant populations which breed consistently throughout the year (often more sedentary, mixed crop-livestock production systems), as modelled here, achieving immunity levels >80% may be challenging due to the continuous presence of young animals. In this context, the GSCE recommends vaccination campaigns at 6-month intervals to maintain flock immunity levels. The timing of vaccination, in any population, should be scheduled to maximise the proportion of immune-competent animals (animals in good body condition) and according to the local agricultural calendar (e.g. accounting for periods of transhumance and availability of funds to pay for vaccination; FAO & OIE, 2015). Where breeding is seasonal, resulting in defined birth peaks, campaigns may be more easily scheduled to maximise the proportion of animals older than 4 months and eligible for vaccination. Previous research has indicated that, in arid regions of West Africa (Senegal), the highest flock immunity levels can be achieved through vaccination between 2 to 4 months preceding a parturition peak when a large proportion of animals are eligible for vaccination (older than 4 months) and additional factors such as periods of transhumance and small ruminant body condition are considered (Hammami et al., 2018; El Arbi et al., 2019). The lack of seasonality in our model is a limitation, however, further work is ongoing to explore the effect of seasonal breeding on flock immunity dynamics in the pastoral flock profiles included here.

The applied analysis demonstrated considerable variation in post-vaccination immunity levels both within and between flock profiles, reflecting reported variation among flock demographics and highlighting the importance of accounting for population demographics in vaccination strategies. In PPRV-endemic regions, detailed, longitudinal, demographic data are rarely available with studies commonly using cross-sectional surveys which require respondents to recall flock dynamics over long periods. Across studies, data can be inconsistent due to annual variations in flock demographics dependent on environmental conditions, differences in the selection and definition of parameters reported and the age-sex stratification of flocks. Through global sensitivity analysis, young male offtake was identified as the most influential parameter driving post-vaccination immunity dynamics however, despite qualitative reports of high young male offtake (Gebre Mariam, 1991; Hassen and Tesfaye, 2014; Tadesse et al., 2014), the parameter is rarely specified within datasets. The lack of high-quality, detailed demographic data to characterise different small ruminant populations can limit the accuracy and practical application of studies incorporating flock demographics. Lesnoff et al. (2014) proposed a framework, and associated tool, to facilitate collection of standardized demographic data in tropical livestock herds. Widespread adoption of this approach would increase the availability of comprehensive, standardized demographic data for diverse small ruminant populations. This would facilitate more accurate assessment of the factors influencing flock dynamics, and improve the quality of studies reliant on demographic data including studies of infectious disease transmission, post-vaccination immunity dynamics and livestock productivity.

The global sensitivity analysis indicated the most important demographic parameters impacting post-vaccination immunity levels in pastoral and agro-pastoral small ruminant populations, offering insights to inform data-collection activities in data-limited settings. The applied analysis demonstrated that the GSCE vaccination programme with annual vaccination, fails to maintain immunity above the targeted 70% threshold in small ruminant flock profiles if breeding is non-seasonal. This supports the findings of previous studies that, where births occur consistently throughout the year, two vaccination rounds per year may be more effective. This study offers insights into the influence of flock demographics on post-vaccination immunity dynamics, however increased availability of detailed demographic datasets is required to enable more accurate characterisation of small ruminant flocks.

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A MULTI-HOST AND MULTI-ROUTE TRANSMISSION MODEL TO ASSESS THE EFFECT OF CONTROL MEASURES ON BOVINE TUBERCULOSIS DYNAMICS IN IRELAND

Y. CHANG*, S. WIDGREN, J.A. TRATALOS, S.J. MORE, M.C.M. DE JONG AND N. HARTEMINK

SUMMARY

Bovine tuberculosis (bTB) presents a complex challenge in many countries including Ireland. bTB transmission involves several contributing factors, including wildlife infection (e.g., in badgers), infected livestock movements, persistence of *Mycobacterium bovis* in the environment, and residual infection in herds due to imperfect screening tests. Quantifying the relative contribution of these factors remains a challenge, hindering the evaluation of different intervention strategies. This study aims to disentangle these contributions and assess eradication strategies. We developed a two-host transmission model that includes the above-mentioned transmission routes. The model suggests that cattle-to-cattle transmission contributes most to new cattle infections at the individual level, while badgers can play an important role in spreading bTB between herds. However, measures that only target badgers, such as badger vaccination or selective culling, when combined with existing control, might not be sufficient to achieve eradication. We suggest a multifaceted approach targeting multiple transmission routes to achieve eradication.

INTRODUCTION

Bovine tuberculosis (bTB) is a longstanding endemic disease caused mainly by *Mycobacterium bovis*. It can infect various mammals, including cattle and humans, and it has several wildlife reservoirs such as deer, badgers (*Meles meles*), and wild boar (*Sus scrofa*) (Broughan et al., 2013). Milk pasteurization substantially reduces the risk of human infection, with only 88 human cases reported in Europe in 2019 (ECDC, 2019). bTB has a detrimental impact on the cattle industry, with an estimated annual cost of over $3 billion globally (Steele & Thoen, 1995). This ongoing economic burden is the primary motivation for bTB eradication.

For many years, bTB eradication has primarily relied on a test-and-removal scheme for cattle. Regular testing of herds is conducted using the single intradermal comparative cervical tuberculin (SICCT) test, and animals that test positive are slaughtered. Herds in which positive animals are detected (often known as a herd breakdown) lose their official TB-free status.

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Additionally, those herds are subjected to movement restrictions until they pass two follow-up tests, which are administered at approximately 60-day intervals (Brooks-Pollock et al., 2014; DAFM, 2018). Despite the success of this strategy in several countries such as Australia, the Netherlands and several northern European countries (More et al., 2015; Orrico et al., 2022), bTB remains persistent in some countries, including Ireland and the United Kingdom, in part due to the presence of infected wildlife such as badgers.

The persistence of bTB has multiple causes, as bTB transmission can potentially involve multiple hosts and routes. At a local level, *M. bovis* can be shed into the environment, where it may survive for an extended period, depending on the substrates and environmental conditions (Rodríguez-Hernández et al., 2016; Allen et al., 2021). Therefore, even after the removal of positive cattle, reinfection can occur within a herd due to the presence of *M. bovis* in the environment. Additionally, badgers usually reside near pastures (Krebs et al., 1998; Woodroffe et al., 2021), sharing contaminated environments with cattle. Badgers can become infected from one herd and may subsequently spread bTB to neighbouring herds, leading to contiguous spread in a local area (Chang et al., 2023). This local transmission can be influenced by the spatial heterogeneity in cattle-badger contacts (Chang et al., 2023). Furthermore, infected cattle might remain undetected due to the imperfect sensitivity of the diagnostic test (Conlan et al., 2012) and continue to shed *M. bovis*. Further, the trade of infected but undetected animals can spread bTB between herds and introduce infections to previously uninfected areas (Gilbert et al., 2005; Gopal et al., 2006; Palisson et al., 2016).

This combination of interacting factors, including the long-term survival of *M. bovis* in the environment, wildlife reservoirs, residual infections, and cattle trade movement, collectively present a challenge to bTB eradication in Ireland. The contributions of these causes are likely to be spatially heterogenous (Akhmetova et al., 2023) and are currently poorly quantified (Green et al., 2008). This knowledge gap hampers the assessment of control measures as their effectiveness can be influenced by the relative contribution of each of the transmission routes that they target.

This study aims to investigate the contribution of badgers, cattle and cattle trade movements to the transmission of bTB at the level of both the individual cattle infection and the herd breakdowns. Additionally, we aim to evaluate the efficacy of various additional interventions for mitigating bTB spread at a regional level. To achieve this, we extended a bTB local transmission model, developed by Chang et al. (2023), by incorporating movement-mediated transmission using real trade movement data. We assessed several interventions, in addition to those within the existing eradication programme, including badger vaccination, badger selective culling and cattle vaccination. Furthermore, we implemented a novel rewiring movement algorithm to evaluate the impact of risk-based trading. By assessing these additional interventions, we aim to gain insights into the potential of these additional interventions to control bTB transmission.

MATERIALS AND METHODS

The model was developed by adapting and writing C and R code within the existing R package SimInf (Widgren et al., 2016; Widgren et al., 2019).

Model formulation

Local transmission: In this study, a local area is defined as a herd along with its associated badger territories and directly neighbouring herds. Within each local area, our local transmission model incorporates both within-herd/within-territory transmission and interspecies transmission (between badgers and cattle). This local transmission model is adapted from a previous study (Chang et al., 2023), with two types of subpopulations, cattle herds and badger social groups. Each subpopulation has its own spatial unit, which corresponds to a specific location, such as a farm location or a badger territory location (represented in Fig.1 by the circle and the rectangle respectively). Since two species co-habit in a region, the study area can be visualized as an overlay of two layers, a farm location map and badger territory map, where a farm can overlap with several territories and a territory can overlap with several farms.

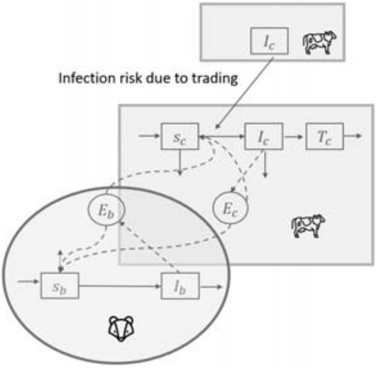

Figure 1. A schematic representation of the bTB transmission model, illustrating transmission dynamics within a local area and between-herd transmission via movement. Badger territory and farm are the two spatial units in the local transmission. The transitions between animal compartments are represented by the solid lines and environmental transmissions are represented by the dashed lines

The model utilizes a stochastic Susceptible (S) – Infectious (I) compartmental model with two environment compartments (E_c and E_b for two spatial layers) to simulate the dynamics of transmission within a spatial unit. All the transmission events are modelled as occurring indirectly via the environment. Infected animals are considered to shed infectious material immediately after infection (that is, we assumed that there is no latent period). Susceptible cattle or badgers can become infected following exposure to *M. bovis* shed by animals of its own species. Susceptible animals can also be exposed to *M. bovis* shed by animals of the other species, whenever spatial units of cattle and badgers overlap. The amount of exposure from the other species is determined by the ratio of the overlapping area to each spatial unit area, as defined in the between-species connection matrix. Decay of the infectious material in the environment is modelled as a deterministic process, with a constant decay rate. To maintain a stable population size, the natural death rate and birth rate of each species are assumed to be equal, and all newborn animals are assumed to be susceptible to infection.

In contrast to the previous model (Chang et al., 2023), this model incorporates the current control scheme in Ireland, which involves a cattle test-and-removal scheme and movement restrictions for infected herds. In the model, all herds are scheduled for periodic screening tests using SICCT on a random date with a fixed interval of 365 days. Test outcomes are assumed to follow a binomial distribution, with test sensitivity representing the probability of successfully detecting positive cases. A positive test result occurring in at least one animal in a herd signifies a herd breakdown, triggering the immediate removal of all test-positive cattle in the model. These removed animals are replaced with newly bought susceptible cattle to maintain a constant number of cattle in a herd. In addition, movement restrictions are implemented during the period following the detection of test-positive cattle. During this restricted period, herds experiencing a breakdown are banned from trading activities. The trading restriction is only lifted when two consecutive negative tests are achieved. For the implementation of risk-based trading, a risk classifier has been added as a compartment that tracks the number of days that each herd remains bTB test-negative. This risk-classifier serves as the basis for comparing herd-level risk between herds.

Global transmission: In this model, intra-area (between subpopulation) transmission can occur due to shared environments between the two species or due to cattle trade movements. The spatial overlap between species not only leads to between-species transmission (Chang et al., 2023), but also establishes indirect connections, creating a chain-like network that links all herds and badger territories. This mechanism accounts for both between-herd (via cattle-badger-cattle) and between-territory (via badger-cattle-badger) transmission. Furthermore, infected but undetected cattle can be introduced to previously uninfected local areas through trading, potentially leading to long-distance bTB spread. We assume that there is no between-herd transmission via sharing equipment and fences, and no between-territory transmission via badger movement.

We incorporate the movement-mediated transmission into the model based on observed trading patterns. The timing of cattle trades, including the exact date for each trade, is determined through a stochastic process implemented using Gillespie's algorithm, similar to other transitions in the model. When a trade occurs, we select trading partners and their trading intensities (the number of cows in each trade) based on the trading frequency derived from trade movement data. To determine the number of infectious cattle involved in a trade, we sample from a binomial distribution. The sample size is the total number of traded cows, and the probability of selected infectious cows is determined by the proportion of infectious cattle to the total cattle population in the selling herd. Once infectious cattle are selected, the model increments the number of infectious cattle in the buying herd and the number of susceptible in the selling herd. Conversely, it incrementally decreases the number of infectious animals in the selling herd and the number of susceptible animals in the buying herd. This modelling approach captures the risk of infection through trade movement while ensuring that the total population of both the selling and buying herds remains constant after trading.

Interventions: The current intervention, test-and-removal with movement restriction, serves as the default scenario in this study. Additionally, we identify several interventions targeting different routes, as listed below.

We first investigate four interventions targeting movement-mediated transmission:

(1) Default (D): Infected herds are movement-restricted until they test negative for two consecutive tests conducted 60-days apart.
(2) Risk-based trading (RBT): Herds can only trade with herds having an equivalent or a lower risk classifier than their own. Buying cattle from outside the study area is redirected to herds within the study area that have an equivalent or a lower risk classifier.
(3) Pre-movement testing (PMT): Cattle are tested prior to movement unless both the animal and the herd of origin were bTB tested in the preceding six months.
(4) Movement ban (MB): All trade movements are banned in this study area.

To implement risk-based trading, we developed a rewiring algorithm that assesses the herd status before cattle movement and rewires trades involving high risk. When a trade occurs, we compare the risk classifier of the selling herd to that of the buying herd. If the selling herd has a higher risk level, we randomly select a new selling herd from the study area with a lower risk classifier.

Secondly, we investigate interventions targeting badgers:

1) Default (D): No badger interventions.
2) Badger vaccination with 50% coverage (BV50): 50% of badgers are vaccinated with BCG vaccine, modelled by reducing transmission rate parameters (Chang et al., 2023).
3) Badger vaccination with 100% coverage (BV): All badgers are vaccinated to assess the maximum potential of badger vaccination. (Note: this scenario may not be feasible in reality but is used for exploratory purposes.)
4) Selective culling (SC): Badgers are tested once a year on average and positive badgers are removed (Menzies et al., 2021). Removed infected badgers are assumed to be replaced by susceptible badgers to maintain a constant badger population.

Thirdly, we investigate interventions on cattle farms.

1) Default (D): Cattle are annually tested using SICCT, and all positive cattle are removed and replaced by susceptible cattle. Herds with positive tests are banned from trading activities until two consecutive negative tests are achieved.
2) Cattle vaccination (CV): All the cattle are vaccinated, assuming a 40% reduction in cattle susceptibility.
3) Improve Farm Biosecurity (IFB): This intervention enhances farm biosecurity measures, such as the use of badger-proof around cattle housing and feeding stores, to reduce the transmission rate parameter from badger to cattle by 50%. (Note: an exploratory scenario)

Data

We utilized data from a previous badger vaccination trial in Kilkenny County conducted from 2009 to 2012 (Aznar et al., 2018), offering insights into cattle and badgers at the same time and place. Cattle-related information, incidence data, and locations for 2009-2012 were sourced from the Animal Health Computer System (AHCS) and the Land Parcel Identification System (LPIS), maintained by the Irish Government's Department of Agriculture, Food, and

Marine (DAFM). Initial infectious cattle in the model were based on the number of cows that tested positive in 2009.

Cattle movement data were extracted from the Animal Identification and Movement system (AIM) of DAFM. Movement data informed a herd-to-herd connection matrix, capturing trading patterns among herds.

Badger data for 255 social groups from Co. Kilkenny during 2009 to 2013 (Aznar et al., 2018 and Chang et al., 2023) provided initial values for infectious and susceptible badgers. Badger territory overlays and farm locations were used to construct a connection matrix, indicating shared areas between farms and badger territories relative to their total areas.

Parameters

There are two types of parameters in the model: global parameters and local parameters (Table 1). Global parameters remain constant for all the subpopulations, including transmission rate, decay rate and death rate parameters, etc. We determined these global parameters by adopting estimations from a previous local transmission model (Chang et al, 2023). For example, the decay rate parameter of *M. bovis* is estimated to be 0.004 per day, indicating a high level of persistence in the environment. The local parameters define parameters specific to each subpopulation including the between-species connection matrix and between-herd connection matrix.

Table 1. Global parameters

Parameter	**Description**	**Value (unit: per day)**
$\beta_{c,c}$	Transmission rate parameter cattle to cattle	1e-5
$\beta_{b,c}$	Transmission rate parameter badgers to cattle	4e-6
$\beta_{b,ub}$	Transmission rate parameter badgers to unvaccinated badgers	9e-5
$\beta_{c,ub}$	Transmission rate parameter cattle to unvaccinated badgers	5e-4
$\beta_{b,vb}$	Transmission rate parameter badgers to vaccinated badgers	5.1e-4
$\beta_{c,vb}$	Transmission rate parameter cattle to vaccinated badgers	4.4e-4
μ	*M. bovis* decay rate parameter	0.004
φ	shedding rate parameter $\frac{\mu^2}{(1-e^{-\mu}+\mu)}$	0.002
γ_b	Infectious badger's death rate	1/365
α_c	Cattle background death rate	9.13e-4
α_b	Badger natural death rate	7.52e-4
SE	Sensitivity of the skin test	0.8 (unitless)

RESULTS

Relative contributions of routes on cattle infections and herd breakdowns

We use the model to simulate transmission dynamics and quantify the roles of various mechanisms within the study area (Fig.2). As the model tracks *M. bovis* shed by cattle or badger (in E_c and E_b layers), we can examine new cattle infections and identify the source of each cattle infection (referred to as "cattle level" in Fig.2). Additionally, when a herd breakdown occurs, which is defined as bTB infection occurring in cattle in a previously bTB free herd, we can also analyse the source of each herd breakdown ("herd level" in Fig.2).

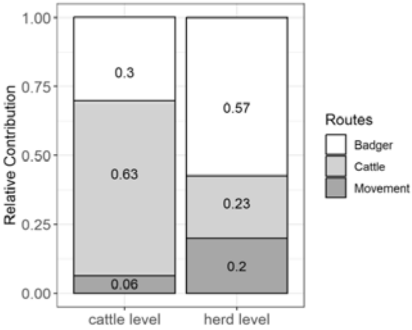

Figure 2. Relative contribution of transmission routes to cattle infections ("cattle level") and herd breakdowns ("herd level")

New cattle infections (cattle level) and new herd breakdowns (herd level) can be classified into three sources:

1) cattle route: infections result from exposure to E_c. This route involves infected (but not yet detected) cattle in the herd who can continue to shed *M. bovis* in the environment (E_c layer). This concept is similar to "residual infection" but with the consideration of environmental transmission. Additionally, previously infected cattle that have been removed also contribute to this route, because the environment (E_c layer) still harbours *M. bovis* from their excreta.

2) badger route: infections result from exposure to E_b where badger territories are connected to a herd. This route relates to infectious badgers (that are currently present) and historically infectious badgers (that are no longer present), which each contributed to the *M. bovis* in the environment (E_b layer).

3) movement route: Infected and infectious (but undetected) cattle might be moved to a previously non-infected herd.

For new cattle infections (at the cattle level), our model results indicate that the cattle route contributes the most, accounting for 63.4% (with 95% quantiles 61.3% - 65.7%) of all transmission events. The movement-mediated transmission plays a minor role, contributing only 6.3% (with 95% quantiles 5.8% - 7.2%). Badger-to-cattle transmission accounts for another 30.4% (with 95% quantiles 28.7% - 31.8%) of the new cattle infections.

In terms of new herd breakdowns (at the herd level), badgers connect neighbouring herds and introduce new infections to herds, resulting in 57.4% (with 95% quantiles 54.1% - 60.2%) of all new herd breakdowns in our model. The contribution of movement-mediated transmission to herd breakdowns (20%) is more than twice that of its contribution to cattle-level infections (6.3%). Residual infections and historically infected cattle contribute to 22.7% of new herd breakdowns.

Intervention impact

Our model shows that the default scenario, including test and removal with movement restriction for breakdown herds, leads to a herd incidence of 14% (Scenario1 in Fig.3). However, it is important to note that all additional interventions, targeting a single route, cannot eradicate bTB. For example, badger vaccination, whether at 50% or 100% coverage, leads to a modest absolute reduction in herd incidence (1~2%, as indicated in Scenario 5 and 6 in Fig.3). This limited effect of badger vaccination is due to its impact only on reducing badger's susceptibility but not the infectivity (Aznar et al., 2018). Consequently, badger vaccination can decrease bTB prevalence among badgers and indirectly reduce the badger-to-cattle transmission. In comparison, selective culling can directly reduce badger-to-cattle transmission by decreasing the infectivity of badgers (Scenario 7 in Fig.3).

According to our results, risk-based trading, pre-movement testing, and even movement ban, together with current measures, cannot eliminate bTB (Scenario 2, 3 and 4 in Fig.3). The reason is that badgers, residual infections and the survival of *M. bovis* in environment enable the persistence of bTB in a region. Cattle vaccination and improving farm biosecurity, which focus on protecting cattle and farms, appear to be relatively effective, reduce the herd incidence by half to one-third (Scenario 8 and 9 in Fig.3).

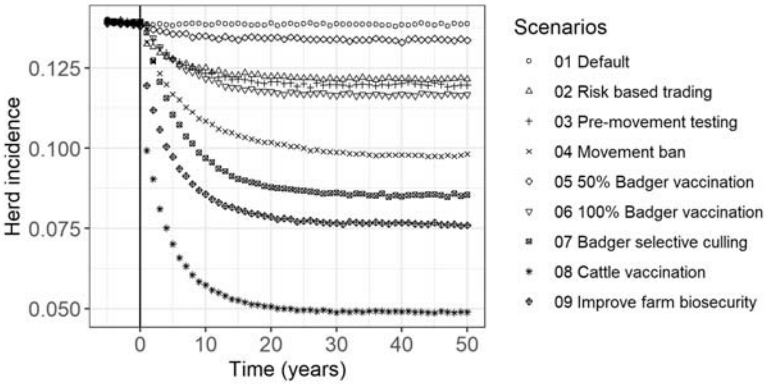

Figure 3. The effect of interventions on herd-level annual incidence

DISCUSSION

Bovine tuberculosis has a complicated infection ecology and is difficult to control in many countries, including Ireland. Current control measures, based on cattle test-and-removal and movement restriction, have proved insufficient to eradicate bTB from countries such as Ireland, where wildlife is involved in transmission. In addition to wildlife involvement, other factors like residual infection, the movement of infected (but undetected) cattle due to imperfect diagnostic testing, as well as persistence of *M. bovis* in the environment, also contribute to the bTB transmission within and between farms. However, the relative contribution of these routes is poorly understood and is likely to vary among different spatial contexts, which poses challenges for evaluating additional intervention strategies. Therefore, this study developed a multi-host and multi-route transmission model to quantify the relative contribution of routes to cattle infections and herd breakdowns, whilst considering local influencing factors such as relative badger density and cattle movement patterns.

According to our model, cattle contribute significantly to new cattle infections (45~65%) in this study area. This aligns with previous modelling study that found cattle to be the primary source of cattle infections (Donnelly & Nouvellet, 2013). Furthermore, whole genome sequencing (WGS) studies also suggested that cattle-to-cattle transmission is more frequent than badger-to-cattle transmission (Van Tonder et al., 2021; Akhmetova et al., 2023). However, the direction of between-species transmission in WGS studies varied across different study areas, reflecting the important influence of spatial contexts. Our model includes local factors such as badger density and movement patterns and can therefore be used to investigate how these local factors influence bTB transmission dynamics and the contribution of different transmission routes in different areas. Finally, cattle movement accounts for 20-36% of herd breakdowns, consistent with previous studies indicating that movement plays a lesser role than local transmission (Green et al., 2008; Clegg et al., 2015; Palisson et al., 2016).

Although badgers may play an important role in herd breakdowns, our modelling demonstrates that badger vaccination alone, combined with current intervention, cannot eradicate bTB. A previous study showed that with a full coverage of badger vaccination, the average value of herd-level R in this study area decreased to 0.85, but in 30% of herds R was greater than 1 (Chang et al., 2023). However, the herd-level regional R depends on the network structure between high-risk herds and low-risk herds in a region. If high-risk herds are clustered with other high-risk herds, bTB can sustain itself in these high-risk areas and can further spread to low-risk areas through cattle movement or connected badger territories, thus sustaining bTB in a endemic state in a region. This could explain why other models that did not account for spatial heterogeneity often yield more optimistic results for badger vaccination. For example, previous studies have suggested that 40% coverage for badger vaccination can eradicate bTB in badgers (Wilkinson et al., 2004; Aznar et al., 2018).

In this study, the assumptions underpinning local transmission are equivalent to those from a previous study (Chang et al., 2023), where the rationale and impact for these assumptions were extensively discussed. In local transmission, transmissions occur indirectly via the environment, assuming animals are distributed homogenously within a farm or a territory, resulting in an even distribution of *M. bovis* over each unit. These assumptions can lead to an overestimation of inter-species transmission, and an underestimation of within-species transmission. Furthermore, since between-herd transmission is assumed to occur only via a "cattle-badger-cattle" route or via cattle movement in the model, ignoring other potential between-herd transmission mechanisms (such as transmission between neighbouring herds via infected tools or contact between cattle over the fence) can lead to an overestimation of the badger contribution on herd level. However, even with an assumption favouring between-species transmission, badger vaccination alone still cannot eradicate bTB when combined with existing control measures. This emphasizes the importance of a multifaceted approach to controlling bTB. Further studies are needed to explore multifaceted control measures and to conduct sensitivity analysis on how uncertainties in parameters and data influence the conclusions.

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CAN THE DANISH VETERINARY AUTHORITIES MEET THEIR KEY PERFORMANCE INDICATOR TARGETS IN THE EVENT OF A FOOT-AND-MOUTH DISEASE OUTBREAK?

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SUMMARY

In the face of an epidemic, effective animal health policy requires a good understanding of how the availability of resources influences control efforts, with resource requirements likely to vary across epidemics. In case of a severe FMD epidemic, this study suggests that, with the current resource levels, the Danish Veterinary Authorities would be unable to meet several current predefined key performance indicator targets for the contingency planning.

INTRODUCTION

Disease spread simulation models can provide valuable insights into the dynamics and control of foot-and-mouth disease (FMD) epidemics, especially in countries that have not experienced a recent FMD outbreak (Dórea et al., 2017; Marschik et al., 2021a,b), such as Denmark (Boklund et al., 2013; Conrady et al., 2023). In a previous study by Conrady and colleagues (2023), the European Simulation Disease Spread Model (EuFMDiS) was used to evaluate the epidemiological and economic impact of a potential FMD outbreak in Denmark. According to these estimates, the costs of an FMD outbreak in Denmark would range from EUR 2.5 billion to EUR 10.0 billion, depending on the nature of the outbreak and control measures implemented. The results indicated that limited availability of human resources can be a serious constraint to the rapid and effective implementation of mitigation measures and may have a negative impact on the outbreak size. Only a limited number of FMD simulation models incorporate resources, such as human capacity and laboratory capacity components, to study how resources for control influence an outbreak. Such models, including EuFMDiS, are able to evaluate whether resources for operational activities such as surveillance, culling, disposal, cleaning and disinfection, and/or vaccination and laboratory capacities are sufficient to eradicate the epidemic without delay and/or whether additional resources may be required (Boklund et al., 2007; Bradhurst et al., 2021; Marschik et al., 2021a; Conrady et al., 2023).

Studies of past FMD outbreaks in the United Kingdom (UK) (Davies, 2002), the Netherlands (Bouma et al., 2003) and Japan (Nishiura and Omori, 2010) have identified several critical bottlenecks in resource adequacy such the number of staff required to perform

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operational activities (McLaws et al., 2009), were major obstacles in the disease control efforts.

To facilitate FMD control in Denmark, the Danish contingency plan includes predefined key performance indicators (KPIs). Modelling studies can provide valuable information for veterinary public authorities regarding potential bottlenecks in outbreak management and improve future resource planning to support disease preparedness.

The aims of the study presented here were (i) to investigate if the predefined KPIs in the Danish contingency plan are reachable given current resourcing levels; (ii) to identify areas of operational activities where the available human resources and laboratory capacities may be insufficient to meet predefined KPIs in Denmark, and (iii) to estimate the resources needed to respond to an FMD outbreak in Denmark while meeting predefined KPIs.

MATERIALS AND METHODS

The EuFMDiS-DK model (Bradhurst et al., 2021; Conrady et al., 2023) was used to simulate a range of FMD outbreaks in Denmark and analyse the reachability of the Danish KPI's. The spread of FMD of a type O's pan-Asia strain in early autumn was simulated in 1,000 randomly selected large commercial dairy herds and 1,000 randomly selected large commercial-scale weaner pig herds without a specific-pathogen-free (SPF) system in place (scenario *A*). The purpose of the scenario *A* was to cover the diversity of production within dairy herds and weaner herds in Denmark. A former study by Conrady and colleagues (2023) indicated that the chosen index herds (i.e. were infection was seeded) significantly influenced the course of a simulated FMD epidemic in Denmark. From the 1,000 randomly selected farms, we selected as seeding herd one specific large commercial dairy herd in medium-density livestock area causing a large epidemic size of 145 infected farms, and in a weaner herd with a herd size of 3,200 pigs resulting in 172 infected farms during the silent phase (scenario *B*). Scenario *B* was chosen to represent an outbreak during the silent spread phase of a reasonable worst case scenario. The simulation results of scenarios *A* and *B* were compared regarding the ability to achieve the KPIs.

For both scenarios *A* and *B*, the model initially simulated FMD spread without any mitigation strategies to the end of a 21-day period (defined as the silent spread phase) until the first FMD-case was detected. From the day of first detection onwards, basic control strategies were modelled. These basic control strategies included a three-day standstill period for livestock movements at the national level (i.e. day 21-24), establishment of a 3-km protection zone (PZ) with assumed 98% compliance for direct contacts and 80% for indirect, and a 10-km surveillance zone (SZ) with assumed 95% compliance for direct contacts and 70% for indirect around each infected herd. Note that restrictions on contact between herds, including animal movements, products, etc. are not assumed to be 100% effective, with the compliance levels reflecting the extent to which number of contacts are reduced. Control activities include surveillance visits, tracing of contacts onto and off infected holdings, culling and disposal of confirmed infected cases, and cleaning and disinfection of infected holdings. Surveillance activities were modelled to take into account reporting of suspect cases, both true (i.e., actual cases of FMD) and false (i.e., clinical cases that are not actually FMD) with the assumption based on the UK 2001 experience of 2.34 false reports for every true report, with the false cases being randomly distributed at 42% in PZs, 36% in SZs and 22% elsewhere in the free zone (FZ). All herds in PZs are also subject to scheduled surveillance visits. Herds delivering or receiving animals to or from infected herds were assumed to be visited and/or tested, based on backward and forward tracing of contacts on to and off infected herds within a 14-day trace window. Positively confirmed infected farms were depopulated and disinfected. On top of the basic control measures, we also simulated for both scenario *A* and *B*, additional mitigating measures, which here meant the pre-emptive culling of dangerous contact herds linked to infected herds through tracing was simulated. This involves culling animals on these holdings without first confirming FMD and therefore surveillance visits and laboratory resources to confirm the infection are not required, although disposal, cleaning, and disinfection of these herds is still necessary. The following KPIs in the Danish Contingency Plan were investigated assuming the available laboratory capacities and human resources described in Table 1. KPI 1-6 were analysed for both FMD introduction scenarios *A* and *B*, with the basic and additional mitigation measures, using the laboratory capacities and human resources described in Table 1 including the composition of team defined based on performance of activities for a herd size of 90 cattle, 1,600 pigs, and 17 small ruminants. Additionally, to analyse how many resources may be needed in Denmark to reach the predefined KPI's, the same simulations were ran but with unlimited resources. The simulation results are presented as median (1st Quartile, 3rd Quartile; shown as Q1;Q3 in the following chapter).

KPI 1: The maximum case reporting time should not exceed ~ 1.5 days (37 hours) including notification, sampling, dispatch, and confirmation with a PCR test.

KPI 2: Culling of an infected herd must start no later than 1.5 days (36 hours) after the declaration of FMD.

KPI 3: Surveillance visit all farms in PZ and SZ zones within 7 days and 14 days, respectively.

KPI 4: Killing of a dangerous contact herd should start not later than 2 days (48 hours) after the declaration of FMD.

KPI 5: Initial cleaning and disinfection must be completed no later than 2.6 days (62.4 hours) after the killing.

KPI 6: The disposal of carcasses must be completed no later than 2 days (48 hours) after killing the animals.

Table 1. A selected input data used in the model.

Parameters		Value
Surveillance team[a] (including sampling)	Clinical suspicion Clinical suspicion Surveillance in zones Surveillance in traced herds	1 veterinarian and 1 technician
Culling team[a]		1 veterinarian, 4 technicians, and 1 truck driver
Disposal team[a]		1 veterinarian, 4 technicians, and 1 truck driver
Cleaning and disinfection team[a]		1 veterinarian and 9 officers from the Danish Emergency Management Agency
Min/Max number of surveillance teams		8/65
Min/Max number of culling teams		3/37
Min/Max number of disposal teams		4/34
Min/Max number of decontamination teams		4/41
Min/Max number of vaccination teams		7/72
Days for herd surveillance visits[b]		0.2–0.7 days (herd type-dependent)
Days to cull a herd[b]		0.25–0.8 days (herd type-dependent)
Days to dispose of a herd[b]		0.2–0.5 days (herd type-dependent)
Days to decontaminate a holding[b]		2–4 days (herd type-dependent)
Days to vaccinate a herd[b]		0.2–0.7 days (herd type-dependent)
Daily ELISA capacity		3570/day
Daily PCR capacity		286/day
ELISA sensitivity		0.86–0.99
ELISA specificity		0.97–0.99
PCR sensitivity		0.95–0.99
PCR specificity		0.99–0.99
Clinical sensitivity of non-vaccinated animals		0.5–0.98 (species-dependent)
Clinical specificity of non-vaccinated animals		0.70
Number of days to report suspect premises after clinical signs		0–19 days (herd type-dependent)
Probability of reporting suspect cases		0.80–0.97 (herd type-dependent)
Ratio of false suspect premises reports to true reports		2.34:1
Time needed for a direct trace (days)		0–3 days (species-dependent)
Time needed for an indirect trace (days)		1–5 days (species-dependent)
Effectiveness of direct tracing		96–99% (species-dependent)
Effectiveness of indirect tracing		55–80% (species-dependent)

[a] Composition of team defined based on performance of activities for a herd size of 90 cattle, 1,600 pigs, and 17 small ruminants. Like Boklund et al., 2017, we do not distinguish between the composition of teams in terms of staff qualifications, e.g. whether veterinarians are public employees or from private practice. Administrative staff only related to work in local crisis centre, and associated costs component assigned to costs of control centres.

[b] E.g. 0.5 represents half a day and 1.25 one and a quarter days.

RESULTS

In scenario *A*, starting in 1,000 randomly selected cattle or weaner herds, the predicted median epidemic size was 4 (i.e., number of infected farms) and the predicted total costs were 3.7 billion EURO, with the operational costs for outbreak management accounted for only 1% of the total costs with most of the costs (99%) were due to export restrictions. All KPI was predicted to be reachable on average, except KPI 1, which would be delayed by of 4.5 days (6; 8).

In scenario *B*, starting in farms predicted to result in large epidemics, the predicted epidemic size (i.e., number of infected farms) was 268 on average and the results indicated that KPI 1 is not reachable, with a delay of 38 days (7; 116). KPI 2 would be not reachable with an average time needed of 5 days (5; 6) i.e. average delay of 3.5 days. KPI 3 would not be reachable for both protection and surveillance zones (PZ and SZ). Specifically, visits for PZ would not be reachable on time, with a delay of 12 days (i.e. median: 19, 7; 89) and visits in SZ would be delayed of 25 days (i.e. median: 39, 5; 111). KPI 4 would be reachable within the predefined value of 2 days. Independent of the scenario, additional mitigation measures would reduce KPI 1. KPI 5 and KPI 6 would be delayed by 3.5 days (1; 48) and 4 days (1; 49), respectively. There was not a statistically significant difference in the reachability of KPIs across dairy or pig species within scenario *B*. Figure 1 indicated that, for example, KPI 3 in PZ would be attainable, even in the worst-case scenario, if more resources become available (simulation runs with unlimited resources). Overall, the simulation with unlimited resources shows that all KPI's could be attained, except for KPI 1. KPI 1 would still needed 3 days to manage the worst-case scenario of scenario *B* to fulfil KPI 1. To reach the other KPIs, surveillance and culling activities were identified as bottlenecks, suggesting more resources are required. Specifically, it was predicted that approximately 16% more surveillance teams and 38% more culling teams were needed, compared to the assumed currently available resources. With these additional resources, while operational costs would be 5.8 times higher than the baseline with currently assumed resources for outbreak management, the total FMD outbreak cost would be 4.47 times lower (7.9 billion EURO), due to effective outbreak response.

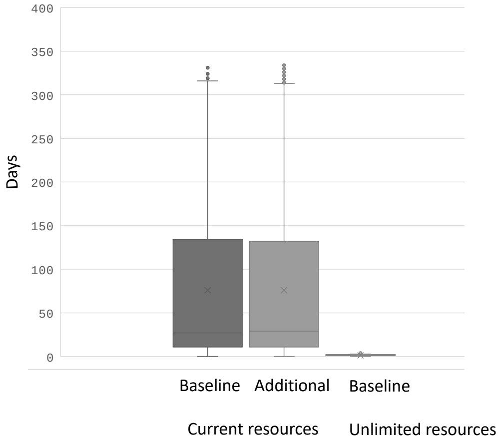

Figure 1. Predicted numbers of days required to perform all surveillance visits in protection zones (PZ) for the baseline mitigation and additional mitigation measures with current available resources for outbreak response and with unlimited resources for scenario 2

DISCUSSION

The aim of the simulations presented here was to assist veterinary authorities in the contingency planning of required human resources to respond effectively to an FMD outbreak in Denmark by investigating the predefined KPI targets and providing recommendation for future investment. Given estimated currently available human resources, the Danish Veterinary Authorities would be able to deal with small-to-medium outbreaks. However, they would be unable to reach all their predefined KPI targets to control larger outbreak FMD outbreaks. Our simulation results demonstrate that, of the operational activities, surveillance and culling were the bottlenecks and most important factors influencing the epidemic size. Increasing the number of surveillance and culling teams, was predicted to increase operational costs, but still significantly lower the overall cost of the outbreak. A disadvantage of our assessment is that, although we differentiated the average required working time per operational activity and per production type (e.g., culling of weaner pig herds vs. dairy herds), we did not consider that the effectiveness of operational activities per operational team can vary. Further, only two control strategies were investigated here, the basic strategy and additional culling of contact farms without further clinical investigation or testing. In situations with very large epidemics, as described in Scenario *B*, further additional measures could be expected to be implemented, for example pre-emptive culling of farms surrounding infected farms, or vaccination. Future work could include investigate of KPI's of such scenarios. Nonetheless, the simulation results presented in this study will help guide the Danish Veterinary Authorities in their investment planning for an effective outbreak response capability and/or their re-evaluation of their current KPI targets.

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DISEASE TRANSMISSION

ANALYSIS OF BETWEEN-PREMISES MOVEMENT DYNAMICS OF PIGS SHIPPED ACROSS 20 U.S. STATES

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SUMMARY

Effective disease control relies on restricting animal movement to prevent the spread of disease in livestock systems. Here, we analysed three years of between-premises pig shipments across 20 U.S. states. We constructed temporal networks, measured movement distances and loyalty, and applied network-based control actions. The median movement distance was 74.37 km; intrastate and interstate median distances were 52.71 km and 328.76 km. Our results of network-based targeted percolation showed that targeting 25% of farms with the highest degree of betweenness effectively reduced potential epidemics. Overall, we answered longstanding questions about the interstate and intrastate movements of swine in the U.S. We also compared actual movements network metrics to published county-level simulated swine shipment networks and demonstrated that simulated networks overestimated the in-degree and out-degree and underestimated the total degree. Ultimately, we highlight the relevance and need for creating a national movement data repository to enhance U.S. disease control preparedness.

INTRODUCTION

It is believed that in the U.S., approximately 1 million pigs are transported between commercial farms and markets and across state lines in a single day (Brodersen, 2020). The extraordinary complexity of these animal and vehicle movements, which change daily, presents a formidable challenge for decision-makers who need to choose and implement effective disease control measures under time constraints (Lee et al., 2017; Machado et al., 2021). Network analysis assesses how diseases spread via these movements (Ferdousi et al., 2019; Passafaro et al., 2020; Galvis et al., 2021). Some European studies using actual data and network modeling have demonstrated that between-farm animal movements accounted for a staggering 99% of African swine fever (ASF) transmission (Andraud et al., 2019), thus highlighting the importance of this approach and the use of real population and movement data. U.S. studies need more real data to achieve these insights (Ferdousi et al., 2019). Although some studies of animal movements have been used to assess the potential spread of ASF in the U.S., this research relied on synthetic, simulated data (Ferdousi et al., 2019). Indeed, the authors noted that real pig movement data would heighten the value of presumptive network modeling to better plan for foreign animal disease (FAD) outbreaks and to understand better and identify large-scale movement patterns. The U.S. swine industry is vertically integrated and comprises approximately 64,871 premises, housing 72 million pigs in premises

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specializing in one or more production phases (e.g., farrow-to-wean, growing pig) (Passafaro et al., 2020). Commingling has been previously linked to a heightened risk of disease transmission (Aragon et al., 2019). Movement flows that rely on many premises as sources to fill nursery or finisher premises result in contact networks with low animal movement loyalty, leading to a reduction in the number of regular connections between the same premises, thus increasing the chances for disease dissemination (Hammami et al., 2022).

This paucity of animal movement data substantially hinders our ability to fully understand how endemic diseases spread within complex, multi-state swine production systems (Galvis et al., 2022; Sykes et al., 2023). Therefore, before FADs are introduced in the country, it is vital to collect, catalogue, and evaluate the quality of animal movement and population data (e.g. farm capacity and animal movement). To address the lack of comprehensive movement data and to better prepare for disease outbreaks, we formed a consortium of multiple states and sectors to develop epidemiological models to combat swine infectious diseases and other animal health threats (Machado et al., 2023). Leveraging the consortium, we have amassed multi-state animal movement data and present a large-scale animal movement network analysis of between-premises shipments from 3,973 premises, moving 391,625,374 pigs across 20 U.S. states and six production companies. Our study compares the number of animal movements by premises' production types and swine production companies, investigating premise-to-premise intrastate and interstate shipment distances and the mixing of movements at the destination premises. Finally, we used a temporal network to examine the effect of node removal in curbing disease spread trajectories.

MATERIALS AND METHODS

Via our stakeholder's consortium, we collected 282,185 premises-to-premises swine movements from six U.S. swine production companies representing movements occurring between January 1st, 2020, and January 15th, 2023. In total, we collected shipment data of 3,973 premises and 391,625,374 pigs shipped across 20 U.S. states: Missouri, Iowa, Wyoming, Illinois, Nebraska, Oklahoma, Indiana, North Carolina, Mississippi, Kansas, Texas, Colorado, Virginia, South Carolina, Pennsylvania, Ohio, New York, Arizona, Utah, and Michigan.

We used the premises geolocations to up-scale between-premises movements and calculate the number of movements within and between counties and states. For state-level movements, we calculated the number of intrastate and interstate shipments and the number of shipped pigs. The results are depicted in thematic maps (Fig.1). The distance from the source to the destination premises was calculated, considering the distances of intrastate and interstate movements for each company and production type. The distances were transformed into an empirical cumulative distribution function (eCDF). An eCDF plot shows the data from the lowest to the highest values and compares them to their percentiles.

In addition, we use commingling to refer to any mixing of animals at the destination premises, regardless of production phase (e.g., at nurseries, finishers, and sow farms). To quantify the number of mixing sources, we used a premises-level network that considered the origin and destination production types. We also examined our multi-state contact network degree distribution to test the scale-free hypothesis. The scale-free hypothesis was tested by fitting the distribution of degrees k to a power law, defined as $p(k) = ak^{-\alpha}$, where α is the scale parameter and a, a proportionality term (Barabási et al., 1999; Passafaro et al., 2020). The data points used in these analyses were averages and standard deviations of each degree value across premises. In-degree and out-degree distributions were fitted to a range of distributions to compare the fit to the data and theoretical power-law distributions. We used goodness-of-fit tests based on the Kolmogorov-Smirnov statistic and calculated log-likelihood ratios between the candidate distributions. We used p-values to determine the significance of the results over 10,000 bootstrapping simulations. In-degree and out-degree distributions fit a power law if the p-value exceeds 0.05 (Clauset et al., 2009). Finally, we have also measured network loyalty, which was defined as the fraction of edges preserved in two consecutive time-frames, calculated by the Jaccard index, which provides a value from 0 to 1. A loyalty of 0 denotes all edges having changed in the consecutive time frame(s), and a loyalty of 1 represents all edges remaining the same (Valdano et al., 2015).

We used cumulative contacts between premises to indicate the outbreak size and the effectiveness of network-based targeted percolation. Network percolation is a method that approximates the effects of countermeasure actions, such as farm closure or movement restrictions, which effectively reduce disease dissemination by targeting premises based on their network role (Nöremark et al., 2011). We ranked premises in descending order and ran five removal scenarios, in which 5%, 10%, 15%, 20%, and 25% of the total number of premises were removed. The percentage removal was proportional to the number of premises in each production company. For example, 5% removal was 100 out of 1,997 premises for company A. Percolation was also performed without any premises ranking. This scenario was used to mimic regular random surveillance activities and to benchmark with network-based interventions. To examine the expected reduction of outbreak size achieved by targeting premises based on their network roles, we adapted the method proposed by Payen et al. (2019), to calculate the out-going contact chains as a proxy for expected outbreak size, named hereafter as "spread cascade model" (Payen et al., 2019; Cardenas et al., 2021; Machado et al., 2021). Here, spread cascade size reduction was calculated for the percolation scenario.

RESULTS

The daily number of shipped pigs had a median of 356,848 (interquartile range [IQR]: 57,350-468,642, maximum: 2,359,236), and 156 shipments (IQR: 41-311, maximum: 453). The states with the highest intrastate movements were North Carolina, Iowa, and Oklahoma, with 88,749, 17,505, and 15,717 shipments, and 322,713,497; 7,001,558, and 4,861,502 shipped pigs, respectively (Fig.1). The top three states with the highest between-state movements were: North Carolina to Virginia, with 3,011 shipments and 11,152,229 pigs; North Carolina to South Carolina, with 2,282 shipments and 6,311,509 pigs; and Illinois to Iowa, with 1,510 shipments and 3,038,413 pigs (Fig.1).

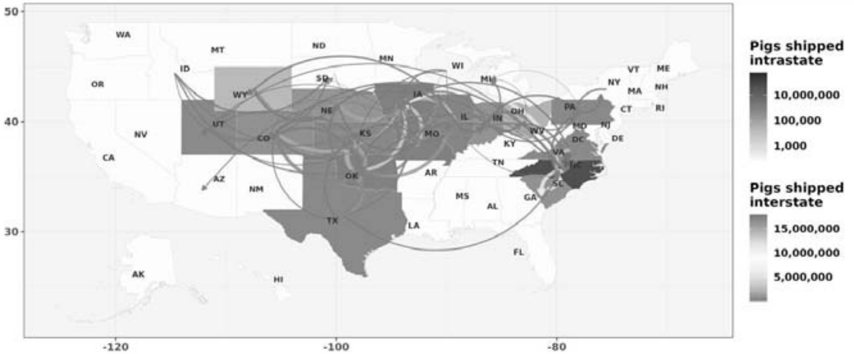

Figure 1. The intrastate and interstate movement of pigs. The shaded gray background shows the number of intrastate pig shipments, while the color and width of the arrow lines represent the interstate shipments. The number of shipped pigs is on a log10 scale

The median distance pigs were shipped was 74.37 km (IQR: 33.79-187.76 km, maximum: 3,415.22 km) (Fig.2A). Intrastate movements had a median distance of 52.71 km (IQR: 26.24-104.15 km, maximum: 534.00 km), while interstate movements had a median distance of 328.76 km (IQR: 191,438-705,680 km, maximum: 3,415.22 km) (Fig.2B). Comparing shipment distances, companies (A, E, and C) shipments were at short distances, with median distances of 56.1 km (IQR: 28.4-11 km, maximum: 3,415 km), 54.1 km (IQR: 30.5-113 km, maximum: 1,046 km), and 33.7 km (IQR: 19.8-46.6 km, maximum: 132 km), respectively. However, companies F, D, and B shipments were long distances, with median distances of 264 km (IQR: 108-567 km, maximum: 1,603 km), 91.7 km (IQR: 33.6-245 km, maximum: 1,250 km), and 56.8 km (IQR: 37.4-74.8 km, maximum: 166 km), respectively (Fig.2D). Regardless of production type, the median number of different premises sources mixing at destination premises was 2 (IQR: 1-4, maximum: 144).

Commingling in wean-to-finisher premises was higher than in finisher, sow, and nursery farms. Different premises sending pigs from sow into wean-to-finisher exhibited a median of 4 (IQR: 2-7, maximum: 24), and sow sending pigs into finisher had a median of 4 (IQR: 2-10, maximum: 22), followed by sow to finisher and finisher to sow farms with a median of 3 (IQR: 2-6, maximum: 24) and median of 3 (IQR: 2-5, maximum: 21), respectively.

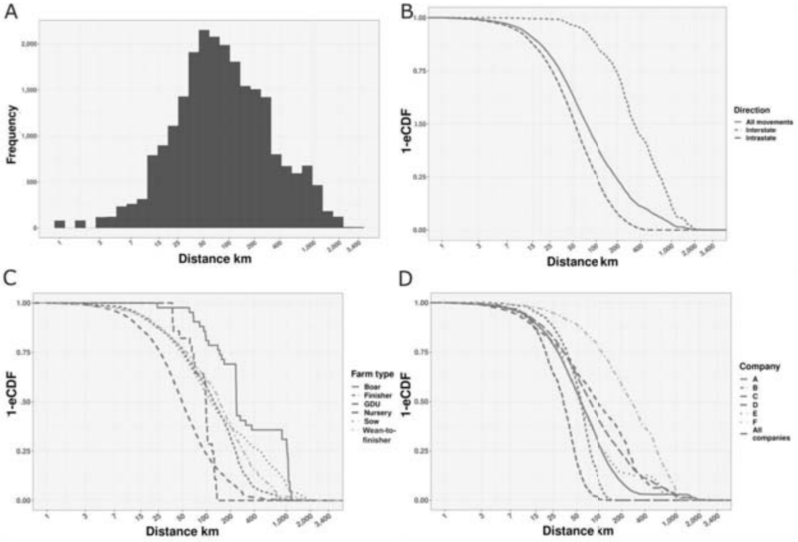

Figure 2. Between-premises movement distances. A) The histogram of between-premises shipment distances. B) Intrastate and interstate empirical cumulative distribution function (eCDF). C) The eCDF by production type. D) The eCDF by company

The overall network median in-going loyalty was 0.69 (IQR: 0.60-0.75, maximum: 0.96), and out-going loyalty was 0.52 (IQR 0.36-0.81, maximum: 0.91). Sow and nursery out-going loyalty was the highest, in comparison with wean-to-finisher and finisher loyalty. Regarding in-going loyalty, finisher, sow, and nursery had similar distributions, with a median of 0.71 (IQR 0.63-0.77, maximum: 0.94), 0.68 (IQR 0.61-0.73, maximum: 0.88), and 0.77 (IQR 0.68-0.82, maximum: 0.96), respectively, and wean-to-finishers and finishers exhibit greater loyalty towards their incoming movements than outgoing movements. We also demonstrated that the out-degree distribution followed a power-law distribution with an exponent of $\alpha = 3.34$ (p-value = 0.75). However, the in-degree distribution did not follow a power-law distribution and only fitted to an $\alpha = 4.1$ (p-value = 0.083).

The best network parameters to reduce cascade spread in descending order were degree, betweennesses, cluster coefficient, and PageRank (Fig.3) Cascade reductions were most effective when 25% of nodes ranked by degree were removed, limiting possible spread to 1.23% of premises (IQR: 0.66% -2.38%, maximum: 7.10%). Node removal based on betweennesses was the next most effective, with a median reduction of 1.70% (IQR: 0.87%-3.21%, maximum: 20.42%). Cluster coefficient and PageRank reduced cascade to a median of 4.87% (IQR:2.15%-4.96%, maximum: 53.63%) and 8.09% (IQR: 2.57%-16.64%, maximum: 56.40%), respectively. As expected, random node removal had the most negligible effect, reducing cascades to a median of 12.01% (IQR: 3.42%-26.34%, maximum: 65.68%).

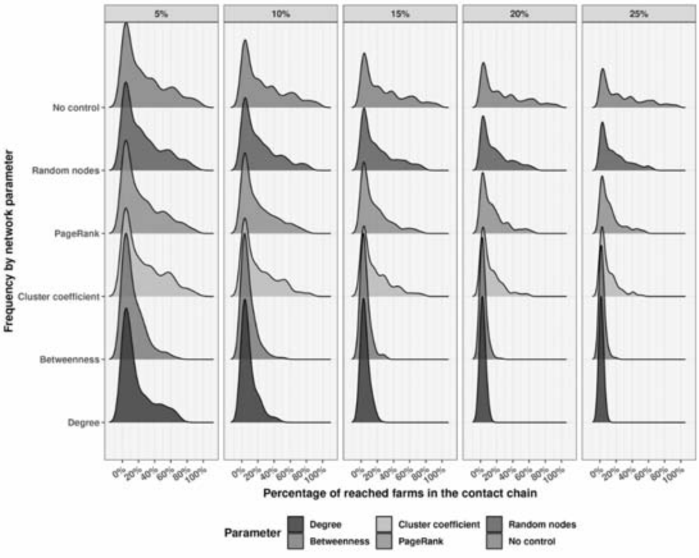

Figure 3. Density distribution of network-based node percolation removal. The ridgeline plot in the top panel shows the percentage of removed premises (5%, 10%, 15%, 20%, and 25%) for network parameters (y-axis). The x-axis shows the percentage of premises within cascades after node removal

DISCUSSION

We demonstrated that most pig shipments were intrastate, with a median of 52 km between the source and destination premises, while long-distance interstate shipments occurred over distances greater than 1,000 km. Shipment patterns significantly differed across companies and production types, with the most significant differences observed in commingling and between premises movement loyalty. The median number of pig shipments of different sources mixing at destination premises varied between two and four. In-going loyalty was 69%, and out-going was 52%, indicating lower loyalty for outgoing movements. The out-degree distribution fitted a power law distribution, exhibiting scale-free topology, which suggests the presence of highly connected premises (hubs) with the capacity to drive super-spread disease events. On the other hand, such scale-free topology is ideal for network-based target control tactics, as shown by the network-based targeted percolation approach, in which targeting farms by degree was highly effective in reducing potential epidemics.

An in-depth understanding of national movement patterns is a key element for epidemic control in highly integrated swine production systems. In the same vein, examining interstate and intrastate pig shipment data is crucial to informing national disease response plans and developing novel tactics against endemic and emerging diseases (Cardenas et al., 2022; Hammami et al., 2022; Sykes et al., 2023). While we demonstrated that most movements occurred over short distances and were intrastate, we noted significant differences in shipment distances among companies. In the same way, low out-going loyalty was significant in all companies' finisher, nursery, and wean-to-finisher. Such low loyalty, when associated with sizeable out-going contact chains, may increase their risk of becoming super-spreaders. Similarly, premises with low in-going loyalty and high commingling are expected to act as super-receivers with a higher risk of infection (Acosta et al., 2023). This suggests that there is significant variation in the topology of the swine production network across countries, which is likely to directly affect the effectiveness of movement restriction strategies to control the spread of infectious diseases.

We demonstrated that the U.S. swine network out-degree distribution followed a scale-free distribution; thus, most premises are poorly connected, and a few have very high connections. Such network property is rare among natural systems (Broido and Clauset, 2019) and has been a characteristic of the swine network (Relun et al., 2016; Passafaro et al., 2020). Given the degree distributions observed in this study, we expected that network-based control and prevention strategies would significantly impact containing movement-related dissemination events. Indeed, we showed that a median of 19.1% of premises would likely be infected if no movement restriction control actions were implemented; however, when targeting 25% of the premises based on degree and betweenness, we were able to reduce the spread to 1.23%, and 1.7% of infected premises, respectively.

Finally, in the U.S., between-state livestock shipment data is collected via certificates of veterinary inspection (CVIs) (Gorsich et al., 2019), while within-state shipment data are collected but not required to be shared with animal health officials (Cabezas et al., 2021). Previous studies describing U.S. swine contact networks have used interstate certificates permitting the movement of animals in some states (Gorsich et al., 2019; Passafaro et al., 2020; Cabezas et al., 2021). However, due to the lack of large-scale, real, between-farm swine movement data, Passafaro et al. (2020), adapted a Bayesian Markov chain Monte Carlo model used to simulate cattle shipment networks (Lindström et al., 2013) to simulate swine shipment networks. These simulated networks included predictions of shipment sizes, number of nodes, and edges and were used to generate maps of movement distributions at the county level. Here, we use our between-premises movement data to compare real data and simulated results (Passafaro et al., 2020). Our comparison indicated significant differences in the in-degree, out-degree, betweenness, and outgoing number of pigs values between actual and simulated data (Kruskal-Wallis Rank Sum Test, p-value <0.05). The simulated data overestimated the in-degree and out-degree by 20 to 22 degrees, respectively, and underestimated the total degree of 43 and 44 counties of 100 counties. Ultimately, we argue that while simulated networks may be helpful, the results fail to represent the complexity of real-world commercial swine network topology. Therefore, we highlight the relevance and need for real movement data for better disease control preparedness and suggest caution in using simulated datasets.

This study offers crucial insights into the network structure of pig movements in the U.S., where most movements were limited to a 74 km radius of the origin premises. We highlighted the presence of low movement loyalty and high commingling, specifically for finisher farms. The proposed spread cascade model, focusing on out-going contact chains, supports targeting farms with a high degree of betweenness for control measures to enhance the efficiency of infectious disease surveillance and management.

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IMPACTS OF CATTLE MOVEMENT NETWORK ACTIVITIES ON THE SPREAD OF *SALMONELLA* DUBLIN BETWEEN CATTLE FARMS IN DENMARK BETWEEN 2010 AND 2020

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SUMMARY

The network analysis of data from the period 2010-2020 revealed the failure of between-farm movement restrictions in Denmark to reduce salmonella transmission.

INTRODUCTION

Salmonella Dublin (*S.* Dublin) differs from other Salmonella serovars by being host-adapted to cattle. Zoonotic infections may be acquired by direct contact with cattle, or faecal matter, or by ingesting unpasteurised milk products or consuming contaminated beef products (Henderson and Mason, 2017). More than 10,000 human Salmonella cases occurred in Denmark in the period 2013-2023. Around 2-3% of those were identified as S. Dublin. To safeguard animal and public health, voluntary surveillance efforts in the cattle sector (i.e. collaborative projects, diagnostic surveillance of all cattle herds and communication campaigns) were implemented in 2000 and progressed to a national eradication programme in 2008 with the aim of eliminating *S.* Dublin from the Danish cattle population by the end of 2014. Despite elaborated mitigation efforts, such as cattle movement restrictions between *S.* Dublin test-positive and test-negative cattle farms since 2010, regionalisation in 2013-2017 prohibiting the transportation of animals from the high-prevalence regions in Jutland to low-prevalence regions, and mandatory control efforts in test-positive farms, an increase in the apparent prevalence from around 5.5% to around 11% and observed new cases of infected farms have been observed since 2015 in the dairy sector.

Hence, it is important to improve our understanding of the roles of movement-related spread and local spread between cattle farms, as *S.* Dublin bacteria can survive outside of the host for long periods of time. Study of the networks of livestock movements provides a means for understanding the dynamics of diseases through the networks and defining effective mitigation measures (Bigras-Poulin et al., 2007; Dubé et al., 2009; Martinez-López, et al., 2009; Bajardi et al., 2012; Pinior et al., 2012a,b; Pinior et al., 2015; Lebl et al., 2016; Lentz et al., 2016; Vidondo and Voelkl, 2018; Pozo et al., 2019; Knific et al., 2020; Tratalos et al., 2020; Cardenas et al., 2022; Hammami et al., 2022). Several studies have demonstrated how

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information about the topology of cattle movement networks and associated animal products can aid risk assessment analysis and the design of targeted, effective and efficient surveillance and control strategies. For example, in Germany (Lentz et al., 2009; Pinior et al., 2012a,b; Pinior et al., 2015; Lebl et al., 2016), Denmark (Bigras-Poulin et al., 2007; Mweu et al., 2013), Sweden (Nöremark et al., 2011), Austria (Puspitarani et al., 2023), Switzerland (Vidondo and Voelkl, 2018; Schirdewahn et al., 2021), France (Dutta et al., 2014; Hammami et al., 2022), Slovenia (Knific et al., 2020), Italy (Natale et al., 2009), Spain (Pozo et al., 2019), The United Kingdom (Ortiz-Pelaez et al., 2006; Guinat et al., 2016), Ireland (Tratalos et al., 2020), Uruguay (VanderWaal et al., 2016) and Brazil (Menezes et al., 2020).

Thus, the objectives of this study were: *i)* to gain insight into the structure of the trade network in Denmark between cattle farms by performing a network analysis on cattle movement data from the period 2010-2020; *ii)* to quantitatively estimate the effect of cattle movements and local transmissions on the spread of *S.* Dublin infections based on previous 1-month and 4-month movement networks; and *iii)* to simulate the spread of *S.* Dublin infections on monthly networks using an epidemiological compartment model driven by between-farm cattle movements.

MATERIALS AND METHODS

We constructed static, directed and weighted trading networks based on individual cattle movements between source and destination cattle farms in each month from the period 01/2010 to 12/2020, resulting in a total of 132 trade networks. For each of the 132 networks, different network properties were calculated both separately for infected farms (i.e. farms considered likely infected due to test-positivity or contact to test-positive farms) and non-infected cattle farms (i.e. farms considered likely not infected due to being test-negative or being a type of farm with known very low *S.* Dublin occurrence, such as extensive beef farms), and combined for both infection categories. Furthermore, we conducted multiple logistic regression analyses on 1-month to 4-months movement networks to get valuable insights into the relationships between local and animal movement transmissions and the odds of infection in the following 6 months, while controlling for independent variables (farm size, month of trade, total neighbourhood (number of all cattle farms in a 5 km-radius around each farm), and production categories).

The spread of *S.* Dublin was simulated based on observed movement activities and a range of hypothetically set of movement network activities (α) between cattle farms to investigate the impact of potential changes in the degree of movement activities on the spread of *S.* Dublin at the monthly level during (2015-2017) and after (2018-2020) the regionalisation period. The movement network activity α is defined as the mean edge frequency of a network (i.e., how often a certain edge between a node pair was active divided by the length of the 3-year period), such that $0 < \alpha < 1$. Subsequently, for each α (i.e., across degrees of cattle movement), outbreak probability was calculated based on the proportion of the simulation runs (n=1,000) that resulted in an *S.* Dublin spread beyond the starting node. The relative outbreak size was calculated as the proportion of infected farms relative to the total number of potentially infectable nodes in the movement network, and outbreak duration was simply the number of months the outbreak persisted. Initially, all non-infected farms in the dataset were assigned in S_i (the susceptible compartment) and, at the randomly chosen time, one randomly selected index farm was set to I_i (the infected compartment). In other words, all starting times and farms had the same selection probability. Following an animal movement, the receiving node would be become infected according to the probability function shown in Eq.(1):

$$p^e = P(X > 0) \sim B(w, p) = 1\text{-}(1\text{-}p)^w \quad (1)$$

where $B(w, p)$ is a binomial function depending on the transmission probability $p = \{0.25, 0.50, 0.75\}$ and w is the edge weight (i.e., the number of cattle moved). A detailed description of the susceptible (S_i), infectious (I_i) and recovered (R_i) simulation approach on movement networks is described by Lebl et al. (2016). Further, we introduced targeted and randomly implemented intervention measures by removing nodes from the cattle movement networks to analyse the associated impact on the spread of *S.* Dublin. Targeted removal of nodes was performed based on the highest betweenness of the farms (betweenness ≥ 100,000).

RESULTS

The network analysis indicated that disease control measures regarding movement restriction such as between regions (regionalisation) within the period 2013-2017 have had no marked impact on the analysed network properties for infected farms, whereas an effect was observed on the average path length of non-infected farms. In the year 2020, highly heterogeneous patterns were observed when comparing infected and non-infected farms in terms of the modularity, degrees, betweenness, density and closeness (Fig.1).

The infected farms had higher trade activities and traded more within their clusters than with the rest of the network based on the determined density and clustering coefficients in the monthly networks. All monthly networks had a negative assortativity, meaning that low-degree nodes are more likely to trade with high- than other low-degree nodes. Moreover, only 1.8% of the connections persisted (referred to as in-loyalty) between farms from 2010 to 2020. For each year, the proportion of nodes in the strong and weak component was quite similar in each of both infection categories, whereby we observed, with time, a decrease in the network component size, which means that the overall connectivity or interconnectivity of the network were decreasing for both the non-infected and infected cattle networks in Denmark. The highest predicted odds ratio for farms changing *S.* Dublin infection status in the surveillance programme from ‘likely not infected’ to ‘likely infected’ was found for the first month of trade following a cattle movement, whereas the odds farms changing *S.* Dublin infection status due to local transmission were roughly constant over post-movement months, based on previous 1-month to 4-month cattle movement data (Fig.2).

Running the infectious disease simulation on monthly movement networks during and after regionalisation indicated that the outbreak probability in the entire network across all simulation runs and trade activities was between 5% and 13%, respectively. The relative outbreak size was, on average, 3% (n=38 infected farms per simulation iteration) depending on the transmission probability and the outbreak duration was, on average, approximately half a year. The simulation model showed that targeted removal of the 1% cattle farms with the highest betweenness (i.e., ≥ 100,000) from the movement network would achieve an 88.7% reduction in the spread compared to the removal of random cattle farms.

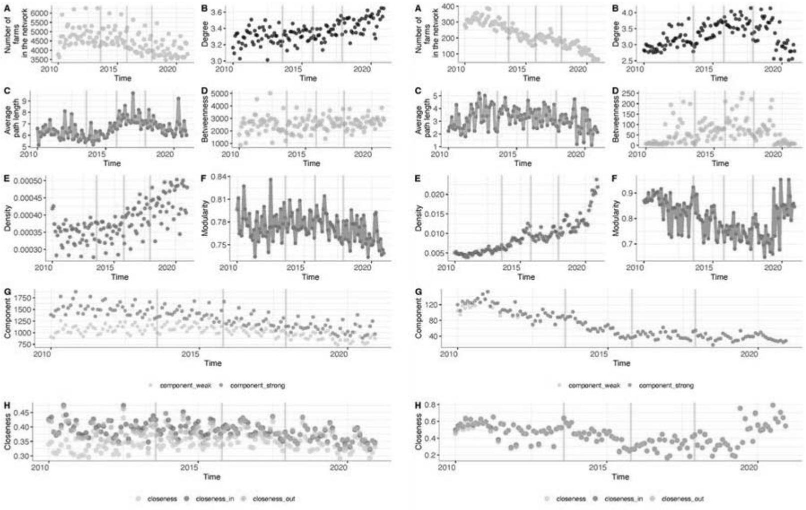

Figure 1. Network properties of non-infected farms (left side) and *S.* Dublin infected farms (right side): (A) the total number of farms that moved cattle. (B) Average degree. (C) Average path length. (D) Betweenness. (E) Density. (F) Modularity. (G) Component. (H) Closeness. Vertical lines in each of the plots are dates of main implementations of disease control measures related to movement restrictions between different regions in addition to movement restriction between infected and non-infected farms since 2010, with the following implementations reflected: *i*) 15/07/2013, when regionalisation was implemented, prohibiting the transportation of animals from Jutland to the Islands; *ii*) 20/10/2015, when regionalisation criteria were revised, expanding the low-prevalence area to include certain parts of Jutland; *iii*) 29/11/2017, when regionalisation was discontinued and movement restrictions were extended to all test-positive farms

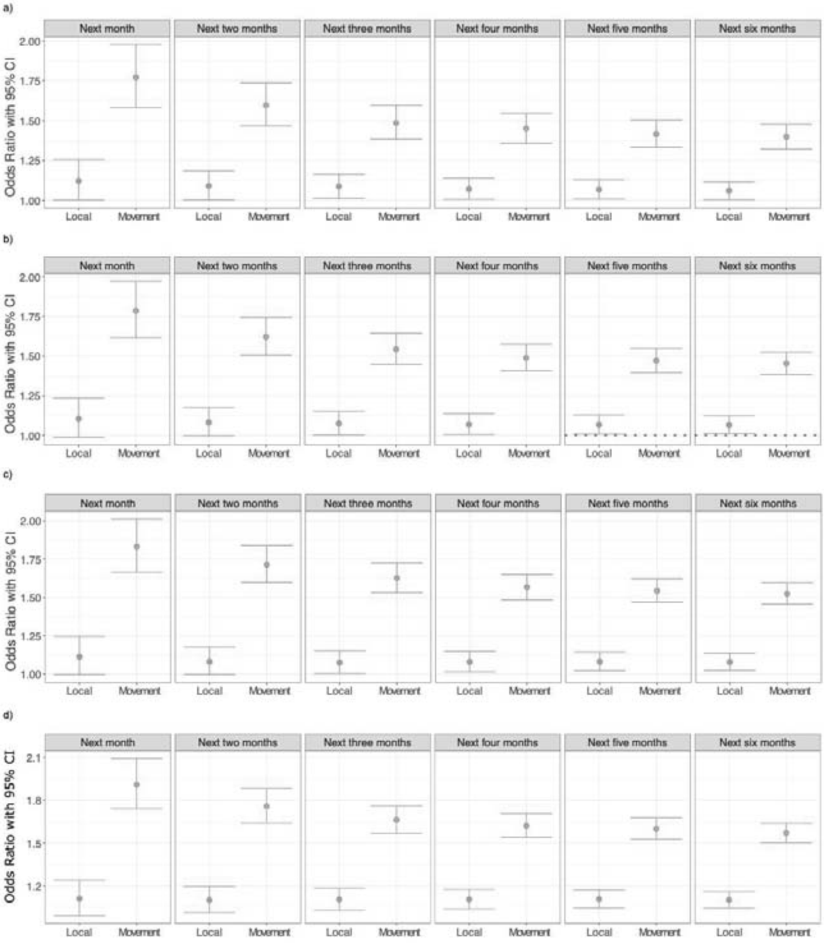

Figure 2. The odds ratio of *S*. Dublin infection due to local (radius of 5 km) and cattle movement transmissions, across months one through six based on the movement period of the previous (A) month, (B) 2 months, (C) 3 months, and (D) 4 months. Adjusted for farm size, cattle movement month, production category of farms and total neighbourhood (total number of farms in a radius of 5 km around the infected farm). Note that local transmissions included all premises (i.e., including farms without cattle movements to other premises)

DISCUSSION

Similar trends in the network properties were observed for both infected and non-infected cattle farms, suggesting that the implemented movement restrictions in the Danish control programme unexpectedly had a uniform effect on trading patterns during that period (Fig.1). In general, the identified network properties in Denmark were higher, and the Danish cattle farmers were more active with respect to movement of cattle, relative to other European cattle trade networks (Christley et al., 2005; Natale et al., 2009; Rautureau et al., 2011; Nöremark et al., 2011; Dutta et al., 2014; Vidondo and Voelkl, 2018; Knific et al., 2020). Nonetheless, our determined network properties concur with previous observations in Denmark, where the range of in- and out-degree exponent values of approximately 2 were obtained (Bigras-Poulin et al., 2007; Mweu et al., 2013). Similar degree values have been reported from analyses of British 2.10 (Christley et al., 2005), Italian 2.26 (Natale et al., 2009) and French 2.58 (Rautureau et al., 2011) cattle networks. The comparison between former network analyses from Denmark in addition to the comparison of the monthly network snapshots in the current study suggest a reduced overall connectivity or interconnectivity of the Danish cattle network over the period (Fig.1). In general, direct comparison between studies is difficult due to different time periods, included holding types and study aims. In contrast to the present study, the former Danish studies only analysed the cattle movement data without focusing on a specific disease and did not combine movement data with specific animal disease surveillance data.

Our study found that the odds of becoming 'likely infected' via cattle movements were greater than the odds of becoming 'likely infected' via local transmission. We also found that the density and clustering coefficients were higher for 'likely infected' farms compared to 'likely non-infected' farms, and that clusters may be used to enhance surveillance and help the inference of the origin of an outbreak. However, the identified temporal variability and in-loyalty of the Danish cattle network in this study suggested that identification of the possible origin of the source of spread and detection of secondary infected cattle farms will be particularly difficult. It is also important to note that our study aggregated movements at the monthly level, which can potentially overrepresent a network's connectivity. This overrepresentation of connectivity can result in overestimation of the following: *i*) network properties, *ii*) local and cattle movement transmission effects, and *iii*) the size, probability, and duration of outbreaks based on both observed movement activities and the hypothetically increased and decreased movement activities. It should also be noted that the surveillance programme of the Danish cattle farms does not allocate *S*. Dublin infected farms to the 'likely infected' status and *S*. Dublin non-infected farms to the 'likely not-infected' status with perfect sensitivity and specificity. This is both due to non-perfect diagnostic test procedures and due to the fact that farms according to the current legislation are allocated to the 'likely infected' surveillance status if they receive animals from a farm in the 'likely infected' status, even if no transmission of bacteria actually occurred through the animal contact. In the used data, this is mainly relevant for farms in multi-site business structures allowing for movement between farms owned by the same farmer, despite one or more farms within the business structure being test-positive. This may also lead to a risk of overestimation of the effect of cattle movement in the previous month on spread of infection, as some of the status-changes may have been purely administrative.

Despite these limitations, based on our findings, we suggest that current surveillance and control measures could be adapted to focus on reducing the risk of moving infected cattle between farms by implementing more focused within-herd control measures to reduce the prevalence of infected farms, and either more restrictive movement restrictions (currently mainly relevant for multi-site business structures and therefore difficult to implement), pre-movement mitigation measures such as animal or animal group testing and/or quarantine of moved animals within a 1-month window to help reduce the movement-related spread of *S.* Dublin between farms.

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HIGHLY PATHOGENIC AVIAN INFLUENZA H5N8 SPREAD: DOES POULTRY FARM ACTIVITY AND PROXIMITY MAKE A DIFFERENCE?

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SUMMARY

Our study investigated the spread of highly pathogenic avian influenza (HPAI) H5N8 in French poultry farms using genetic data. Duck farm activity and proximity were associated with the reproduction number. Results guide policies to control HPAI, suggesting thresholds for farm layout changes and predictive models for vaccination planning.

INTRODUCTION

The global spread of highly pathogenic avian influenza (HPAI) viruses poses a significant threat to animal health, with profound economic implications for the poultry production sector and wildlife conservation efforts. Over the past decade, Europe has experienced four major epidemics of HPAI, reaching a devastating peak during the 2021-22 wave with 2,771 poutry farm outbreaks, and the subsequent 2022-23 wave becoming particularly impactful for wild birds with 4,116 cases (European Food Safety Authority et al., 2023). Effectively managing HPAI epidemics is now a critical challenge for the sustained well-being of the European poultry sector and wildlife conservation.

Numerous risk factors have consistently emerged as a pivotal factor associated with the occurrence of HPAI outbreaks (Gilbert and Pfeiffer, 2012). Notably, poultry farm density (Boender et al., 2007; Chaudhry et al., 2015) and the close proximity between farms (Nishiguchi et al., 2007; Kim et al., 2018) have been previously suggested as potential contributors to the spread of HPAI. Consequently, intervention strategies targeting the reduction of poultry farm density have been explored using modelling studies (Lambert et al., 2023), including reactive or preventive culling around infected premises (Andronico et al., 2019; Bauzile et al., 2023) and imposing a ban of restocking on emptied farms (Dorigatti et al., 2010). However, the extent and threshols at which farm density and proximity might limit virus spread remain to be precisely defined, highlighting the necessity to explore preventive measures further.

During the winter 2020-2021, France experienced a substantial surge in HPAI H5N8 outbreaks, notably affecting duck farms located in Southwestern France, totalling 468

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outbreaks (Lambert et al., 2022). During this wave, a significant number of poultry sequences from affected farms were successfully obtained, providing a unique opportunity to conduct a detailed quantification of virus transmission dynamics between poultry farms in Southwestern France using a Bayesian phylodynamic framework. Furthermore, our study capitalizes on a comprehensive dataset allowing to distinguish empty and full operational poultry farms during the outbreaks. This enables us to assess the association between poultry farm activity level, proximity and virus transmission dynamics over time. This multifaceted approach aims to provide nuanced insights into the effectiveness of preventive measures, ultimately contributing to the development of more targeted startegies for controlling HPAI spread in the region.

MATERIALS AND METHODS

Data selection

Genetic sequences: In the winter 2020-21, a total of 468 poultry farm outbreaks were reported from December 2nd, 2020 to March 20th, 2021 in France. Among these outbreaks, 83.1% (381/468) occurred in duck farms located in South-western France. The outbreak count peaked in Janurary and then declined over the study period, consistent with implemented farm-level interventions (Fig.1). During this period, the National Reference Laboratory at ANSES sequenced a total of 432 samples, representing 92.3% of all reported outbreaks. To identify introduction events into these outbreaks, we merged and aligned these sequences with those available on GISAID (https://gisaid.org) in Europe from September 7th, 2020 to May 1st, 2021 (n = 613 sequences) using MAFFT v7.49 (Katoh and Standley, 2013). To ensure data quality, we removed duplicate sequences with similar genetic diversity (threshold of 0.001) with the same sampling date, geographical coordinates, species and country. Based on this alignment, we inferred a maximum likelihood phylogenetic tree using the HKY+Γ_4 substitution model with 1,000 bootstrap replicates using RAXML-NG (Kozlov et al., 2019). Notably, all sequences from France formed a distinct phylogenetic clade, with the exception of one sequence that was excluded from further analysis (Fig.2). This curation resulted in a final dataset of 381 sequences, providing a representative depiction of the distribution of poultry farm outbreaks (Fig.1).

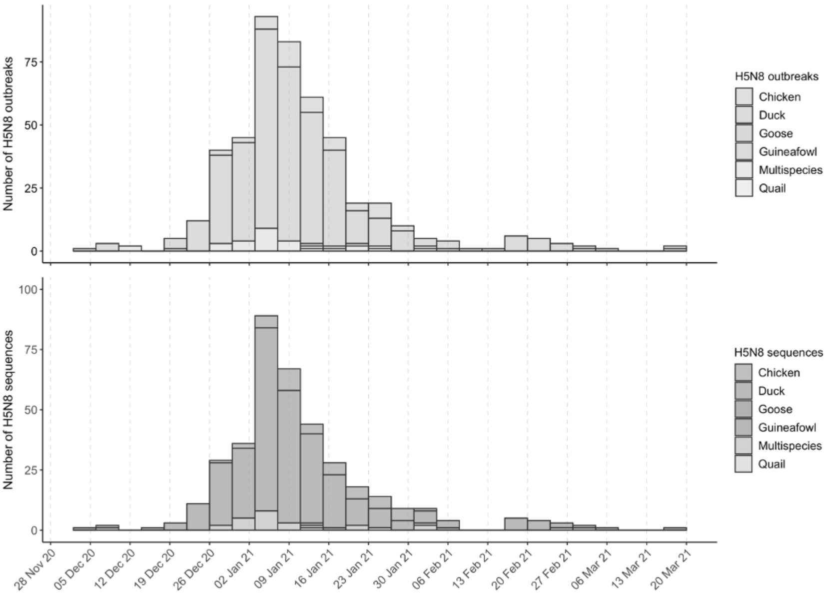


Figure 1. Temporal distribution of the H5N8 outbreaks and sequences in France in 2020-21

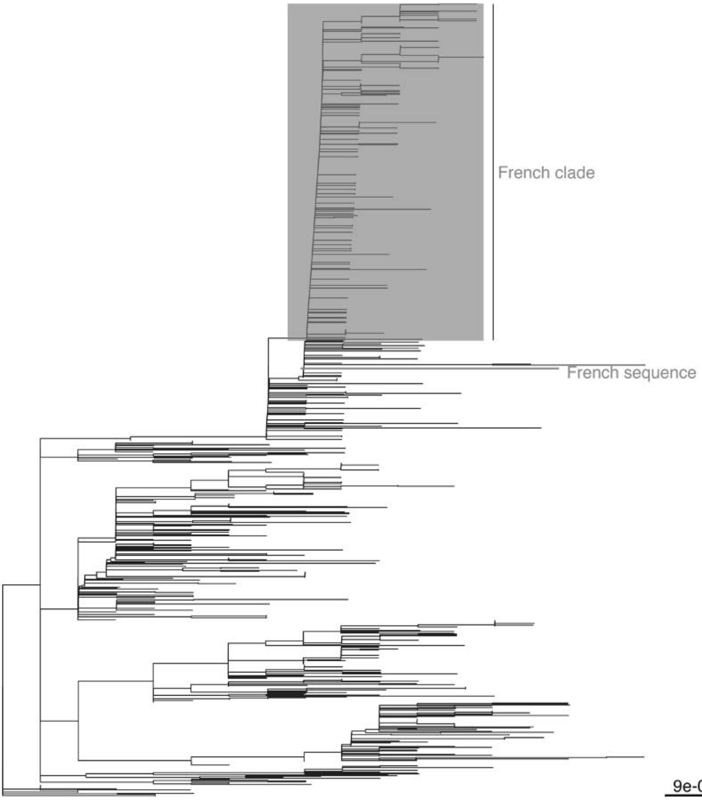
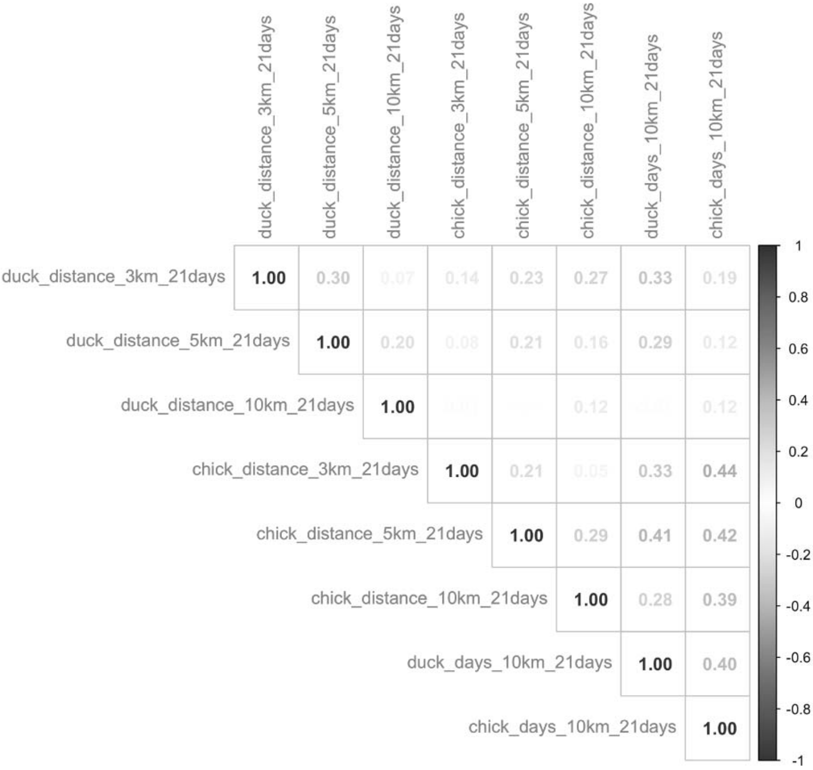


Figure 2. Maximum likelihood phylogenetic tree of French H5N8 sequences with European context in 2020-21

Number of activity days and distance variables: For each poultry farm outbreak, we calculated the cumulative number of activity days of farms in buffer zones of 3 km, 5 km and 10 km, as well as within 7 days, 14 days and 21 days prior to the reported time. Additionally, we calculated the mean distance of active farms within the same buffer zones and timeframes. The term “farm” refers to a building unit, and the term “active” refers to units with poultry inside. This excludes those that are empty either due to the delay between production cycles (typically around three weeks between two flocks) or due to culling measures (specifically if they are infected or if at risk of infection). Correlations between pairs of variables were examined using Pearson’s correlation coefficients and considered significant if their absolute value was < 0.70 (Dohoo et al., 2003). Ultimately, we selected eigth variables for further analysis (Fig.3). The chosen variables include: the cumulative number of activity days of duck farms within 10 km and 21 days, the cumulative number of activity days of chicken farms within 10 km and 21 days, the mean distance of duck farms within 3 km and 21 days, the mean distance of duck farms within 5 km and 21 days, the mean distance of duck farms within 10 km and 21 days, the mean distance of chicken farms within 3 km and 21 days, the mean distance of chicken farms within 5 km and 21 days and the mean distance of chicken farms within 10 km and 21 days. These variables are temporally represented in Fig.4. Notably, duck farms showed an average of 660 cumulated days of activity and the duck farm distance an average of 1.8 to 6.4 km. Chicken farms showed an average of 99 cumulated days of activity and the chicken farm distance showed an average of 0.9 to 5.8 km.



	duck_distance_3km_21days	duck_distance_5km_21days	duck_distance_10km_21days	chick_distance_3km_21days	chick_distance_5km_21days	chick_distance_10km_21days	duck_days_10km_21days	chick_days_10km_21days
duck_distance_3km_21days	1.00	0.30	0.07	0.14	0.23	0.27	0.33	0.19
duck_distance_5km_21days		1.00	0.20	0.08	0.21	0.16	0.29	0.12
duck_distance_10km_21days			1.00	[illegible]	[illegible]	0.12	[illegible]	0.12
chick_distance_3km_21days				1.00	0.21	0.05	0.33	0.44
chick_distance_5km_21days					1.00	0.29	0.41	0.42
chick_distance_10km_21days						1.00	0.28	0.39
duck_days_10km_21days							1.00	0.40
chick_days_10km_21days								1.00

Figure 3. Correlation coefficients between pairs of explanatory variables

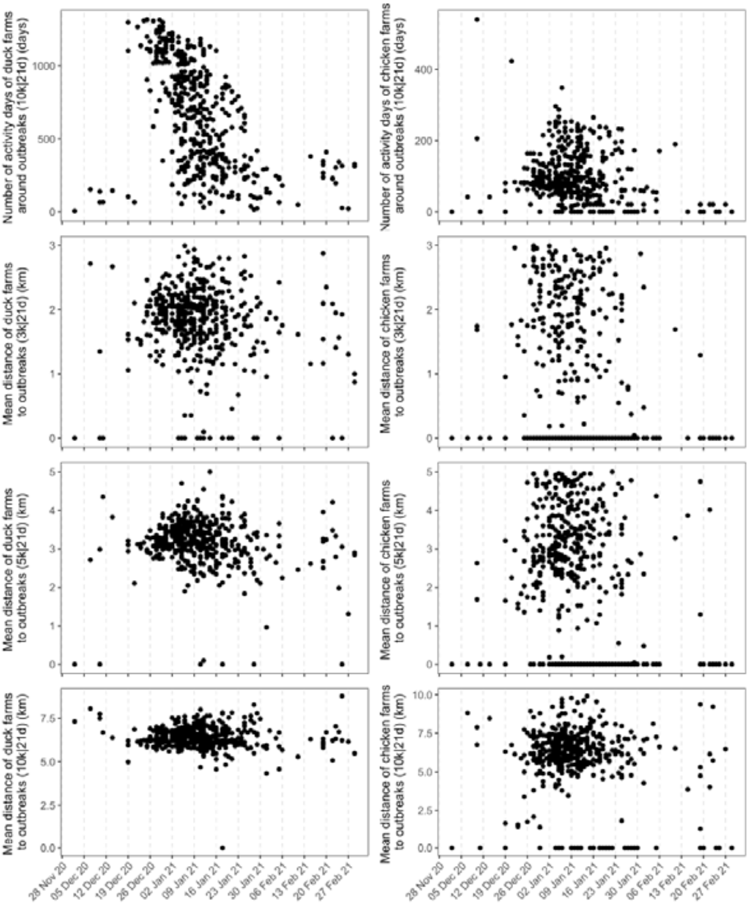


Figure 4. Temporal distribution of the explanatory variables

Phylodynamic analysis to estimate transmission parameters

We conducted Bayesian phylodynamic analyses using the 381 sequences sampled between December 2nd, 2020 and March 20th, 2021 in France, using BEAST v2.7.5 (Bouckaert et al., 2014). To infer transmission dynamics, we used the birth-death skyline model (Stadler et al., 2013), allowing for piecewise changes in the reproductive number (R_e) over time. Our model configuration involved weekly intervals for R_e, with the incorporation of a smoothing parameter designed to gently smooth out the R_e estimations over time. The R_e was approximated by a prior distribution around a median of 1 (lognormal(0,1)). We assumed that no poultry farm outbreaks could have been missed considering the severity of the clinical signs, especially in ducks, and the recurrent nature of HPAI waves, making farmers more likely to report. The sampling proportion was deinfed across two time intervals, fixed at 0 before the first reported poultry farm outbreak and at 0.81 after (381/468), representing the ratio between the number of sequences (n = 381) and the number of reported poultry farm outbreaks (n = 468). The origin of the epidemic was approximated by a prior distribution around one week prior to the first reported outbreak to align with previous epidemiological estimates (lognormal(-1.4, 0.05)) (Vergne et al., 2021). The model was coupled with a HKY+Γ_4 nucleotide substitution model and a relaxed molecular clock model with median rate of 10^{-3} substitution/site/year (lognormal(0.005, 1)). The infectious duration of farm units (e.g. the time from infection to sampling/culling) was approximated by a prior distribution around a median of 7 days to match previous epidemiological estimates (lognormal(52, 0.6)) (Andronico et al., 2019; Vergne et al., 2021). Posterior phylogenetic trees and model parameters were inferred using Markov Chain Monte Carlo (MCMC). MCMC was run for 500 millions generations and was sampled every 50,000 steps. Convergence was assessed using Tracer v1.7 (Rambaut et al., 2018), ensuring that the estimated sampling size values associated with the posterior model parameters were > 200. Post-processing involved discarding the initial 10% of the MCMC samples as burn-in. The maximum clade credibility (MCC) tree was obtained using TreeAnnotator v2.7.5 (Bouckaert et al., 2014) and annotated using the ggtree package in R v4.2.3 (R Core Team, 2013).

Statistical analysis to test for associations with poultry farm activity days and distance

Multivariable logistic regression analysis was used to identify variables statistically associated with a poultry farm outbreak having a $R_e > 1$. Each poultry farm outbreak was assigned the mean R_e estimated for the time intervals of the outbreak. A binary outcome was created to distinguish farm outbreaks with a $R_e > 1$ from those with a $R_e <= 1$. The multivariable logistic regression model incorporated the eight explanatory variables and variable selection was performed through backward-forward elimination with a significance level set at p-value < 0.05. Regression coefficients were expressed as odds ratios (OR) with corresponding 95% confidence intervals (CI). All statistical analyses were performed using R v4.2.3 (R Core Team, 2013).

RESULTS

Phylodynamic estimates of the reproductive number (R_e)

Bayesian phylodynamic analyses estimated the median time to the most recent ancestor of the 381 samples as December 2nd, 2020 (95% high posterior density interval, HPD: November 30th, 2020 - December 4th, 2020), with an average evolutionary rate of 9×10^{-3} substitutions/site/year. The infectious period of poultry farm units was estimated at a median of 6.6 days (95% HPD: 5.5-7.7). The birth-death skyline model indicated an increase in R_e starting from December 12th, 2020, reaching median of 2.2 (95% HPD: 0.2 – 4.4). Subsequently, from December 19th, 2020 the R_e showed a further increase to a median of 2.5 (95% HPD: 1.4-4.1) (Fig.5). From December 26th, 2020, the R_e gradually decreased, reaching a median of 1.6 (95% HPD: 1.0-2.4) and then consistently dropped to median values below 1 until February 6th, 2021. There was a subsequent increase in R_e, up to a median of 1.2 (95% HPD: 0.1-2.8) during the week of February 6th to 13th, 2021.

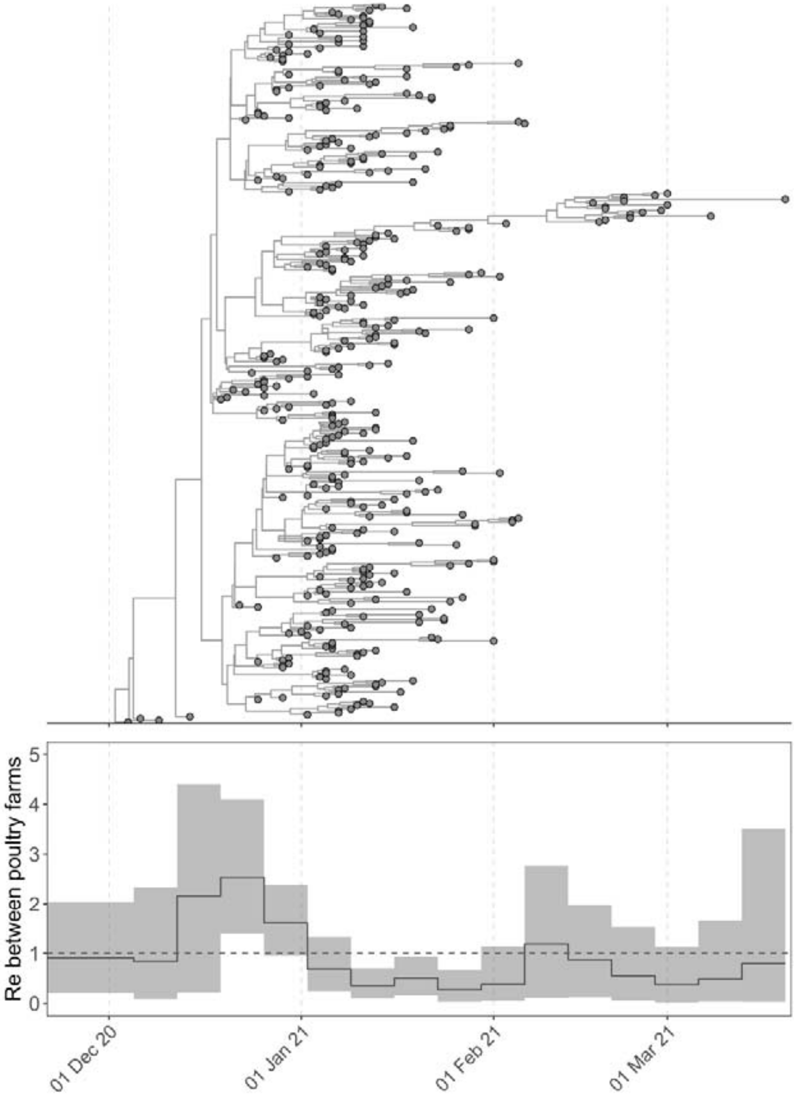


Figure 5. Maximum clade credibility tree aligned with the phylodynamic estimates of the reproductive number (R_e)

Estimating the effect of poultry farm activity days and distance

In the multivariable logistic regression analysis, three explanatory variables were found to be significantly associated with the likelihood of having $R_e > 1$ (Table 1). For each additional day of duck farm activity, the odds of $R_e > 1$ increased by a factor of 1.007 (95% CI 1.006-1.009). Conversely, for each additional day of chicken farm activity, the odds of $R_e > 1$ decreased by a factor of 0.98 (95% CI 0.97-0.99). Additionally, for each unit increase in distance, the odds of $R_e > 1$ decreased by a factor of 0.39 (95% CI 0.18-0.81).

Table 1. Explanatory variables statistically associated with the likelihood of farms with a $R_e > 1$ in the multivariable analysis (p-value < 0.05)

Variables	Odds ratio	95% Confidence intervals	p-value
Cumulative number of activity days of duck farms within around outbreak (10 km 21 days)	1.007	1.006-1.009	< 0.001
Cumulative number of activity days of chicken farms around outbreak (10 km 21 days)	0.98	0.97-0.99	< 0.001
Mean distance of duck farms to outbreak (10 km 21 days)	0.39	0.18-0.81	0.01

DISCUSSION

Our study provides a comprehensice exploration of the transmission dynamics of H5N8 in poultry farms in France, leveraging genetic data to quantify spread pattersn. The insights derived from our findings play a pivotal role in identifying factors influencing the effective reproduction number R_e, offering valuable information for policy decision-making in the face of futre HPAI outbreaks.

Our results indicate that prolonged activity of duck farms is positively associated with the occurrence of $R_e > 1$ in poultry farm outbreaks, while prolonged activity of chicken farms is inversely associated with $R_e > 1$. Moreover, greater distances of duck farms from outbreak locations are associated with a decreased likelihood of $R_e > 1$ in poultry farm outbreaks. The application of threshols based on farm activity and average distance between farms is a promising approach for shaping recommendations regarding the structure of poultry production systems.

The parameters inferred in our study serve as essential inputs for the parametrization of predictive models of HPAI spread, allowing for the evaluation of current vaccination programs and other preventive measures in the country. The increasing availabity of virus genetic data has opened avenues for phylodynamic analysis, and our study, focusing on factors associated with HPAI transmission dynamics, applied a birth–death skyline model. This approach provides a unique perspective that complements traditional epidemiological methods and also represents a significant advancement, allowing for accurate reconstruction of changes in epidemiological parameters over time based on sequence data.

Implementing a reduction in the number of days of activity of ducks farms and increased idstances between them, as suggested by our findings, may pose real-world challenges. However, exploring the extension of delay between production cycles as short-term measures during high-risk periods could be considerd, provided adequate financial compensation is provided to farmers. The "Plan Adour", an poultry industry-driven plan implemented from December 15th, 2020 to January 15th, 2023 preventing farmers from restocking during this high-risk period in the main affected departments seems to have been successful in reducing the number of outbreaks in the region (Lambert et al., 2022), showing promising for future prevention strategies.

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ZOONOSES

UNCOVERING MECHANISMS BEHIND THE SPATIO-TEMPORAL EMERGENCE OF USUTU VIRUS IN THE NETHERLANDS

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SUMMARY

The Netherlands has recently seen the emergence of West Nile and Usutu virus. We aim to uncover which factors have shaped the spatio-temporal patterns of Usutu virus emergence. A temperature-dependent, age-structured stochastic metapopulation model was developed to simulate Usutu virus transmission between *Culex pipiens sl.* mosquitoes and birds in the Netherlands between 2016-2022. Random Forest models were fitted to predict relative abundance of birds and mosquitoes using mosquito trap count data, bird point count data and environmental predictors. Bird dispersal was parameterised by analysing Dutch ring-recovery data for blackbirds. Using an Approximate Bayesian Computation approach, the model can be fitted to multiple summary statistics based on several sources of surveillance data. Here, we demonstrated the ability of our framework to correctly estimate relevant parameters from simulated data. This framework can be adapted to study other mosquito-borne pathogens, enhance surveillance programmes and evaluate the impact of future scenarios on arbovirus transmission.

INTRODUCTION

Arboviruses, viruses transmitted by arthropods such as mosquitoes and ticks, are becoming increasingly widespread across the world, including in Europe (Ryan et al., 2018). In the past decade both Usutu and West Nile virus have emerged in the Netherlands (Rijks et al., 2016; Sikkema et al., 2020). The first case of Usutu virus in the Netherlands was detected in April 2016, with a substantial increase of reported dead blackbirds observed later that year and in subsequent years (Rijks et al., 2016; Montizaan et al., 2019). Several other European countries have recently seen the emergence of Usutu virus, including Belgium (Garigliany et al., 2014), and Germany (Becker et al., 2012). Both viruses are transmitted in a cycle between mosquitoes and birds, with *Cx. pipiens* likely being the most important vector species in Europe (Nikolay, 2015). Blackbirds have been particularly impacted by Usutu virus, but the virus has been detected in several other bird species (Nikolay, 2015).

Understanding which factors have shaped the emergence of Usutu virus in the Netherlands is important to assess the risk of future outbreaks, plan potential intervention strategies and improve the efficiency of surveillance schemes. Bringing together information on the biology

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of virus transmission, mosquito and bird population distributions, bird dispersal, and environmental conditions with outbreak surveillance data make it possible to obtain a mechanistic understanding of Usutu virus transmission, which can then be applied to regions where outbreak data is lacking. However, such efforts are often hampered by limited availability of outbreak data. This is especially true for wildlife diseases, where information on host and vector dynamics can be challenging to collect. Combining several sources of limited, incomplete, or 'noisy' data with complex transmission models requires the use of novel inference approaches as standard likelihood-based methods quickly become impractical in these situations.

In this study, we make use of a range of surveillance data sources collected as part of an extensive bird surveillance network in the Netherlands. These include RT-PCR and serological testing of live birds, reports of dead blackbirds, RT-PCR testing of dead birds and bird population trends. These data are available since the start of the observed emergence of Usutu virus in 2016 and show that the virus spread northwards across the country in a period of three years. We aimed to better understand which factors have shaped the spatio-temporal patterns of Usutu virus emergence in the Netherlands, by bringing together a range of different surveillance data into a Bayesian framework using a data-driven transmission model. We were particularly interested in the efficiency of virus overwintering and in possible mechanisms underlying the South-to-North gradient observed. Mechanisms we explored included variation in environmental suitability, regional differences in immunity from prior transmission, and local virus introduction events. In this paper, we present a highly data-driven model of Usutu virus transmission including information on mosquito and bird abundance, bird dispersal, and local temperature. We assess the ability of our inference framework to estimate the parameters of interest using simulated data mimicking the data collected in real-life.

MATERIALS AND METHODS

Model overview

We created a stochastic compartmental metapopulation model to simulate transmission of Usutu virus between *Culex pipiens* mosquitoes and blackbirds in the Netherlands. Due to the majority of infections having been detected in blackbirds, abundance and dispersal were estimated for this species. As other species may display similar abundance, dispersal, and competence characteristics, the modelled host population represented blackbird-like birds. Both the host and vector populations were divided into compartments based on their infection status: susceptible, exposed, infected, recovered, and dead compartments for birds, and susceptible, exposed, and infected compartments for mosquitoes. The temporal resolution of the model was one day. We used a 5 by 5 km grid structure and assumed that birds and mosquitoes were homogeneously distributed within a grid cell. Virus transmission occurred within a grid cell and cells were connected through bird dispersal. Given the size of the grid cells, movement of mosquitoes between cells was ignored. To allow for different dispersal patterns across life stages and seasons, we divided the transmission season into breeding season (March to June) and summer (July to October) and the blackbird population into adult and juvenile. For an overview of the model structure, see Fig.1.

Model design and implementation

The model was implemented using the SimInf package (Widgren et al., 2019). Birds can get infected when they get bitten by an infectious mosquito. Mosquitoes can get infected when they bite an infectious bird. We assumed host frequency-dependent transmission, following from the assumption that the vector biting rate is independent of host density, and the biting rate experienced by hosts increases with vector density. The main extension we made to their model was the inclusion of a spatial component. We did not explicitly include mosquito larval stages as we obtained adult mosquito population sizes directly from a statistical model (see Input data).

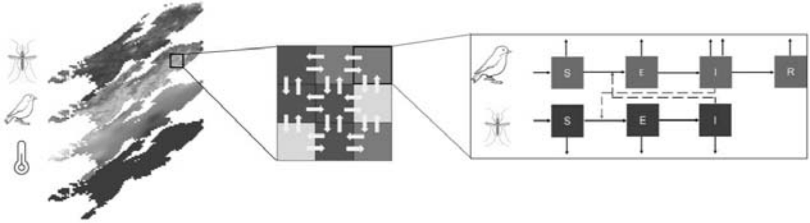

Figure 1. Model overview. The country is divided into equally sized grid cells, which vary with respect to bird and mosquito abundance as well as temperature. Birds disperse between cells. Transmission occurs within a grid cell. In the SEIR-model, dark arrows represent transitions between infection states, while dashed arrows represent transmission. S = susceptible, E = exposed, I = infectious, R = recovered. Non-connected outward pointing arrows represent death of the animal. Non-connected inward pointing arrows represent births

The model consisted of both stochastic and deterministic elements. Transitions between compartments were modelled as continuous-time discrete Markov chains using the direct Gillespie stochastic simulation algorithm. Bird birth events were processed deterministically to ensure an equal number of births each year, independent of changes in the population size due to virus transmission. This allowed for the recovery of the host population size after increased death rates due to infection. Mosquito emergence and death rates were also processed deterministically, to ensure the mosquito population sizes always followed the predicted abundance (see Input data). Thirdly, seasonal movements of birds were included deterministically. These deterministic processes are executed at the end of each time step for which they were planned.

The equations Eq.(1-13) for the modelled system describe susceptible (Sm), exposed (Em), and infectious (Im) mosquitoes, where the total adult mosquito population is Nm = Sm + Em + Im, and susceptible (S), exposed (E), infectious (I), recovered (R) and dead (D) birds. The bird population was split into two age classes - those under 1 year old (juvenile birds: JB) and those above 1 year of age (adult birds: AB). For each age class, the total live bird population in grid cell i is Ni = Si + Ei + Ii + Ri.

Mosquito population:

$$\frac{d\,S_{M,i}}{dt} = \delta_M(t) * N_{M,i} - b(T) * q * p_{B-M} * S_{M,i} * \left(\frac{N_{JB,i}}{N_{JB,i}+N_{AB,i}} * \frac{f_{ii}*I_{JB,i} + \sum_j f_{ji}*I_{JB,j}}{f_{ii}*N_{JB,i}+\sum_j f_{ji}*N_{JB,j}} + \frac{N_{AB,i}}{N_{JB,i}+N_{AB,i}} * \frac{f_{ii}*I_{AB,i} + \sum_j f_{ji}*I_{AB,j}}{f_{ii}*N_{AB,i}+\sum_j f_{ji}*N_{AB,j}}\right) - \mu_M(T) * S_{M,i} - \sigma(t) * S_{M,i} \quad (1)$$

$$\frac{d\,E_{M,i}}{dt} = b(T) * q * p_{B-M} * S_{M,i} * \left(\frac{N_{JB,i}}{N_{JB,i}+N_{AB,i}} * \frac{f_{ii}*I_{JB,i} + \sum_j f_{ji}*I_{JB,j}}{f_{ii}*N_{JB,i}+\sum_j f_{ji}*N_{JB,j}} + \frac{N_{AB,i}}{N_{JB,i}+N_{AB,i}} * \frac{f_{ii}*I_{AB,i} + \sum_j f_{ji}*I_{AB,j}}{f_{ii}*N_{AB,i}+\sum_j f_{ji}*N_{AB,j}}\right) - u(T) * E_{M,i} - \mu_M(T) * E_{M,i} - \sigma(t) * E_{M,i} \quad (2)$$

$$\frac{d\,I_{M,i}}{dt} = u(T) * E_{M,i} - \mu_M(T) * I_{M,i} - \sigma(t) * I_{M,i} \quad (3)$$

Juvenile bird population:

$$\frac{d\,S_{JB,i}}{dt} = \delta_B(t) * N_{AB,i} + \rho * R_{JB,i} - b(T) * q * p_{M-B} * I_{M,i} * \left(\frac{N_{JB,i}}{N_{JB,i}+N_{AB,i}} * \frac{f_{ii}*S_{JB,i}+\sum_j f_{ji}*S_{JB,j}}{f_{ii}*N_{JB,i}+\sum_j f_{ji}*N_{JB,j}}\right) - \mu_{JB} * S_{JB,i} \quad (4)$$

$$\frac{d\,E_{JB,i}}{dt} = b(T) * q * p_{M-B} * I_{M,i} * \left(\frac{N_{JB,i}}{N_{JB,i}+N_{AB,i}} * \frac{f_{ii}*S_{JB,i}+\sum_j f_{ji}*S_{JB,j}}{f_{ii}*N_{JB,i}+\sum_j f_{ji}*N_{JB,j}}\right) - \varepsilon * E_{JB,i} - \mu_{JB} * E_{JB,i} \quad (5)$$

$$\frac{d\,I_{JB,i}}{dt} = \varepsilon * E_{JB,i} - \gamma * I_{JB,i} - \mu_{JB} * I_{JB,i} - v * I_{JB,i} \quad (6)$$

$$\frac{d\,R_{JB,i}}{dt} = \gamma * I_{JB,i} - \mu_{JB} * R_{JB,i} - \rho * R_{JB,i} \quad (7)$$

$$\frac{d\,D_{JB,i}}{dt} = \mu_{JB} * \left(S_{JB,i} + E_{JB,i} + I_{JB,i} + R_{JB,i}\right) + v * I_{JB,i} \quad (8)$$

Adult bird population:

$$\frac{d\,S_{AB,i}}{dt} = a * S_{JB,i} + \rho * R_{AB,i} - b(T) * q * p_{M-B} * I_{M,i} * \left(\frac{N_{AB,i}}{N_{JB,i}+N_{AB,i}} * \frac{f_{ii}*S_{AB,i}+\sum_j f_{ji}*S_{AB,j}}{f_{ii}*N_{AB,i}+\sum_j f_{ji}*N_{AB,j}}\right) - \mu_{AB} * S_{AB,i} \quad (9)$$

$$\frac{d\,E_{AB,i}}{dt} = a * E_{JB,i} + b(T) * q * p_{M-B} * I_{M,i} * \left(\frac{N_{AB,i}}{N_{JB,i}+N_{AB,i}} * \frac{f_{ii}*S_{AB,i}+\sum_j f_{ji}*S_{AB,j}}{f_{ii}*N_{AB,i}+\sum_j f_{ji}*N_{AB,j}}\right) - \varepsilon * E_{AB,i} - \mu_{AB} * E_{AB,i} \quad (10)$$

$$\frac{d\,I_{AB,i}}{dt} = a * I_{JB,i} + \varepsilon * E_{AB,i} - \gamma * I_{AB,i} - \mu_{AB} * I_{AB,i} - v * I_{AB,i} \quad (11)$$

$$\frac{d\,R_{AB,i}}{dt} = a * R_{JB,i} + \gamma * I_{AB,i} - \mu_{AB} * R_{AB,i} - \rho * R_{AB,i} \quad (12)$$

$$\frac{d\,D_{AB,i}}{dt} = a * D_{JB,i} + \mu_{AB} * \left(S_{AB,i} + E_{AB,i} + I_{AB,i} + R_{AB,i}\right) + v * I_{AB,i} \quad (13)$$

Model parameters

Parameter values were constant, time-dependent (t) or temperature-dependent (T) (table 1). Most values were obtained from literature, however some were based on calculations. Similar to Wonham et al. (2004), we assumed that mosquitoes only bite birds and have no preference with regards to bird species. Therefore, the proportion of bites that are on competent hosts is equal to the proportion of the total bird population that are competent (here represented by blackbirds). This proportion was calculated each day and location, by setting it to 100% in the location where the blackbird density was largest. In all other locations this proportion was equivalent to the relative blackbird density compared to the cell where this was largest. The bird death rate was assumed to be constant over space and time, while births occurred seasonally. We used a Leslie matrix to calculate the annual number of births assuming the population remained constant in the absence of infection. Births were distributed uniformly across the period where newborns start leaving their nests assuming a hatching period of 14 days and a further 14 days until they fledge the nest (Birdfact, 2023). Ageing from juvenile to adult bird occurred instantaneously on the last day of April, the day before births start.

Input data

Bird abundance: Relative bird abundance was estimated for each grid cell using a species distribution model (manuscript in preparation). Random Forest models were fitted to blackbird point count data (n=262,380 observations) from the Netherlands (Meetnet Urbane Soorten & Meetnet Agrarische Soorten, Sovon) and France (Common Bird Monitoring Scheme) in the period 2001-2016. A set of environmental and climatic predictors was used; these included temperature, precipitation, land use, altitude and soil data. These resulting estimates reflected relative abundance estimates, as the exact size of the total blackbird population is unknown.

Bird dispersal: Two movement patterns were distinguished in the model, representing foraging behaviour and seasonal movement. Foraging-type movement takes place in an area around the bird's home base after which birds return to their home location. This means that birds visit other grid cells but were assumed not to move to other cells. In the model, this is reflected as a contribution to the force of infection experienced in other grid cells (quantified using parameter f_{ji}). In addition to daily foraging, we also included seasonal movement that represents processes such as the search for a new nest location or for a food-rich area. Seasonal movement took place during the change from breeding to summer season in early July and assumed that birds move to a different grid cell. We quantified both the daily and seasonal blackbird dispersal patterns by fitting dispersal kernels to ringing and recovery data collected by Vogeltrekstation NIOO-KNAW. Blackbirds ringed and recovered in the Netherlands were used for this analysis. We estimated dispersal kernels for each combination of age group and season. We constructed movement matrices by sampling from these dispersal kernels and assuming rotational symmetry.

Mosquito abundance: Relative *Culex pipiens* abundance was estimated for each day and grid cell using an approach similar to bird abundance estimates (manuscript in preparation). Data on female mosquito trap counts was obtained from the National Mosquito Survey between 2010 and 2013 (Ibañez-Justicia et al., 2015) and the MODIRISK project (Bortel et al., 2022). Count data of *Culex pipiens* and *Culex torrentium* was included, because no distinction was made between these species in the National Mosquito Survey. Random Forest models were fitted to these female mosquito count data including a set of land cover, environmental, and climatic predictors. Because the total population size is unknown, estimates reflect relative abundance and are not on the same scale as relative blackbird abundance. This species distribution model was used to predict the relative number of female adult mosquitoes for each location and day. We used these daily abundance predictions to estimate vital dynamics in the mosquito population. Mosquito death rate was assumed temperature-dependent (table 1) and therefore differed over space and time. The number of emerging mosquitoes was estimated for each location and day such that, together with the time-dependent mortality rates, the resulting population size follows the abundance predicted by the abundance model.

Table 1. Parameters and their values used in the model. T indicates temperature-dependence, t indicates time-dependence

Parameter	Description	Value	Source
Blackbirds			
$\delta_b(t)$	Birth rate	1 May – 15 June: 0.05 / day. Else: 0	Value: calculated. Timing: (van den Bremer and van Turnhout, 2021)
μ_{JB}	Mortality rate, juvenile	0.0059 / day	(van den Bremer and van Turnhout, 2021)
$_{AB}$	Mortality rate, adult	0.0011 / day	(Robinson et al., 2010)
$p_{m\text{-}b}$	Transmission probability, mosquito to blackbird	0.88	(Wonham et al., 2004)
ε	Intrinsic incubation period	0.667 / day	(Rubel et al., 2008; Kuchinsky et al., 2022)
γ	Recovery rate	0.25 / day	(Komar et al., 2003)
	Disease-induced mortality rate	NA	To be estimated
ρ	Rate of immunity loss	0.0014 / day	(McKee et al., 2015)
f_{ji}	Fraction of birds in cell *j* dispersing to *i*	Variable	Calculated, see 'Input data'
a	Ageing rate	On 30th April: 1. Else: 0	(van den Bremer and van Turnhout, 2021)
Culex pipiens			
$\delta_m(t)$	Emergence rate	Variable	Estimated from abundance data
$\mu_m(T)$	Mortality rate [daily]	$(0.0025 * T^2 - 0.094 * T + 1.0257) / 10$	(Rubel et al., 2008), data from (Reisen, 1995)
$b(T)$	Biting rate	$0.344/(1+1.231*\exp(-0.184*(T-20)))$	(Rubel et al., 2008), data from (Reisen et al., 2006)
q	Proportion of bites that are on competent host	Variable	Calculated, see 'Model parameters'
$p_{b\text{-}m}$	Transmission probability, blackbird to mosquito	NA	To be estimated
$u(T)$	Extrinsic incubation period	For $T>15\ ^\circ C$: $0.0093T-0.135$, else 0	(Rubel et al., 2008), data from (Reisen et al., 2006)
$\sigma(T)$	Rate of entering diapause	1 Sept – 15 Oct: 0.05. Else: 0	(Field et al., 2022)

Temperature data: Average daily temperature data in the period 2016-2022 were obtained from the Royal Meteorological Institute (KNMI), with a 1x1 km spatial resolution. Abundance and temperature data were resampled into the model grid using bilinear interpolation.

Inference

We used an Approximate Bayesian Computation approach using a Sequential Monte Carlo sampler (ABC-SMC) to calibrate our model to surveillance data. This iterative algorithm enhances the basic ABC algorithm by incorporating two main steps: weighted resampling of simulated particles and a gradual reduction in tolerance. In ABC-SMC, estimation of the posterior distribution is achieved sequentially by constructing intermediate distributions in each iteration, converging towards the posterior distribution. Our specific implementation of the algorithm improves upon Del Moral et al.'s, (2006) original algorithm in three ways: (1) an adaptive threshold schedule selection based on quantiles of distances between simulated and observed data (Drovandi and Pettitt, 2011; Del Moral et al., 2012) (2) an adaptive perturbation kernel width during the sampling step, dependent on the previous intermediate posterior distribution (Toni et al., 2008; Beaumont et al., 2009), and (3) the capability to use multiple criteria simultaneously. We used five different criteria, one for each type of surveillance data: seroprevalence in live birds, PCR prevalence in live birds and PCR prevalence in dead birds for each month and region (north, middle, south) as well as the annual relative host population size and the relative number of reported dead birds per year within each region.

The following parameters were estimated: 1) abundance scaling parameter, this converts the host and vector abundance to be on the same scale, meaning that their ratio reflects the vector-to-host ratio, 2) overwintering efficiency, the relationship between prevalence before and after winter, 3) disease-induced mortality rate, 4) transmission probability bird-to-mosquito, 5) historical force of infection (FOI) by region (North, Middle, South), to study the possibility of tranmission prior to first detection leading to immunity already being present in 2016. To validate the ability and accuracy of our inference algorithm we simulated data mimicking the surveillance data sources available but with known parameter values. Six distinct parameter sets were used for this analysis.

RESULTS

Model input

Blackbird abundance was highest in the South-East and East of the Netherlands (Fig.2A). With regards to the dispersal kernels, Weibull distributions provided the best fit for the majority of age-season combinations and were therefore used for all dispersal patterns. We found no evidence of age differences in daily dispersal (Wilcoxon rank sum test, p=0.27) and therefore used the same daily dispersal distance for juvenile and adults. Daily dispersal was higher in breeding season compared to summer (Wilcoxon rank sum test, p=0.03) (Fig.2B) resulting in the following dispersal parameters: breeding season: shape=0.44 & scale=329.4, summer: shape=0.46 & scale=63.4. Contrary to daily dispersal, there was some evidence of seasonal dispersal being higher in juveniles than adults (Wilcoxon rank sum test, p=0.04). Estimates for seasonal movement were therefore separate for juveniles (shape=0.64 & scale=668.2) and adults (shape=0.39 & scale=459.4). Mosquito abundance varied between years, and was highest around August-September (Fig.2C), just before diapause was initiated.

Validation of inference approach

Across the six simulated datasets 6/7 (86%) to 7/7 (100%) true parameter values fell within the 95% highest density interval of the posterior distributions. We calculated concordance coefficients to quantify the agreement between parameter's true values and the estimated median values from the posterior (Fig 3). Contrary to the Pearson correlation coefficient measuring any linear relationship, this specifically measures the strength of the relationship *y*=*x*. A value of 1 indicates perfect agreement, while 0 indicates poor agreement. We found that the overwintering efficiency and disease-induced mortality were best approximated by the posterior median. The transmission probability host-to-mosquito tended to be somewhat overestimated in most of the simulated datasets. The inference framework was unable to stimate the values of the historical FOI in all three regions within the range of the chosen prior.

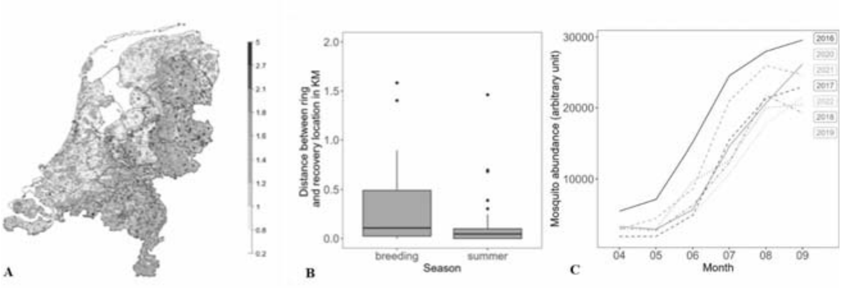

Figure 2. A. Relative blackbird abundance map. B. Distance between ringing and recovery location of blackbirds that were recovered in the same season as they were ringed. This was used as a proxy for daily dispersal distances. C. Monthly estimates of the relative abundance of *Culex pipiens* mosquitoes in the Netherlands for each year

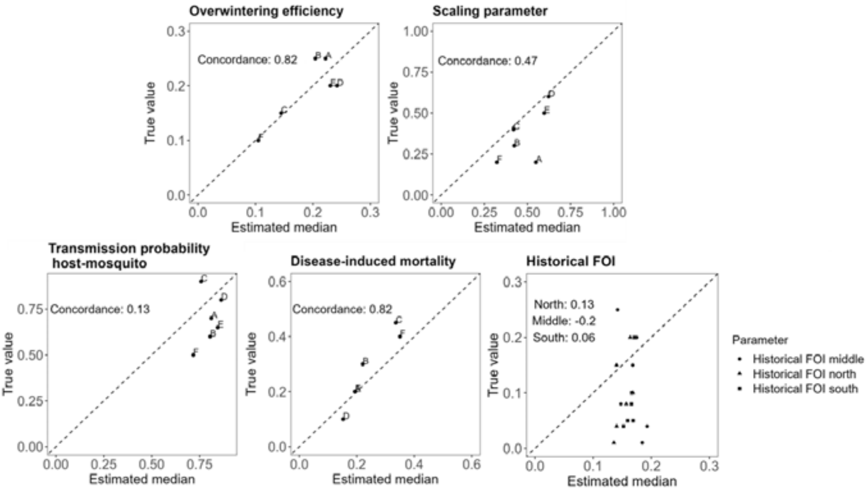

Figure 3. Concordance between parameter's true values and the estimated posterior median values across six simulated datasets (A-F)

DISCUSSION

Computational and theoretical advances increasingly enable data-driven analyses of disease transmission. The ability to study virus emergence is often limited by the availability of data, both in terms of demographic data as well as surveillance data, especially for wildlife diseases. We developed a data-driven simulation model and a Bayesian inference method to study the emergence of Usutu virus in the Netherlands. We brought together information on virus transmission, bird distribution, temporal mosquito distribution, bird dispersal, and temperature in a spatially-explicit model. The validation approach using simulated datasets similar to the real-life datasets indicated that our model framework is able to correctly estimate the parameters of interest, but is unable to detect the impact of low levels of pre-existing immunity. This suggests that if undetected transmission prior to 2016 was low we are unable to detect its impact on transmission in subsequent years.

The validation of our inference framework showed that the posterior distributions captured the true values well. As this could also be due to uninformative, wide posterior distibutions, we also assessed the ability of the posterior medians to approximate the true values. This indicated that low levels of transmission prior to the modelled years was not identifiable. Both the scaling parameter and transmission probability were somewhat overestimated which may reflect some degree of correlation making identifiability imperfect. This challenge is common when estimating multiple parameters that have a similar impact on the data patterns used and is affected by the amount of information available in the data. However, as both parameters are used in simulations and output calculation, such as the basic reproduction number, this does not necessarily prevent the model from fitting the data well as long as both parameters are used together.

Our model framework contained a high level of detail and complexity compared to other Usutu and West Nile virus models, and was well grounded in location-specific data on mosquitoes, birds, and their movements. While local movement of birds has been studied before in the context of WNV such as in Bhowmick et al., (2023), this has not been combined with bird abundance data. The majority of Usutu and West Nile virus models focus on one transmission season, and when multiple years are studied, mosquito overwintering is often included without explicit considerations about the implications for the proportion of infectious mosquitoes (Rubel et al., 2008; Bhowmick et al., 2020). By combining information from multiple years into one simulation we were able to explicitly study the relationship in prevalence between transmission seasons. This could shed some light on possible persistence mechanisms and their efficiency, an important factor in understanding arbovirus transmission dynamics. Several overwintering routes have been suggested in literature, but their relative importance is still unclear (Blom et al., 2023). Additional complexities we have not explored include variation in Usutu virus strains, changing *Culex pipiens* biting preferences during the season (Kilpatrick et al., 2006), or alternative mosquito and host species. It remains challenging to reflect the complex reality of a multi-host system in a data-driven transmission model as it is unclear which and to what extent other species contribute to transmission (Nikolay, 2015). Although we did not explicitly include virus reintroductions through migrating bird species, these are in part captured in the overwintering efficiency parameter. Further work is needed to disentangle local overwintering from reintroductions. High-quality phylogenetic data could possibly contribute to our understanding of these mechanisms.

Next steps include the fitting of the model to real-life data rather than simulated data. As we use the same summary statistics when fitting to the real data as we used to validate our inference framework, we expect to be able to approximate parameter values with sufficient level of certainty. The results from the model fitting will shed a light on the contribution of different mechanisms to the pattern of emergence that we observed, especially with regards to regional differences in suitability, prior immunity and virus introductions. After obtaining a good fit to the data we can calculate spatiotemporal estimates of the basic and effective reproduction number as well as the prevalence. This framework could also be adapted to study other mosquito-borne pathogens, enhance surveillance programmes and evaluate the potential impact of future change scenarios on arbovirus transmission. This highlights the range of possible applications of the framework developed here to enhance our understanding of mosquito-borne disease epidemiology.

ACKNOWLEDGEMENTS

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ZOONOSES IN AUSTRIA – A COMPREHENSIVE ASSESSMENT BASED ON PUBLISHED LITERATURE

A. VOGL, A. JOACHIM, A. KAESBOHRER AND A. DESVARS-LARRIVE*

SUMMARY

This work aimed to describe the web of zoonotic interactions in Austria. A systematic literature search identified 246 relevant publications spanning 1975–2022, from which information about zoonotic agents was extracted. Overall, 198 zoonotic agents were evidenced across 155 animal host species, 12 invertebrate species, 32 categories of food, and six environmental media. Network analysis revealed that human, chicken, cattle, horse, cat, dog, red fox, and wild boar were the most influential hosts in the network of zoonotic agent sharing, potentially serving as bridges for zoonotic agent spread among host communities. Similarly, Usutu virus, West Nile virus*, Salmonella, Campylobacter jejuni, Escherichia coli, Campylobacter coli, Listeria monocytogenes, Toxoplasma gondii*, Influenza A virus, and *Leptospira* demonstrated a high zoonotic potential. Trend analysis revealed a growing focus on the environment in zoonotic disease research in Austria. This study exemplified how structuring and analysing literature data can further our understanding of zoonotic disease dynamics.

INTRODUCTION

Zoonoses are caused by pathogens naturally transmissible between humans and animals. Places where humans and animals or animal products interact create interfaces that facilitate zoonotic pathogen transmission (Heymann and Dixon, 2013). Anthropogenic-driven changes, such as habitat destruction, shifts in land use (Plowright et al., 2021), agricultural intensification (Jones et al., 2013), and animal domestication (Fournié et al., 2017), along with the rising significance of companion animals and livestock, contribute to the expansion of human-animal interfaces. This expansion is further compounded by the resulting growing animal-human contacts, increased interactions with wildlife due to hunting (Abrantes and Vieira-Pinto, 2023), the proliferation of exotic companion animals, and the increasing consumption and illegal trade of wild animals (Milbank and Vira, 2022). These interactions create new opportunities for zoonotic pathogen transmission, evolution, and emergence.

Austria has a growing population of nine million people, with a population density of 108 inhabitants/km^2. Its fauna encompasses approximately 45,870 distinct species, of which 626 are vertebrates, including 110 mammalian and 418 avian species (Geiser, 1998). Moreover, of 3.9 million Austrian households, 35% own pets while there are approximately 53,300 cattle,

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one million pigs, and five million poultry kept in 154,953 agricultural operations. Additionally, 133,000 hunting permits are issued annually. Along with multiple slaughterhouses, food processing plants, and zoos, these numbers underline the importance of the human-animal interfaces at the national scale.

A comprehensive understanding of circulating zoonotic agents, their hosts, vectors, environmental reservoirs, and the key interfaces where spillover events may occur is essential for developing effective integrated One Health monitoring, prevention, and control of zoonoses (Ghai et al., 2022). However, information about zoonotic agents circulating in Austria is dispersed across scientific papers, reports from the Austrian Agency for Health and Food Safety (AGES), Reports from the Federal State Veterinary Services, and student theses. The main objectives of this work were to i) compile and structure data on zoonotic agents in Austria that is available in the literature, along with information about their hosts, vectors, food and environmental matrices, and ii) build the web of zoonotic interactions to characterise the various interfaces through which zoonotic spillover may occur. Additionally, this study aimed to pinpoint research trends and research gaps on zoonotic agents in Austria.

MATERIALS AND METHODS

Search strategy

Between 17 July and 23 August 2022, a systematic literature search was conducted using the query ("Zoono*" AND ("Austria" OR "Österreich")) in the following databases: PubMed®, Scopus, and vetmed:seeker (internal database of the University of Veterinary Medicine Vienna, Austria), including articles published between the inception of the databases and the date of the search. Furthermore, the publication database of the AGES (https://www.ages.at/en/research/publication-database) was searched using the keyword "zoono*". Additional papers found in the reference section of reviews that provided relevant information were also considered. Retrieved publications were deduplicated in the reference manager Citavi (Swiss Academic Software, 2023) before the following selection processes.

Paper selection

Titles and abstracts were first screened for relevance using the following inclusion criteria: the publication presented data pertaining to at least one zoonotic disease or agent that was investigated or documented in Austria and the infectious agent was identified as zoonotic in the paper. Publications were excluded if: i) they did not investigate or describe a zoonotic disease/agent that was identified as such; ii) research was not conducted in Austria; iii) publications did not describe natural occurrence of the zoonotic agent; iv) publications described disease physiology; or v) they dealt with treatment or methods for infectious agent detection. Book chapters, posters, literature reviews, statistical forecasts, and conference proceedings were excluded. Regarding antimicrobial resistant bacteria, papers were included if they specifically explored the animal-human interface and/or the authors referred to zoonotic transmission. To prevent duplication of data, diploma-, Master's-, and doctoral theses were not included if a peer-reviewed research paper published the same data.

In a second step, the full texts of the previously selected titles/abstracts were screened using the inclusion/exclusion criteria described above. Publications were excluded if they were not in the German or English language or did not describe the situation in Austria. When a publication dealt with multiple countries, it was included if it provided specific information on zoonotic agents in Austria.

Data extraction

The following data was extracted from the selected publications: *i) Publication data*; *ii) Type of study*; *iii) Investigated zoonotic agent:* type (bacterium, virus, parasite, fungus, prion, or other), common and scientific names as mentioned in the information source; *iv) Investigated host:* host category (human, companion animal, livestock, wildlife), common and scientific names as mentioned in the information source, if the zoonotic agent or antibodies against it was/were detected in the host, i.e., seropositive (confirmed by the presence of antibodies), positive (direct detection of the agent), or negative; *v) Investigated vector:* common and scientific names as mentioned in the information source, and if the zoonotic agent was detected in the vector (positive/negative); *vi) Investigated environmental matrix:* type, and if the zoonotic agent was detected (positive/negative); *vii) Investigated food matrix:* the specific type of food investigated, the origin of the food product (animal or plant), and if the zoonotic agent was detected (positive/negative); *viii) Epidemiological context:* study year, federal state(s), whether the zoonotic agent was mentioned as emerging in Austria, and whether specific professional activities were deemed to carry an elevated probability of exposure.

Data process

Quality control, data cleaning, and taxonomic validation: First, the data underwent quality control and cleaning procedures where the unique values of each field were checked using the R function *unique()*. Errors detected were manually reviewed against the original data source and corrected or removed as needed. Furthermore, for each animal host, vector, and zoonotic agent, common and scientific names as well as taxonomic classification were resolved against the NCBI Taxonomy database (Schoch et al., 2020) using the R package “taxize” (Chamberlain and Szöcs, 2013).

Data analysis and visualisation: The dataset was used to create a network representing the web of zoonotic interactions, i.e. depicting the zoonotic agents and their compartments (hosts, vectors, food sources, and environment) as nodes linked by edges, which indicated occurrence of the agent in the compartment. The full network was simplified to a bipartite zoonotic agent-host network, which was subsequently projected into a one-mode network of zoonotic agent sharing among Austrian animal and human hosts. Edges were weighted by the number of shared zoonotic agents between two species. To account for sampling bias, we used the total number of studies as an estimate of scientific research effort for each host and regressed each edge weight by the Box-Cox transformed number of studies of the least studied host of each edge. The residuals were subsequently rescaled so that the lowest weight value was 1 (Luis et al., 2015). We calculated the following centrality metrics: degree, betweenness, and closeness (Newman, 2018). To calculate weighted betweenness and closeness centrality, the weights were transformed into costs by dividing 1 by the weight (Newman, 2001). Network analysis was performed using the R packages "igraph" (Csardi and Nepusz, 2006) and "bipartite" (Dormann et al., 2008).

RESULTS

Selected publications, data records, and publication timing

The search identified 2,186 publications. After 542 duplicates were removed, 1,644 publications were screened, with 1,269 excluded at the title/abstract screening stage as they were not eligible. This left 375 publications, of which 16 could not be retrieved, so that 359 full-text articles were assessed for eligibility, of which 229 met criteria for final inclusion. In addition, 17 publications were found in excluded review articles, leading to a total of 246 publications that were ultimately included in this study.

The final dataset was a *.csv. file with 2,128 rows and 48 data fields. Each row represented one investigated zoonotic agent along with the results of the investigation in the animal host(s), vector(s), environment, or food matrix(-ices). All included publications were published between January 1975 and August 2022. We evidenced a 17.8-fold increase in the number of publications on zoonoses in Austria between the first (1975–1997) and the second half (1998–2022) of the study period. To provide context for this result, it was compared with global data: a PubMed search using the terms (zoono* OR "zoono* disease*") from 1975 until 23 August 2022 (without restricting the search to Austria) generated a total of 64,282 results and revealed an increase of the same order (18-fold).

Zoonotic agents

Between 1975 and 2022, 228 unique zoonotic agents were investigated in Austria. Ten genera collectively accounted for ~40% of the selected literature: *Salmonella*, *Escherichia*, *Listeria*, *Echinococcus*, *Orthoflavivirus*, *Brucella*, *Toxoplasma*, *Campylobacter*, *Trichinella*, and *Leptospira*. Overall, 198 zoonotic agents were evidenced: 190 (96.0%) were directly or indirectly detected in 155 different vertebrate hosts (including humans), 26 (13.1%) were detected in 12 different invertebrate (vector) species, 21 (10.6%) were detected in 32 categories of food, and twelve (6.1%) were detected in six types of environmental media (some agents have been evidenced in more than one compartment) (Figure 1). Eight zoonotic agents were described as emerging in Austria over the period 1975–2022 (i.e. first discovered in Austria or first autochthonous cases), including: Usutu virus (USUV, birds, 2001), *Rickettsia helvetica* (*Ixodes ricinus*, 2005), *Anisakis* (human, 2009), *Brucella canis* (dog, 2010), *Rickettsia conorii* subsp. *raoultii* (dog, 2015), West Nile virus (WNV, horse, 2016), *Thelazia capillipaeda* (domestic cat, 2018), and *Baylisascaris procyonis* (racoon, 2019).

Web of zoonotic interactions and vertebrate host-zoonotic agent interface

The majority of zoonotic agents were studied in wildlife hosts, which accounted for 76.9% of the 221 animal species investigated. In total, zoonotic hosts were identified in 111 wildlife species, eight livestock species, and 36 companion animal species (including exotic animals), encompassing 81 Aves, 41 Mammalia, 29 Lepidosauria, three Testudinata, and one Actinopteri.

The bipartite network analysis revealed that the human node exhibited the highest degree centrality (*k* = 88), followed by cattle (*k* = 38), dog (*k* = 30), domestic cat (*k* = 23), red fox (*k*

= 19), pig (k = 17), and chicken (*k* = 15). This pattern suggested high zoonotic agent richness associated with these hosts. Furthermore, the nodes USUV, WNV, *Salmonella* sp., *Campylobacter jejuni*, *Escherichia coli*, *Campylobacter coli*, *Listeria monocytogenes*, *Toxoplasma gondii*, Influenza A virus, and *Leptospira* spp. displayed a high degree centrality ($k \geq 10$), revealing a greater host plasticity. Similarly, the node *Ixodes* exhibited high degree centrality (*k* = 16), with multiple connections to *Rickettsia, Borrelia,* and *Babesia* species, implying a central role in the epidemiology of these bacteria. Finally, some zoonotic agents, e.g. *Salmonella enterica*, Influenza A, USUV and WNV, along with their respective hosts were connected to the network largest connected component by few host or vector nodes (e.g. *Culex* for USUV and WNV).

Within the scientific research effort-adjusted host network of zoonotic agent sharing, degree centrality was positively correlated with betweenness centrality and closeness centrality (Kendall's Tau ranging from 0.21 to 0.47, all p-values < 0.001). Human, chicken, cattle, horse, domestic cat, dog, red fox, and wild boar were the most influential nodes in this network of zoonotic agent sharing, appearing in the top 10 actors by at least two centrality metrics (Table 1). In addition, the common vole, yellow-necked field mouse, and racoon dog showed high betweenness centrality, suggesting that they may act as bridges between host communities (without necessarily transmitting zoonotic agents across these communities).

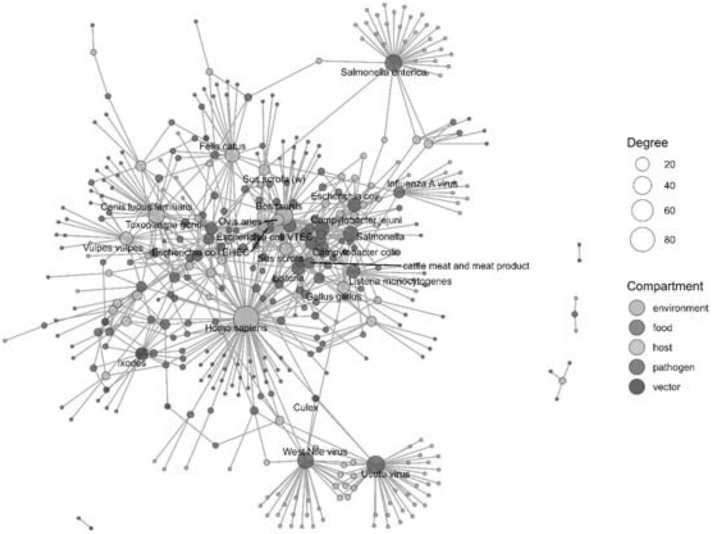

Figure 1. Networks representing the web of zoonotic interactions in Austria, 1975–2022. Size of the node represents the node degree (i.e. the number of links a node has). Only nodes with degree > 10 are labelled, as well as the *Culex* node to illustrate the Results description

Table 1. Top ten actors in the host network of zoonotic agent sharing by node centrality metrics, Austria, 1975–2022

Degree	Weighted betweenness	Weighted closeness
Human (106)	Human (0.710)	Human (3.06)
Chicken (61)	Wild boar (0.287)	Cattle (2.91)
Wild boar (56)	Chicken (0.127)	Chicken (2.88)
Horse (56)	Cattle (0.116)	Pig (2.82)
White stork (55)	Dog (0.023)	Sheep (2.76)
Hooded crow (55)	Common vole (0.013)	Dog (2.68)
Jackdaw (55)	Yellow-necked field mouse (0.013)	Red fox (2.59)
Long-eared owl (55)	Domestic cat (0.013)	Turkey (2.57)
Snowy owl (55)	Racoon dog (0.013)	Horse (2.52)
Western marsh harrier, Eurasian eagle-owl, ural owl, lammergeier (55)	Red fox (0.012)	Domestic cat (2.50)

Food system-zoonotic agent interface

Investigated food products originated from 23 different sources, encompassing plant-based foods (5.6 % of all types of food investigated) and animal source foods, such as livestock- and horse-derived food products (65.6% of the studies investigating food products), game-derived food products (13.8%), and fish products (e. g. herring, mackerel, salmon, trout; 3.1 %). Only one publication investigated honey. Meat and meat products yielded the majority of positive results (45.9%) while studies focusing on plant-based foods comprised only 6.9% of zoonotic agent-positive food products. Zoonotic agents identified in food were mainly of the genera *Listeria* (33.7% of evidenced zoonotic agents in food), *Escherichia* (8.8%), and *Salmonella* (22.9%). Out of the 21 identified zoonotic agents in food, all were bacteria except for three parasites (*Anisakis, Echinococcus*, and *Trichinella spiralis*) (Figure 2).

Environment-zoonotic agent interface

Across the selected publications, 21 invertebrate species were investigated for zoonotic agents, including mosquitoes (47.8% of the studies investigating vectors), ticks (39.1%), sand flies, gastropods, and fleas (4.3%, each). Twelve arthropod vectors, belonging to the genera *Aedes, Ctenocephalides, Culex, Dermacentor, Haemaphysalis, Hyalomma, Ixodes,* and *Uranotaenia*, were found to be carrier of zoonotic agents.

Eight distinct environmental matrices were investigated for the presence of zoonotic agents, of which six were found positive with bacteria (*Listeria monocytogenes, Salmonella* sp., *Escherichia coli,* and *Mycobacterium* sp.) or parasites (*Cryptosporidium; Giardia,* and *Toxocara*). Investigated environmental media included public spaces, surfaces and tools in food processing environments, as well as “natural” matrices (e.g. water, sandboxes).

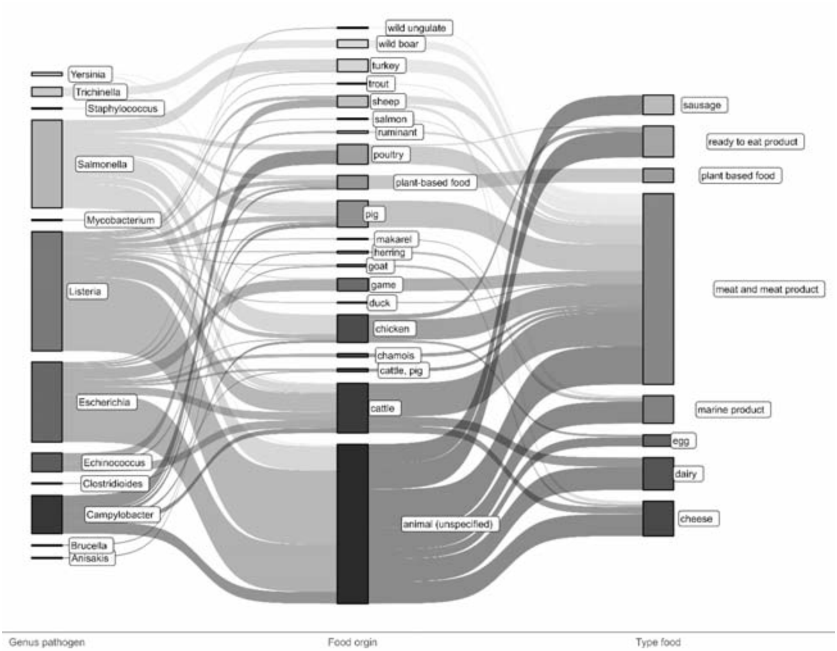

Figure 2. Sankey graph of the food system-zoonotic agent interface in Austria, 1975–2022. The zoonotic agents´ NCBI-resolved genera are shown on the first axis, the food origin is displayed on the middle axis, and the food category is specified on the right axis. The height of each bar is proportional to the number of times a zoonotic agent was reported

Trends in scientific interest

There has been a noticeable upward trend in the scientific interest in Austria for the three types of zoonotic agents, bacteria, viruses, and eukaryots (Figure 3A), with bacteria garnering most attention. We observed an upward trend across all compartments, as recognised by the traditional One Health triad, i.e., animal, human, and environment (Destoumieux-Garzón et al., 2018), followed by a subsequent decrease in the number of studies investigating animals (from 2015) and humans (from 2010). The environmental aspect was not considered in studies on zoonotic diseases in Austria until 1997 but subsequently demonstrated the most gradual increase in scientific interest (Figure 3B).

DISCUSSION

Our findings unveil the complexity of the zoonotic agent-host-environment-food interfaces in Austria. Notably, this work demonstrates that the majority of zoonotic agents are capable of infecting both human and diverse animal species across various taxa. They evolve within multi-host multi-agent ecological communities, which aligns with the established principles in parasite community ecology (Combes, 2001). Ten zoonotic agents accounted for ~40% of the published research on zoonotic diseases in Austria, of which seven are subjected to compulsory surveillance and reporting in humans and/or animals (Bundeskanzleramt Österreich, 2005).

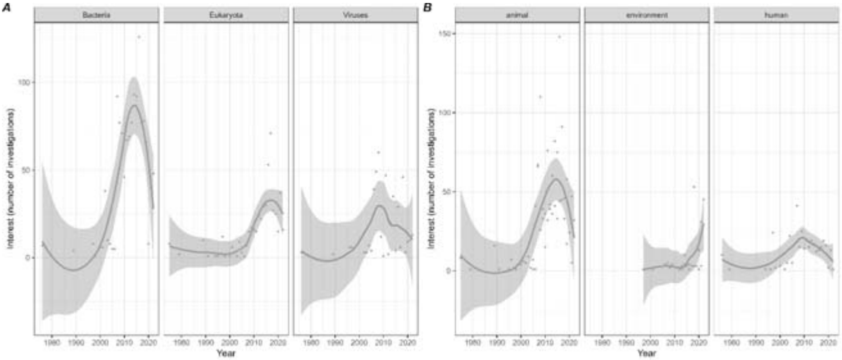

Figure 3. Trend in research interest, as measured by the number of studies involving **A.** each category of zoonotic agents and **B.** each compartment, as recognised in the traditional One Health triad. Note that a single publication might encompass multiple compartments and/or zoonotic agent categories

Results of the network analysis revealed that multiple different compartments (animal hosts, food, environment) can contribute to the maintenance and spread of zoonotic agents in host populations (including humans). Moreover, some livestock (e.g. cattle, chicken), companion animal (e.g. dog and domestic cat), and wildlife (e.g. common vole, yellow-necked field mouse, racoon dog, and wild boar) species play a crucial role as bridge hosts (Caron et al., 2015), acting as links through which zoonotic agents can potentially be transmitted from maintenance (generally wild) host populations or communities to target populations (generally livestock or humans) that are "protected" through public health or biosecurity measures (Cleaveland et al., 2001; Jones et al., 2013). Zoonotic spillover events typically occur at interfaces where maintenance and target hosts meet, i.e. within a shared environment, especially ecotones, common grazing pastures, and water points (Caron et al., 2013; Wiethoelter et al., 2015) or through the consumption of comtaminated food. At the animal-human interface, zoonotic infection through consumption of contaminated food is a major public health risk, with *Listeria*, *Salmonella*, and *Escherichia coli* being the most reported agents in food products across the included publications.

The analysis evidenced the importance of wildlife-livestock-human interfaces in the circulation and spread of zoonotic agents in Austria. Among the ten most studied diseases at livestock-wildlife interfaces globally, nine are zoonotic (Wiethoelter et al., 2015), of which six (salmonellosis, trichinellosis, leptospirosis, brucellosis, toxoplasmosis, and echinococcosis) belong to the top ten most studied zoonotic diseases in Austria. An example of spillover from wildlife to livestock that poses a risk to public health involves free-ranging red deer and grazing cattle, both carrying *Mycobacterium tuberculosis* variant caprae in the Alpine region (Fink et al., 2015). Furthermore, the two sub-systems involving USUV and WNV, along with their avian hosts, illustrate zoonotic pathogen maintenance in a specific wildlife host community. This community is epidemiologicaly connected to potential mammalian hosts through the *Culex* vector, which plays a central role, serving as a primary amplification vector of WNV or acting as a bridge vector in transmitting both orthoflaviviruses to humans (Kilpatrick et al., 2005) and horses (Magallanes et al., 2023).

Veterinarians, farmers, slaughterhouse and agricultural workers, military personnel, and hunters, who interact directly and frequently with livestock, game species, or contaminated environments, are commonly considered to be at higher risk of zoonotic infections (Adam-Poupart et al., 2021). However, the general population is also exposed to zoonotic agents, e.g. through companion animals, often with limited awareness of the risk (Stull et al., 2015). Notably, several non-traditional pet (NTP) species, such as *Testudo hermanni*, *Boa constrictor*, and *Morelia viridis*, can potentially carry *Salmonella* sp., the main zoonotic risk associated with NTP in Austria, which supports the findings of other studies (Varela et al., 2022).

From 1975 to 2022, Austria saw the emergence of eight zoonotic agents, averaging one emerging infectious disease (EID) every six years. Major changes in land-use globally have expanded human-wildlife interfaces, with humans encroaching on natural habitats (Daszak et al., 2000), further increasing the risk of wildlife-to-human (and reciprocally) spillover events (Taylor et al., 2001; Jones et al., 2008). This heightened risk is reflected in the Austrian literature, with a strong focus on wildlife hosts in zoonotic disease research, comprising 76.8% of the investigated hosts. Moreover, climate change increases the risk of extreme weather events, thereby amplifying the potential for infectious disease outbreaks (Baker et al., 2022). For example, the emergence of tularemia in western Austria has been linked to the effects of global warming on local temperatures and precipitation patterns (Seiwald et al., 2020). Additionally, land-use changes, driven by socio-economic factors including a growing human population, significantly and adversely affect the country´s ecosystem services. The Austrian urban area has almost doubled in the last 50 years. However, only 0.8% of the Austrian forests is dedicated to the conservation of biodiversity and maintenance of natural forest dynamics, while the majority is managed primarily for economic purposes (Schirpke et al., 2023). These factors reduce the ecosystem's resilience to stress (Essack, 2018). Yet, despite the growing interest in the subject, very few publications address zoonotic agents within the environmental context, a gap which is also observed on a global scale (Essack, 2018).

The lack of a readily accessible and structured dataset on zoonotic agents investigated and circulating in Austria poses significant challenges in identifying spillover risk. Organising data and then applying a network analysis approach can assist in shaping targeted national mitigation and surveillance activities for zoonotic diseases through a One Health approach, by identifying where efforts should focus (pathogens, hosts, ecosystems, regions) and facilitating efficient allocation of resources. The data can also support the construction of eco-epidemiological models for a specific zoonotic pathogen and its host community (Caron et al., 2015). Network analysis represents a cross-disciplinary method, encompassing infectious disease epidemiology and data science, for unveiling the intricate web of zoonotic interactions involving multiple hosts and pathogens within an ecological system (Poulin, 2010). In addition to presenting interactions between nodes (e.g. hosts and pathogens), network analysis enables the identification of influential nodes that may hold particular epidemiologic significance.

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THE VALUE OF MULTIVARIATE INDICATORS OF RESILIENCE IN EARLY WARNING FOR INFECTIOUS DISEASE OUTBREAKS: A WEST NILE VIRUS CASE STUDY

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SUMMARY

As no vaccine against West Nile Virus in humans is currently available, WNV prevention measures mostly rely on vector control. However, such preventive measures are most effective if implemented before or at the start of an epidemic. The early anticipation of outbreaks is therefore important. Using simulated data, we studied resilience indicators as early-warning signals for West Nile Virus outbreaks. Specifically, we applied multivariate indicators that combine different time series. We found that combining incidence time series and seroprevalence time series provided robust signals prior to West Nile virus outbreaks. This method can help overcome the challenge of large data requirements faced when implementing early-warning systems.

INTRODUCTION

Mosquito-borne diseases such as dengue, chikungunya, and West Nile virus are a leading cause of mortality worldwide and are extremely challenging to control. They account for 17% of infectious diseases according to WHO (WHO, 2014). This threat is likely to increase in the upcoming years because of, among others, climate change and urbanisation. A predicted 50% of the global population will be at risk of mosquito-borne disease in 2050 (Kraemer et al., 2019). To reduce the spread of mosquito-borne diseases, only a few interventions are available, and the timing of implementation is crucial. Interventions mostly focus on reducing the vector population and limiting the contact between hosts and vectors. This is typically done by spraying and using larvicide products, or by promoting the use of mosquito nets and mosquito repellent (Reisen and Brault, 2007). Additionally, these interventions are costly. To prevent unnecessary costs, anticipation of upcoming epidemics is decisive to limit the consequences of mosquito-borne disease outbreaks.

Common anticipation methods rely on the association between risk factors and the risk of an epidemic. These associations are trained on previous data and often use meteorological factors as predictors to estimate the risk of upcoming outbreaks, as mosquito populations highly depend on temperature and precipitation (Racloz et al., 2008, 2012; Semenza, 2015). However, these models are highly context-dependent, requiring the fitting of a new model for each new

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location studied. Thus, a generic, model-free method would be valuable to anticipate future epidemics.

Resilience indicators are such a generic, model-free method used to anticipate critical transitions in complex systems, but they require long, high-resolution time series. The use of resilience indicators to anticipate epidemics has attracted attention in the last decade. These indicators typically rely on the theory of critical slowing down, stating that a system approaching a critical transition such as the start of an epidemic loses its resilience and recovers from external perturbations with increasing difficulty (Scheffer et al., 2009, 2012). In the context of vector-borne diseases, this translates into a longer time for minor outbreaks to resolve. This slow behaviour can be observed in trends of autocorrelation and variance over time calculated in the time series using a rolling window. Such indicators have proven to be able to anticipate upcoming epidemics up to several months in advance, for infectious diseases but also mosquito-borne diseases (O'Regan et al., 2016; Harris et al., 2020; Southall et al., 2021; Delecroix et al., 2023). However, these indicators require long incidence time series prior to the epidemic. As cases are rarely reported in periods of low transmission, data requirements are rarely met in practice.

Mosquito-borne diseases can generate multiple incidence time series as some affect multiple host and vector species. The transmission burden can be monitored in mosquito pools by estimating the proportion of infected mosquitoes (Engler et al., 2013), but also by monitoring cases in humans, livestock, or wildlife through public health and veterinary surveillance systems. Such time series could be combined to improve the current practice of resilience indicators. Multivariate indicators have been investigated in simulation studies and shown to be signalling upcoming critical transitions in the same way univariate resilience indicators do (Weinans et al., 2021). In this study, a plant-pollinator model was investigated as a simple example of a multivariate system undergoing a critical transition. These multivariate indicators rely on data reduction techniques to combine multiple data streams, such as Principal Component Analysis (PCA) and Maximum Autocorrelation Factors (MAF). Common resilience indicators such as autocorrelation and variance are then computed in the obtained combined time series. Similar indicators have been investigated in simulated incidence time series of metapopulation models and could signal upcoming epidemics (Ghadami et al., 2023).

In the present study, we investigate the use of multivariate indicators of resilience as an early warning for upcoming epidemics of vector-borne diseases. We specifically focus on West Nile Virus as a case study, as it affects multiple host species and vector species, and can be monitored in numerous ways. We used a mechanistic model to generate incidence and seroprevalence time series and then computed multivariate resilience indicators.

MATERIALS AND METHODS

Model

We used a compartmental, Ross Macdonald model of West Nile Virus to simulate time series, adapted from Laperriere et al. (2011). This model considers the vector population and three host populations: birds acting as an amplifying host, and humans and horses acting as dead-end hosts (Fig.1A).

The mosquito population was subdivided into susceptible mosquitoes M_S, exposed mosquitoes M_E, and infected mosquitoes M_I. Mosquitoes could get infected with a probability p_M when biting infected birds, which they encountered with a probability $\delta_M k$ (with δ_M the fraction of non-diapausing mosquitoes, and k the biting rate of mosquitoes). We assumed that infected mosquitoes do not recover from the infection and that they are not affected by an additional disease-induced death rate. The population was in equilibrium as the natural birth rate and the natural death rate b_M were considered equal.

The bird, human and horse populations were subdivided into susceptible (respectively B_S, H_S and E_S), exposed (respectively B_E, H_E and E_E), infected (respectively I_B, I_H and I_E) and recovered (respectively B_R, H_R and E_R). The bird and horse populations were in equilibrium as the natural birth rate and the natural death rate (respectively b_B=m_B and b_E=m_E) were considered equal. They were affected by an additional disease-induced death rate v_B and v_E respectively. Additionally, West Nile Virus was maintained in the population via stochastic events of importation of infected birds at a rate Λ_B. We considered that the human population dynamics are insignificant compared to the disease dynamics, and we did not include them in the equations. All hosts could get infected with a probability p_M when getting bitten by an infected mosquito, which they encountered with a probability $\delta_M k\Phi_B$, $\delta_M k\Phi_H$, and $\delta_M k\Phi_E$ respectively (with δ_M the fraction of non-diapausing mosquitoes, k the biting rate of mosquitoes, and Φ_B, Φ_H and Φ_E mosquito-to-host ratios).

We used the parameter values provided in Laperriere et al. (2011), with a constant temperature of T=22°C and a constant day length of D=12 hours. Intrinsic stochasticity was added to the model using a branching process implemented using the Gillespie algorithm (Gillespie, 2007). Simulations were run in R 4.2.3 using the package SimInf (Widgren et al., 2019).

Perturbation-recovery experiments

To study critical slowing down, we performed perturbation-recovery experiments using the West Nile virus model described previously, for different fixed values of R_0. Five infected birds (resp. mosquitoes) were introduced in the disease-free population at $t=0$, and the return time to the disease-free equilibrium (i.e. no infected individuals) was measured. To improve the precision of the measure of the recovery time, the exponential decay of a perturbation was measured. The number of infected individuals per time step was measured and transformed into a logarithmic scale. The slope of a linear regression between the log number of infected and time was considered to represent the decay rate of a perturbation. The inverse of this number is the recovery time from a perturbation.

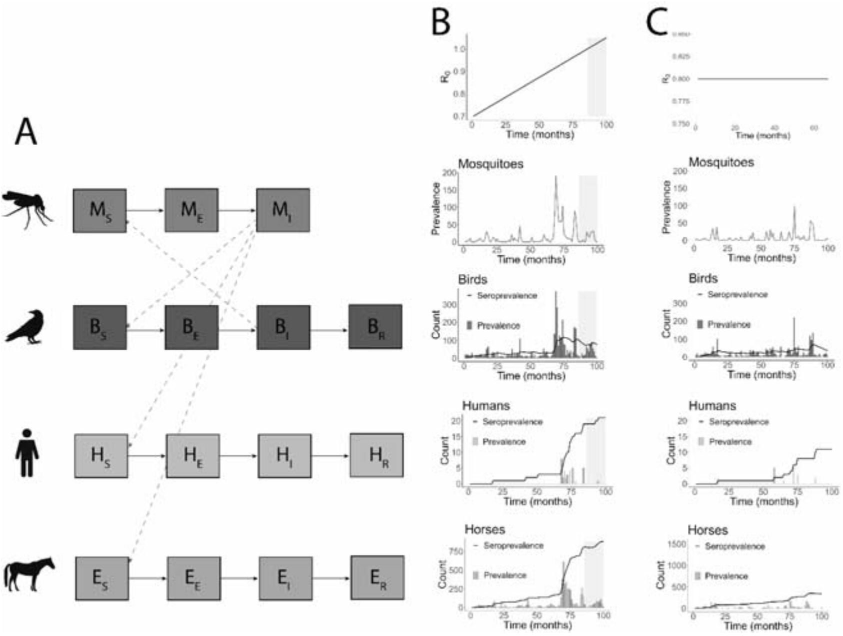

Figure 1. WNV model and simulated data (A) WNV compartmental model used in the analyses, adapted from (Laperriere et al., 2011). (B) Example of simulated data with an upcoming epidemic, simulated by increasing R_0 from 0.7 to 1.05. The shaded region indicates where R_0 is above 1. (C) Example of simulated data with no upcoming epidemic, simulated with a stable R_0=0.8. In (B) and (C), the time series were aggregated per month to improve the readability of the figure

Data simulations

For our analyses, we assumed a scenario where mosquito prevalence, bird prevalence and bird seroprevalence are monitored with high precision. We used these three variables as our data for the analyses. The biting rate was chosen as a control parameter as it is known to be influenced by temperature, which mimics the emergence of West Nile due to increasingly suitable climatic conditions (Paz and Semenza, 2013).

We simulated time series using two different approaches for two different purposes. Our first approach was to run the model over time under different fixed R_0 values under the critical threshold (i.e. <1) and analysed each time series separately. We call these data "fixed R_0 runs". These data served to test the behaviour of our indicators for different values of R_0. Each of these time series was simulated over 70 weeks, with a weekly resolution, for 100 values of R_0 between 0.2 and 1. For each value of R_0, 100 stochastic repetitions were simulated.

In our second approach, we simulated an approaching epidemic due to increasingly suitable conditions in a single long run, with the biting rate increasing slowly over time. These so-called "emergence time series" were used to measure the performance of the indicators as an early warning for upcoming epidemics. In these time series, R_0 gradually increased from 0.7 to 1.05 over 428 weeks with a weekly resolution (Fig.1B). As a control, we simulated data under the assumption of a fixed R_0 over time, leading to no major outbreak (Fig.1C). We used $R_0 \approx 0.8$ for that scenario. We simulated 100 stochastic repetitions for each outcome.

To explore the limitations of the multivariate indicators of resilience as an early-warning sign for upcoming epidemics, we down-sampled the data from the full emergence time series. These down-sampled time series were used to test the performance of resilience indicators in data-poor scenarios. To that extent, the full time series were down-sampled by reducing the resolution, to reach a fixed total amount of data. Thus, the R_0 gradient remains unchanged, but the number of data points decreases.

Resilience indicators

After detrending the simulated time series, we calculated the indicators of resilience over time. A rolling window of 50% of the size of the time series was used to assess the trends in the indicators over time (Dakos et al., 2012a). We then evaluated the strength of the trend in the indicators over time using the Kendall tau correlation. We used an implementation of the indicators in Matlab (Van Nes, 2017).

We used a subset of the multivariate indicators described in Weinans et al. (2021). These indicators rely on different techniques to combine multiple time series. Some indicators use the maximum or average of a univariate indicator among all time series. For instance, the maximum variance keeps the maximum value of variance for each window. Conversely, two more elaborate data reduction techniques were used to combine all the time series (PCA and MAF). PCA indicators were calculated in time series of the system projected in the direction of highest variance, corresponding to the first component identified using the principal component analysis. MAF indicators were calculated in time series of the system projected in the direction of the highest autocorrelation, using a MAF analysis (Max Autocorrelation Factor analysis) (Weinans et al., 2019).

We quantified the performance of the indicators as early-warning signals using ROC (receiver operating characteristic) curves. Emergence time series with both outcomes (increasing R_0 leading to an epidemic, and fixed R_0 with no upcoming epidemic) were used to calculate the true positive and true negative rate for different cut-off values of the Kendall tau. The Area Under the ROC Curve (AUC) was used to estimate the prediction performance of the different indicators under different data scenarios.

RESULTS

Perturbation recovery experiments

To investigate critical slowing down in the model, perturbation recovery experiments were performed by introducing infected birds (Fig.2A) and mosquitoes (Fig.2B) to disturb the disease-free equilibrium. In both cases, the recovery time increased as R_0 approached the critical threshold, indicating a loss of resilience as the system approaches suitable conditions for an epidemic to spark.

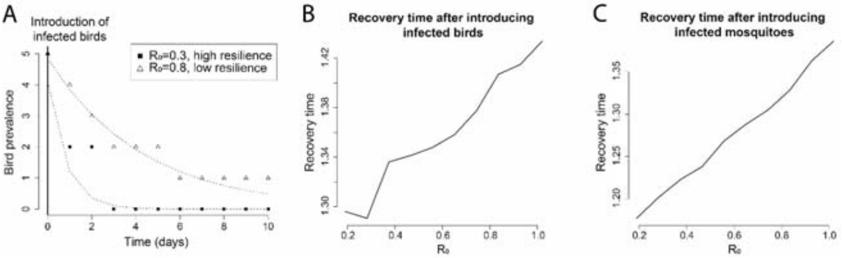

Figure 2. (A) Example of two perturbation-recovery experiments with birds, in a case of high and low resilience. The points represent the observations, and the dotted line indicates the fitted, exponential return rate to the disease-free state. (B) Return time to the disease-free state after perturbing the system by introducing infected birds for different values of R_0. (C) Return time to the disease-free state after perturbing the system by introducing infected mosquitoes for different values of R_0

Multivariate indicators of resilience for different R0

The slowing down observed in the perturbation recovery experiments (Fig.2) was reflected by an increase in multivariate indicators of resilience in our simulated fixed time series (Fig.3). Especially variance-based indicators such as MAF variance, mean variance, max variance and PCA variance displayed a strong increase as R_0 increased. Autocorrelation based indicators such as MAF autocorrelation, mean autocorrelation and max autocorrelation displayed a weaker increase. Additionally, autocorrelation-based indicators sometimes were high for low values of R_0. Due to the scarcity of cases far from the epidemic threshold, the time series contained long stretches of consecutive zeros, resulting in high autocorrelation, but this obviously did not reflect any measure of the recovery speed of the system.

Performance of the indicators as an early warning for upcoming epidemics

The area under the ROC Curve was used as an estimation of the performance, as it takes into account both the true positive rate (ability to predict accurately an upcoming epidemic) and the true negative rate (ability to predict that no upcoming epidemic is approaching). The performance of each multivariate indicator was estimated in our simulated emergence time series, with increasing R_0 for the true positives and stable R_0 for the true negatives (Fig.4). The variance-based indicators, especially mean variance, maximum variance and MAF variance were the best-performing ones with respective AUCs of 0.90, 0.89 and 0.86. Conversely, autocorrelation-based indicators were among the worst-performing ones. Explained variance, maximum absolute cross-correlation and mean autocorrelation respectively yielded an average AUC of 0.36, 0.70 and 0.71.

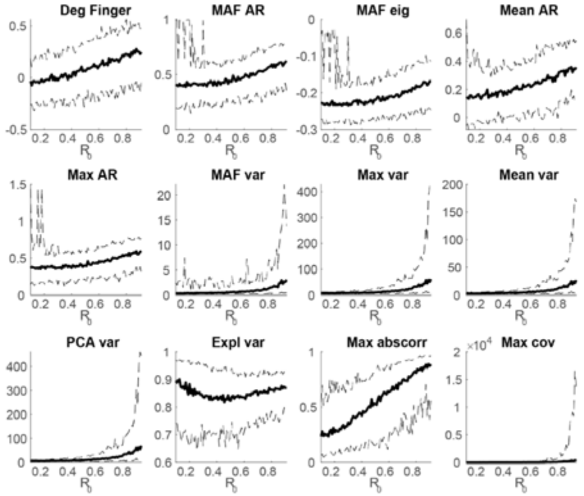

Figure 3. Multivariate indicators of resilience, calculated from separate time series generated for each level of R_0 (i.e. fixed R_0 runs, see Methods). The solid line indicates the median estimation over all the repetitions, and the dotted lines are the 2.5 and 97.5 percentiles

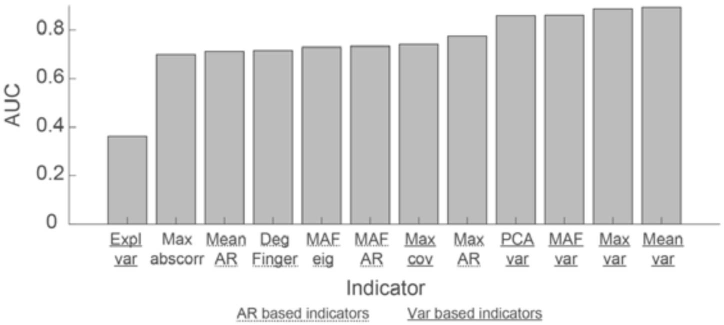

Figure 4. Performance of the different multivariate indicators of resilience using the AUC

Required amount of data for multivariate indicators compared to univariate indicators

The performance of multivariate indicators of resilience was compared to the performance of univariate indicators of resilience in down-sampled time series (Fig.5). The comparison was reproduced for different numbers of data points obtained by subsampling the original time series and reducing the resolution (Fig.5A). For autocorrelation-based indicators, the performance of both univariate and multivariate indicators decreased when the resolution of the data decreased (Fig.5B). This result is consistent with previous studies (Dakos et al., 2012b; Weinans et al., 2021). For variance-based indicators, the performance was not affected by the number of data points (Fig.5C). Additionally, variance-based multivariate indicators outperformed the univariate indicators regardless of the number of data points, while for autocorrelation-based indicators this was only true above 300 data points.

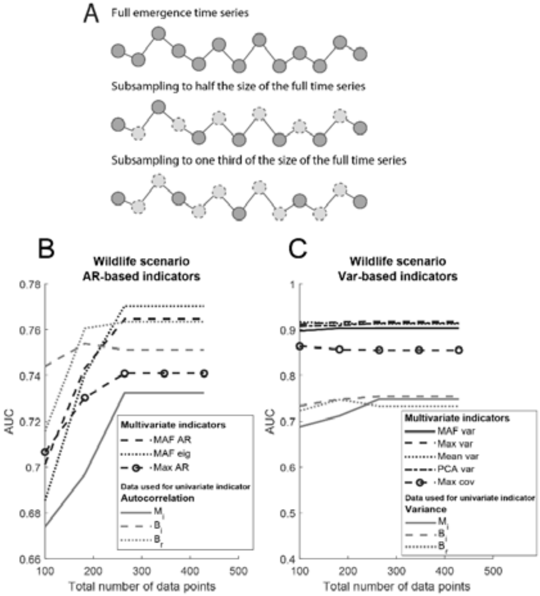

Figure 5. Performance of the best-performing multivariate indicators of resilience for different numbers of data points, estimated using the AUC. (A) Illustration of the down-sampling method. The dark grey dots represent data points used in the calculations, and the light grey dots with dotted lines are the data points we omitted for the calculation. (B) Performance of autocorrelation-based indicators for multivariate indicators and autocorrelation for univariate indicators. (C) Performance of variance-based indicators for multivariate indicators and variance for univariate indicators

DISCUSSION

In simulated data of West Nile Virus, multivariate indicators of resilience could discriminate time series with an upcoming epidemic from time series with no upcoming epidemic. Specifically, variance-based indicators estimated from wildlife data (i.e. birds and mosquitoes) could best anticipate upcoming epidemics. These results suggest that monitoring mosquito prevalence and bird prevalence and sero-prevalence in combination with the calculation of real-time multivariate indicators of resilience could be used as an early warning for upcoming epidemics of West Nile virus, and more generally vector-borne diseases.

Variance-based indicators outperformed autocorrelation-based indicators and remained robust in data-poor contexts. This is consistent with previous studies which identified variance or related indicators as the best performing when researching the performance of resilience indicators to anticipate epidemics (O'Regan et al., 2016; Miller et al., 2017; Brett et al., 2018). In the present study, the poor performance of autocorrelation-based indicators was likely due to the excess of zeros when R_0 is far from the critical threshold, leading to a high autocorrelation and thus obscuring the overall trend in autocorrelation. Additionally, the performance of autocorrelation-based indicators decreased when the resolution of the time series was reduced, in agreement with previous studies (Dakos et al., 2012b; Weinans et al., 2021). Multivariate variance-based indicators of resilience showed stable performance, even when reducing the resolution of time series. This was especially true for indicators using the average of univariate indicators, consistent with previous research (Weinans et al., 2021). As data requirements are a major factor limiting the use of resilience indicators in practice, multivariate indicators hold potential to improve the performance of resilience indicators as an early warning of upcoming epidemics using fewer data.

However, several factors can hamper the performance of multivariate indicators of resilience. Resilience indicators can only be used as an early-warning sign for epidemics caused by a slowly changing underlying factor, and their performance will be limited if the change happens too fast (Dakos et al., 2015; Delecroix et al., 2023). Additionally, our findings may be limited, as the model does not consider seasonal fluctuations nor imperfect observation processes. Seasonal fluctuations could hamper the signal of multivariate indicators of resilience. However, previous studies have shown that resilience indicators remain reliable, even with data subject to seasonality (Miller et al., 2017). Imperfect observation processes were also not taken into account, although they play an important role when monitoring wildlife prevalence and seroprevalence. Highly variable reporting rates affect the performance of resilience indicators, according to previous research (Brett et al., 2018). Consequently, although multivariate indicators of resilience loss hold potential to improve the current practice of early warning for vector-borne diseases, these findings should be confirmed in real data.

Seroprevalence time series were identified as an extra source of data to be combined with other widely used prevalence data when calculating resilience indicators. However, such data are mostly informative about the past state of infection, which could lead to bias in informing early warnings (Chaintoutis et al., 2014; Donnici et al., 2022). Further analyses are necessary to determine if seroprevalence time series are subject to slowing down. Additionally, cross-reactivity sometimes causes errors in seroprevalence data (Lustig et al., 2018). Seroprevalence data used cautiously, and in combination with prevalence time series, could help prevent misleading results.

Further analyses investigating other monitoring schemes, for instance, with horse and human data, could be compared with the monitoring scheme considered in this study, to identify the most informative data sources. However, as birds and mosquitoes are part of the enzootic cycle, and thus a direct measure of the ongoing spread of the pathogen, they are likely to outperform equine and human monitoring schemes, as these are an indirect measure of the ongoing transmission. Nonetheless, comparing different performance measures such as true positive rate, true negative rate and lead time of the warning could indicate the most relevant monitoring scheme to predict future outbreaks of West Nile Virus.

ACKNOWLEDGEMENTS

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RISK QUANTIFICATION

RISK FACTORS FOR RACE-RELATED FATALITY IN JUMP RACING THOROUGHBREDS IN GREAT BRITAIN (2010-2023)

S. ALLEN[*], S. TAYLOR, J. GIVEN AND K. VERHEYEN

SUMMARY

Identifying risk factors for fatality in jump racing can help improve racehorse safety and welfare. This study examined data from all jump starts made on British racecourses between January 2010 and April 2023, with steeplechase and hurdle starts analysed separately. Risk factors for fatality were evaluated using mixed-effects logistic regression modelling. The overall fatality rate was 5.9 per 1,000 steeplechase starts (95% CI: 5.5-6.3) and 4.5 per 1,000 hurdle starts (95% CI: 4.3-4.8). In both race types, increasing horse age was associated with higher odds, and softer ground with lower odds of fatality. For steeplechase starts, other risk factors were field size, horse sex and country where the horse was trained. Season, trainer performance and intensity of racing at the racecourse were associated with odds of fatality in hurdle starts. Population impact measures indicated that racing on going softer than good could substantially reduce fatalities in jump racing.

INTRODUCTION

Race-related fatalities are a major concern for racing jurisdictions worldwide, as each looks to enhance the safety and welfare of racing Thoroughbreds. In Great Britain, approximately 35% of horse races are held over jumps (British Horseracing Authority, 2023), with jump racing carrying an inherently greater risk of fatality compared to flat racing (McKee, 1995; Williams et al., 2001; Reardon, 2013). Jump racing consists of three types: steeplechasing where horses compete over larger fences, hurdling where horses jump over smaller flexible hurdles, and National Hunt flat racing where inexperienced jump racehorses run on a hurdle course but without hurdles. The most recent study of race-related fatality in British jump racing estimated the incidence of fatality at 6.2 per 1,000 steeplechase starts and 4.6 per 1,000 hurdle starts (Reardon, 2013). The relatively high risk of fatality in jump racing is of concern for the welfare of jump racehorses and is increasingly jeopardising the sport's social license to operate (Butler et al., 2019).

Identifying risk factors for fatality is an important step in addressing this important welfare concern, as it will enable the racing industry to implement effective changes to improve racehorse safety. Previous studies investigating jump racing fatalities have identified several

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risk factors, including increasing age, longer race distances and firmer going (Wood et al., 2001; Williams et al., 2001; Parkin et al., 2005; Henley et al., 2006; Boden et al., 2007; Reardon, 2013). Prior racing intensity and season were also associated with fatality (Boden et al., 2007; Reardon, 2013). With no recent studies of fatality in jump racing, the purpose of this work was to provide an update on the current factors associated with the odds of fatality in British Jump racing.

MATERIALS AND METHODS

The study population included all Thoroughbred racehorses that competed in either a steeplechase or hurdle race on a British racecourse between 01 January 2010 and 30 April 2023. Information describing all starts, including horse demographics, race, course, trainer and jockey information was extracted from Weatherbys' racing database (Weatherbys, 2023). Details of all race-related fatalities were extracted from the British Horseracing Authority's (BHA) veterinary database. Race-related fatality was defined as any race-day event that resulted in the sudden death or euthanasia of a horse within 48 hours of them starting a race. This included all causes of fatality and cases of elective euthanasia. Fatalities were recorded by official BHA veterinary officers.

For this study, starts made in steeplechase and hurdle races were analysed separately. The unit of observation was a race start, i.e., a horse that crossed the starting tape, and one horse could have multiple starts during the study period. Incidence of fatality was expressed as the number of deaths per 1,000 starts. Exposure variables were extracted from the racing database and additional performance variables were calculated. Age-related factors included current age (in years), age at first race start and the number of years in racing. Horse sex was categorised in two groups: male (colts, stallions and geldings) and female (fillies and mares). The firmness of the racing surface or going was initially categorised in six groups: firm, good-to-firm, good, good-to-soft, soft and heavy. Performance variables were calculated using data prior to the current race start and included the number of previous race starts, percentage wins, percentage placed and average performance score (30 points awarded for a win, 20 for a place, 10 for a completed run, 0 for a start in which the horse failed to finish). As the racing database only stores information for starts made in Great Britain (GB) and outside GB if the race includes a GB-trained horse, performance variables for non-GB participants were set to missing as the available performance history may be incomplete.

A total of 90 potential risk factor variables were evaluated (47 horse-related, 26 race-related, 6 course-related, 6 trainer-related, 5 jockey-related). Exposure variables were initially screened using univariable logistic regression and those with a likelihood ratio test (LRT) P value <0.25 were carried forward for consideration in the multivariable model. Linearity of continuous variables was assessed and if non-linearity was identified (either through LRT or graphical assessment), the variable was categorised based on quartiles. After ranking remaining variables by the Akaike information criterion (AIC) and log likelihood values, models were built using a forward selection process, starting with the variable with the lowest AIC. Variables were retained if the LRT P values were ≤0.05.

All variables carried forward for multivariable modelling but not included in the final models were resubmitted to check for potential confounding. Confounding was present if the addition of a variable altered the estimates by >10%, in which case the confounding variables were retained. Biologically plausible two-way interactions between variables in the final model were assessed by comparing models including and excluding interaction terms using the LRT. Mixed-effects logistic regression modelling was used throughout to account for horses making multiple starts during the study period. Due to computational constraints, it was not possible to include multiple random effects in the same model.

For identified risk factors, population attributable fractions were calculated to estimate the potential change in fatality risk under various hypothetical situations, such as reducing field size, decreasing the firmness of going and retiring older horses from competition.

Institutional ethical approval for this work was obtained from the RVC's Clinical Research and Ethical Review Board (URN 2023 2182-2)

RESULTS

Risk factor analysis for race-related fatality in steeplechase races

There were 141,922 steeplechase starts on British racecourses during the study period, of which 836 resulted in fatality. This equates to an overall incidence of 5.9 fatalities per 1,000 steeplechase starts (95% CI 5.5-6.3). The study population included 19,134 horses competing in 19,126 races at 42 racecourses. In total, 2,390 trainers and 1,684 jockeys contributed to the steeplechase dataset.

Identified risk factors for fatality in steeplechase racing are presented in Table 1. Of the 32 variables carried forward to the multivariable build, 5 were retained in the final model. Increased odds of fatality were observed with advancing horse age, in male horses compared to females, for horses trained outside GB compared to within, with increasing number of runners (field size) and when racing on good ground compared to good-to-soft going. Starts made on heavy ground had lower odds of fatality compared to those made on good-to-soft going. No significant interaction terms were identified

The model outputs indicated significant variation in the odds of fatality between horses ($P<0.001$), with 51% of variation not explained by the model attributable to the horse. There was also evidence of significant variation between trainers ($P<0.001$) and racecourses ($P=0.004$), although, they were only responsible for 2% and 1% of the unexplained variation, respectively.

Risk factor analysis for race-related fatality in steeplechase races

There were 242,486 hurdle starts on British racecourses during the study period, of which 1,096 resulted in fatality. This equates to an overall incidence of 4.5 fatalities per 1,000 hurdle starts (95% CI 4.3-4.8). The study population included 37,499 horses competing in 26,109 races at 42 racecourses. In total, 1,323 trainers and 1,343 jockeys contributed to the hurdle dataset.

Identified risk factors for fatality in hurdle racing are presented in Table 2. Of the 51 variables carried forward to the multivariable build, 6 were retained in the final model.

Increased odds of fatality were observed with advancing horse age, when racing on ground firmer than good-to-soft, for starts made in spring (March to May) compared to winter

(December to February), for horses trained by the top 25% of trainers (by win rate) and for racecourses with the greatest number of races in the previous 90 days. Racing on ground softer than good-to-soft was associated with lower odds of fatality. Lower odds of fatality was also observed in races restricted to novices compared to those that were not. No significant interaction terms were identified.

The model outputs indicated significant variation in the odds of fatality between horses (P<0.001), with 52% of variation not explained by the model attributable to horse. There was also evidence of significant variation between trainers (P<0.001), however, only 2% of the unexplained variation was attributable to trainer.

Table 1. Results of logistic regression analysis investigating risk factors for fatality in horses competing in steeplechase races in Great Britain between 01 January 2010 and 30 April 2023. Horse ID is included as a random effect to account for multiple starts made by the same horse

Risk factor	Odds ratio[a]	Wald P-value	95% confidence interval for the odds ratio	Likelihood ratio test P-value
Number of runners				0.001
per extra runner	1.03	<0.001	1.01 – 1.04	
Country of trainer				<0.001
Great Britain	1.00 (Ref)			
Not Great Britain	1.96	<0.001	1.38 – 2.80	<0.001
Going				<0.001
Firm	2.06	0.35	0.45 – 9.41	
Good to Firm	1.21	0.26	0.87 – 1.68	
Good	1.21	0.04	1.01 – 1.45	
Good to Soft	1.00 (Ref)			
Soft	0.85	0.15	0.68 – 1.06	
Heavy	0.64	0.01	0.47 – 0.89	
Age at race				
per extra year	1.09	<0.001	1.03 – 1.15	0.003
Sex				
Female	1.00 (Ref)			
Male	1.38	0.05	1.00 – 1.90	0.042

Table 2. Results of logistic regression analysis investigating risk factors for fatality in horses competing in hurdle races in Great Britain between 01 January 2010 and 30 April 2023. Horse ID is included as a random effect to account for multiple starts made by the same horse

Risk factor	Odds ratio	Wald P-value	95% confidence interval for the odds ratio	Likelihood ratio test P-value
Going				<0.001
Firm/Good to Firm	1.50	<0.001	1.15 – 1.95	
Good	1.24	0.01	1.05 – 1.46	
Good to Soft	1.00 (Ref)			
Soft	0.73	<0.001	0.59 – 0.89	
Heavy	0.51	<0.001	0.37 – 0.69	
Season				0.03
Winter (Dec-Feb)	1.00 (Ref)			
Spring (Mar-May)	1.30	<0.01	1.08 – 1.56	
Summer (Jun-Aug)	1.11	0.37	0.88 – 1.40	
Autumn (Sep-Nov)	1.09	0.38	0.90 – 1.34	
Age at race				<0.001
per extra year	1.14	<0.001	1.09 – 1.20	
Novice race				0.046
No	1.00 (Ref)			
Yes	0.86	0.04	0.74 – 1.00	
Trainer win rate				0.02
≤15%	1.00 (Ref)			
>15%	1.25	<0.01	1.06 – 1.48	
Incomplete data	1.29	0.17	0.89 – 1.86	
Number of races held at the racecourse in the preceding 90 days				0.02
0 to 27	1.00 (Ref)			
28 to 41	1.21	0.05	1.00 – 1.45	
42 to 62	1.10	0.31	0.92 – 1.31	
63 or more	1.38	<0.01	1.11 – 1.71	

Measures of population impact

Despite increasing horse age being associated with greater odds of fatality in both steeplechase and hurdle racing, population attributable fractions suggest that retiring horses from racing at 14 years would have little effect on the overall rate of fatality. For this hypothetical scenario, the population attributable fraction was 0.2% (95% CI -0.4-0.8%) for steeplechase racing and 0.05% (95% CI -0.2-0.3%) for hurdle racing. This equates to 1 fewer death per 100,000 steeplechase starts and 1 fewer death per 500,000 hurdle starts. For a hypothetical scenario of not racing on ground firmer than good-to-soft, the population attributable fraction was 12.7% (95 CI: 6.6-18.4%) for steeplechase racing and 17.8% (95% CI 11.7-23.5%) for hurdle racing. This equates to 75 fewer deaths per 100,000 steeplechase starts and 80 fewer deaths per 100,000 hurdle starts.

DISCUSSION

Understanding the factors associated with race-related fatality is essential for the implementation of risk-mitigating strategies needed to improve the safety and welfare of jump racehorses. This study revealed that overall fatality rates have remained relatively static in steeplechase and hurdle racing for the past 30 years. The identified risk factors are broadly consistent with previous investigations of race-related fatality and, encouragingly, some are modifiable.

The incidences of fatality described in the current paper are similar to previously reported estimates. Using start data from January 1990 to December 1999, Henley et al., (2006) estimated the overall risk of fatal injury at 6.7 per 1,000 steeplechase starts (95% CI 6.2-7.2) and 4.9 per 1,000 hurdle starts (95% CI 4.6-5.3). A later evaluation of start data from January 2000 to December 2009, estimated the incidence of fatality at 6.2 per 1,000 steeplechase starts and 4.6 per 1,000 hurdle starts (Reardon, 2013). The relatively static rates of fatality emphasise the importance of improving our knowledge of the factors associated with race-related fatality.

Increasing horse age was associated with greater odds of fatality in both the steeplechase and hurdle model. This is consistent with previous investigations of all-cause fatality (Williams et al., 2001; Henley et al., 2006), catastrophic musculoskeletal injury (Bailey et al., 1998) and sudden death (Lyle et al., 2012; Bennett and Parkin, 2022). Catastrophic musculoskeletal injuries are the most common cause of race-related fatality. The effect of age may reflect the accumulation of bony microdamage, and the structural deterioration of tendons, over time, which predispose a horse to catastrophic musculoskeletal injury. As in the current study, Henley et al. (2006) also found that males had higher odds of fatality compared to females.

The firmness of the racing surface was associated with fatality, with decreased odds of death observed for starts made on softer ground in both models. Similar associations between going and race-related fatality have been observed in British flat racing (Rosanowski et al., 2018) and across all race types (Williams et al., 2001; Henley et al., 2006). Conversely, an investigation by Lyle et al. (2012) indicated that starts made on soft and heavy going had greater odds of sudden death compared to those made on good ground. After controlling for the effect of going, season was also independently associated with fatality in hurdle starts, with the greatest odds of fatality observed in Spring. This period covers the end of the core jump racing season, and the effect may be attributable to the increased competitiveness of races at this time of year. Starts made in hurdle races restricted to novice horses were at lower odds of fatality. This may also relate to the competitiveness of the race, with inexperienced horses potentially raced less intensely.

Field size was only associated with fatality in steeplechase racing, with a greater number of runners increasing the odds of fatality. Larger field sizes have previously been associated with greater odds of catastrophic musculoskeletal injury (Bailey et al., 1997) and fatal distal limb fracture (Parkin et al., 2004), however, other studies have shown no effect of field size on odds of fatality (Bailey et al., 1998; Henley et al., 2006; Georgopoulous and Parkin, 2016; Zambruno et al., 2020).

Although multiple risk factors have been identified, implementing these findings to bring about a reduction in fatality is not straightforward as many only have a marginal effect or are uncommon. Population attributable fractions are useful for assessing the potential change in fatality rate under different hypothetical scenarios. Evaluation of selected risk-mitigating strategies suggested it would be more beneficial to focus efforts on creating a softer racing surface rather than imposing restrictions on older horses.

The limitations of this work primarily relate to incompleteness of data collated within the racing database. Most of the unexplained variation in the odds of fatality was attributed to horse. As such, future work should look to evaluate other horse-related factors, not considered in the current study, such as training regime, injury history and medications record.

This study has identified some potentially modifiable risk factors for fatality in Jump racing in Great Britain. Outputs from this work can be used to inform interventions that reduce the risk of fatality in steeplechase and hurdle racing.

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RISK FACTORS FOR THE INTRODUCTION OF *SALMONELLA* SPP. IN DAIRY HERDS

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SUMMARY

Salmonella spp. infections in animals are a concern due to their zoonotic nature, welfare effects and economic impact on the livestock industry. Based on bulk milk ELISA testing on antibodies against *Salmonella* spp., we aimed to identify risk factors for the introduction of *Salmonella* spp. in diary herds. Effects of putative risk factors for becoming newly infected were studied using a multivariable population average logistic regression (PA-GEE) model. Identified risk factors included short distance (<500m) to another herd where antibodies were detected, larger herd size, on farm movement of >2 cattle from other herds in the previous year, keeping pigs on-farm, being located in postal code areas with >2% surface water, peat and sandy loam and high soil moisture, and being located in the province of Fryslân. The obtained information on risk factors for becoming newly infected can be used to improve surveillance and control of salmonellosis in dairy herds.

INTRODUCTION

Salmonellosis in dairy cattle is an infectious disease caused by bacteria of the species *Salmonella*. Clinical signs of salmonellosis in cattle include fever, diarrhoea, pneumonia, arthritis, abortion and mortality (La Ragione et al., 2013). In the Netherlands, salmonella infections are usually caused by *Salmonella enterica* subsp. *Enterica* serogroups B (including serovar Typhimurium) and serogroup D (including serovar Dublin). Salmonellosis in dairy cattle is a concern due to decreased welfare, economic losses and its zoonotic nature (Nielsen et al., 2010; 2013; Nielsen and Dohoo, 2012; Mangat et al., 2019; Kudirkiene et al., 2020).

Dutch dairy processors require herds delivering milk to their plants to participate in a Salmonella programme. This programme has been implemented since 2009, and aims to stimulate control of *Salmonella* spp. in dairy herds as well as to improve farm management in order to reduce the prevalence of *Salmonella* spp. in cattle. A complete description of this programme can be found in Santman-Berends et al., (2021). In short, bulk milk of participating herds is tested three times a year, namely in February/March, June/July and October/November (hereafter: test rounds), for antibodies against *Salmonella* spp. serogroups B and D. Based on these bulk milk results, the herds are assigned a herd level (1, 2 or 3). The preferential level, level 1, is assigned after two consecutive bulk milk tests without any antibodies. A herd is classified as level 2 when antibodies are detected in two consecutive rounds, and a herd is

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classified as level 3 when antibodies are detected in at least four out of five consecutive rounds. Level 2 and 3 herds are obliged to take control efforts and progress towards level 1 for which they need two consecutive negative bulk milk tests by the terms of delivery of their dairy processor. Throughout 2022, on average 97 percent of participating dairy herds were assigned level 1, reflecting a low salmonellosis herd level prevalence in the Netherlands.

Transmission of *Salmonella* spp. between cattle occurs primarily through the faecal-oral route (La Ragione et al., 2013). Infected cattle may shed bacteria, either asymptomatically or during clinical illness, contaminating the environment or infecting other cattle directly. The probability of introduction of *Salmonella* spp. in a herd can be influenced by several factors including cattle movements, contact with other herds, use of infected slurry, insufficient biosecurity for visitors and sharing of equipment (reviewed by Pedersen et al., 2023). To enable targeted surveillance, it is important to identify risk factors for the introduction of *Salmonella* spp. in a herd based on regularly collected and centrally managed data. With such information targeted measures can be taken to reduce the transmission of *Salmonella* spp. within and between herds, and these measures can be adapted when needed. The aim of this study was, therefore, to identify risk factors for introduction of *Salmonella* spp. into dairy herds based on regularly collected and centrally managed data.

MATERIALS AND METHODS

Data collection

In this study, bulk milk test results obtained between 2018-2021 from all 15,847 dairy herds within the Dutch Salmonella programme were included. Bulk milk samples were tested by in-house ELISA for antibodies at a single laboratory (Qlip, Zutphen, The Netherlands). For each test round, herds were assigned to one of three different classification groups based on their test results: (1) herds in which no antibodies were detected in the current test round, as well as in the previous three test rounds, were classified as susceptible; (2) herds in which antibodies were detected in the current test round, but not during the previous three rounds, were classified as newly infected; and (3) herds in which antibodies were detected in any of the three previous test rounds, regardless of whether antibodies were detected in the current test round, were classified as persistently infected or recovered (hereafter: persistently infected/recovered). As we needed results of bulk milk tests of three rounds to classify herds, herds were classified only for the period 2019-2021. Furthermore, we classified a herd at risk if the herd was susceptible in the previous test round for which a test result in the present round was available.

For all herds, data on putative risk factors to become newly infected were obtained from private and public organisations that routinely collect these data. Data on cattle movements, distance to neighbouring herds (dairy cattle, non-dairy cattle and pigs), net return on milk (fat and protein corrected 305-day milk production minus variable costs), herd size, annual growth in herd size and location were obtained at herd level from the Dutch enterprise agency (RVO, The Hague), CRV (Arnhem), Qlip (Zutphen) and Royal GD (Deventer). We received this data per quarter of the year, where we used the data from the first, second and fourth quarter of the year for each test round. Dominant soil type, relative soil moisture (RSM) and proportion of water surface area were obtained at the level of the two-digit postal code area from Wageningen Environmental Research (Wageningen). For the dominant soil type and proportion of water surface area we received one value per postal code area for the study period, while for RSM we received a value per quarter of the year. For the latter we used the data from the first, second and fourth quarter of the year for each test round.

Seven factors were categorical variables or were categorized in biologically relevant categories: (1) number of dairy herds within 500 metres, (2) on farm movement of cattle from other herds in previous year, (3) proportion of water surface area, (4) RSM, (5) test round, (6) province and (7) dominant soil type (see Table 2 for the exact categories). Furthermore, four numerical variables (i.e. distance to closest pig herd, net return on milk, herd size and annual herd size growth) were categorized into four categories: lowest 10th percentile, 11th to 50th percentile, 51th to 90th percentile and the highest 10th percentile. Lastly, one numerical variable (i.e. trend over time) was included. Prior to data analyses, all data were anonymized by an independent company (IntroFocus Data Transformation Services (IDTS), Deventer, The Netherlands).

Statistical analyses

Pearson correlation coefficients were determined to check for multicollinearity. Univariable analyses were not performed, since all factors were deemed to be relevant with a sufficient number of observations. A multivariable population average logistic regression model (PA-GEE) with binomial distribution, a logit link function and independent correlation structure was used to associate the different factors to the probability of becoming newly infected. In this analysis we corrected for within herd clustering of observations over time. As a dependent variable we included new infection (yes or no) in the herds at risk per test round. Thus, herds classified as persistently infected/recovered were excluded from the analysis as they were not at risk of becoming newly infected in that round. The final model was obtained by backward elimination, with a p-value of 0.10 as a threshold for stepwise removal. Three two-way interactions that were considered biologically plausible were tested: (1) on farm movement of cattle from other herds in the previous year and herd size, (2) on farm movement of cattle from other herds in the previous year and annual herd size growth, and (3) dominant soil type and RSM. All statistical analyses were performed using Stata 17 (StataCorp, 2021) and a significance level of alpha = 0.05 was used.

RESULTS

A total of 149,625 test rounds from 15,847 dairy herds were included in the analyses. Of these herd test rounds, in 147,010 the herd was classified as susceptible and in 2,615 herd test rounds the herd was classified as newly infected. Across the study period, on average 13,654 herds were classified as at risk per round. Of these herds, 0.85-4.10% became newly infected between rounds (Table 1).

All Pearson correlation coefficients were below 0.3. All factors from the full PA-GEE model were retained in the final PA-GEE model (Table 2), with a significant two-way interaction between dominant soil type and RSM. In the final model, data for at least one of the factors was missing for 1,5% of the herd test rounds, and therefore these herd test rounds were not included in the model.

Table 1. Summary of the number of herds within the Dutch Salmonella Programme that were at risk to become newly infected with *Salmonella* spp. serogroups B and D, per test round

Year and round	Herds at risk	Number of newly infected herds	Percentage of newly infected herds
2019 – Round 1	13,728	139	1.01%
2019 – Round 2	14,183	138	0.97%
2019 – Round 3	14,233	583	4.10%
2020 – Round 1	13,603	152	1,12%
2020 – Round 2	13,595	140	1.03%
2020 – Round 3	13,606	449	3.30%
2021 – Round 1	13,251	113	0.85%
2021 – Round 2	13,313	131	0.98%
2021 – Round 3	13,370	374	2.80%

The odds of becoming newly infected decreased over time (OR = 0.95), and was four times higher at each third round of screening during the year, as compared to the first round (OR = 4.00, Table 2). Furthermore, the odds of becoming newly infected was almost three times higher if there was at least one newly infected or persistently infected/recovered dairy herd within 500 metres compared to no dairy herds within that distance (OR = 2.68). The odds of becoming newly infected did not increase if there were only susceptible dairy herds within 500 meters compared to no dairy herds (OR = 0.93, Table 2). Other factors that were associated with higher odds of becoming newly infected included having distance of 0 kilometre to the nearest pig herd (in other words: on-farm presence of pigs) compared to no pig herd within a radius of 2.46 kilometre (OR = 1.64), and on farm movement of >2 cattle from other herds in the previous year compared to no cattle (OR = 1.17, Table 2). At an RSM of >0.54% the odds of becoming newly infected where higher when being located on peat or sandy loam, compared to sand (OR = 1.28 and 1.30, respectively). Furthermore, the odds of becoming newly infected were also higher with a water surface area of >2% (compared to ≤2%, OR = 1.15, Table 2). Compared to the Dutch average, higher odds of a new infection was found in herds with a low net return on milk (OR = 1.20) whereas a highest net return on milk was associated with lower odds of a new infection (OR = 0.81). Furthermore, a larger herd size (OR = 1.65), a lower herd size growth (OR = 1.21) and being located in the province of Fryslân (OR = 1.85, Table 3) were associated with higher odds of becoming newly infected. Factors that were associated with lower odds of becoming newly infected, compared to the Dutch average, were a smaller herd size (OR = 0.69), a larger herd size growth (OR = 0.89) and being located in the province of Zuid-Holland (OR = 0.70).

Table 2. Risk factors associated with becoming newly infected with *Salmonella* spp. serogroups B and D. Analyses are performed on bulk milk screenings of 15,847 dairy herds in the study period 2019-2021

Variable	Category	Odds ratio (95% CI)	β (SE)	p-value
Round	Round 1 (Feb/Mar)	(Ref)		
	Round 2 (Jun/Jul)	1.37 (1.14-1.66)	0.32 (0.10)	**0.001**
	Round 3 (Oct/Nov)	4.00 (3.55-4.50)	1.39 (0.06)	**<0.001**
Dairy herds within 500 metre	None	(Ref)		
	Only susceptible dairy herds	0.93 (0.86-1.03)	-0.06 (0.05)	0.166
	At least one infected/ recovered dairy herd	2.68 (2.30-3.12)	0.98 (0.08)	**<0.001**
Distance to nearest pig herd in kilometre	0	1.64 (1.27-2.11)	0.49 (0.13)	**<0.001**
	>0-0.93	0.98 (0.84-1.14)	-0.02 (0.08)	0.750
	>0.93-2.46	1.02 (0.89-1.18)	0.02 (0.07)	0.744
	>2.46	(Ref)		
On farm movement from other herds in the previous year	None	(Ref)		
	1 or 2 cattle	1.14 (0.98-1.32)	0.13 (0.08)	0.095
	>2 cattle	1.17 (1.07-1.29)	0.16 (0.05)	**0.001**
Annual herd size growth [a]	10% lowest	1.21 (1.08-1.34)	0.19 (0.06)	**0.001**
	40% lowest	1.00 (0.93-1.08)	0.00 (0.04)	0.959
	40% higher	0.89 (0.82-0.96)	-0.12 (0.04)	**0.002**
	10% highest	0.93 (0.84-1.04)	-0.07 (0.06)	0.216
Herd size [a]	10% smallest	0.69 (0.60-0.79)	-0.37 (0.07)	**<0.001**
	40% smaller	0.78 (0.72-0.84)	-0.25 (0.04)	**<0.001**
	40% larger	1.13 (1.05-1.21)	0.12 (0.04)	**0.001**
	10% largest	1.65 (1.49-1.82)	0.50 (0.05)	**<0.001**
Water surface	≤2%	(Ref)		
	>2%	1.15 (1.01-1.30)	0.14 (0.06)	**0.031**

95% CI = 95% confidence interval, β = logistic regression coefficient, SE = Standard Error. p-values ≤ 0.05 are marked in bold. QIC (Quasi information criterion) = 20433.04. $r^2 = 0.022$, scale parameter = 1

[a] The Dutch average has been taken as the reference.

Table 2 (cont). Risk factors associated with becoming newly infected with *Salmonella* spp. serogroups B and D. Analyses are performed on bulk milk screenings of 15,847 dairy herds in the study period 2019-2021

Variable	Category	Odds ratio (95% CI)	β (SE)	p-value
Province [a]	Drenthe	0.93 (0.76-1.13)	-0.08 (0.10)	0.438
	Flevoland	1.20 (0.87-1.65)	0.18 (0.16)	0.265
	Fryslân	1.85 (1.65-2.07)	0.62 (0.06)	**<0.001**
	Gelderland	0.95 (0.83-1.08)	-0.05 (0.07)	0.439
	Groningen	1.00 (0.82-1.22)	0.00 (0.10)	0.980
	Limburg	0.84 (0.57-1.26)	-0.17 (0.20)	0.403
	Noord-Brabant	1.07 (0.92-1.25)	0.07 (0.08)	0.353
	Noord-Holland	1.07 (0.90-1.28)	0.07 (0.09)	0.451
	Overijssel	0.97 (0.85-1.11)	-0.03 (0.07)	0.706
	Utrecht	1.08 (0.89-1.31)	0.08 (0.10)	0.444
	Zuid-Holland	0.70 (0.57-0.86)	-0.36 (0.11)	**0.001**
	Zeeland	0.72 (0.48-1.07)	-0.33 (0.20)	0.103
Net return on milk [a]	10% lowest	1.20 (1.06-1.35)	0.18 (0.06)	**0.005**
	40% lower	0.98 (0.91-1.06)	-0.02 (0.04)	0.657
	40% higher	0.94 (0.87-1.02)	-0.06 (0.04)	0.126
	10% highest	0.81 (0.71-0.93)	-0.21 (0.07)	**0.002**
	Unknown	1.11 (1.00-1.24)	0.11 (0.06)	0.056
Dominant soil type for relative soil moisture ≤0.54%	Sand	(Ref)		
	Light clay	0.95 (0.60-1.51)	-0.05 (0.23)	0.835
	Peat	0.86 (0.48-1.54)	-0.15 (0.30)	0.619
	Loam	1.51 (0.57-4.04)	0.41 (0.50)	0.408
	Sandy loam	0.80 (0.57-1.12)	-0.22 (0.17)	0.194
	Heavy clay	0.90 (0.63-1.28)	-0.11 (0.18)	0.545
Dominant soil type for relative soil moisture >0.54%	Sand	(Ref)		
	Light clay	0.91 (0.71-1.15)	-0.10 (0.12)	0.419
	Peat	1.28 (1.00-1.62)	0.24 (0.12)	**0.049**
	Loam	0.42 (0.17-1.04)	-0.87 (0.47)	0.061
	Sandy loam	1.30 (1.11-1.53)	0.26 (0.08)	**0.002**
	Heavy clay	1.13 (0.94-1.35)	0.12 (0.09)	0.207
Trend over time		0.95 (0.94-0.97)	-0.05 (0.01)	**<0.001**

95% CI = 95% confidence interval, β = logistic regression coefficient, SE = Standard Error. p-values ≤ 0.05 are marked in bold. QIC (Quasi information criterion) = 20433.04. $r^2 = 0.022$, scale parameter = 1

[a] The Dutch average has been taken as the reference.

DISCUSSION

Based on the results of the bulk milk tests in the Dutch Salmonella programme, we concluded that 0.85-4.10% of the Dutch dairy herds became newly infected per test round in 2019-2021. The apparent herd-level incidence of *Salmonella* spp. in dairy herds in the Netherlands is therefore low. Our study identified several risk factors for becoming newly infected in the context of a metapopulation with a low herd-level prevalence. These included, among others, distance to an infected herd, distance to a pig herd, on farm movement from other herds in the previous year, farm size and geographical location of the herd.

The relatively low incidence observed in our study period is similar to the proportion of herds (2.6%) that seroconverted each quarter in the Danish surveillance programme for *S.* Dublin (Nielsen et al., 2007). It should be noted however, that Nielsen et al. (2007) included each and every conversion from a negative to a positive bulk milk test. In our study, we restricted our analyses to herds considered to be at risk to become newly infected after at least three test rounds in which no antibodies were detected in bulk milk. Given the imperfect sensitivity of the bulk milk ELISA, we assumed that three negative bulk milk test rounds were required to consider a herd likely to be free of prior infection. Even though the apparent herd-level incidence of *Salmonella* spp. in dairy herds is relatively low in the Netherlands, we observed large regional differences. Similar regional differences in incidence were observed in Denmark (Nielsen et al., 2007), coinciding with a large variation in the apparent herd-level prevalence between regions (Ersbøll and Ersbøll, 2010; Ersbøll and Nielsen, 2011). The regional differences that were found will probably be the result of measures that differ per region and that were not included in our study e.g. herd management, landscape and history.

In our study, several environmental factors were identified as risk factors. This coincides with the regional differences in the apparent herd-level prevalence in the Netherlands. Herds located in postal code areas with a higher proportion of surface water area had higher odds of becoming newly infected. Studies in Ireland and the USA identified frequent access to watercourses (i.e. surface water) as a risk factor for the presence of antibodies to *Salmonella* (Fossler et al., 2005; O'Doherty et al., 2014) and several case-control studies have associated (infected) surface water with *Salmonella* infections (Hooper, 1970; Vaessen et al., 1998; Lathi et al., 2010; Ågren et al., 2016). Our results on the effect of soil type and surface water area on the odds of becoming newly infected corroborate the results of previous studies in the Netherlands in the nationwide Salmonella programme (Brouwer et al., 2013). A study conducted on data from a voluntary Salmonella Control Programme in the Netherlands, in which currently approximately 40% of Dutch dairy herds participate (Santman-Berends et al., 2021), also found similar effects of soil type and surface water area (Weber et al., 2010). In these studies, relative soil moisture was not included as a factor. It has been shown that *Salmonella* spp. survive longer in loam soil compared to sand soil (Phan-Thien et al., 2020), and in soil with a higher humidity (Zibilske and Weaver, 1978).

Having an infected neighbouring herd was a significant risk factor in our study. This result is supported by previous studies (Wedderkopp et al., 2001; Nielsen et al, 2007) where a similar factor was found in the Salmonella surveillance programme in Denmark. Presence of pigs at the same location as the dairy herd, resulted in higher odds to become newly infected. In contrast, in a Dutch study on risk factors for clinical salmonellosis due to *S.* Typhimurium, a serovar infecting both cattle and pigs, the presence of pigs was not found to be a significant risk factor (Veling et al., 2002). However, in that study the introduction of pig manure did increase the odds of clinical salmonellosis (Veling et al., 2002). A case-study in Denmark showed that *Salmonella* was transmitted between cattle and pig herds by people, equipment and other physical sources (Langvad et al., 2006). This means that if a farmer has both pigs and cattle, there might be a risk of transmission of *Salmonella* spp. from pigs to cattle, especially without sufficient biosecurity between the different animal species. Whether the dairy herds in our study with on-farm pigs became newly infected due to *S.* Typhimurium, or due to another *Salmonella* serovar, could not be determined, given that the bulk milk ELISA results do not distinguish between the different serovars of *Salmonella* spp. serogroups B and D.

On farm movement from other herds in the previous year was found to be a risk factor. Other studies have showed that the purchase of cattle is associated with the introduction of *Salmonella* in the herd or with presence of antibodies in bulk milk (e.g. Vaessen et al., 1998; van Schaik et al., 2002; Nielsen et al., 2007; Brouwer et al., 2013; Perry et al., 2023). Introduction of *Salmonella* spp. in the herd can be due to the introduction of an infected animal. In addition, naïve cattle newly introduced in an infected herd may become infected and infectious, subsequently resulting in an increase of the spread of infection in the herd. However, given that we classified herds as newly infected after at least three negative test rounds, on farm movement from other herds is a more likely explanation of the observed effect of cattle introduction. In the nationwide Dutch Salmonella programme, post movement testing is not obligatory and on farm movement from other herds does not directly lead to a change of level in the programme. A Dutch voluntary Salmonella Control Programme aims to enable low-risk trade of cattle, to alert farmers to an infection with *Salmonella* spp. in their herd, and prevent human exposure to *Salmonella* spp. (Weber et al., 2010). In this programme, all cattle that have been introduced from herds without the preferred herd status (in this case ‘Salmonella unsuspected’) are required to be tested by serology within eight weeks of introduction.

The larger the herd, the higher the odds of becoming newly infected. This result has also been found by other studies (Vaessen et al., 1998; Nielsen et al., 2007; Weber et al., 2010). Herd size might be a confounder of herd management, just as net return on milk and growth in herd size. Other studies have found management as risk factors for the presence of antibodies to *Salmonella* spp., including grazing, manure disposal on land where cattle graze, shared equipment with other herds and poor biosecurity for visitors (e.g. van Schaik et al., 2002; Veling et al., 2002; Perry et al., 2023). These management factors were not investigated in our study, since data on these factors are not automatically collected and centrally managed. The seasonal pattern found in our study, indicates that there are seasonal factors at play, that we did not measure in our study. Seasonality in the incidence of salmonella infections and clinical salmonellosis in cattle has been observed previously (e.g. Nielsen and Dohoo, 2012; Webb et al., 2017; Nickodem et al., 2023), but the biological mechanisms behind these observations remain to be elucidated. Likavec et al. (2016) hypothesized that season is a proxy measure of potential risks such as meteorological factors and change in diet and farm management practices.

A limitation of our study is that it focused on dairy herds, since non-dairy herds do not participate in the Dutch Salmonella programme. Non-dairy herds can be a potential source of infection to dairy herds. However, previous studies showed that the prevalence in non-dairy herds is typically lower than in dairy herds, and follows the decreasing prevalence of *Salmonella* spp. that is observed in dairy herds (Santman-Berends et al., 2021). Furthermore, in our study we classified herds as newly infected only after three test rounds where no antibodies against *Salmonella* spp. were detected. We assumed that antibodies found in bulk milk after only one or two negative test rounds, may have been due to a pre-existing infection in the herd. Some of these infections may have in fact been new infections and such herds may have been misclassified as persistently infected. Another limitation of this study is that in the Dutch Salmonella programme, no distinction is made between the different serovars of *Salmonella* spp. serogroups B and D. This means that two neighbouring infected herds, may in fact have been infected with different serovars, meaning that the infections are unrelated. However, despite this limitation, a strong effect of infections in neighbouring herds was observed in this study, presumably reflecting the effect of local spread of infection as well as the effects of unmeasured common risk factors between neighbouring herds.

CONCLUSIONS

The results of this study indicate that, in addition to on farm movement of infected cattle, local transmission plays an important role in the between-herd transmission of *Salmonella* spp. Factors that facilitate survival of *Salmonella* spp. in the environment such as soil type and humidity seem to be of importance, and may even become more important in the future due to the changing climate and water management. Identification of risk factors for becoming newly infected with *Salmonella* spp. based on regularly collected data, can improve the surveillance and control of *Salmonella* spp. both in the Netherlands and elsewhere. With the identified risk factors, potential risk areas can be identified and targeted surveillance can be implemented to further reduce transmission of *Salmonella* spp. in dairy herds.

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QUANTIFYING HUMAN TOXOPLASMOSIS RISK FROM PORK CONSUMPTION IN DENMARK: A CONCEPTUAL FRAMEWORK FOR TISSUE CYST-BASED ANALYSIS

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SUMMARY

A quantitative risk assessment model was developed to provide a biologically representative approach to quantify the risk of human toxoplasmosis by using tissue cysts (clusters of infectious bradyzoites) as exposure units, rather than individual bradyzoites. The model with the most recent data on salting and heating, estimated the cumulative prevalence of *T. gondii* infection in the Danish population to be in the range of 7-15%, depending on the underlying model assumptions. Human infections occurred from consumption of undercooked pork, dry-cured sausages, and smoked pork. Scenario analyses demonstrated that the model was sensitive to both data on salting and to the model assumptions. Moreover, the risk of infection attributed to consumption of dry-cured sausages was highly uncertain and required careful interpretation. Supported by sensitivity analyses, Beta (1, 2) appears to be the most appropriate prior, as it best represented the prior beliefs on tissue cyst survival probability applied in the model.

INTRODUCTION

Toxoplasma gondii, a protozoan parasite, can infect all warm-blooded animals, including livestock (Tenter et al., 2000). Humans may become infected with *T. gondii* through many different sources including consumption of infected meat. In pregnant women, about 33-63% of the infections have been attributed to undercooked and processed products (Cook et al., 2000). Most human infections are asymptomatic; however, some immunodeficient individuals may develop severe health problems. Dormant infection in humans may persist for life and potentially reactivate in immunosuppressed individuals, causing visual problems (ocular toxoplasmosis) and sometimes even result in death in persons infected with HIV due to encephalitis. Acute infection in pregnant women may cause abortions, stillbirths, or in the transmission of infection to the fetus resulting in congenital toxoplasmosis (CT). Some infants with CT may show signs and symptoms at birth, while others may develop signs and symptoms such as hearing loss, cognitive impairment, or serious eye infections, later in life (Tenter et al., 2000). The incidence of CT in Denmark is low (3 cases in 10,000 livebirths) compared to some western European countries for example the Netherlands (20 cases in 10,000 livebirths) (Nissen et al., 2017). In Denmark, the annual incidence of ocular toxoplasmosis, a proxy for infections mostly acquired postnatally, was also low (0.09 cases per 100,000 inhabitants, in 2019)

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(Marstrand et al., 2021). Despite the low incidence of human toxoplasmosis in Denmark, the disease leads to serious life-long illness; its risk of transmission should therefore be kept low (Tenter et al., 2000).

Like many other livestock animals, pigs can become infected and carry tissue cysts in their meat (Dubey et al., 1986). Tissue cysts remain infectious in pork that is raw or undercooked, and in processed products that are not sufficiently heat-treated, or cured without adequate salting i.e., level of Sodium Chloride concentration (NaCl%) for a sufficient duration. Hence, there is a risk of *T. gondii* transmission from consuming pork. However, in case of certain processed products such as ready-to-eat (RTE) dry-cured sausages that are not heat-treated prior to consumption, the curing process during the production stages of these products involves practices that may potentially inactivate *T. gondii* cysts. This is supported by evidence from recent research studies conducted in the United States that used industry standard concentration of salt and demonstrated inactivation of *T. gondii* cysts at low concentrations (1.3% NaCl) within 4-6hrs into the fermentation process (Hill et al., 2018; Fredericks et al., 2019). This finding is significant in the context of *T. gondii* in RTE dry cured sausages, as a few risk factor studies on human population have categorized these products as high-risk products (Cook et al., 2000; Jones et al., 2009).

Researchers have developed Quantitative Microbial Risk Assessment (QMRA) models to estimate the risk of transmission from high-risk pork products. Conventional QMRA models for *T. gondii* use bradyzoites, as exposure unit, further assuming uniform distribution across infectious portions of meat (Opsteegh et al., 2011; Deng et al., 2020). However, biologically, bradyzoites are clustered inside tissue cysts and this has led some researchers to believe that this approach may lead to an overestimation of the infection risk (Crotta et al., 2017). Furthermore, the recent QMRA models have not assessed the impact of using the most recent data on salting concentrations in attributing dry-cured products as a source of human *T. gondii* infections. Hence, the objectives of this study were to develop a model based on tissue-cysts using updated salting concentrations and to apply this model to identify high-risk pork products and estimate the *T. gondii* risk of infection in the Danish population.

MATERIALS AND METHODS

Model framework

To assess the risk of consuming an infected 100g portion of pork in Denmark, a "farm to fork" QMRA model was developed. As a first step in the model, the true prevalence of *T. gondii* infections in finishers was estimated using Bayesian Monte Carlo methods applied to observed data in order to estimate the number of infectious 100g portions. The data were derived from a recent seroprevalence study on Danish finisher pigs, where the true prevalence in conventional and organic finishers was found to be 1.6% (95% PI: 0.1-4.6%) and 6.3% (95% PI: 0.3-14.2%), respectively (Olsen et al., 2020). Thereafter, number of tissue cysts in an infectious portion was quantified using bioassay data on mice from an experimental study (Dubey et al., 1986). Data from this study consisting of five commercial meat cuts from naturally infected pigs fed to mice were used to determine the probability of a portion from an infectious pig being infectious using a Beta distribution as a conjugate prior. Next, Monte Carlo simulation was used to estimate the number of tissue cysts in a random portion from an infected pig, taking into account the prevalence of tissue cysts in different meat cuts and the weight of the different meat cuts. In the simulation involving a million portions, a Beta distribution was used to model the prevalence of infectious meat cuts, and a Poisson distribution was used to model the number of tissue cysts in each infectious meat cut. Finally, the number of tissue cysts in a random portion from an infected pig was estimated by sampling from the Poisson distributions for each meat cut, weighted by the weight of the meat cut, and averaging the number of tissue cysts sampled from all the meat cuts.

In the model, the simulated pork portions in the above step were assumed to represent the products present in the consumption data sourced from the Danish National Survey of Diet and Physical Activity (DANSDA) (Pedersen et al., 2015). All the pork products in the DANSDA survey were broadly categorized into five product-types, based on the information on how they were produced and handled prior to consumption. Hence, the pork portions in the model comprised of the following: (i) fresh pork portions that were raw at the time of purchase but cooked prior to consumption, (ii) cold cuts which included sufficiently heat-treated products (≥ 75°C), including those that were pre-boiled products such as hotdogs and similar products, (iii) dry-cured sausages that were cured salted but not heat-treated during processing or before consumption (iv) smoked products that were salted and heated to a maximum of 56°C during processing and (v) bacon products that were salted and thoroughly cooked.

Following the estimation of number of tissue cysts in infectious portions in the five products, the model accounted for the reduction in tissue cysts in these portions during various product preparation stages such as heating and salting, as well as freezing based on the bioassay data on mice from various experimental studies (Dubey et al., 1990; Kotula et al., 1991; Dubey et al., 1997). For this part of the model, we hypothesized that the treatments reduced the tissue cyst dose in an infectious portion by a certain factor, which was derived mathematically, assuming the reduction factor to be the same in the different product categories. Therefore, the model estimated the number of infectious portions that remained after treatment i.e., at preparation and before consumption.

In the final step, a dose-response model was developed to predict human infection from consuming a portion with at least one tissue cyst. The model's "single hit parameter" (value = 0.46) i.e., the dose-response parameter, which is the probability that a single cyst results in infection upon ingestion, was adapted from bioassay experiments on mice using oocysts (Marinović et al., 2019). This substitution was made based on the authors' judgement, as the single hit parameter value for tissue cyst was unavailable, and since oocysts, like tissue cysts, are individual infectious units, these were considered relevant as single exposure units. In this step of the model, for the million simulated portions, the total infection probability (P_i) of each portion was determined. For this, the probability of a human being infected was multiplied with the prevalence of infected animals, where the latter represented the probability that the portion originated from an infected animal. Upon contracting *T. gondii*, individuals are assumed to be infected for life (Tenter et al., 2000). Therefore, the "waiting time until first infection" was utilized to describe the human infection probability. Using Monte Carlo samples of the previously determined probabilities (P_i), waiting time distributions were created for multiple individuals. The DANSDA consumption data on pork which included information on 3,946 individuals between ages 4 to 75 was used to determine how often, over a year, a person of a specific age was likely to consume pork (Pedersen et al., 2015). Additionally, the probability that each consumed pork portion results in an infection was determined using a Bernoulli distribution, linking back to the P_i values derived in the previous step. Through simulations, the age at which each individual first became infected was tracked. If an individual did not become infected during their simulated lifespan (capped at 80 years), a placeholder value was used to signify the absence of infection. This process was repeated multiple times, to derive a "age of first infection" values in 2,000 simulated individuals.

Scenario analyses

In the scenario analyses, the modelling step for salting treatment was modified. In scenario I, a previously published logistic mixed effects regression model Eq.(1) was fitted directly to the model using an inverse logistic equation to estimate the reduction in tissue cysts (Deng et al., 2020).

$$\frac{1}{1 + e^{-(11.4 - 2.6 \times \mathrm{NaCl\%} - 0.62 \times \mathrm{T} - 0.016 \times \mathrm{D} - 0.068\, (\mathrm{T} \times \mathrm{D}))}} \tag{1}$$

where, NaCl% = Concentration of Sodium Chloride (range = 2-6%), T = Temperature = 21℃, D = Duration = 21 days

In an alternate scenario II, the existing data on NaCl%, temperature and duration in the salting part of the model was fitted with some additional data on NaCl% (range = 1.3-2.8%), temperature (23.4C°) and duration (range = 0.13-0.63 days) from recent mice bioassay studies, which demonstrated killing of tissue cysts under low salting conditions (Fredericks et al., 2019, Hill et al., 2018).

Sensitivity analyses

In the QMRA model, the probability of a portion being infected in the following steps i.e., originating from an infected pig, and after the application of salting and heating treatments was determined using a Beta distribution. In the baseline model, a Jeffreys prior, Beta (0.5, 0.5) was applied at an average hit probability of 0.46. However, in the sensitivity analyses the effect of applying the weakly informative Beta (1,1) and Beta (1,2) priors was also explored. The effects of these two priors were also investigated for scenarios I and II.

Furthermore, in the sensitivity analyses, the uncertainty in the single hit probability parameter on the predicted cumulative prevalence in humans was evaluated by selecting a lower (0.30) and a higher value (.60) in the baseline model and the two scenarios while also varying the Beta priors.

RESULTS

In the baseline scenario model, the cumulative prevalence of *T. gondii* infection was estimated to reach 14.3% in the Danish human population at the age of 75 years. Additionally, the model predicted the risk of *T. gondii* infection to occur from consumption of undercooked portions of pork, dry-cured sausages, and smoked pork products (Fig.1).

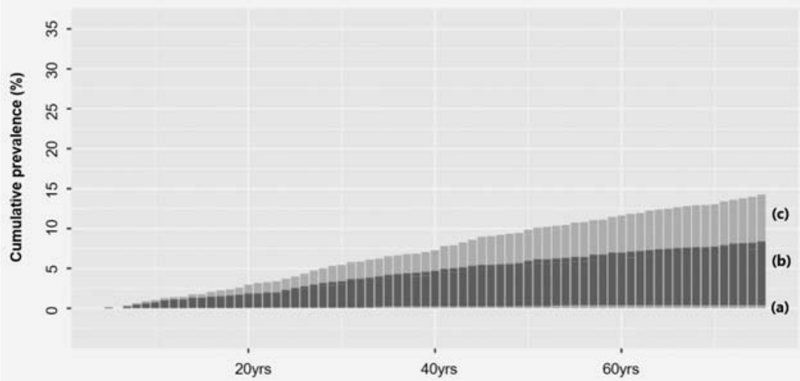

Figure 1. Cumulative prevalence of *T. gondii* infection in humans by product type. A = smoked pork products, b = dry-cured pork sausages, c = undercooked pork) and age (in years) as predicted by the baseline model at an average hit probability of 0.46 using a Beta (0.5, 0.5) prior

Scenario and sensitivity analyses

The results of scenario analyses showed the predicted cumulative prevalence of *T. gondii* to be highest in the baseline scenarios, closely followed by scenario I, and was lowest in scenario II, as shown in Fig.2.

In the sensitivity analyses considering all combinations of Beta priors and hit probabilities, the predicted cumulative prevalence was found to be lowest with the Beta (0.5, 0.5) prior, followed by Beta (1, 1), and Beta (1, 2). However, an exception occurred in scenario II with a hit probability of 0.30, as the prevalence using the Beta (1, 1) was marginally higher at 10.9%, compared to 10.4% with the Beta (1, 2) (Fig.2). Additionally, the values for the predicted cumulative prevalences were comparable between Beta (1, 1) and Beta (1, 2) priors across the scenarios. The predicted cumulative prevalence values at a hit probability value of 0.46 across varying Beta priors are shown in Table 1.

Table 1. Predicted prevalence of *T. gondii* infection in humans with a hit probability of 0.46, based on different underlying Beta priors under different salting scenarios

Scenarios	Beta (0.5, 0.5)	Beta (1, 1)	Beta (1, 2)
Baseline	14.3%	21.2%	22.7%
I	14.2%	15.1%	15.9%
II	6.7%	10.8%	11.5%

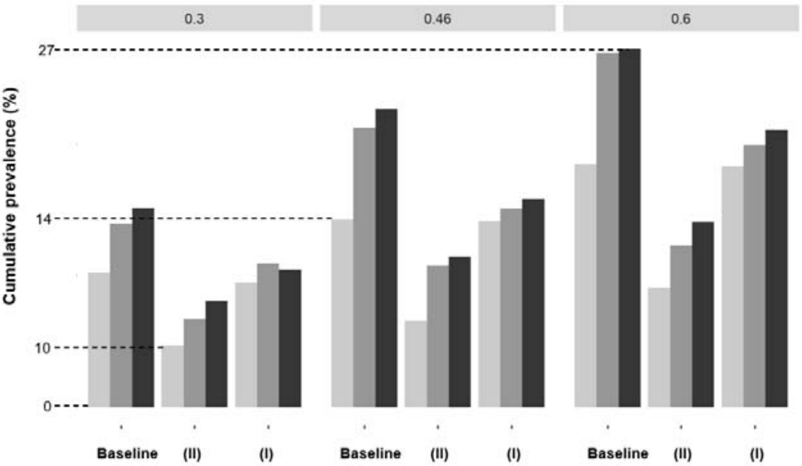

Figure 2. Sensitivity analyses outcomes for predicting *T. gondii* cumulative prevalence with variable Beta priors and hit probabilities (0.3, 0.46, and 0.6) in Baseline, Scenario I, and II models. Vertical bars correspond to Beta prior distributions, arranged from left to right as follows: light grey = Beta (0.5, 0.5), mild grey = Beta (1, 1), and dark grey = Beta (1, 2)

In the model, as the hit probability value varied from a baseline average value of 0.46 to lower (0.30) and higher (0.60) values, the predicted cumulative prevalence correspondingly decreased and increased, respectively (Fig.2).

DISCUSSION

In this study, a conceptual “farm to fork” framework for estimating the risk of *T. gondii* infection in humans from consumption of tissue cysts has been presented. The conventional QMRA model originally developed by Dutch researchers is commonly applied in risk assessments on *T. gondii* (Opsteegh et al., 2011; Deng et al., 2020). Some researchers have argued that the Dutch QMRA model based on bradyzoites, overestimates the risk of *T. gondii* infection in humans. Hence, in response to this, a recent study proposed a mathematical framework for developing a QMRA using tissue cysts. However, the model was only developed to estimate the probability of a 100g portion of fresh pork to contain a viable tissue cyst (Crotta et al., 2017). Hence, the model presented in the present study is conceptually distinct from the existing published QMRA models, making the results not directly comparable to those of previous studies. However, in line with the recent results published from the Dutch QMRA model utilizing more industry-standard data on salting, this study also demonstrates that the QMRA model is sensitive to the underlying salting data (Deng et al., 2020). However, in comparison, the present study has additionally applied some more recent data on the salting parameters (NaCl%, temperature and duration) from recent bioassay experiments on mice. Additionally, the QMRA model in this study only considers values for the salting parameters that are considered industry standard for the preparation of mildly cured products used in the model from the DANSDA consumption data.

Like in the previously published studies, the model identifies portions of undercooked pork, dry-cured sausages, and smoked products as high-risk products (Opsteegh et al., 2011; Condoleo et al., 2018; Deng et al., 2020). However, these results are in contrast to some recent experimental results obtained from bioassay tests in mice (Fredericks et al., 2019, Hill et al., 2018). In these studies, mice fed with portions of dry-cured sausages, during various stages of fermentation process, did not become infected from dry-cured sausages prepared at low salt concentration (NaCl% = 1.3%) and short duration (within 4-6 hrs). Hence, these recent results suggest, dry-cured sausages may be safe for consumption. In the model scenario II, when these recent experimental data were added to existing data in the baseline model, the predicted cumulative prevalence was approximately twice as low. Moreover, the risk from consumption of dry-cured sausages was approximately four times lower than in the baseline model (results not shown). The data available from recent two published studies on dry-cured sausages suggest that these products may be free of *T. gondii* tissue cysts. However, these data also together with the previously published data on salting are not sufficient to estimate the risk posed from these products to be negligible. Nevertheless, it is to be noted that the data for the model parameters such as salt concentrations and exposure temperatures, which influence the efficacy of salt in reducing cyst viability, were uniformly applied to the five product types. Hence this assumption may have led to an overestimation of the risk associated with dry-cured sausages. Therefore, the risk from dry-cured sausages should be interpreted with caution, especially when these products are prepared using high concentrations of salt.

In the QMRA model, Beta priors were employed to represent prior beliefs about the probability of tissue cyst survival following different treatments (heating and salting). In the baseline model scenario, a Beta (0.5, 0.5) (Jeffreys) prior which favours more extreme probabilities (closer to 0 or 1) over probabilities closer to 0.5, was applied to the existing data on tissue cyst survival probabilities. Additionally, in the sensitivity analyses, a Beta (1, 1) prior, indicating equal probabilities between 0 and 1, was applied along with a weakly informative Beta (1, 2) prior, which introduced a bias towards lower probabilities. Despite being frequently assumed to be a non-informative prior, the Beta (0.5, 0.5) distribution can produce posteriors with narrower confidence intervals than the Beta (1, 1) distribution when used as a prior for data with rare outcomes due to the high prior weight given to probabilities close to zero. Whereas, the Beta (1, 2) prior is deliberately designed to reflect the prior belief of lower survival probabilities. Hence, theoretically, the Beta (0.5, 0.5) and Beta (1, 2) priors may converge on similar prevalence estimates if the underlying data robustly supports low survival rates. However, the observed differences in the prevalence estimates indicate that the data may not be sufficiently strong to counteract the influence of the prior. Hence, we prefer to use the Beta (1, 2) prior because it explicitly weights the model towards lower survival probabilities based on prior knowledge, which is consistent with empirical data that indicates a tendency towards lower tissue cyst survival following treatment. This suggests a decreased likelihood of consuming infectious portions, leading to fewer infections. Moreover, the similarity in prevalence estimates under the Beta (1, 1) and Beta (1, 2) priors suggests that the model inference is more robust between these priors, which further underlines the utility of a Beta (1, 2) prior for scenarios where the underlying probability is expected to be low but not so low as to be considered negligible.

In conclusion, this study introduces a farm-to-fork conceptual QMRA model that aligns with the biology of the *T. gondii* parasite. The model predicts the risk of *T. gondii* infection from consuming undercooked portions, dry-cured sausages, and smoked pork products. Implementing scenarios on salting revealed that the model is sensitive to salting parameters. Therefore, the underlying data on salting for various products in the QMRA models should always adhere to industry standards. Additionally, while products such as dry-cured sausages may be considered safe for consumption, more data is needed to demonstrate their negligible impact on infection risk.

ACKNOWLEDGEMENTS

This work is a part of the Industrial PhD funded by the innovation Fund Denmark (7038-00129B) and the SEGES pig research centre.

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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

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

EXECUTIVE COMMITTEE 2023-2024

D. Brodbelt (President), P.A. Robinson (Senior Vice-President), T. Vergne (Junior Vice-President), F. Dórea (Honorary Secretary), E. Fischer (Honorary Treasurer)

Honorary Auditors: K. Howe & K. Verheyen

LIFE MEMBERS

J.M. Booth (deceased), M.J. Clarkson, J.D. Collins (deceased), P. Cripps, G. Davies, J.T. Done (deceased), R.G. Eddy, P.R. Ellis, E.A. Goodall, G. Gettinby (deceased), L.E. Green, K.S. Howe, M.E. Hugh-Jones, W. Martin, D. Mellor, 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**
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 ....

....

....

....

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