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AFRICAN SWINE FEVER

THE ROLE OF VEHICLE MOVEMENTS AND WILD BOARS IN THE SPREAD OF AFRICAN SWINE FEVER VIRUS IN THE 2019 KOREAN EPIDEMIC

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SUMMARY

Although African swine fever (ASF) is a major concern for global food security, the understanding of the main drivers of ASF virus (ASFV) transmission is limited due to the lack of epidemiological data in most affected areas. By combining data generated by ASF surveillance on pig farms and in wild boars and nationwide GPS-tracked data for vehicles associated with pig production and health, this study assessed the role of vehicle movements and wild boar populations in the spread of ASFV to pig farms in Korea. Movements of vehicles from infected premises were associated with a higher probability of ASFV incursion into a farm than proximity to ASFV-infected wild boar populations. While ASFV could spill-over into farms from infected wild boars, vehicles played a significant role in spreading infection between farms, despite rapid on-farm detection and culling, highlighting the need for interventions targeting both farm-to-farm and wildlife-to-farm interfaces.

INTRODUCTION

African swine fever (ASF) is causing the worst pandemic ever having affected livestock populations, due to its impact on the global pork and food systems as well as swine health and welfare. In the absence of vaccines and treatments, ASF control heavily relies on on-farm biosecurity, early detection and containment of infected premises. It is therefore essential to identify, and target, the main ASFV transmission routes. However, only a few studies have aimed to assess the contribution of different transmission routes in ASF epidemic areas (Vergne et al., 2016; Iglesias et al., 2017; Vergne et al., 2017; Ma et al., 2020), likely due to a lack of detailed epidemiological data. While those studies have improved the understanding of risk factors for ASFV infection, their findings are limited by possible biases in farm and wild boar case data due to underreporting of outbreaks, and the absence of information about contact patterns between farms.

After a viral introduction, potentially infectious contacts between farms may significantly contribute to viral dissemination (Craft, 2015). Notably, vehicle movements for animals, people, feed, or medical supplies may create conditions for large epidemics amongst livestock farms (Green et al., 2006; Fournie et al., 2013; Kim et al., 2018). However, the role of vehicle

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movements in shaping ASF epidemics has not been assessed, despite their likely epidemiological importance.

In 2019, the Republic of Korea experienced its first ASF outbreak in both domestic pigs and wild boars in the northern-most part of the country. ASFV infection was confirmed in 14 pig farms (infected premises, IPs) (Yoon et al., 2020), from Sep 17 to Oct 9, and in 26 wild boars from Oct 3 to Nov 20. By combining ASF case data and vehicle movement data generated by a nationwide GPS tracking system, this study assessed the contribution of vehicle movements and wild boars to ASFV spread to pig farms during the 2019 Korean epidemic.

MATERIALS AND METHODS

Data

The Animal Plant and Quarantine Agency (APQA) provided the domestic pig farm registry and farm case data (i.e. farm GPS coordinates, dates of reporting, detection and culling).

GPS-tracked vehicle movement data were collated from the Korean Animal Health Information System (KAHIS). By law, vehicles used for livestock farming activities, such as private and governmental veterinary services, feed, manure, livestock transport, are required to be installed with a GPS device. It transmits a signal to KAHIS when it is located on the site of a farm registered in the government database. All movements made by vehicles that entered IPs between Aug 28 (i.e. 20 days before the first IP report) and Oct 16 (i.e. a week after the last IP report) 2019 were considered in this study, based on the following assumptions. First, given the low number of symptomatic pigs in IPs at the time of reporting and estimated incubation period in pigs (4–13 days) (Guinat et al., 2014), it was assumed that the length of time from infection of a herd to reporting was fewer than 20 days. Additionally, considering that vehicles were required by law to be disinfected before entering farms, and at the entrance to and exit from a city, town, or village, it was assumed that ASFV-contaminated vehicles were infectious fewer than 1 week.

Wild boar case data were provided by the Ministry of Environment. Since the first report of ASFV infection in domestic pigs, surveillance efforts in wild boars were progressively increased by incentivizing financially wild boar hunting, trapping and carcass reporting, and by testing all wild boars caught or found dead for ASFV, across the country.

Spatial clustering in wild boar cases

This study assessed whether wild boar cases were spatially clustered using an elliptic version of the spatial scan statistic in SatScan software (Kulldorff M, 2009).

Bayesian modelling approach

A model of ASFV transmission was fitted to the farm case data to test the hypothesis that exposure to vehicle movements from IPs and ASFV-infected wild boars were the main sources of infection for domestic pig farms. A vehicle was considered as 'potentially contaminating' if it entered farm i within d days after having visited farm j when farm j was infectious. For a given farm on a given day, the overall force of infection was modelled as the sum of (i) the risk of infection resulting from visits by potentially contaminating vehicles (P_V), (ii) the risk resulting from exposure to wild boars in the spatial clusters of ASFV-positive wild boars (P_W),

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and (iii) two levels of background risk: (i) across the country (P_{B_1}) or (ii) in the affected municipalities (P_{B_2}).

The force of infection for farm i on day d, $F_{i,d}$, was then modelled as Eq.(1):

$$F_{i,d} = P_V n_{i,d}^V + P_W S_i^W + P_{B_1} + P_{B_2} A_i \qquad (1)$$

where $n_{i,d}^V$ was the number of potentially contaminating vehicle movements farm i received on day d, S_i^W (1 if farm i was located in the spatial cluster, 0 otherwise) and A_i (1 if farm i was located in the affected municipalities, 0 otherwise) were indicator variables.

Since infection dates were not observed for IPs, infection dates (I_i), and therefore the time between infection and reporting (D_i), were updated in each iteration by using a data augmentation technique, which has been successfully adopted to infer transmission dynamics from incomplete epidemic data (Walker et al., 2010; Cauchemez et al., 2011; Chapman et al., 2018). With $F_{i,d}$, I_i, and D_i, the likelihood of the model given the data was expressed as Eq.(2):

$$L = \prod_i e^{\sum_{d=1}^{I_i-1} -F_{i,d}}(1 - e^{-F_{i,I_i}})f(D_i; \alpha, \beta) \prod_j e^{\sum_{d=1}^{d_{last_j}} -F_{j,d}} \qquad (2)$$

The first term represented the probability that IP i avoided the infection until a day before its infection date I_i, and became infected on day I_i, and the probability density function of the duration between infection and reporting in farms, expressed as a Gamma distribution, $f(D_i; \alpha, \beta)$, with α and β as mean and variance. The last term was the probability that non-IP j did not become infected during the study period. Some farms were depopulated through culling or government purchase during the study period, and therefore no longer at risk of infection. Thus, for those farms, d_{last_j} was defined as the date at which it was depopulated. For farms that were not subject to those preventive measures, d_{last_j} was the last date of the study period.

Parameters were estimated using a two-stage Metropolis-Hastings Markov chain Monte Carlo (MCMC) algorithm and a data augmentation technique (Walker et al., 2010; Cauchemez et al., 2011; Chapman et al., 2018). Models with variable combinations of the above risk variables were compared based on the deviance information criterion (DIC).

RESULTS

Description of the epidemic

The study population included all 6,340 registered domestic pig farms. APQA confirmed ASFV infection in 14 domestic pig farms, located in four contiguous municipalities, including Ganghwa island (n=5/35, 14.3%), Gimpo (n=2/20, 10.0%), Paju (n=5/93, 5.4%), and Yeoncheon (n=2/80, 2.5%) (Yoon et al., 2020).

Since July 25, swill for pigs on Korean farms had to be heat-treated in facilities approved by the government. Then, following the detection of the first IP, swill feeding was completely banned across the country. Transport of pig manure from affected municipalities was also banned at the same time. Over the course of the epidemic, six 48-hour standstill periods (i.e. a ban on movements of livestock, people, vehicles, and supplies to farms and slaughterhouses)

were enforced across the country, or in affected municipalities (Fig. 1). Nationwide active surveillance was also conducted on registered farms by screening pigs for clinical signs indicative of ASF (e.g. fever, loss of appetite, or sudden death) and testing suspected cases. IPs, herds located within a radius of 3 km around IPs, and herds with an epidemiological link to IPs (e.g. same ownership) were culled within three days following confirmation. As the epidemic developed, the government further culled or purchased remaining herds in affected municipalities as a preventive measure. By Oct 16, 2019 (i.e. one week after the last IP report), 143 (62.7%) out of 228 herds in affected municipalities had been depopulated.
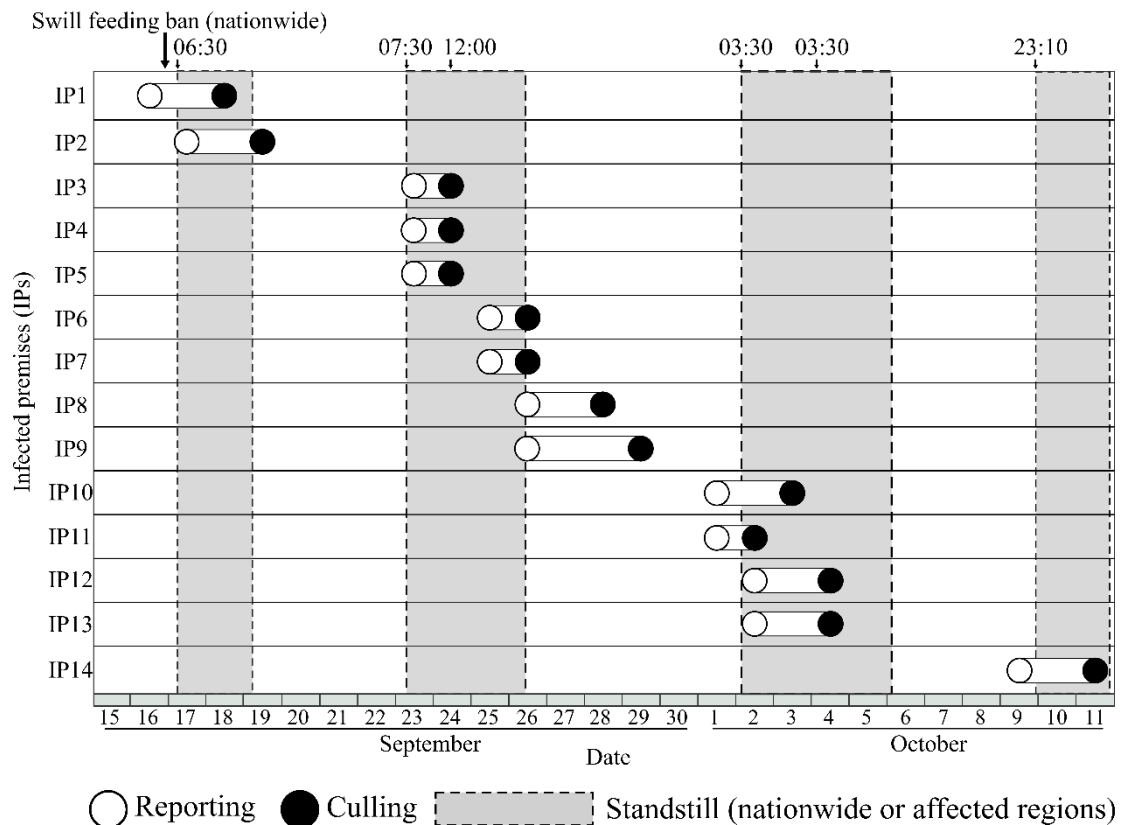
Figure 1. The timeline of the reporting and culling of infected premises (IPs), and control measures.

Eleven IPs were detected through the report by farmers of clinical signs indicative of ASF, and three through active surveillance. At the time of reporting, no more than five pigs showed clinical signs indicative of ASF. All IPs were registered, except for one backyard farm. Most IPs (n=10, 71.4%) raised more than 1,000 pigs. Ten IPs were breeding-fattening farms, two were breeding farms, and another two were fattening farms. In nine IPs, clinical signs were observed in sows, which tested positive for ASFV. The distance between any two IPs ranged from 3.3 to 83.9km, with a median of 28.5 km.

Vehicle movement patterns

During the study period, 208 vehicles visited IPs, and made 12,671 visits to 832 farms (IPs or non-IPs). When only considering movements made by a vehicle within three days following the visit of an IP, 156 vehicles made 2,824 farm visits, with a median of 5.5 farm visits per vehicle (interquartile range: 2–16). Of those farm visits, 255 (9.0%) involved other IPs, and 2,569 (91.0%) involved 360 non-IPs (5.7% of farms in the country). Amongst movements

between IPs, 96 (37.6%) movements started from a farm within the 20 days preceding the report of a suspected infection, and reached, within three days, another farm before it reported a suspected infection. All these movements occurred amongst five IPs within Ganghwa island (63, 65.6%), or amongst six IPs outside the island (33, 34.4%).

Spatial clustering of ASF-positive wild boars

Between Sep 21 and Nov 20, 1,292 wild boars were tested across the country, and all positive wild boars were in Paju (n=6/57, 10.5%), Yeoncheon (n=8/130, 6.2%), or Cheorwon (n=12/398, 3.0%) (Fig. 2). Spatial point pattern analysis identified two clusters of ASFV-positive wild boars. Wild boars caught or found dead within Clusters 1 (10.7km^2) and 2 (1,209.4km^2) were 21.8 and 37.2 times as likely to be infected with ASFV than those outside these clusters, respectively (all p-values <0.001). While there was no pig farm in Cluster 1, six IPs and 112 non-IPs were in Cluster 2 (Fig. 2). For all IPs, the distance between an IP and the nearest ASFV-positive wild boar ranged from 1.3 to 37.0km.
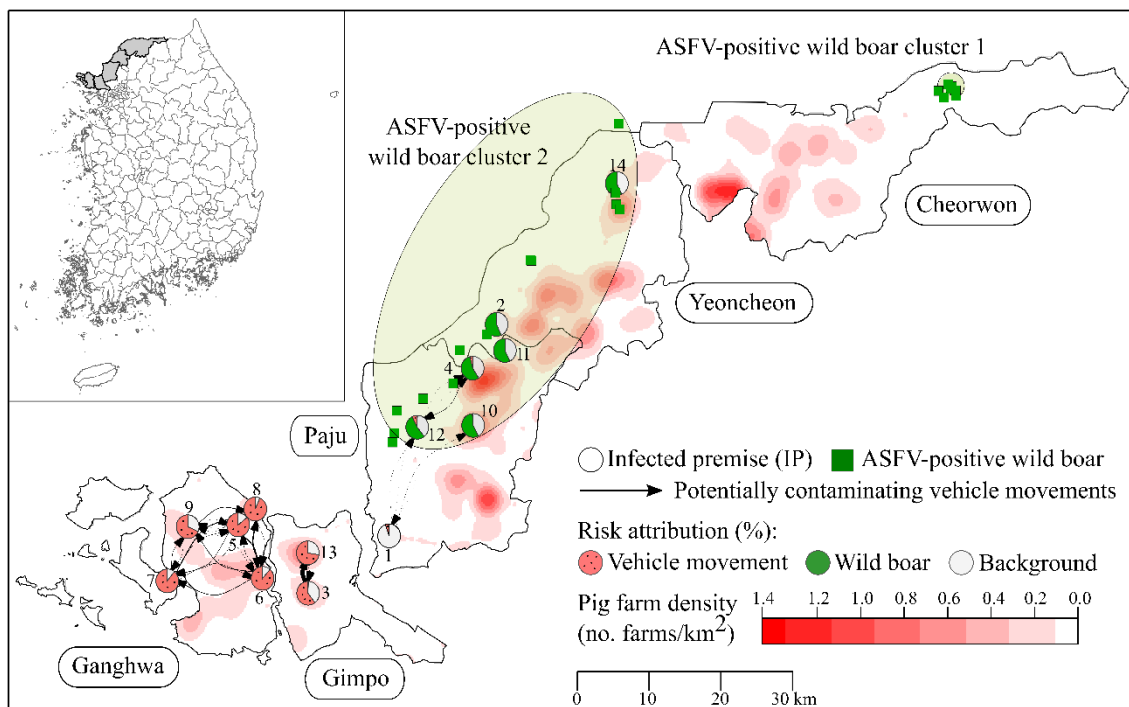
Figure 2. The spatial distribution of IPs, non-IPs, ASFV-positive wild boars, and potentially contaminating vehicle movements between IPs. The duration of vehicle infectiousness was set here to three days. Circles represent IPs, with their numbers representing the order of reporting dates. The pie charts show the estimated contribution of different transmission routes to the infection of each IP. Edge width is proportional to the number of potentially contaminating vehicle movements between IPs, weighted by the probability that an exit IP was infectious at the time of the vehicle departure. Edge arrows represent the direction of vehicle movements. Pig farm density is shown in shades of red. Squares represent the location of ASFV-positive wild boars, with ellipses representing their spatial cluster.

<u>The model</u>

The results presented below assumed that a vehicle remained infectious for three days after having left an infected premise. Changing this parameter value to one or six days did not have a major impact on the interpretation of the results.

When compared using the DIC, the model accounting for both vehicle movements and exposure to wild boars in the spatial cluster was preferred over models accounting for only one, or none, of those sources of infection (Table 1). Indeed, exposure to those factors substantially increased the risk of farms becoming infected. The daily probability of infection of a farm increased 11.1-fold (95% highest density interval (HDI): 1.1–39.3) following the visit of a potentially contaminating vehicle, compared to a farm which was not visited by such a vehicle. For a farm in the spatial cluster of ASFV-positive wild boars, the daily probability of becoming infected was 2.5 (95% HDI: 1.0–7.7) times as high as for a farm outside this cluster.

Table 1. Posterior parameter estimates and posterior predictive length of time between infection and reporting

Parameters	MCMC output	
	Median (95% HDI[a])	G-R[b]
Full model		
Potentially contaminating vehicle movement (P_V)	53.9 (7.4-113.4) x10^{-4}	1.00
Wild boar cluster (P_W)	8.2 (0-19.0) x10^{-4}	1.00
Background (country, P_{B_1})	0.03 (0-0.1) x10^{-4}	1.00
Background (epidemic region, P_{B_2})	5.4 (1.1-11.2) x10^{-4}	1.00
Mean of the Gamma distribution (α)	3.7 (1.0-8.8)	1.00
Variance of the Gamma distribution (β)	44.6 (5.2-113.5)	1.00
Length of time between infection and reporting (D)[c]	4.3 (1.0-15.8)	
DIC[d]		
Full model	275.8	
'Vehicle movement' model	277.8	
'Wild boar' model	285.8	
Null model	284.6	

[a] Highest density interval
[b] Gelman-Rubin convergence diagnostic
[c] The distribution was obtained by simulating values from the Gamma distribution, based on parameters α and β randomly sampled from their joint distribution.
[d] The deviance information criteria from Celeux (2006).

The contribution of different transmission routes to ASFV incursion into IPs varied with the spatial location of those farms, when estimated based on the best-fitted model. Vehicle movements were the most likely route of ASFV introduction into IPs in the southwestern epidemic region, whereas wild boars were estimated to be the main source of infection for pig farms in the northeastern epidemic region (Fig. 2). Indeed, the density of movements of potentially contaminating vehicles greatly differed between both regions. When accounting for the posterior predictive probability that an IP was already infected when a vehicle left it, the estimated number of potentially contaminating vehicle movements was 36.6 between IPs, and 891.6 from IPs to non-IPs. Of those movements between IPs, 94.3% reached IPs in the southwest (4.3 visits/IP), and 5.7% reached IPs in the northeast (0.3 visits/IP), suggesting that a dense network of potentially contaminating vehicle movements was formed among a small group of farms, although the length of time from onset of infection to reporting was 4.3 days (median, 95% HDI: 1.0–15.8) (Table 1). To prevent infected farms from spreading ASFV to

more than 1 other farm through vehicle movements, both the average number of vehicles visiting a farm in a day, and the average number of farms visited by a vehicle in a day should be limited to 1.3 (Fig. 3).
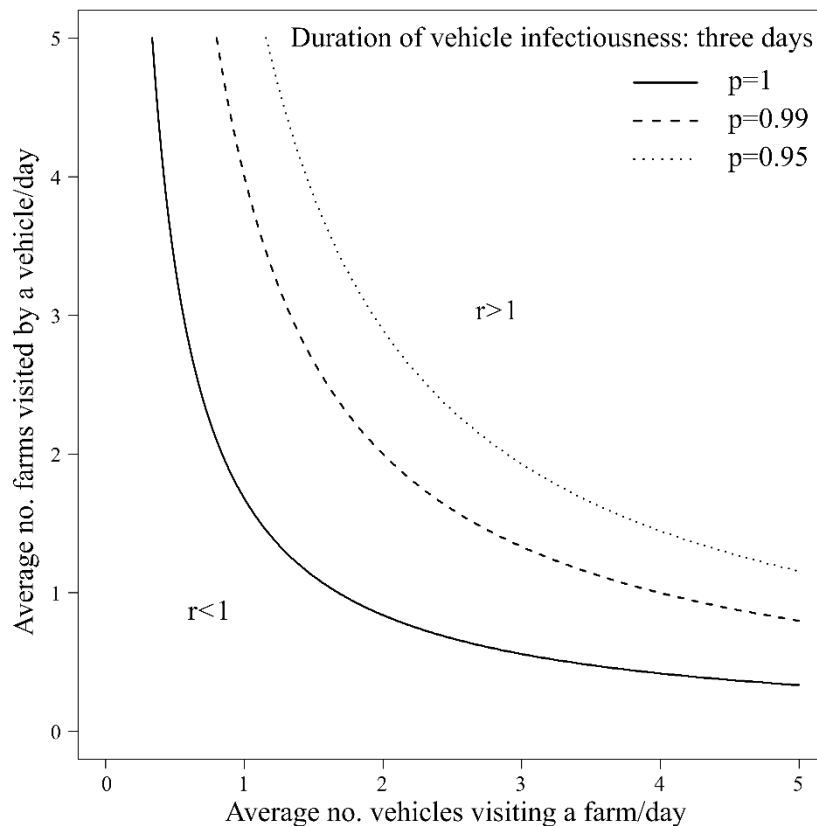
Figure 3. The expected number of secondary farm cases (r) caused by one infected farm through the movements of vehicles. r is computed as a function of the average daily number of vehicles visiting a farm (x-axis) and the average daily number of farms visited by a vehicle (y-axis). Different lines represent different thresholds for the proportion of iterations in which r was lower than one (p=1, 0.99, or 0.95). Vehicles were assumed to remain infectious for three days after leaving an infected farm.

DISCUSSION

Our study investigated the role of vehicle movements from IPs and ASFV-infected wild boars in the spread of ASFV to pig farms during the 2019 Korean epidemic. This was made possible by the availability of (i) data on vehicle movements generated by the integrated GPS tracking system for vehicles associated with pig production and health, and (ii) wild boar case data generated by enhanced ASFV surveillance in wild boars. The model accounting for the influence of both vehicle movements and exposure to wild boars better explained the epidemic pattern, suggesting that both transmission routes contributed to the spread of ASFV in the Korean pig population.

Interestingly, the model suggested that the contribution of both transmission routes varied spatially. First, the movements of contaminated vehicles were likely the main route of ASFV introduction for IPs in the southwestern epidemic region. Indeed, there were a large number of

vehicle movements between the five IPs on Ganghwa island (i.e. IPs 5–9) around one to nine days before they reported suspicion of ASFV infection. The reports from these five IPs were made within a period of three days, and only a small number of pigs showed clinical signs ($\leq$ 5). Possibly low effectiveness of vehicle disinfection measures on the island may have further promoted the contribution of vehicle movements to ASFV spread. Indeed, according to epidemiological investigations, IPs on Ganghwa island appeared to have insufficient disinfection facilities for vehicles and personnel. Moreover, vehicles moving between farms within the island were likely to bypass those bases because most vehicle disinfection bases on the island were near two bridges connected to the mainland.

However, it is unclear as to how the virus reached the southwestern epidemic region. There were no recorded potentially contaminating vehicle movements from other affected municipalities to Ganghwa island and Gimpo, even when the length of time during which a vehicle remained infectious was extended to six days. Also, no wild boar was caught or found dead, and therefore available for ASFV testing in both municipalities. While this does not exclude the possibility that ASFV circulated in the wild boar populations, it may also indicate that there were a relatively small number of wild boars, and therefore the risk of ASFV spread from wild boars to domestic pigs was very low. Alternatively, ASFV could have been introduced through vehicle movements not captured in this study. This study only accounted for vehicle movements between farms, and not movements from other types of premises (e.g. slaughterhouses), which could have acted as a source of infection.

Our model also suggested that exposure to ASFV-positive wild boars was the main source of infection for pig farms in the northeastern epidemic region. First, IPs in Paju and Yeoncheon, except for IP1, were within a cluster covering almost all of the ASFV-positive wild boars found in those municipalities. Secondly, those IPs were not or only weakly connected to other IPs through vehicle movements, contrary to IPs in the southwest. However, the transmission mode from wild boars to domestic pigs is still unclear. In all but one IP, pigs were kept indoors. Therefore, direct contacts between wild boars and domestic pigs were unlikely. Also, potential vectors (*Ornithodoros* species) have not been reported in the Republic of Korea (Chae et al., 2017; Chae et al., 2019). Although the exact mode of ASFV transmission remains still unknown, the potential for ASFV spread from infected wild boars needs to be addressed by ASF prevention and control efforts.

The background risk accounted for a substantial fraction of the force of infection exerted on several IPs. Swill feeding may not have contributed to this background risk, as it was banned since the start of the epidemic. Also, according to the outbreak investigations, it did not appear to be practiced in any of the IPs. Based on the results of regular inspections at feed factories and manure disposal plant facilities, it is reasonable to assume that the great majority of vehicles for pig production and health were registered. Nonetheless, some vehicle movement patterns not captured in this study could have contributed to disease spread. For instance, the contamination of vehicles while visiting other types of premises (e.g. slaughterhouses) was not considered. Vehicles for personal use was not considered either, although outbreak investigations did not identify any IP connections through such vehicles. Wild boars may have also contributed substantially to this background risk. While our findings strongly suggest that the prevalence of ASFV infection in wild boars was much higher within, than outside, the clusters, it cannot exclude the possibility that the virus might have also circulated, at a lower prevalence, in wild boar populations outside the spatial clusters. This might have been a plausible source of infection for some IPs, for which most of the force of infection was attributed to the background risk.

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This study had several limitations. First, our model did not consider that a farm's susceptibility to ASFV infection may differ from one another. The purpose of some vehicle visits could have involved direct contacts between people, their equipment, and pigs, thereby presenting a higher risk of infection than other visits. Additionally, farms with poor biosecurity could have been exposed to an increased risk of infection when visited by contaminated vehicles. The risk of infection from infected wild boars was likely to vary amongst farms due to different levels of on-farm biosecurity and proximity to wild boar habitats. Also, while the model identified an excess risk of infection for farms in circumscribed areas where ASFV was very likely to circulate more intensely in wild boars than anywhere else in the study area, the spatiotemporal heterogeneity in ASFV prevalence in wild boar populations may have been under-estimated.

Secondly, this study cannot completely exclude the possibility that some wild boar cases were infected by IPs. However, ASFV was likely to have been circulating in wild boars before the infection of pig farms, considering (i) the lower sensitivity of surveillance in wild boars compared to domestic animals, and (ii) surveillance efforts in wild boars progressively increased following disease detection in farms. Also, since the end of our study period (Nov 21 2019), ASFV infection has been confirmed in >700 wild boars and two pig farms (OIE., 2020), suggesting that ASFV could be sustained in wild boars, in the absence of viral circulation in domestic pigs.

In conclusion, our study suggested that vehicle movements have the potential for causing large chains of transmission between farms, while the circulation of ASFV in wild boar populations is likely to pose a continuous risk of viral spill-over into pig farms. The nationwide GPS vehicle tracking system provided a unique opportunity to study the role of vehicle movements in the viral dissemination among IPs, based on a contact network of pig farms. Although the length of time from infection to reporting was estimated to be short, and several movement standstill periods were enforced, a large number of vehicles had already visited IPs during their estimated infection period, suggesting that restrictions of vehicle movements need to be prioritized where high on-farm biosecurity standards cannot be guaranteed. Contact tracing, if available, could be used to target those restrictions and active surveillance to premises which have been in contact with IPs. Likewise, the maintenance of high on-farm biosecurity standards, and the effectiveness of disinfection of vehicles visiting farms should also be ensured, especially in areas where ASFV is circulating in wild boar populations.

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PARTICIPATORY RISK MAPPING OF AFRICAN SWINE FEVER OUTBREAKS IN EUROPE

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SUMMARY

Risk assessments for complex animal diseases, such as African swine fever, require an understanding of underlying risk factors and are complemented through disease status evaluation by persons with relevant knowledge. Here we present a novel participatory mapping system (Participatory risk mapping network for animal diseases, PRMNAD) that allows timely and spatially explicit retrieval of expert opinion in the context of ongoing epidemics and facilitates the identification of potential risk factors. Application of PRMNAD to African swine fever in Europe during a pilot study mapped high-risk regions for disease occurrence and recorded typically considered risk factors to examine efficient heuristic disease spread classification strategies.

INTRODUCTION

African swine fever (ASF) is an internationally spreading, viral pig disease that severely damages agricultural pork production, economy and social welfare in affected regions. ASF virus (ASFV) has a narrow host range, only infecting members of the Suidae family, including wild and feral species, such as the European wild boar. Each year the disease moves along for kilometres and invades previously disease-free territories (EFSA, 2020; Lamberga et al., 2020), requiring instantaneous decision-making by local authorities and stakeholders to implement adequate and efficient ASF control. These decisions have to be made in regards to resource allocation for surveillance efforts and implementation of prevention and control measures, such as fencing or searching and removal of wild boar carcasses.

Whilst the risk of ASF incursion into previously disease-free regions appears to increase with increasing proximity to ASF infected areas overall (Vergne et al., 2016; Vergne et al., 2017; Podgorski and Smietanka, 2018; Podgorski et al., 2020), it is unclear through which pathways ASF infected territories expand and which disease free regions adjacent to ASF outbreak clusters will be affected next. In addition to distance-dependent spread, ASF has been observed to exhibit human-mediated transmission jumps, whereby new ASF outbreaks are

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detected hundreds of kilometres away from previously known diseased areas in a distance-independent manner (Linden et al., 2019; Mazur-Panasiuk et al., 2019; Cukor et al., 2020).

For ASF management, efficient and risk-based decision-making is important. This requires anticipation of ASF spread in the near future. Assessing the risk of disease dissemination in a spatially and timely explicit manner would allow the design of efficient control strategies that could be tailored to local contexts.

To assess future ASF spread and to determine currently disease-free regions that are at high-risk of ASF incursion a comprehensive knowledge of ASF risk factors is essential. For example, ASFV susceptible European wild boar are a mobile and dispersed wildlife species that plays an important role in the short distance dissemination and persistence of ASF in Europe (Depner et al., 2016; Lange and Thulke, 2017; Podgorski and Smietanka, 2018). Therefore, in areas where wild boar are present, risk factors related to their biology and behaviour need to be considered for the assessment of ASF spread. Many additional, potential ASF risk factors have been considered previously, including disease aspects of the environment, host, animated or unanimated vectors and risk factors in relation to the pathogen itself (EFSA, 2017, 2019; Niederwerder et al., 2019; Patterson et al., 2019; Boklund et al., 2020; EFSA, 2020). Yet, our knowledge of complex ASF risk factors is imprecise and incomplete, making it difficult to apply to local contexts in a timely and spatially explicit manner (Boklund et al., 2020). Similarly, in the absence of reliable risk factor information, utilising a process of systematic expert opinion elicitation to determine the regional disease risk status for ASF would involve complex and time-consuming processes (EFSA, 2014).

Here, a 'Participatory risk mapping network for animal diseases' (PRMNAD) is presented to organise risk factor information and facilitate disease management decision-making by capturing individual, spatio-temporal risk assessments from persons with expertise in disease-related knowledge areas. In a pilot study, PRMNAD was applied to examine the currently ongoing spread of ASF in Europe.

MATERIALS AND METHODS

The PRMNAD system

Conceptually, PRMNAD uses spatially- and timely-explicit case report data to map the disease status of pre-defined 10 x 10 km grid regions. A region is considered diseased, if a case was reported within the region. The regional disease status is then combined with potential disease risk information to create an interactive map (Fig. 1). The integrated disease status and risk factor map is subsequently presented to a network of people with defined knowledge in specific areas to identify disease-free regions that are at high risk of disease incursion. The most important underlying risk factors that were considered to determine high-risk regions for disease incursion are recorded. For each time step under examination, the disease status is updated and the assessment is repeated. For the PRMNAD example in the context of ASF presented herein, one month was chosen as a time step. As a result, high-risk disease regions and risk factors that characterise each of these regions are identified for each time step.
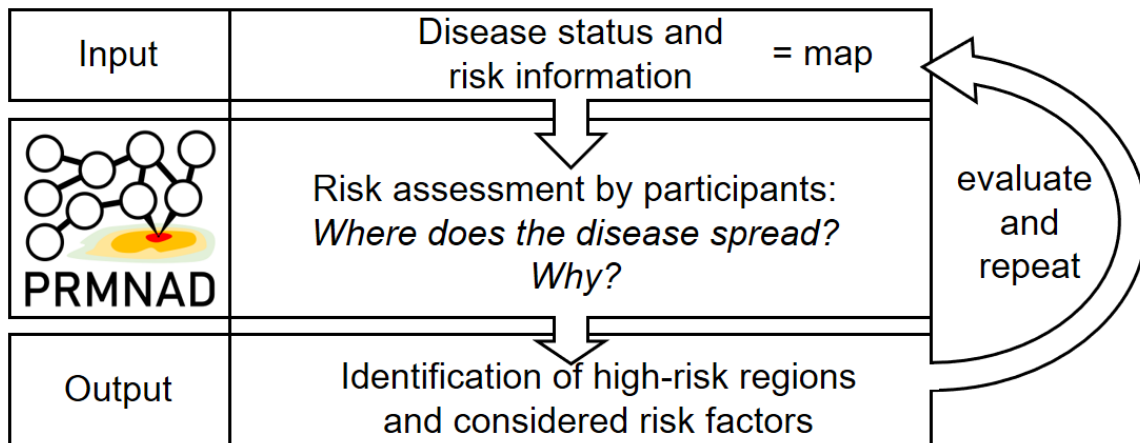
Figure 1. Schematic illustration of the PRMNAD concept.

Implementation of PRMNAD for ASF

To examine the spread of ASF in Europe during the current panzootic that originated from Georgia in 2007 (Rowlands et al., 2008), we applied PRMNAD to ASF by conducting a pilot study. Informed by literature-based identification of potential ASF risk factors, we combined and mapped customised geospatial land cover information from CORINE Land Cover (EEA, 2006), OpenStreetMap (OpenStreetMap contributors, 2020) and GlobCover 2.3 (European Space Agency; ESA Globcover 2005 Project, 2005). We then added ASF disease case reports since 2007 to the map, which were extracted from the Animal Disease Notification System (ADNS) of the European Union and the World Animal Health Information System (WAHIS) of the World Organisation for Animal Health (OIE). By mapping risk factor information, ASF case reports and the regional disease status, we created a map-based risk assessment tool (Fig. 2). EuroBoundaryMap 8.1 and the European Environmental Agency (EEA) reference grid were used to structure the mapped disease status and the land cover information spatially. At each time step (one month here), the mapped information was updated and a new risk map generated.

The uniquely codified grid cells defined the area that was available for assessment, representing Western and Central Europe and a single grid cell demarcated the smallest epidemiological unit of interest, which was a region of 10 x 10 km. For each region, underlying ASF outbreak and case report data for domestic pigs (DP) and wild boar (WB) were used to determine the ASF disease status at monthly assessment time steps. ASF cases or outbreaks that were reported from regions for the first time during the preceding month were described as 'newly infected', whereas regions with ASF reports that occurred more than a month ago were referred to as 'infected'. Differentiated by DP or WB reports, regions were coded with different colours to display the ASF disease status (Fig. 2, disease status is indicated by symbols), resulting in a total of nine possible status for each region: ASF-free, infected both, infected DP, infected DP & newly infected WB, infected WB, infected WB & newly infected DP, newly infected both, newly infected DP, newly infected WB.

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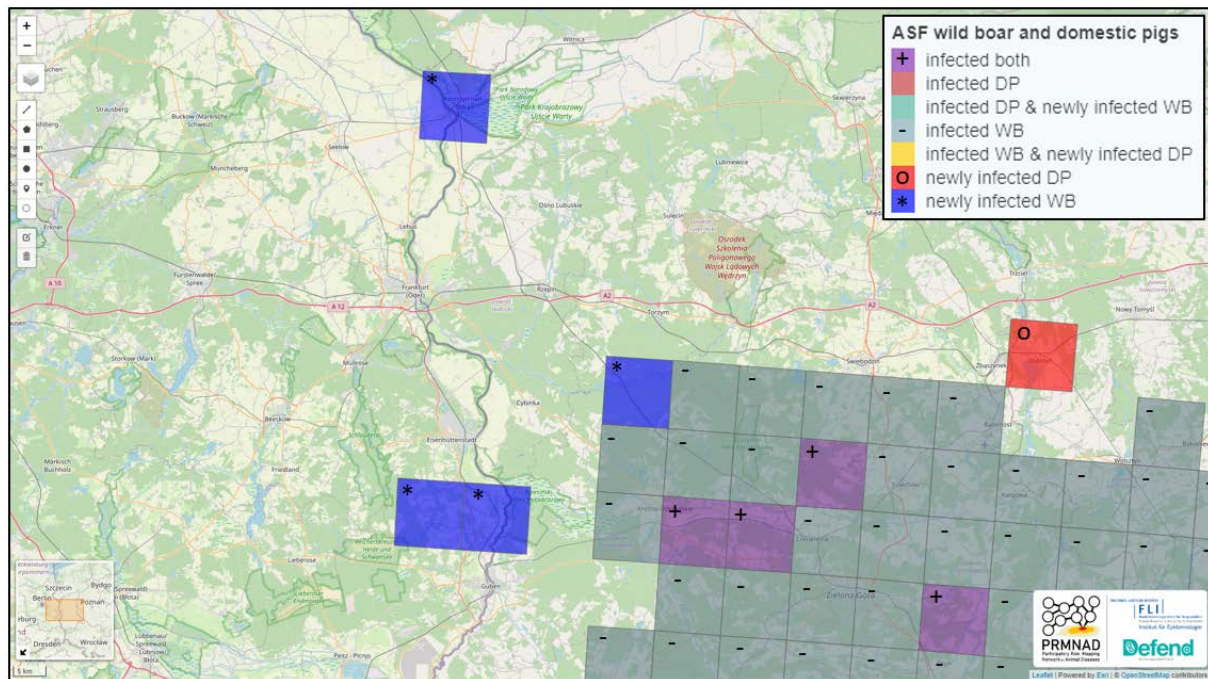
Figure 2. Interactive PRMNAD map for African swine fever displaying the disease status of 10 x 10 km regions at the German-Polish border on 5 October 2020.

The interactive mapping format allowed direct integration of region-specific ASF risk information with the disease status through selectable map layers. The aforementioned background maps provided a multitude of potential risk factor data that users could choose to consider, including land cover, traffic networks, jurisdiction, urbanity or any recorded features, such as rest areas or picnic sites. Additionally, data on wild boar occurrence probability (Alexander, 2016), domestic pig density (Gilbert et al., 2018) and EU restriction zones for the corresponding monthly time step were mapped (European Commission, 2020). All grid cells identified as high-risk regions by the users, up to the previous time step, were collectively displayed in the map for consideration of peer opinion. At every monthly time step the updated interactive map was rebuilt and then provided as a HTML-file to PRMNAD users for risk assessment. The map was generated using the statistical programming language R, version 3.6.3 (R Core Team, 2020) with the 'sf' (Pebesma, 2018), 'tmap' (Tennekes, 2018) and 'leaflet' packages (Cheng et al., 2019).

Risk assessments

In total, 22 users were recruited to participate during the study period from February to September 2020 (eight study months). Thirteen users were recruited during study month one and two, eight users during month three and four, and one user during the final study month. User knowledge areas and experiences were recorded. Once a month, users were provided with the updated PRMNAD map to determine previously disease-free regions with high-probability of ASF reports to occur in WB and DP during the following month. Users were asked to select five high-risk regions for ASF in DP and five high-risk regions for ASF in WB. In electronic forms, users recorded the unique grid cell code of their selected high-risk regions. For each selected region, up to five ASF risk factors, upon which users had based their selection decision, were recorded as well. A list of 29 potential environmental ASF risk factors was provided for the users to consider in their decision-making. Some of the considered risk factors

were identified through systematic literature review, but PRMNAD users were also able to suggest non-listed risk factors of their own, if the provided factors did not adequately describe why a decision to select a high-risk region was made. These user-generated potential risk factors were added to the list of potential risk factors to consider.

PRMNAD data

Monthly-updated ASF case reports informed the disease status for each 10 x 10 km grid cell. The gridded European study area encompassed mainland and excluded sea, resulting in a total of 100,650 regions, equivalent to a total area of approx. 10,065,000 km^2. Consequently, disease status data consisted of 100,650 unique region codes, with an assigned disease status for each region and month of the study. Monthly PRMNAD assessments resulted in user-submitted high-risk regions, with a possible number of five WB and five DP region selections per user. Each selected high-risk region was associated with the underlying risk factors that had been considered for their selection and with the country that contained the majority of its area. PRMNAD user selected regions were validated by checking that they met two conditions. First, the recorded cell code had to be included in the study area. Second, the prevailing disease status of the grid cell had to be ASF-free for WB or DP before selection of the region as a high-risk area for the respective species type. PRMNAD user selected grid cells that met these conditions were considered valid. Therefore, PRMNAD data represented a set of the selected region codes with their underlying risk factors for WB and DP, and for each time step (one study month). The PRMNAD outputs were evaluated in relation to the true disease status that emerged the following time step (month). R statistical programming language version 3.6.3 (R Core Team, 2020), the package 'epitools' (Aragon, 2020) were used for data analysis and GraphPad Prism version 5 for Mac OS X for generating the bar graphs.

RESULTS

PRMNAD user reporting

The 22 recruited PRMNAD participants worked as scientists and their expertise collectively covered veterinary medicine, epidemiology, geospatial analysis, networks, parasitology, wild boar biology and machine learning. Sixteen participants had prior experience working with ASF in their field of expertise, but six did not. Participants reported that they spent around 10 to 20 minutes to decide which high-risk regions to select each month. Collectively, PRMNAD users conducted and submitted 725 individual regional risk assessments for ASF in WB and DP across Europe. Of these, 712 were found to be valid, with 342 recorded for DP and 370 for WB. According to the start month of each participant during the 8-month study period, 114 active user-months were accumulated, theoretically accounting for 570 regional ASF assessments for WB and DP. The overall response rate of retrieved assessments was 0.625 (712/1140) overall, 0.600 (342/570) for DP and 0.649 (370/570) for WB.

Evaluation of PRMNAD assessments

Of the 712 valid assessments, ASF cases were reported the following month in 74 (0.104) grid cells, of which 19/342 (0.056) were for DP and 55/370 (0.148) for WB.

Country allocation of high-risk regions

PRMNAD-selected high-risk regions were identified in 18 countries overall, in 11 countries for DP and in 16 countries for WB. Most high-risk regions, in which DP ASF outbreaks were newly reported during the study period, were in Romania, Poland and Bulgaria (Fig. 3A), while high-risk regions with new ASF outbreaks in WB were mostly selected in Poland, Hungary and Romania (Fig. 3B). In these most frequently identified countries, the number of correctly selected ASF high-risk regions was also generally highest (Fig. 3). An exception were few selected Slovakian regions adjacent to the Hungarian border, where ASF cases in wild boar were reported a month after selection (Fig. 3B). For DP, in 13 out of 205 (0.063) identified high-risk regions in Romania and in 6 out of 83 (0.072) identified regions in Poland, ASF outbreaks in DP were reported the following month (Fig. 3A). For WB, in 31 out of 221 (0.140) identified high-risk regions in Poland, in 17 out of 52 (0.327) identified regions in Hungary, in 5 out of 12 (0.417) identified regions in Slovakia (mostly near the Hungarian border) and in 1 out of 24 (0.042) identified regions in Romania, new ASF cases were reported in WB one month after selection (Fig. 3B).
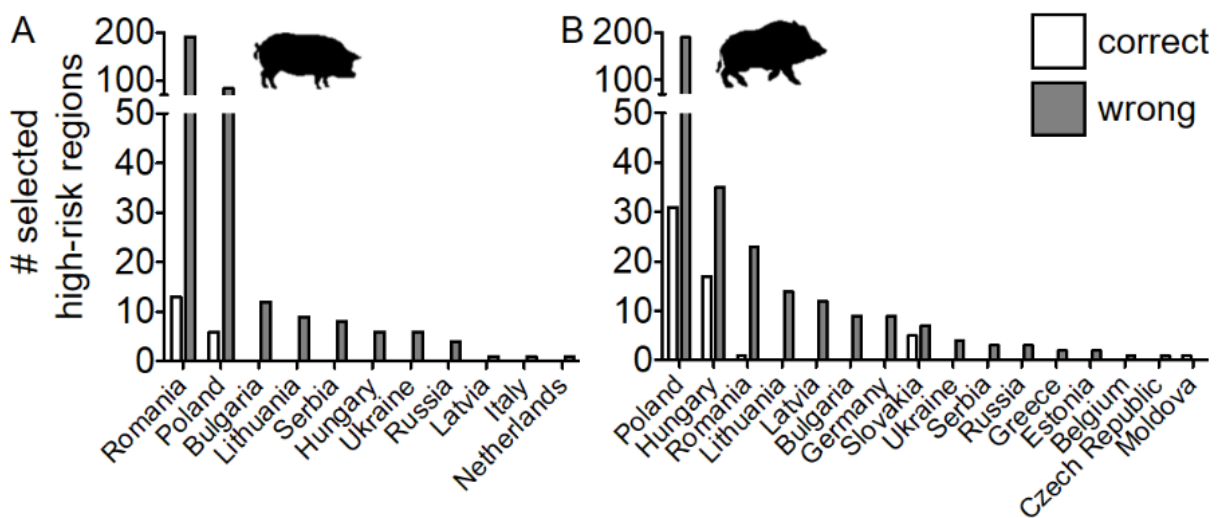
Figure 3. Occurrence (correct) or absence (wrong) of ASF outbreak reports for either domestic pigs (A) or wild boar (B) in selected high-risk regions of the indicated countries one month after the selection decision was made.

Risk factors considered

Decisions to select high-risk regions were mostly based on risk factors. In this study, the most frequently considered risk factors also yielded the most frequent correct selection of high-risk regions for which ASF outbreaks were indeed reported a month later. PRMNAD users predominantly accounted for 'nearness of ASF outbreaks in DP' (13/224, 0.058), 'road connectedness with outbreak areas' (2/43, 0.047) and 'nearness of ASF outbreaks in WB' (3/30, 0.1) as risk factors when selecting high-risk regions for ASF in DP (Fig. 4A). To determine ASF high-risk regions for WB, users mostly considered 'nearness to ASF outbreaks in WB' (33/240, 0.138), 'nearness to the epidemic front' (13/68, 0.191) and 'connectedness with outbreak areas through broad-leafed forest edges' (7/26, 0.269) as risk factors (Fig. 4B).
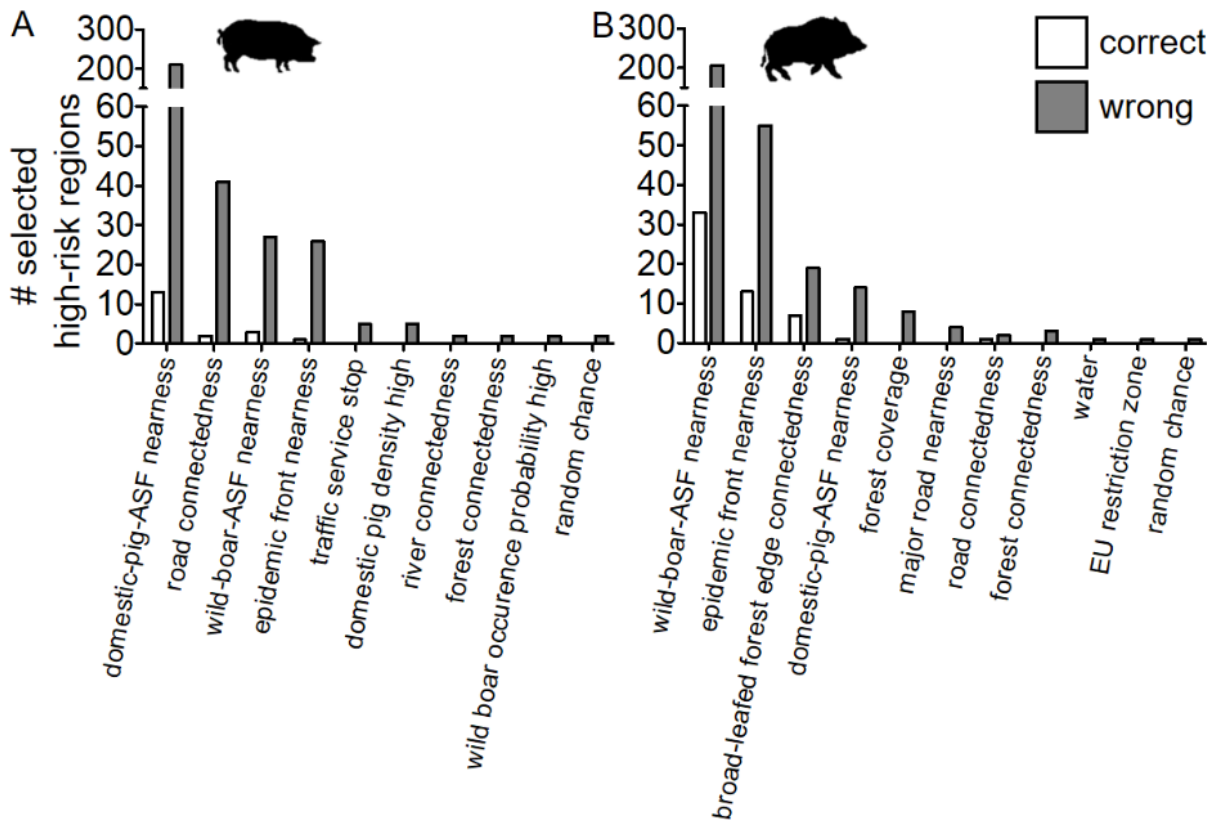
Figure 4. Subsequent occurrence (correct) or absence (wrong) of ASF outbreak reports for either domestic pigs (A) or wild boar (B) in selected high-risk regions when the indicated risk factors were considered for the selection decision.

DISCUSSION

PRMNAD integrates spatio-temporal animal disease reporting data with potential risk factor information through an interactive mapping format to facilitate participatory evaluation of disease spread risks. Here we developed and demonstrated the PRMNAD concept and examined the current ASF epidemic in Europe during an 8-month pilot study in 2020. We showed how PRMNAD could be implemented to access and report expert opinion, but also tested risk factors and disease spread classification strategies.

PRMNAD risk assessments fundamentally rely on participatory inputs from a network of users. It could be anticipated that recruited user numbers, their knowledge areas and user response rates may be important factors that influence PRMNAD performance. It also appears feasible that established PRMNAD networks could be useful for continuous contributions to specific disease problems in the future. Therefore, participant numbers, knowledge areas and response rates are discussed to describe possible regulating factors in expert-based, spatio-temporal disease risk assessment systems.

In this study of ASF spread in Europe, 22 users were recruited for participation. Overall, their recorded knowledge areas were judged to be well aligned with the focus of the PRMNAD. The proportion of selected high-risk regions could be used to estimate a participation rate of around 60% for this study. The PRMNAD participation rate could help to understand user acceptance and factors influencing user recruitment as well as their continuous engagement,

likely necessitating the provision of clear benefits for the user. These benefits could include regular disease risk or forecasting reports.

It may be assumed that a large number of users or very high participation ratios would be necessary to obtain reliable results. Studies that used groups of persons to harness the 'wisdom of crowds' for estimating weights (e.g. estimating the weight of an object or an animal) or numbers (e.g. estimating the number of beads in a jar) found that with increasing participant numbers the average estimate became more precise (Galton, 1907; Kao et al., 2018; van Dolder and van den Assem, 2018; Keck and Tang, 2020; Palan et al., 2020). This might suggest that assessment errors homogenously varied around a specific value and therefore having many participants could be beneficial to obtaining a precise estimate through balancing the effect of errors in the individual judgement. Whilst object weight estimation experiments could be explained through common experiences among humans to estimate weights, the capacity to assess disease spread likely relies much more on specific knowledge and experiences that are unique to persons or communities with specific interests in animals and their disease mechanisms. In other words, PRMNAD could capture the intuitive or analytical assessment abilities of animal disease spread that people with experiences in related knowledge areas may have developed.

Although a relatively small number of participants had been recruited for this study, consensus with regards to the selection of countries at risk of ASFV introduction and with regards to the underlying risk factors was high (Fig. 3 and Fig. 4). This observation indicates that low user numbers may be sufficient to saturate PRMNAD system output, suggesting that PRMNAD could operate meaningfully with a small number of users. Diminishing errors through aggregation of multiple inaccurate estimates is a well-known statistical effect, which in some contexts is already achieved when combining judgements from a small group of individuals (Hogarth, 1978; van Dolder and van den Assem, 2018). Nevertheless, this observation highlights the importance of describing and recording the knowledge areas of PRMNAD users at a greater level of detail. If only a small number of users are allowed to contribute to PRMNAD risk assessments, it is likely that their type and level of expertise would strongly influence the assessment outcome. This aspect should be further developed beyond the scope of this study to help understand PRMNAD outputs better. This study helped describe user numbers, their knowledge areas and user response rates as PRMNAD characteristics, but their possible effects in this context require further experimental testing.

This pilot study harnessed expert knowledge to identify high-risk regions for ASF disease occurrence in Europe and potentially associated risk factors. Evaluation of expert selection decisions that identified ASF high-risk regions in the context of real-world disease reporting provided insights. Complex ASF risk factor knowledge is deemed incomplete and uncertain (Costard et al., 2013; Chenais et al., 2019), but analysing expert selection of high-risk regions in conjunction with the considered underlying risk factors revealed potential disease spread classification strategies. For example, it appeared that during this study, the following strategy was useful for the selection of high-risk regions where WB ASF cases would be reported during the following month: to identify regions near recent ASF reports in WB, near the ASF epidemic front, connected through broad leaf forest edges with ASF case locations (Fig. 4B), and particularly in Poland or Hungary (Fig. 3B). Such simplified disease risk classification strategies may suggest that PRMNAD users largely exploited efficient cognitive decision-making processes known as heuristics to select high-risk regions for ASF outbreaks (Gigerenzer and Gaissmaier, 2011). Complexity and uncertainty of the presented ASF information was judged to be high and users spent little time to decide upon their selection

(between one and two minutes per high-risk region), indicating that heuristic decisions were involved. Adaptive heuristic decisions, sometimes referred to as 'gut-decisions', ignore part of the available information and may outperform strictly rational approaches in situations of high uncertainty, complexity and variance (Snook et al., 2004; Gigerenzer and Gaissmaier, 2011; Keck and Tang, 2020), as is the case of hardly predictable animal diseases such as ASF (Linden et al., 2019; Mazur-Panasiuk et al., 2019; Cukor et al., 2020; Dixon et al., 2020). Nevertheless, the appropriate application and relevance of heuristic decision-making processes in PRMNAD systems require further investigation.

In summary, PRMNAD quickly collated spatially-explicit opinions of users with defined knowledge areas, potentially harnessing efficient heuristic decision-making processes and 'wisdom of the crowd' effects. PRMNAD further supplied disease spread classification strategies to complement stochastic disease risk assessments and ultimately offered the capacity to funnel and integrate collective human knowledge to support the management of complex animal diseases, such as ASF.

ACKNOWLEDGEMENTS

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AVIAN INFLUENZA

SPATIAL PATTERNS OF AVIAN INFLUENZA IN WILD BIRDS FROM DENMARK, 2006-2020

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SUMMARY

We investigated factors affecting avian influenza virus (AIV) detections in Danish wild birds using data from the passive and active AIV surveillance in wild birds from 2006-2020. We used this data and machine learning (ML) algorithms along with landscape and environmental variables to develop predictive models of AIV occurrence in Denmark. We furthermore assessed potential accessibility bias in the passive AIV surveillance data submitted by the public. The passive AIV surveillance data was biased regarding accessibility to areas compared to random locations within Denmark. ML models differed in their predictive power and were used to predict the risk of AIV presence throughout Denmark. Our results suggest that landscape variables may affect AIV presence and enabled us to create risk maps of AIV occurrence in Danish wild birds. This may aid future targeted surveillance efforts within Denmark.

INTRODUCTION

Avian influenza (AI) is a contagious disease of birds caused by Influenza A viruses (AIV). AIV can be divided into low pathogenic and high pathogenic pathotypes. Low Pathogen Avian influenza (LPAI) is a persistent problem worldwide and has a widespread occurrence in a variety of bird species. LPAI has the potential to mutate into High Pathogenic Avian Influenza (HPAI), which can cause great economic losses when transmitted to farmed birds (Rao et al., 2009; Monne et al., 2014). Furthermore, some HPAI strains have zoonotic potential with high case-fatality for humans (Lai et al., 2016), thus it is crucial to monitor and prevent the spread of AIV in both wild and farmed birds.

Data from surveillance programmes can be a valuable means to monitor the distribution of AIV and to evaluate potential risks for both wild and farmed birds, and many countries have implemented such programmes (Buscaglia et al., 2007; Hesterberg et al., 2009; Bevins et al., 2014; Machalaba et al., 2015). Data from these surveillance programmes can be used in models to identify areas and time periods where AIV is more likely to occur, which can aid in optimising further AIV surveillance.

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Mandatory AIV surveillance programmes within the European Union (EU) are performed to detect AIV in both wild and domestic birds (Brouwer et al., 2018). HPAI and some LPAI subtypes are notifiable diseases in the EU, and all positive samples must be reported. The passive surveillance programme includes all types of poultry farms, other birds in captivity and wild birds, and it is compulsory to target moribund or dead found wild birds. Furthermore, as surveillance of poultry farms is mandatory (European Union, 2005), a risk based approach is recommended (Brouwer et al., 2018), for instance stratification according to proximity of water bodies or poultry farm density. Similarly recommended is a risk based approach for wild birds, targeting species that are susceptible to infection with AIV (Brouwer et al., 2018).

We obtained data from the Danish national surveillance program from 2006 to 2020, and assessed surveillance data submitted by the public (passive surveillance) by investigating if human accessibility to areas could be a potential bias. We furthermore used machine learning (ML) algorithms to investigate whether landscape variables could predict the occurrence of AIV and used these ML algorithms to create prediction maps of AIV occurrence and identify high-risk areas in Denmark. This may aid future targeted surveillance efforts within Denmark.

MATERIALS AND METHODS

AIV surveillance data

We obtained precise location data (UTM coordinates) from passive AIV surveillance from 2006-2020, and data at the postal code level from active AIV surveillance data from 2007-2019. Passive AIV surveillance data were from dead and diseased wild birds submitted by the public, whereas the active AIV surveillance data were based on healthy birds submitted by hunters, captured for sampling or ringing, or from bird dropping samples. Data were obtained from Statens Serum Institut, The Danish National Veterinary Institute and the Danish Veterinary and Food Administration. All data were checked for errors, and erroneous locations (such as coordinates not being within Denmark) were omitted from our analyses.

Passive AIV surveillance data was generated from samples reported by the public, thus we suspected that these data could be biased regarding accessibility by the public to areas where wild birds might be found. To assess this potential bias, we compared various accessibility variables to random locations within Denmark (See sections "Landscape and environmental variables" and "Bias in the passive AIV surveillance data" below).

Landscape and environmental variables

We used the raster package (Hijmans, 2019) in R 3.5.2 (R Development Core Team, 2018) and the freely available Corine land cover data (100 x100m pixels; European Environment Agency, 2018) to extract land cover types for each observation in the passive AIV surveillance data. We furthermore used the Corine land cover data to define wetlands within Denmark (Corine land cover types: inland marshes, peat bogs, salt marches, salines, intertidal flats) and used ArcMap 10.6.1 (Environmental Systems Research Institute, 2017) to calculate the distance to nearest wetland for each location in the passive AIV surveillance data. We additionally obtained a freely available shapefile of the Danish coast line (Danish Map Supply; Kortforsyningen, 2020) and added a 1 km buffer around the coast line. We then calculated distance to nearest buffered coastline from our passive AIV surveillance locations.

The active AIV surveillance data was reported at the postal code level, thus instead of distances, we calculated the area of wetlands, coast and city within a postal code. Area of city within a postal code could be used as a measure of population density. As with the passive AIV surveillance data, we used the 100 m^2 resolution Corine land cover data (European Environment Agency, 2018) to calculate area of wetland and area of coast, and the freely available Gridded Population of the World dataset (raster with 1 km^2 resolution; Socioeconomic Data and Applications Center, NASA, 2015) to calculate area of city (pixel cells with ≥ 200 inhabitants/km^2). We used the raster package (Hijmans, 2019) in R 3.5.2 (R Development Core Team, 2018) to calculate areas.

Bias in the passive AIV surveillance data

To investigate variables related to public accessibility, we obtained a freely available shapefile of all roads in Denmark (Danish Map Supply; Kortforsyningen, 2020) and calculated distance to nearest road for our passive AIV surveillance observations. We furthermore calculated distance to cities as well as population density at each location, using the Gridded Population of the World raster dataset (raster with 1 km^2 resolution; Socioeconomic Data and Applications Center, NASA, 2015). Population density at a location was simply extracted from this raster. We defined a city to be a raster grid cell with ≥ 200 inhabitants/km^2, and we then calculated the distance from our passive AIV surveillance locations to the nearest grid cell with ≥ 200 inhabitants/km^2. To investigate whether accessibility to areas created a bias in the location of the passive AIV surveillance data, we generated random locations within Denmark and for each of these locations, we calculated the distance to nearest coast, distance to nearest roads, population density at the location and distance to nearest city, using the same methods described above. We used ArcMap 10.6.1 (Environmental Systems Research Institute, 2017) to perform all our distance calculations.

Statistical analysis

We compared accessibility variables from the passive AIV surveillance data to accessibility variables from random locations within Denmark using a Kolmogorov-Smirnov test in R 3.5.2 (R Development Core Team, 2018).

To investigate whether our calculated landscape variables could be associated with AIV occurrence, we used the ML algorithm boosted regression trees (BRT; Elith et al., 2008) in R 3.5.2 (R Development Core Team, 2018) using the packages caret (Kuhn et al., 2018) and gbm (Greenwell et al., 2019). We ran BRT models separate for the passive and active AIV surveillance data. As the BRT method does not account for random effects, we could not use multiple observations from the same location or postal code at different months and years. Instead, we summarised our data to contain only unique coordinates/postal codes. If the location or unique postal code at any time during the study period had observations positive for AIV, this location/postal code were designated as a positive occurrence, otherwise it was designated as a negative occurrence.

Variables included in the passive AIV surveillance BRT models were the landscape predictors described above – distance to wetlands, distance to coast, and Corine land cover (European Environment Agency, 2018) – whereas variables for the active AIV surveillance model were area of coast, area of wetlands and area of cities. In addition, we included Fourier transformed environmental data derived from images from a MODIS satellite v5 time series for 2001-2012 (only available from EDENext partners; EDENext, 2014) for the passive AIV surveillance BRT model. The temporal Fourier Analysis describe environmental cycles

(temperature, vegetation phenology etc.) as the sum of a series of sine curves with different amplitudes and phases (Scharlemann et al., 2008). We could not include these environmental MODIS predictors for the active AIV surveillance model, as these predictors were not available at a postal code level, but only in a resolution of $1km^2$. The environmental MODIS predictors were middle infra-red index, daytime land surface temperature, night time land surface temperature, the normalised difference vegetation index, and the enhanced vegetation index. We used the following Fourier processed outputs for each of the above variables: mean, minimum, maximum, variance in raw data, combined variance in annual, bi-annual, and tri-annual cycles as well as amplitude, phase and variance of annual bi-annual and tri-annual cycle (EDENext, 2014). In addition to the MODIS data, we used freely available environmental data from Worldclim and Bioclim (WorldClim, 2005), including altitude, precipitation and annual mean temperatures. See Kjær et al. (2019) for further details about the environmental predictors used.

For both the passive and active AIV BRT models, we set the data as Bernoulli distributed (logistic regression for 0-1 outcome), and to validate the models, we conducted a 5-fold repeated cross validation (CV) scheme with 10 repetitions. This validation scheme split the data into 5 subsets, and within each run of the model one subset was withheld. The models were then used to predict the "unknown" withheld data subset, and we then estimated the prediction error from these predicted subsets. BRT model performance can be hampered by class imbalance (Batista et al., 2004), thus we compared different methods for handling unbalanced data: tomek with the majority removed and tomek with both classes removed (Batista et al., 2004), rose (package ROSE; Lunardon et al., 2014), smote (Batista et al., 2004), down-scaling and up-scaling. We used area under the curve (AUC) for the receiver operating characteristic (Elith et al., 2008), to determine the best balancing model (as well as a model using unbalanced data) and used 5-fold repeated CV with 10 repetitions for each method. We used a tuning grid to optimise model parameters and varied the parameters interaction depth, learning rate and minimum number of observations per node, but kept the number of trees constant at 1,500 trees (Elith et al., 2008). Once we had selected a final model with the highest AUC score, we plotted the residuals to check for spatial autocorrelation and used the models to predict AIV occurrence throughout Denmark.

To predict throughout Denmark, for the passive AIV surveillance BRT model we used Corine land cover in a 1 km^2 resolution (European Environment Agency, 2018), and created 2 additional raster files, one in which the value of each pixel was the distance to nearest coast and one in which the value was the distance to nearest wetlands. We only predicted to landcover types observed in the passive AIV surveillance data. For the active AIV surveillance BRT model, we calculated area of coast, wetlands and cities for each postal code within Denmark. We used R 3.5.2 (R Development Core Team, 2018) for all calculations.

RESULTS

Passive and active AIV surveillance data

We did not divide the passive or active AIV surveillance data into AIV subtypes, as positive data were sparse, thus we solely treated an observation as being AIV positive or AIV negative. For the same reason, we did not divide the data into bird species.

From the passive AIV surveillance, we obtained 2,089 observation entries, with 1,601 unique site locations. Out of the 2,089 observations, 189 (9.0%) were positive for AIV. When

summarising the observations for our BRT model, 143 of the 1,601 unique sites (8.9%) were AIV positive (Fig. 1). When testing for bias in the passive AIV surveillance data, we found significant differences for all accessibility variables when we compared the 1,601 unique locations to 1,601 random locations (all P < 0.0001, Fig. 2). Dead birds submitted through the passive AIV surveillance system were generally from areas with low population densities, but closer to cities and closer to roads than random locations. A majority of the records were additionally found closer to the coast than random locations. However, since positive and negative AIV locations were equally biased, we proceeded with our BRT modelling.
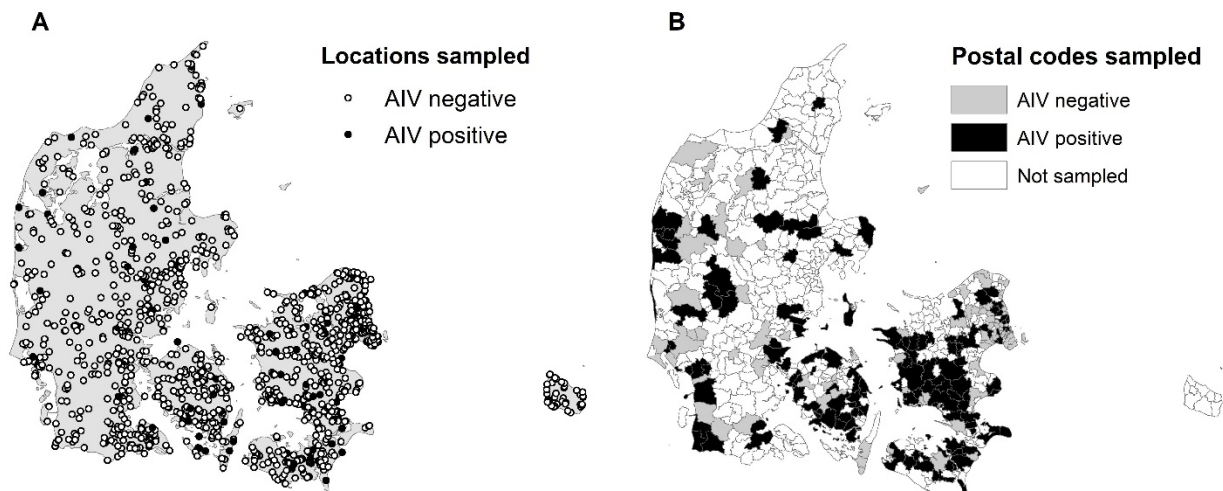
Figure 1. Summarised occurrence of avian influenza virus (AIV) in the A) passive AIV surveillance data (2006-2020) and the B) active AIV surveillance data (2007-2019) in Denmark.

We obtained 8,912 observations from the active AIV surveillance system, from 233 unique postal codes, 1,066 (12.0%) of these observations were AIV positive. Summarising the active AIV surveillance data for BRT modelling resulted in 103 AIV positive (44.2%) out of the 233 postal codes (Fig. 1).

Predictive ML models

Our passive AIV surveillance data were highly unbalanced with 92.4% AIV negative observations and 7.6% AIV positive observations. This number differs from the original numbers calculated in the section above, as some locations were not within the boundaries of the MODIS, Worldclim and Bioclim rasters and were thus omitted from the modelling process. Due to this imbalance, we tested the different balancing methods described in the methods section, and found that the SMOTE balancing method produced the highest AUC-score (Table 1). The final model using the SMOTE balancing method yielded an accuracy of 0.85, a sensitivity of 0.34 and a specificity of 0.90 (Table 1). The accuracy of this BRT model was lower than the proportion of the majority class (0.924 AIV negative) in the unbalanced original dataset, thus performing more poorly than if all observations were predicted to be AIV negative (all other balancing methods produced similar accuracies lower than 0.924). Thus any predictions based on this model should be viewed with caution.
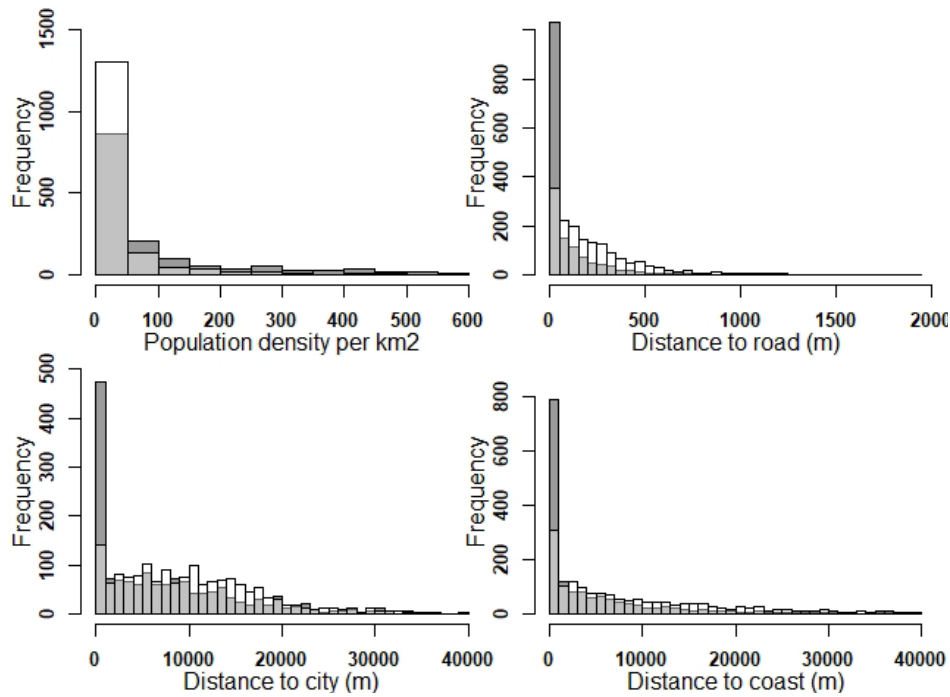
Figure 2. Histograms of passive avian influenza virus (AIV) surveillance locations and random locations within Denmark. The histograms depict locations recorded through the passive AIV surveillance in Denmark, 2006-2020 (dark grey) and random locations in Denmark (white) in relation to population density, distance to nearest city (≥200 inhabitants/km2), distance to coast and distance to nearest road. All x-axes have been truncated to omit large outliers.

We did not observe the same imbalance in our active AIV surveillance data as 44.2% of the data were AIV negative whereas 55.8% of the data were AIV positive, thus, we did not use a balancing method. The final active AIV surveillance BRT model had an AUC of 0.63, an accuracy of 0.65, a specificity of 0.51 and a sensitivity of 0.76 (Table 1).

The most important predictors in the passive AIV surveillance model were variables related to day- and night-time temperatures, land cover and distance to wetlands (Fig. 3), whereas wetlands ranked highest, followed by city and lastly coast for the active AIV surveillance model (Fig. 4).

Table 1. Boosted regression tree (BRT) modelling results for the passive and active avian influenza virus (AIV) surveillance models. Area under the curve (AUC), accuracy, specificity and sensitivity scores for the final BRT AIV surveillance models are shown. Also shown are AUC results for the different balancing methods as well as the final parameters used in the models selected from a tuning grid

Data	Balancing method	AUC	Model results	Parameters of final model
Passive AIV surveillance	Unbalanced	0.70		
	Down-scaled	0.69		
	ROSE[a]	0.51		
	SMOTE[b]	0.71	Accuracy: 0.85 Sensitivity: 0.34 Specificity: 0.90	Interaction depth: 1 Learning rate: 0.01 Min. observations in node: 3
	Tomek-both[b]	0.67		
	Tomek-majority[b]	0.70		
	Up-scaled	0.70		
Active AIV surveillance	Unbalanced	0.63	Accuracy: 0.65 Sensitivity: 0.76 Specificity: 0.51	Interaction depth: 1 Learning rate: 0.01 Min. observations in node: 2

[a]Lunardon et al., 2014, [b]Batista et al., 2004

Corine landcover
1: Continuous urban fabric
2: Discontinuous urban fabric
3: Industrial or commercial units
5: Port areas
6: Airports
7: Mineral extraction sites
10: Green urban areas
11: Sport and leisure facilities
12: Non-irrigated arable land
16: Fruit trees and berry plantations
18: Pastures
20: Complex cultivation patterns
21: Land principally occupied by agriculture, with significant areas of natural vegetation
23: Broad-leaved forest
24: Coniferous forest
25: Mixed forest
26: Natural grasslands
27: Moors and heathland
29: Transitional woodland-shrub
35: Inland marshes
36: Peat bogs
37: Salt marshes
39: Intertidal flats
41: Water bodies
42: Coastal lagoons
44: Sea and ocean

Figure 3. Partial dependence plot of the top 5 most important variables in the passive avian influenza virus (AIV) surveillance boosted regression tree (BRT) model. Partial dependence plots illustrate the marginal effect of the selected variables on the response (logit) after integrating out the other variables. The BRT modelling process creates dummy variables for

the factor land cover and thus a partial dependence plot is not available, instead the predicted probability of presence plotted against landcover is shown. Temperature variables are from the MODIS Fourier processed data. Phase denotes the timing of the peaks in the seasonality cycles, whereas variance denotes the variability in the cycles.

Residual plots showed some spatial autocorrelation for both the passive (Morans I = 1.05, z = 6.95, P < 0.0001) and active AIV surveillance model (Moran's I = 0.046, z = 2.68, P < 0.01, but plotting the spline (cross-) correlograms of the final model residuals showed that the spatial autocorrelation was weak for both models, with a weak negative autocorrelation (correlation coefficients < -0.20) at distances of 300-500 km.
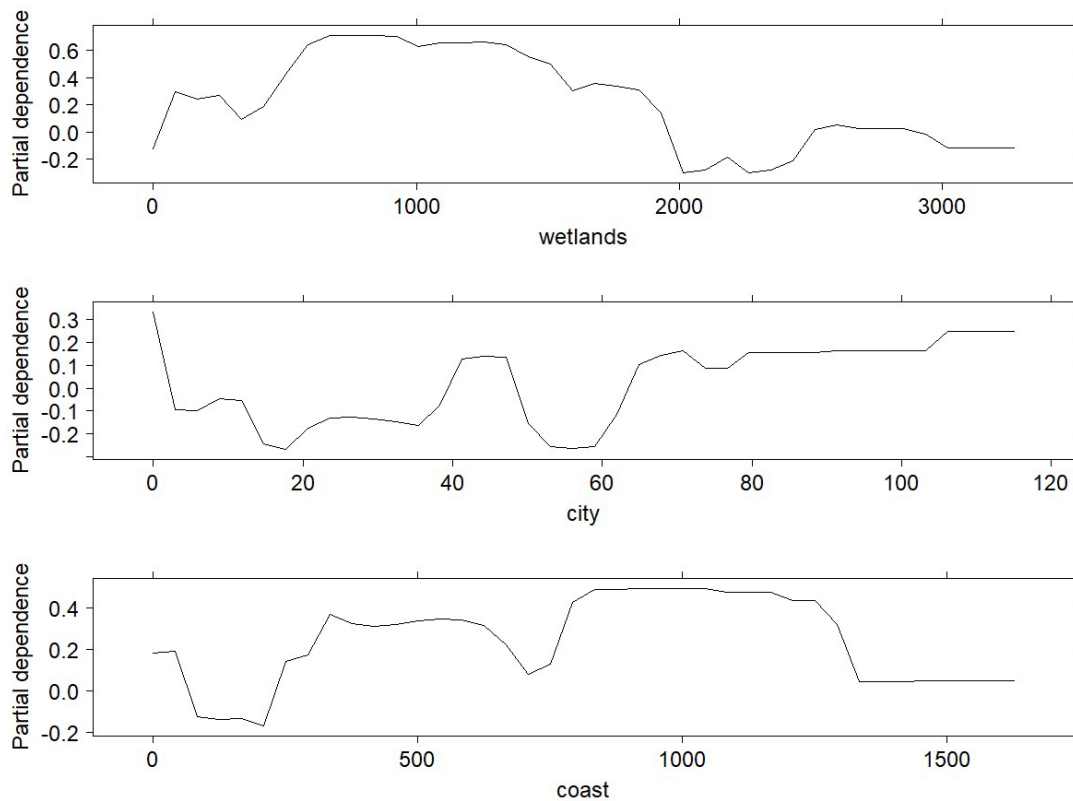
Figure 4. Partial dependence plots of the 3 predictors: area of wetlands, area of city and area of coast in the active avian influenza virus (AIV) surveillance boosted regression tree (BRT) model. Partial dependence plots illustrate the marginal effect of the selected variables on the response (logit) after integrating out the other variables. X-axes are area in units of $100m^2$.
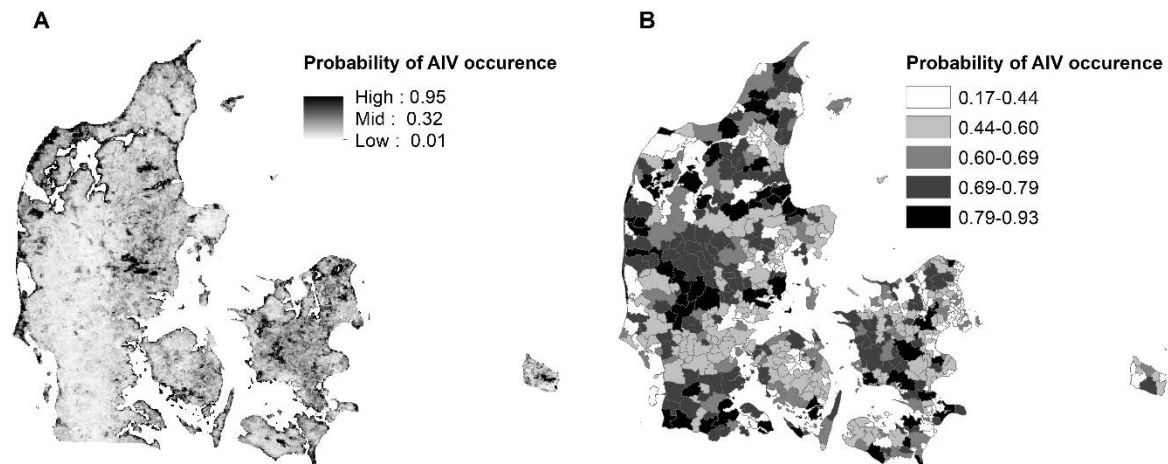
Figure 5. Prediction maps of the probability of avian influenza virus (AIV) occurrence in Denmark. The maps are based on the final boosted regression tree models for A) the passive AIV surveillance model, and B) the active AIV surveillance model.

Final prediction maps for the passive AIV surveillance model showed high probability of AIV occurrence along the Danish coast line and fjords as well as some high probabilities inland in the middle of Jutland and Zealand (Fig. 5A). The lowest probabilities of AIV occurrence were seen in the dry heathlands of western Jutland (Fig. 5A). For the active AIV surveillance model, high probabilities were found throughout the country with the lowest probabilities in the Capital region and several of the smaller islands (Fig. 5B).

DISCUSSION

We developed models to predict the occurrence of AIV in Denmark using machine learning algorithms and environmental variables and created predictive distribution maps of the country. Both models performed relatively poorly, particularly the model for passive AIV surveillance. We also assessed bias in the passive AIV surveillance data by comparing sampling locations to random locations within Denmark.

We found that sampling locations in the passive AIV surveillance data were biased with respect to their accessibility to wild life areas: the majority of the locations were close to roads and larger cities. Sampling locations were furthermore close to the coast, which are areas with public access in Denmark. As passive surveillance depends on the efforts and cooperation of the public, it can be hard to manage. However, conducting public information campaigns could be helpful to increase sampling in areas with insufficient information, which could be a valuable addition to the ongoing surveillance programme.

For both our passive and active AIV predictive models, wetlands ranked high in predictor importance (distance to wetlands for the passive AIV model and area of wetlands for the active AIV model). This finding was expected as wetlands attract waterfowl and migrating birds and therefore might increase the potential for AIV transmission. Several studies have found an effect of landscape on AI occurrence in wild and farmed birds. Ward et al. (2008; 2009) found associations between HPAI outbreaks in Romania and distance to migratory waterfowl sites, distance to rivers or streams and distance to major roads. Similarly to our ML approach, Belkhiria et al. (2018) used ML models and found land cover, distance to coast and other

variables related to climate to be the top ranking predictors in their models for AIV in wild birds in California.

The poor performance of the passive AIV surveillance model and the low specificity of the active AIV surveillance models could indicate that other factors not included in the models might be important for predicting AIV occurrence. It could also be affected by a large degree of stochasticity in the introduction of AIV into Denmark, for instance with migratory birds. There has long been suspicions that migratory birds might transmit AIV (Sullivan et al., 2018; van der Kolk, 2019) and including data on bird migration in our models could potentially improve the models. However, there is no fine-scale data available on bird migration routes in Denmark that would allow us to differentiate between different locations in the same area. Additionally, the low prevalence of AIV-positive locations in the passive AIV surveillance model could be a reason for the poor performance of the model. Without enough AIV presence data, the models may not be able to fully learn what drives AIV and how to differentiate between locations. For the active AIV surveillance model, the postal code scale may not have been fine-scaled enough to determine drivers of AIV presence or absence.

The predictive maps created by the passive AIV surveillance model, and to some degree also the active AIV surveillance model, predicted high-risk areas along the Danish coast and fjords. Some of these areas, especially in northern Jutland, are not well covered in the current AIV surveillance program, and our results suggest that risk-based surveillance should focus on these areas. Interestingly, western Jutland was predicted to be a low-risk area by the passive AIV surveillance model, but the active AIV surveillance model predicted relatively high probability of occurrence in these areas. This could be due to the low specificity of the active AIV surveillance model, as a low specificity can result in many false positives.

Though we did not separate our models into AIV subtypes, HPAI subtypes constituted the majority of the positive cases in the passive AIV surveillance data, whereas the active AIV surveillance data mainly found LPAI subtypes. Our separate passive and active AIV surveillance models can therefore be loosely interpreted as predicting the occurrence of HPAI and LPAI, respectively.

The predictive models and maps produced in this study illustrate some of the shortcomings in the existing Danish AIV surveillance programme. Jutland is sparsely sampled compared to other regions within Denmark, and our model predicted several areas in Jutland with high probability of AIV presence. More data and information regarding the presence of AIV in these areas are required, and our findings provide the motivation for more detailed surveillance in these parts of Denmark.

ACKNOWLEDGEMENTS

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UNRAVELLING EPIDEMIC DYNAMICS OF H5N8 HIGHLY PATHOGENIC AVIAN INFLUENZA IN FRANCE (2016-17) BY INTEGRATING EPIDEMIOLOGICAL AND VIRAL GENOMIC DATA

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SUMMARY

Interactions between viral geographical subpopulations are key drivers of epidemic spread but are not easily addressed using incidence data alone. Phylogeographic methods can unravel these interactions using genomic data. However, most studies either assume populations to be homogeneous or do not explicitly model migration. These shortcomings were recently addressed by the development of the MASCOT model. Adjusting this model to viral genomes (n = 196), we unravelled the dynamics of the 2016-17 H5N8 highly pathogenic avian influenza epidemic in southwest France. Our results indicated the virus emerged in the Tarn département around 14 November 2016 (95% credible interval: 3 - 25 November). We detected multiple migrations between départements and showed that their likelihood was higher between départements sharing borders and before control measures were reinforced nation-wide. This study demonstrated the usefulness of considering viral population structure in epidemiological investigations and the effectiveness of widespread control measures in limiting viral spread.

INTRODUCTION

The latest highly pathogenic avian influenza (HPAI) epidemic in France was concentrated in the southwestern region of the country. It was caused by an H5N8 virus of the lineage 2.3.4.4 (A/Gs/Gd/1/96 clade) of Asian origin that was likely introduced by migratory birds (Bronner et al., 2017). The viral emergence (first incidence) in poultry farms was suspected (based on clinical symptoms) on a duck breeder farm in the Tarn *département* (a French administrative division) on 25 November 2016 and the infection was confirmed (based on lab diagnosis) on 28 November (A. Scoizec, *pers. comm.*). The last case of the epidemic was reported on 23 March 2017 (Guinat et al., 2018). Control measures were implemented immediately after the start of the epidemic. However, based on the manner of outbreak response, the epidemic period can be divided into two parts: 28 November 2016 – 2 February 2017 and 3 February – 23 March 2017. Control measures were considerably reinforced from 3 February 2017 onwards, with the establishment of a large buffer zone (including 463 communes: the smallest French

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administrative division) in which pre-emptive culling was deployed. By the end of the epidemic period, 484 farms had reported infections and approximately 7 million birds were culled (Guinat et al., 2018). The dynamics of the epidemic have been studied widely using epidemiological models based on incidence data. However, these models do not directly capture viral transmission dynamics between geographical subpopulations, which are key drivers of epidemic spread (Frost and Volz, 2013). Phylogeographic methods, on the other hand, can infer these interactions between subpopulations by integrating information on sampling *départements* with genomic data, which track the virus directly (Lemey et al., 2009).

Yet most viral phylogeographic studies either assume viral populations to be homogeneous, or do not explicitly model migration events between subpopulations, which makes results biased (De Maio et al., 2015). Structured coalescent models, on the other hand, explicitly model population structure and viral migration between subpopulations (Hudson, 1990). Despite being more realistic than unstructured models, these models are impractical to use on most data sets due to the sheer volume of computations involved (Vaughan et al., 2014). This problem has recently been addressed with the development of a marginal approximation of the structured coalescent model that is able to infer unbiased parameter estimates, and which is also computationally efficient for data with a large number of subpopulations (Müller et al., 2018). In the present study, we employed this cutting-edge implementation of the structured coalescent model to study the epidemic patterns of the 2016-17 HPAI outbreaks in French poultry. Specifically, we reconstructed a detailed phylogeographic history of HPAI spread between the seven most affected *départements* in southwest France. Further, employing a generalised linear model (GLM) extension of the marginal approximation model (Müller et al., 2019), we combined the genomic data with covariates of spatial spread and tested the effect of multiple potential predictors, including the nation-wide reinforcement of control measures.

MATERIALS AND METHODS

Genomic data, bioinformatics and phylogenetic analyses

HPAI H5N8 viruses were sampled on farms that experienced an outbreak between November 2016 and March 2017, and the samples were then sequenced at the French national reference laboratory for avian influenza (ANSES-Ploufragan, France). Each sequence was geocoded and associated with the *département* where the outbreak was located (Tarn, Aveyron, Lot-et-Garonne, Gers, Landes, Pyrénées-Atlantiques and Hautes-Pyrénées) along with the isolation date. The virus sequences were aligned using the MUSCLE multiple sequence alignment algorithm (Edgar, 2004) implemented in MegaX software with default parameters (Kumar et al., 2018). Viral genomes can cause recombination which, if undetected, may provide spurious genomic signals. We therefore checked for the absence of recombination in our aligned sequences using RDP v4.1 (Martin et al., 2015). We used five different algorithms, namely BootScan, CHIMERA, MaxCHI, RDP and SisScan. None of the algorithms detected any recombination events. Therefore, we used all available sequences (n = 196) across the seven *départements* for downstream analyses.

The marginal approximation of the structured coalescent (MASCOT) model

We conducted an exploratory phylogenetic analysis, based on a quick maximum likelihood method, to ensure the tree was structured appropriately and the samples collected earlier were closer to the root of the phylogenetic tree. This analysis justified the use of the structured and more computationally intensive MASCOT model (data not shown).

In our study, we modelled jointly the coalescing of viral lineages within départements and the viral migration between them using the MASCOT model, which is both computationally efficient and has been found to detect viral transmission dynamics reliably (Müller et al., 2018). This model also estimated the viral population size, called the effective population size (Ne), (Sjödin et al., 2005) in each département, allowing the comparison of the intensity of local spread between départements. Furthermore, to identify associations between time-dependent and time-independent putative explanatory variables and Ne or the migration rates between départements, we used an extension of the MASCOT that integrates a GLM framework (Müller et al., 2019).

Empirical predictors

To explain migration rates between *départements,* we used three predictors—geographic distance (measured as great circle distance) between each *département* (time-independent), whether two *départements* shared borders (time-independent) and the implementation of reinforced control measures (including culling, active surveillance and restrictions on live-duck movements) on 3 February 2017 onwards (time-dependent). Previous virological studies have shown that the temporal distribution of the number of outbreaks is a good predictor of *Ne* (Müller et al., 2019). To explain the *Ne* in each *département*, we therefore used as predictors the observed number of outbreaks in each *département* per week (time-dependent). Similar to an application of this model to the Ebola epidemic in West Africa (Müller et al., 2019), we further added four predictors for which we shifted the observed weekly incidence in the past (for 1 week or 3 weeks) and in the future (for 1 week or 3 weeks) so that each predictor reflected undetected infection before and after the observed epidemic. All non-binary variables were log transformed and standardised so that means and standard deviations equalled 0 and 1, respectively.

Parameter inference

The MASCOT analyses were conducted in a Bayesian framework with 30 million iterations using BEAST v2.6.3 (Bouckaert et al., 2014). A GTR+G nucleotide substitution model with gamma site heterogeneity using four rate categories and a strict molecular clock model were employed to describe virus evolution. The convergence and mixing of the MCMC chains were checked visually in Tracer v1.7.1 (Rambaut et al., 2018). The time-scaled phylogenetic tree with the most likely *département* of each lineage was annotated and visualized in FigTree v1.4.3 (Rambaut, 2016). To explain how much of the data informed the inclusion of a predictor, we estimated inclusion probability (likelihood of a predictor value falling within posterior distribution) (Müller et al., 2019) in R v4.0.3 (R Core Team, 2020).

RESULTS

Origin and trajectory of the HPAI epidemic

The reconstructed evolutionary relationships and the migration history of HPAI viral lineages are shown in Fig.1A in the form of an inferred time-scaled maximum clade credibility phylogenetic tree with different colours representing the most likely *départements*. A change in colour across tree branches represents migration events between *départements*. Figure 1B depicts the weekly observed distribution of the number of outbreaks in each of the seven *départements*. The overall pattern resembles the structure of the phylogenetic tree. The incidence peaks in each *département* overlap with phylogenetic clusters of samples from that

département over time. In other words, the fidelity between the epidemic distribution and the structure of the phylogenetic tree is strong. Tarn was inferred as the most likely source of the epidemic in the region with 99% posterior probability support. The time of the emergence was estimated to be around 14 November 2016 (95% credible interval: 3 - 25 November). Results suggest that the virus spread from Tarn to Aveyron, Lot-et-Garonne, Landes and Gers, and then to Pyrénées-Atlantiques from Landes. Lastly, the migration history inferred considerable viral admixture between Gers and Landes.

Predictors of HPAI migration rate between *départements*

Figure 2A shows the inferred predictors for the migration rates between *départements*. Results indicate that sharing borders (inclusion probability = 0.43) and the first epidemic period (0.46) were positively associated with migration rates. On the other hand, the results also suggest that geographic distance between *départements* was weakly negatively associated with migration rates (-0.17).

Predictors of HPAI effective population size within *départements*

Figure 2B depicts jointly inferred predictors of *Ne* within *départements*. Weekly incidence data shifted three weeks earlier than the observed date better explained (0.92) the *Ne* (thus included) than the other predictors, such as the non-shifted observed weekly incidence predictor (0.55), assumed one week earlier (-0.45), one week later (0.52) and three weeks later (-0.23).

DISCUSSION

The present study investigated the epidemic patterns of the 2016-17 HPAI outbreaks in France using a structured phylogeographic model, which combined genomic data with information on sampling *départements*. We used concatenated whole genome viral HPAI H5N8 sequences from 196 infected samples that were collected from infected poultry farms across seven *départements* between 28 November 2016 and 23 March 2017. The results detected detailed migration events between the seven affected *départements*, in addition to identifying Tarn as the most likely origin of the epidemic. Further, the results identified predictors that best explained the epidemic pattern in terms of the viral migration rate between *départements* and the temporal pattern of the change in the *Ne* within *départements*. The sharing of borders between *départements* and the timing of the strengthening of control measures best explained the migration rates. The weekly incidence per week explained the *Ne* but the predictor that was adjusted to reflect an earlier emergence than observed had the largest positive support.
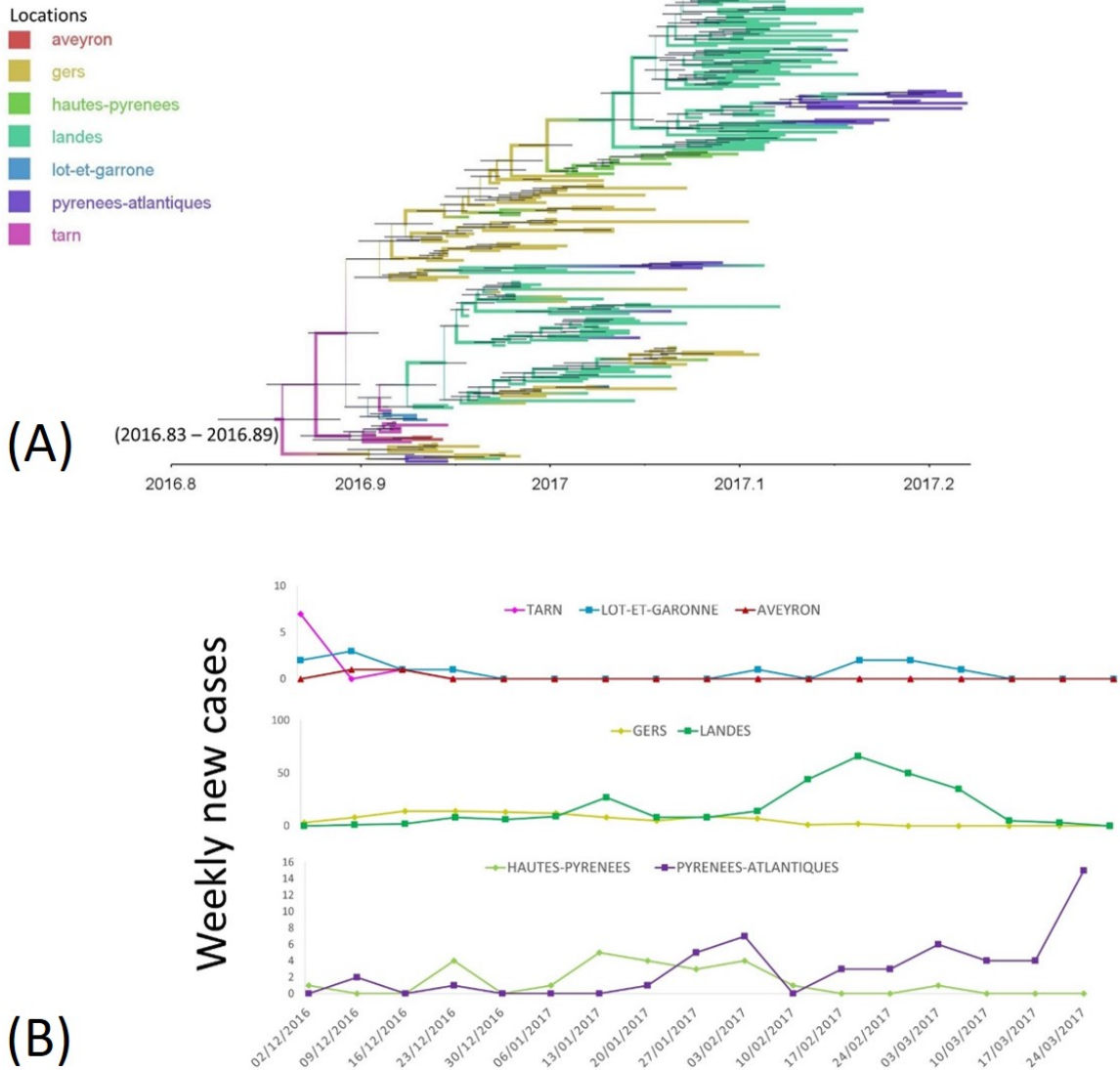
Figure 1. Temporal phylogeography of the HPAI epidemic in southwest France (2016-17). (A) Inferred maximum clade credibility tree of viral sequences. The colour of tree branches indicates their most likely *département* (see legend). A colour change on a branch indicates a virus migration. Horizontal lines at nodes represent 95% highest posterior density interval of node height (estimated time of occurrence of a specific node), which reflects the uncertainty associated with time estimation. The inferred time of the most recent common ancestor to all sequences, which can be interpreted as the time of virus introduction in France, is mentioned within the parenthesis. (B) Weekly incidence by *département*. The x-axis represents time in weeks and overlaps with the time scale of the phylogenetic tree. The colour scheme identifying each *département* mirrors that of the phylogenetic tree.

(A) Predictors for viral migration rates between locations

(B) Predictors for viral effective population size (Ne)
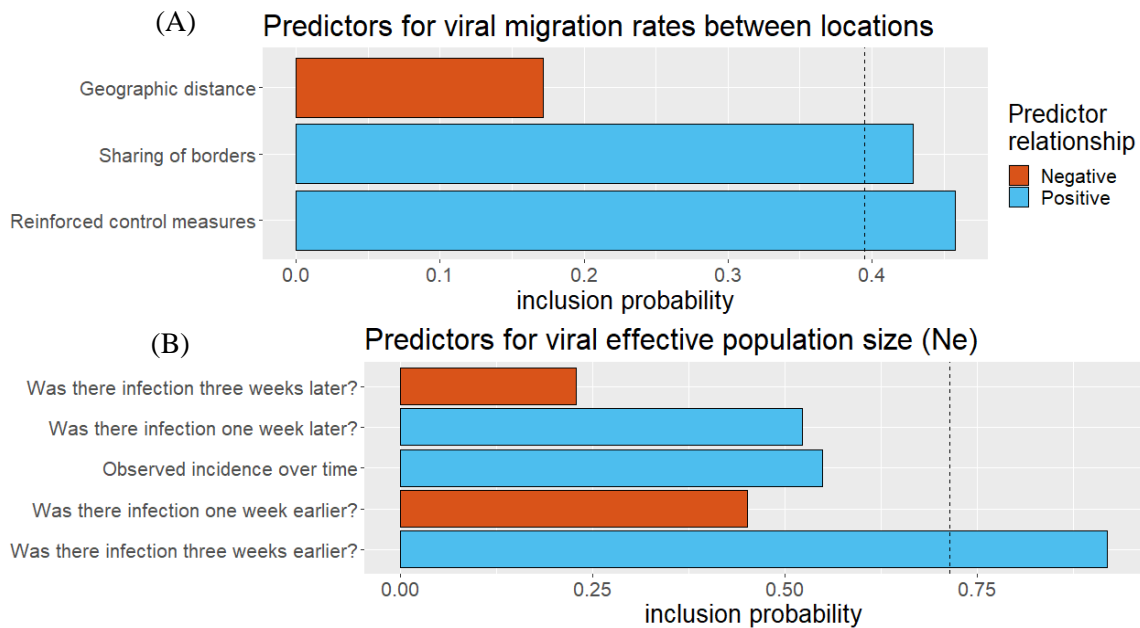
Figure 2. Inferred predictors of the spread of the HPAI epidemic in southwest France (2016-17). (A) Predictors for migration rates between *départements* and (B) predictors for effective population size (*Ne*) within *départements*. The x-axis shows the inclusion probability of the predictors being included in explaining the response variables. The red colour represents predictors for which the median value of the coefficient is negative (inverse relationship with response variables) and the blue colour represents predictors for which the median value of the coefficient is positive (direct relationship with response variables). The magnitude of the coefficients of *Ne* predictors and geographic distance did not provide any direct interpretation as the predictor values (weekly incidence numbers) were standardised. The dashed line corresponds to a Bayes Factor of 10 equivalent to "positive support" in favour of the predictor concerned.

The structured coalescent model identified Tarn as the *département* where the epidemic originated with the highest probability, in comparison to other *départements*. This inference is independently supported by the actual identification of the first incidence in Tarn on 25 November 2016 and the infection was confirmed (based on lab diagnosis) on 28 November. This timing is also congruent with genetically estimated emergence (14 November 2016; 95% credible interval: 3 - 25 November). The difference is partially an outcome of rounding off decimal estimates into a full day during conversion of fractional years into actual dates. While this independent evidence provides ample confidence concerning the estimates, it must be remembered that the ancestral state reconstruction is a difficult process and models are often fraught with implicit assumptions (e.g., assumed exhaustive sample), thus requiring cautious interpretation (Cunningham, 1999; Hahn and Nakhleh, 2016). The reconstructed migration history across the tree depicts a complex viral movement dynamic that could be used to test the dominant hypothesis that the virus spread through the transport of live poultry between farms. This could be achieved by comparing the phylogenetic tree with epidemiological data collected from transport network companies (Guinat et al., 2020) or outbreak investigation surveys (Mulatti et al., 2018).

The sharing of borders between *départements* was positively associated with the migration rate. This suggested that the virus may have spread—perhaps through movements of birds (poultry or wild) or fomites (vehicles, farm equipment or even humans)—between adjacent

départements more frequently than *départements* far from each other. In other words, this predictor could be a function, at least in part, of geographic distance between *départements*, which is supported, albeit weakly, by the geographic distance predictor being inversely correlated to the migration rate. It would be worth exploring in the future whether *département* adjacency (border sharing) translates into actual poultry transport volume and/or frequency (as poultry transport between farms is likely to be a significant contributor to HPAI dispersal between *départements*). The positive correlation between the nation-wide reinforcement of control measures and the migration rate between *départements* suggests that the strengthening of control measures (pre-emptive culling, transport ban and systematic testing before movements) affected viral migration between *départements*. However, the extent of the predictor's influence on the viral migration rate was not clear from the binary predictor. Additionally, the effect of each measure—pre-emptive culling, transport ban and active surveillance—on viral spread will also require investigation.

The weekly number of reported outbreaks had a strong inclusion probability. However, the support diminished and the predictor was not included after the addition of the four offset predictors. This pattern could reflect the fact that the virus was already circulating within poultry in the region a few weeks before the first case was notified. The estimate of the root age, with a wide range encompassing multiple weeks around the observed date of epidemic origin, may also suggest the virus was already introduced a couple of weeks earlier than the first detection in Tarn. It should be noted, however, that Ne change patterns must be interpreted cautiously. Many real world data may not snugly fit the neutral model of sequence evolution that links estimated population size to observed genetic diversity in the coalescent model (Gillespie, 2001). In that case, Ne interpretations may not be meaningful. In the context of the epidemiology of viral pathogens, Ne is not directly proportional to the number of infected hosts in general except during the initial exponential epidemic growth phase (Frost and Volz, 2010). Thus, changes in the Ne within *départements* may not readily reflect the number of infected birds.

Despite using multiple covariates of both migratory rates and Ne, current predictors are not exhaustive. We therefore likely overlooked other potential drivers of viral migration, such as the magnitude and frequency of poultry transports between *départements*, presence of reservoirs (wild birds, backyard flocks, environment) and movements of people. Similarly, to explain the change in the Ne, multiple predictors related to host population size—such as poultry density in different *départements*, density of wild birds and density of humans (potential spreaders of infected material)—were not considered. Furthermore, the predictors adopted in this study were scale-dependent. More exact and high-resolution predictors should be included in these models in the future to test even more specific hypotheses.

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UNRAVELLING DIRECT AND INDIRECT CONTACT PATTERNS OF FRENCH DUCK FARMS USING MULTILAYER NETWORK ANALYSES

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SUMMARY

Live animal movements generate contacts between farms that are direct (via the exchange of live animals) and indirect connections (via the transit of transport trucks). We analysed how networks resulting from French duck movements evolved between 2016 and 2018. The study period included the most intense phase of the Highly Pathogenic Avian Influenza epidemic (H5N8, 2016-2017), where numerous control measures were implemented to curb the epidemic. Results showed that both networks were substantially modified during the most intense phase of the epidemic (January–March 2017) compared to those estimated during the non-epidemic period. However, the topology has since returned to the pre-epidemic configuration. Moreover, the transport truck network formed a larger cluster than the network formed by the exchange of live animals, thus suggesting that it could be an important vector of disease transmission.

INTRODUCTION

In 2016–2017, the emergence and spread of Highly Pathogenic Avian Influenza (HPAI) H5N8 in Europe resulted in a total of 1,108 poultry outbreaks (Alarcon et al., 2018) in several countries. With more than 400 farms affected, France was the hardest hit country in Europe, due to extensive farm-to-farm spread, predominantly in ducks, followed by Hungary (200 infected farms). Producing over 965 million birds per year, the French poultry industry is the fifth-largest in the world. It was severely affected by the HPAI H5N8, with 81% of the farms detected being part of the foie gras sector. This industry involves distinct production stages which may be handled by different farms: rearing (1-day-old ducklings are reared for ≈ three weeks), breeding (1-day to 3-week-old ducks are bred for ≈ 9–12 weeks), and force-feeding (12-week-old ducks are force-fed for ≈ 12 days) (Guinat et al., 2020). Previous studies showed that trade-related transport and movements of fattening ducks have played a crucial role in the early stages of the HPAI H5N8 epidemic (Guinat et al., 2019, 2020).

Network analysis provides an analytical framework to study the contact patterns between farms that are generated by animal movements. Due to the nature of these movements in the foie-gras sector, contacts are unidirectional from one farm to another. In addition to the risk posed by the movements of potentially infected live animals, contaminated trucks used for

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these movements can also play a significant role in the transmission process through the spread of fomites. Investigating both networks is key to understanding the links and potential disease spread between otherwise unconnected farms.

In recent years, numerous studies have used network analysis to describe and inform disease control interventions in various livestock species, including cattle, small ruminants (Kao et al., 2006; Ortiz-Pelaez et al., 2006; Marquetoux et al., 2016) and pigs (Bigras-Poulin et al., 2007; Rautureau et al., 2011, 2012; Thakur et al., 2016; Salines et al., 2017; Porphyre et al.; 2020). Of these, only three studies (Thakur et al., 2016; Salines et al., 2017; Porphyre et al.; 2020) have analysed the coexisting and interacting layers of networks generated by the transit of trucks used for the movements. Findings suggested that farms are more connected through the transit of trucks network than in the animal exchange network. Thus, it emphasized that solely focusing on live animal movements would likely underestimate the risk of disease transmission. Although several studies have analysed the role of live poultry trade networks in the spread of avian influenza (Soares Magalhães et al., 2010; Kurscheid et al., 2017; Guinat et al., 2020), the potential role of transit trucks in HPAI spread has thus far remained unknown.

The objectives of this study were twofold: (i) to characterise the direct and indirect contact patterns resulting from trading of live birds between duck farms in France based on a multilayer approach, and (ii) to investigate variations in the contact networks between the most intense phase of the HPAI H5N8 epidemic from 2016-2017 to non-epidemic periods.

MATERIALS AND METHODS

Data processing and network building

The recorded movements were collected from one of the most important French private transport companies which operates in southwest France. The dataset consisted of movements that occurred between 2016 and 2018. It contained movement characteristics (loading or unloading) and farm details (ID, GPS coordinates). We only considered movements between farms, excluding movements to slaughterhouses as they were not expected to play a significant role in pathogen spread. The dataset was reorganised into truck round records (defined as the loading of ducks on one farm followed by the unloading of ducks on successive farms by a given truck on a given day). A single truck can load birds from one breeding site and unload them at several different force-feeding locations visited successively during its round (Ministère de l'agriculture et de l'alimentation, 2018). The production type of the farms was determined based on the type of movements the farms were involved in, i.e., breeding (for farms only sending ducks: loading), force-feeding (for those only receiving ducks: unloading). Farms with both breeding and force-feeding activities were categorised as mixed farms.

A multilayer network analysis approach inspired by Salines et al. (2017) was taken to describe these movements. One-mode directed networks were created: farms were considered as nodes, and unidirectional contacts between two nodes were considered as links. Two types of connections were considered, generating two types of networks: (i) in the animal introduction network (AIN), links represent the movements of live animals being exchanged between breeding and force-feeding farms; (ii) in the transit network (TN), links represent the rounds of a truck that could have visited several farms to unload different batches originating from one flock.
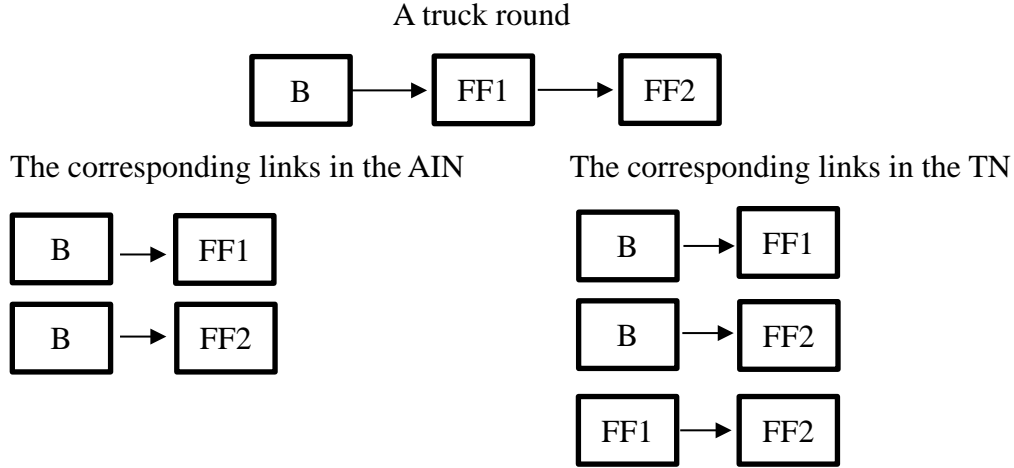
Figure 1. Links in the animal introduction network (AIN) and the transit network (TN) generated from truck round data. B: Breeding, FFx: Force-feeding, with "x" indicating the order the movement occurred in (e.g. FF1 = first movement).

Data analysis

Static network description: Both networks were described using the farm-to-farm directed networks generated with the movement data aggregated yearly for the period from January 2018 to December 2018. Both farm-level and network-level descriptive statistics were computed for each network (Table 1). The distributions of the three main centrality measurements were computed for each farm type: in-degree (the number of different farms from which a farm receives animals), out-degree (the number of contacts originating from a specific farm) and betweenness centralities (the frequency at which a node is located on the shortest path between any two pairs of nodes in the network). We also investigated if there was a correlation between the centrality statistics and the farm's production activities. All networks were generated and analysed using the igraph package (Csardi and Nepusz, 2006) and tidyverse 1.3.0 (Wickham et al., 2019) in R version 4.0.2 (R Core Team, 2020).

Next, we assessed the node *in*-loyalty which measures the tendency of a farmer to purchase animals from the same sellers (for the AIN) and of a transport company to do the same rounds (for the TN) between two consecutive quarters in 2018. The quarterly time window was defined to match the duration between the entry of two successive batches of ducks into a breeding farm. More specifically, the node loyalty index θ at time t measures the fraction of preserved direct contacts of a farm between two consecutive periods; $t-1$ and t. The loyalty index varies between zero and one, with zero indicating that all connections were different between the periods, and one indicating that all links were maintained.

This approach is based on the Jaccard index, and was calculated as follows (Eq. (1)):

$$\theta_i^{t-1,t} = \frac{\left| Y_i^{t-1} \cap Y_i^t \right|}{\left| Y_i^{t-1} \cup Y_i^t \right|} \tag{1}$$

with $Y_i^{t-1,t}$ defined as the set of in-going neighbours of farms i in the quarter $t-1$, and $\theta_i^{t-1,t}$ the loyalty index between the period $t-1$ and t (Schulz et al., 2017).

Impact of the epidemic on the topology of the network: We also analysed the impact of the epidemic on the structure of the network by comparing network statistics of the epidemic period (January – March 2017) with those of the year before (January – March 2016) and the year after (January – March 2018). The statistics and the distribution of betweenness and degree were used to investigate the evolution of topology of the networks over the study period.

Table 1. Description of network analysis terminology as used in the context of animal movement networks, based on material discussed in: (Dutta et al., 2014; Thakur et al., 2016; Salines et al., 2017; Machado et al., 2019).

Term	Level	Definitions
Node	Farm	The unit of interest in the network. In our case, a farm that was active during the study period.
Link	Farm	The unidirectional contact between two farms (in our case, movement of live animals or transit of trucks).
Average path length	Network	The average number of links along the shortest paths between all possible pairs of nodes.
Density	Network	The proportion of actual links present over all possible links in the network.
Diameter	Network	The longest geodesic for all node pairs in the network.
Clustering coefficient	Network	The average proportion of neighbours of a farm that are linked to each other. As a global measure, clustering coefficient quantifies 'cliquishness' within the network.
Assortativity	Network	The Pearson correlation coefficient between the degrees of linked farms.
Strongly Connected Component (SCC)	Network	The subset of networks in which every node can be reached from every other node via one or several directed paths.

RESULTS

Network description

Comparison of the animal introduction network (AIN) and the transit network (TN): In 2018, both networks contained 395 active farms. The overall number of links was around 1.4 times higher in the TN than in the AIN (4978 and 3440 links, respectively). On average, in the TN a farm was in contact with 25 other farms (average degree), while in the AIN, a farm was in contact with 17 other farms. Moreover, any two given farms were separated on average (average path length) by 3.1 links in the TN versus 2.6 links in the AIN. There were 377 SCCs in the AIN, compared to 240 SCCs in the TN, suggesting that the AIN was more fragmented than the TN. However, larger SCCs were detected in the TN. The largest SCC in the TN consisted of 152 (38%) active farms, which is eight times larger than the largest SCC in the AIN.

Table 2. Description of network indicators of the duck movement networks in south-west France, 2018. AIN: animal introduction network; TN: transit network; SCC: strongly connected components.

Parameters	January – December 2018	
	AIN	TN
No. of active nodes	395	395
No. of active links	3440	4978
Average degree	17.4	25.2
Average path length	2.6	3.1
Density	0.02	0.03
Diameter	7	9
Clustering coefficient	0.1	0.2
Assortativity	0.08	0.07
Number of SCCs	377	240
Size of the largest SCC	19 (4.8%)	152 (38.5%)

At the network level, the TN was 45% denser than the AIN, with density statistics of 0.03 and 0.02, respectively. The TN also had a longer diameter (1.28 times longer) compared to the AIN (9 versus 7). The clustering coefficients of the network were two times higher in the TN than in the AIN, suggesting that nodes were more tightly connected in the TN than in the AIN. The assortativity was positive for both networks, indicating that farms were more often linked to farms with similar degrees.

The degree distribution in 2018 for both networks was right-skewed (figure not shown), meaning that the majority of the farms had only a low degree. A significant difference (Kruskal-Wallis test: p-value < 0.0001) was also observed in the centrality values (in-degree and betweenness) between types of farm production activities in both networks. Farms with force-feeding production activities had a median in-degree value of 18 (min-max: 1-44) in the AIN as opposed to farms with mixed production activities which had a median in-degree of 8 (min-max: 0-89). For the TN, these values were 25 (min-max: 1-67) and 12 (min-max: 0-149), respectively. In the AIN, only the mixed production farm type was detected on the shortest path between any two pairs of nodes in the network (betweenness). Its distribution had a median value of 0.0049 (min-max: 0-0.0076) while for the TN it was 0.0036 (min-max: 0-0.0762). For the out-degree centrality, in the AIN, there was a significant difference between the production types, breeding farms and mixed farms (p-value $= 0.021$). However, in the TN, there was no significant difference between farm production types for the out-degree (p-value $= 0.47$).
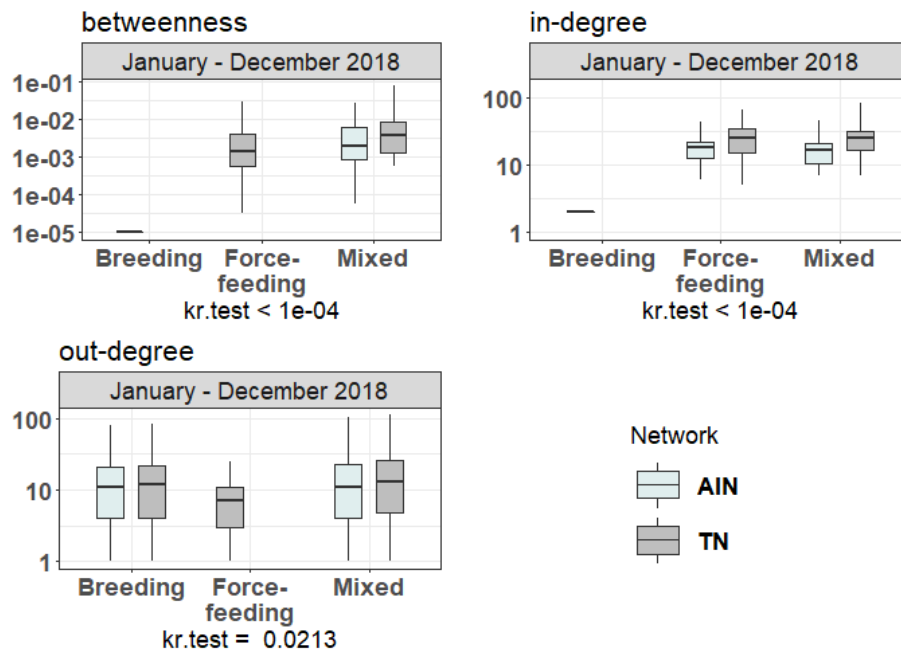
Figure 2. Distribution of betweenness and degree centralities in southwest France, 2018, according to different duck production types for the Animal Introduction Network (AIN) and Transit Network (TN). kr.test: Kruskal-Wallis test whether there is a difference between farm productions type.

The distribution of loyalty values computed in the AIN showed one peak irrespective of the network of the time window. Whatever the network considered (AIN or TN), the average loyalty index was around 15%.
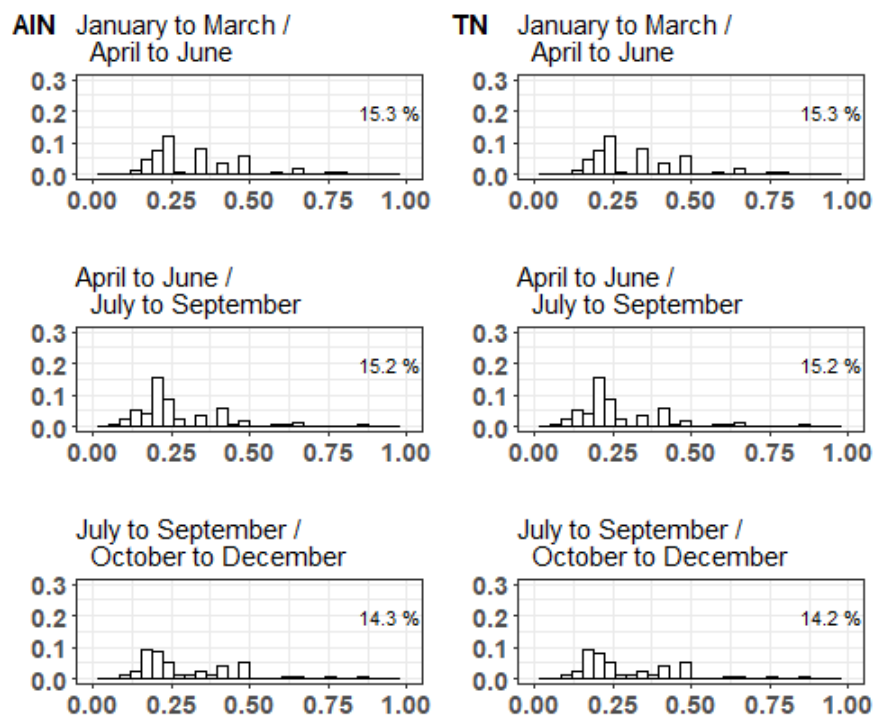
Figure 3. Node loyalty distributions in the duck movement network in southwest France in 2018 using the animal introduction network (AIN) and the transit network (TN). The average

percentage of origins maintained from one period to another is shown in the upper right corner.

Influence of the epidemic on the network: For the period before the epidemic (first quarter of 2016), the AIN contained 2.45 times more active nodes (319) than during the epidemic (130). The number of links in the AIN before the epidemic (896) was 3.7 times higher than during the epidemic (236). A farm on average exchanged animals with six different farms before the epidemic, while during the epidemic, this indicator decreased to four different farms (average degree). The average path length dropped by 43% during the epidemic from 2.3 to 1 link. No largest SCC was detected in the AIN, as each component detected contained less than 1% of active nodes for both 2016 and 2017. The assortativity was 1.4 times higher during the epidemic (0.3) compared to before (0.2). The clustering coefficient decreased from 0.03 to 0 between the pre-epidemic and epidemic period. In the TN, the clustering coefficient remained stable at around 0.16. The size of the largest SCC detected before the epidemic contained 71 active nodes as opposed to two active nodes during the epidemic.

Table 3. Impact of the epidemic on the network. Network indicators of the animal introduction network (AIN) and transit network (TN) of French duck movements for the first quarter (January-March) of each year; SCC: strongly connected components.

Parameters	AIN			TN		
	2016	2017	2018	2016	2017	2018
No. of active nodes	319	130	310	319	130	310
No. of active links	896	236	700	1256	284	998
Average degree	5.6	3.6	4.5	7.8	4.4	6.4
Average path length	2.3	1.0	2.0	5.1	1.4	5.0
Density	0.01	0.01	0.01	0.01	0.02	0.01
Diameter	6	2	6	15	4	18
Clustering coefficient	0.03	0	0.02	0.17	0.16	0.16
Assortativity	0.2	0.3	0.1	0.4	0.2	0.1
Number of SCCs	319	130	309	248	125	236
Size of the largest SCC	1(0.3%)	1(0.8%)	2(0.7%)	71(22.1%)	2(1.5%)	69(22.3 %)

For the period after the epidemic, the number of active nodes and links were more than double that of the period during the epidemic. The number of different farms with which a given farm exchanged animals (average degree) was up by 25% (as compared to during the epidemic), and the average path length was doubled. In the TN, the size of the largest SCC detected went from two during the epidemic to 69 after the epidemic. These results led us to conclude that the epidemic heavily impacted the topology of the network.

Irrespective of the network, both degree and betweenness distributions decreased during the epidemic. For example, the betweenness distribution for farms with mixed production activities was the most impacted during the epidemic with the distribution considerably decreased, thus indicating that these farms were less active. It worth noting that even though the median of the distribution (in-degree, out-degree, and betweenness) has returned to the pre-epidemic level (except for betweenness), the distribution has become more compact. Additionally, breeding farms were located on the shortest path (betweenness) only in the period before the epidemic.
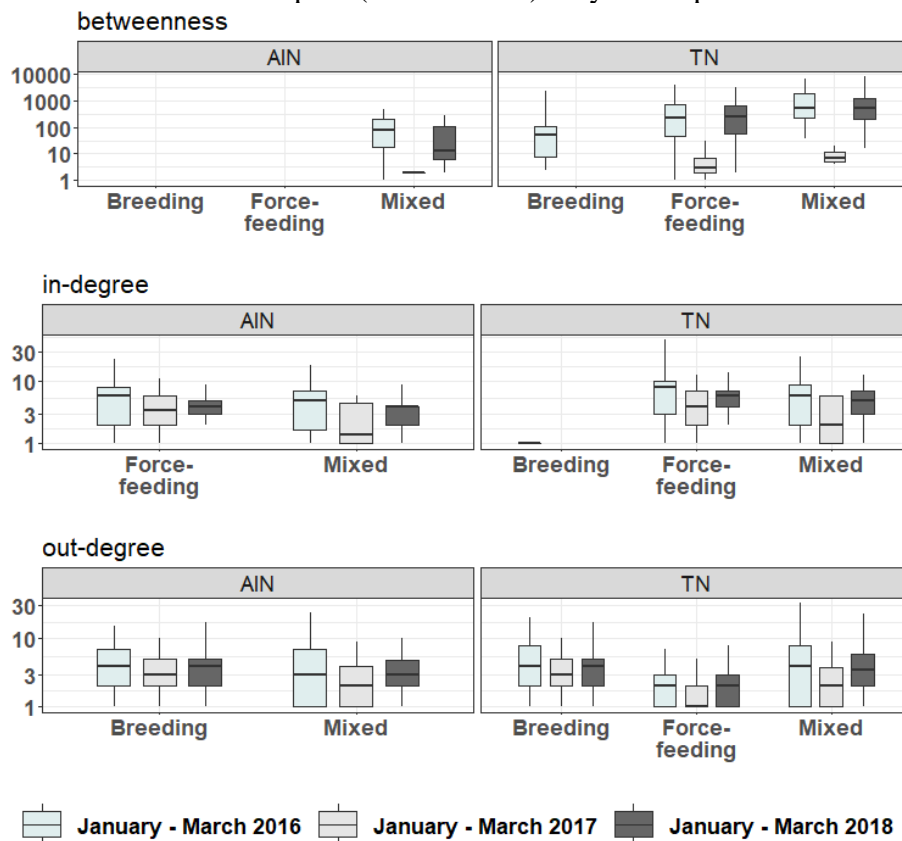
Figure 4. Impact of the epidemic on the centrality distributions.

DISCUSSION

This work described the contact networks of duck farms based on live animal movements and the transit of trucks. We also evaluated variations in the contact patterns before, during and after the HPAI H5N8 epidemic that profoundly impacted the French poultry industry in 2016-2017.

This study showed that farms in southwest France are highly connected through the exchange of birds, which is consistent with a previous analysis of the AIN of ducks in France (Guinat et al., 2020). Most importantly, this study demonstrates that the trucks used to transport live birds are a significant source of inter-farm connectivity. The TN puts many more farms in contact than the AIN as its largest SCC was eight times bigger than the largest SCC in the AIN. The connections within SCCs provide a vector for the transmission of many diseases, including HPAI. These findings are consistent with what has been found in similar studies of hierarchical pig transport networks (Bigras-Poulin et al., 2007; Thakur et al., 2016; Salines et al., 2017). Consequently, knowing the contact pattern of these movements could prove essential in managing disease spread and in estimating the worst-case scenario in terms of the size of an

epidemic (Kao et al., 2006). Our results also demonstrated that node loyalty in both networks was highly volatile. On average, node loyalty from one trimester to the next never exceeded 15%, indicating future contacts might be challenging to predict.

It is essential to assess the risk potential of disease spread from the index farm and to be able to trace animals at a fine-scale to achieve effective control. Network analysis of the contact network shows that the centrality distribution presented here can be used to implement targeted intervention measures (farm-level). This analysis has shown that the network indicators are significantly different for each farm type. Irrespective of the network, mixed production farms appeared more central (betweenness and degree values) than specialised breeding or force-feeding farms. In their dual role, mixed farms have a more significant potential to become infected and to transmit the virus to other farms, to connect different production activities and to form hubs. Hubs can facilitate the spread of disease in a hierarchical network, and as such, they should be targeted by control measures during an epidemic (Rautureau et al., 2011).

After showing a strong impact from the epidemic, in 2018, network statistics returned to the same levels that were recorded before the epidemic. Whatever the time scale studied (yearly or quarterly), the mean degree, average path length, and the size of the largest SCC after the epidemic (2018) returned to values similar to those noted before the epidemic (2016). Due to the high connectivity observed in duck production, attention should be given to improving biosecurity on farms and during transport to limit the risks of pathogen spread. Following the 2017 epidemic, essential measures have been taken to strengthen biosecurity over the production chain. These measures included a ban on successive loadings during a transit round, as well as the careful cleaning and disinfection of trucks and transport cages after each round. Biosecurity at the farm-level has been reinforced following a new national regulation issued in February 2016. These regulations are periodically enforced by audits, carried out by the veterinary health services (Delpont et al., 2020). Systematic virological screening before animals are moved to force-feeding farms also has been implemented since the end of 2017 for long-distance movements (more than 20 km), especially during the high-risk period (Ministère de l'agriculture, 2016). Additional measures that can decrease the size of large components in the TN, such as shortening the length of a transit round, could prove useful in managing future outbreaks. Furthermore, efforts have been made to build an exhaustive database of movement records for both live animal movements and truck transit, which will be integrated into an information system that can be consulted by professionals to facilitate tracking and alert strategies in the case of disease outbreaks.

A potential limitation to the results presented is that the data used were collected from a single transport company. Potential bias in network-level measures (fragmentation and lack exhaustivity) may exist with an underestimation of the true connectivity of farms. To complement these results and gain a more comprehensive view of farms' connectivity networks, the analysis presented here should be applied to a more general level dataset consisting of all transport companies.

In conclusion, we used network indicators to analyse the AIN and TN of ducks in France and assessed how these networks were modified by the 2016-2017 epidemic of HPAI H5N8. This study shed light on the relevance of transit movements in forming new links between farms that otherwise would be unconnected by the exchange of animals. Due to these connections, large components were detected and can be viewed as risk structures for an infectious disease like avian influenza. The relative contribution of these two networks to HPAI transmission events could be assessed using a network k-test (VanderWaal et al., 2016; Guinat et al., 2020). Finally, the networks generated could be used together in an epidemiological

model to take into account the two modes of transmission (direct and indirect) to have an adequate mechanistic view of transmission patterns.

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DAIRY COW PRODUCTIVITY

INCREASED LIVING SPACE FOR HOUSED DAIRY COWS IMPROVED MILK

VOLUME PRODUCTION IN RANDOMISED CONTROL TRIAL

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SUMMARY

Living space is a basic requirement for all farmed animals yet its fundamental effect on dairy cow physiology, health, production and welfare remains unclear. The research reported here relates to the ethics of farming as well as the understanding of animal biology and how livestock interact with their housed environment. The study comprised a year-long randomised control trial to evaluate the impact of increased living space for adult dairy cows. Results indicated that when cows are given an increased amount of living space, their production, in terms of milk volume output, increases. Fundamental physiology is also altered; for example, cows with more space spent more time ruminating. This provides evidence to show that there is potential for current on-farm management practices to be improved sustainably through increasing living space.

INTRODUCTION

In the livestock industry, dairy cows have complex housing scenarios, ranging from outdoor pasture to indoor tiestalls, as well as freestall and free-walk designs (Bewley et al., 2017), with different farms displaying a huge variety of quality in all of these scenarios. Most dairy cows globally are housed for four to six months of the year with an increasing number being housed in year-round systems (March et al., 2014; Barkema et al., 2015; USDA, 2016). The housed environment is likely to have a fundamental impact on health and wellbeing, yet virtually no evidence exists on the long-term effects of specific housing conditions.

Of the housing scenarios, freestalls have become the predominant method of housing in most countries worldwide (Bewley et al., 2017), but this infrastructure is still variable. For example, commercial dairy farms in the United Kingdom (UK) ranged from having 5.4-12.7m^2 total space per cow (Thompson et al., 2020), and Norwegian herds had a range between 4.5-8.9m^2 (Naess, 2010). Research focussed on humans suggests space allowance as the most important characteristic for assessing housing quality (WHO, 2016; Boch et al., 2020), but similar research has not been undertaken in livestock. Much of the focus in housed dairy cows has been centred around optimising the freestall design, including cubicle dimensions (Bickert, 2000), lying surfaces (Bickert, 2000; Bewley et al., 2017) and stocking density (Fregonesi et al., 2007; Charlton et al., 2014; Talebi et al., 2014). Total space allowance should be the simplest recommendation for freestall housing but currently it is extremely varied and based

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on historical building design, such as the United States recommendation of 7.4m^2 from the 1980's (Bickert and Light, 1982). Further worldwide sources indicate recommended space allowances range from 6m^2 to 11m^2 (NFACC, 2009; Red Tractor, 2014; AHDB Dairy, 2018; SPCA, 2018). However, none of these recommendations have a clear scientific basis.

A reason for the lack of scientific evidence around space allowances is that spatial set-ups are further complicated by shed layouts. For example, there are disputes around the ambiguous definition of "loafing spaces" and whether the definition includes passageways (Thompson et al., 2020). Recently, a novel term "living space" has been proposed to allow easy quantification of the 'space above baseline requirements' for all freestall-housed dairy cows (Thompson et al., 2020). However, no trials have yet been conducted to test the effects of increasing floor space allowance whilst maintaining the proportions of other key areas such as freestall stocking density and feed-face length.

Key aspects of dairy sustainability rely on herd performance, thus managing dairy cows in appropriate housing will aid this. Productive performance of dairy cows is based on milk volume per unit of time, and critical to this is the conversion of feed into milk as this drives the profitability of dairy enterprises. When milk production can be increased per unit of feed, dairy enterprises become more sustainable but if the environment affects feed intakes or causes the animal to use energy for behaviours such as thermoregulation or competition for resources, milk production efficiency will be lost. This means that the environment in which these animals are kept is likely to be crucial for the sustainability of the dairy industry.

For the dairy sector to be sustainable it must also adhere to the social pillar, meaning that changes to the industry must be accepted by both farmers and consumers by taking into account animal welfare and wellbeing (Ventura et al., 2016; Jackson et al., 2020). Animal wellbeing is a subjective and complex measure, but the housed environment must provide a positive quality of life for farmed animals (Mandel et al., 2016; Bertocchi et al., 2018).

It is clear that housed environments are varied, and that recommendations are not based on firm scientific evidence. The primary aim of this research was to evaluate the impact of increased living space within freestall housing on the productivity of dairy cows.

MATERIALS AND METHODS

Study Design

A randomised controlled trial was undertaken to assess the impact of living space allowance on milk production and rumination time. This took place at the University of Nottingham in a new, specifically designed cow facility which allowed modification of the internal layout. Ethical permission was granted by the University of Nottingham, and the study was carried out under the Animal [Scientific Procedures] Act 1986.

The study was undertaken for 364 consecutive days. Two groups of animals were formed and (based on sample size calculations) a minimum of 30 individuals per group were required at all times to observe a significant difference of 0.5 kg milk per day. Cows were paired by parity and days in milk (DIM). Due to the length of study, the design was complex with pairs of cows exiting the trial and immediately being replaced with a fresh cow pair (<14DIM). Overall, 150 part-cow-lactations were included in the study (75 pairs). Cows were randomly assigned to a treatment group using Microsoft Excel *RAND()* function (Microsoft, 2016).

The housing was set-up using two identical pens, with the exception of the trial intervention of the total available floor space allowance; a 'high space' group had wider passageways and an indoor loafing space area. The high space group was provided with a total area of $14m^2$ ($6.5m^2$ living space) whereas the control group had $9m^2$ ($3m^2$ living space). All other aspects of the shed were identical, for example each group had access to 39 deep sand freestalls, the same length of feedface, one Lely Astronaut A4 milking robot, identical water access and environmental enrichment.

To ensure no bias was introduced through environmental effects, a cross over design was used; cow groups alongside their environment changed sides every 91 days.

Due to the nature of the trial researchers could not be blinded to study interventions and outcomes, although most outcome measurements were made objectively using sensors or laboratory equipment. Blinding was introduced at the analytic stage with researchers undertaking the statistical analysis being blinded to cow and intervention group identities.

Milk production

Data Collection: Daily data for milk volume and rumination times were sourced from the on-farm 'Time 4 Cows' (T4C) Lely system (Lely, 2019). Data were removed from this analysis from the housing cross-over days (3 days) where cows were disrupted due to readjustment of the environment and movement of all cows within the environment.

Statistical Analysis: All data analysis was undertaken using R statistical software, version R-3.6.1(R Core Team, 2020) , using the following packages: tidyverse (Wickham, 2019), lme4 (Bates et al., 2015), minpack.lm (Elzhov et al., 2016), gridExtra (Auguie, 2017) and sjPlot (Lüdecke, 2020). Graphical assessments were undertaken to visualise data patterns and statistical models were built for final inference.

Milk Volume (Model 1) Analysis: Non-linear models were used to assess and identify differences found in milk production between trial group. Data were incorporated into lactation curve models as described by Ehrlich (Ehrlich, 2013), to produce a single curve for each group. Model complexity and fit were checked. Predicted lactation curves were created for each trial group to visualise differences between intervention group by stage of lactation.

Milk Constituents (Model 2): A monthly milk sample from each cow was analysed for milk protein and fat using an accredited external laboratory (QMMS ltd, Somerset, UK). A value for the total kilograms of constituents produced per cow per day was calculated using the formula: total solid % x 1.03 (cow milk density) x daily milk volume (Rutz et al., 1955). Total solid % was the sum of each cow's fat and protein percentage. Samples where a reading for fat and protein equalled zero were removed from the dataset as a missing result. Non-linear models were used for analysis following methods described above for milk volume.

Rumination (Model 3): Rumination data were collected via sound monitor microphones in Lely neck collars; part of the QWES-HR Lely Tag system (Schirmann et al., 2009; Ambriz-Vilchis et al., 2015). A conventional mixed effects model, Eq.(1), was used to investigate the patterns of rumination time between the trial group and DIM:

$$y_{ij} = A + D_{ij} * T_j \beta_1 + u_j + \epsilon_{ij} \tag{1}$$
$$u_j \sim N(0, \sigma_j^2)$$

where subscripts *i* and *j* denote the *i*th observation of the *j*th cow respectively, y_{ij} = daily rumination time (minutes). A = intercept value; D = the days in milk (continuous) of the *i*th observation; T = trial group (categorical: high vs control space); β_1 = vector of coefficients for $D_{ij} * T$; u_j = random effect to account for residual variation between cow-parity (assumed to be normally distributed with mean = 0 and variance = σ2v); ϵ_{ij} = the residual model error.

Cow-lactation was set as a random effect to account for repeated measures of rumination within cow. Model fit was evaluated using influence, delta-betas and residual analyses (Loy et al., 2017). Predicted lactation curves were created for each trial group to visualise differences between intervention group by stage of lactation.

RESULTS

The results described in this section are preliminary.

Total milk production

A total of 150 cow-parities were included in the study (75 unique cow pairings) with 25,614 daily milk yield observations recorded. Over the 364-day trial period, preliminary findings indicated that animals in the high space group produced a total of ~575,000 litres of milk compared to ~557,000 litres in the control group. For recordings between 1-305 days in milk (24,449 observations), the high space group produced ~556,000 litres and the control group ~539,800 litres.

Total milk volume production (Model 1)

From Model 1, predictions were made for the lactation curves of the trial groups, as seen in Fig.1a. Cows in the high-space group produced 13,602 L of milk within the first 305 DIM, substantially more than the control group which produced 13,224 L, a difference of 378 L or 1.24 L/day (P<0.05). Model fit was deemed to be good.

Milk constituent model (Model 2)

Milk constituent prediction curves from Model 2 for both trial groups (Fig. 1b) illustrate that a cow in the high space group produced 988 kg solids in the first 305 DIM compared to 968 kg in the control group (P<0.05), the difference being 20 kg of milk solids or 66 g/day. Model fit was deemed to be good.

Rumination model (Model 3)

Results of the rumination model show that cows in the high space group had significantly higher rumination times than cows in the control group (~10-14 minutes per day, P<0.05), although the effect became slightly smaller as lactation progressed. Predictions of the model are shown in Fig.1c. Model fit was again good.
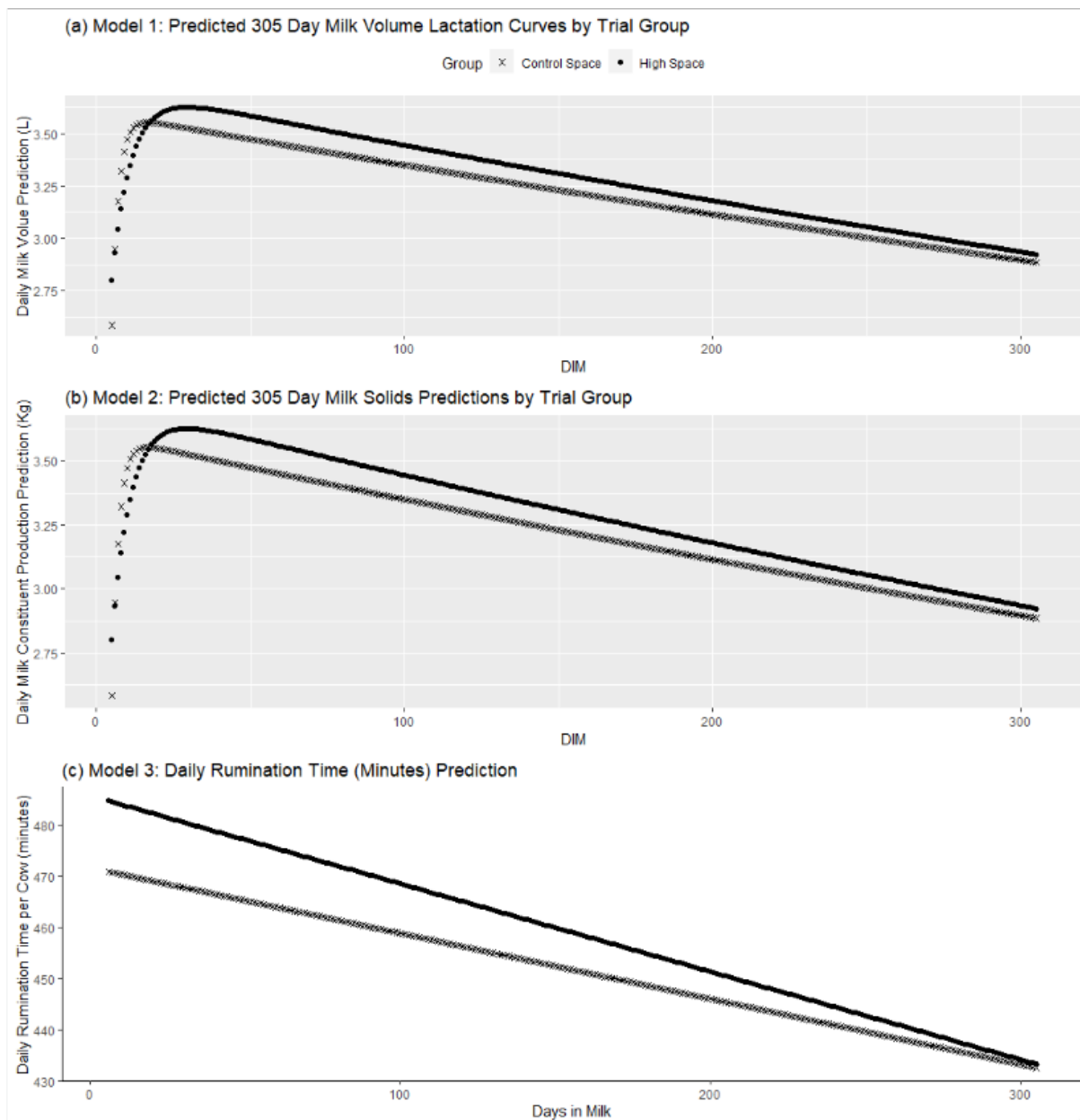
Figure 1. (a) Model 1: milk volume predictions by days in milk. (b) Model 2: milk constituent predictions by days in milk. (c) Model 3: daily rumination time predictions by days in milk.

DISCUSSION

Our preliminary results indicate that the living space available to cows has an important impact on milk volume, milk constituents and rumination times. This suggests that despite there being no differences in other management practices, including feeding, the provision of additional space has a substantial impact on cow physiology and productivity. Further measurements taken during the research (yet to be analysed) may throw light on the underpinning mechanisms behind these changes. Our findings to some extent agree with previous research into dairy cow housing design. For example, when stocking density is reduced giving cows greater access to cubicles, similar observations are reported with milk volume performance (Fregonesi and Leaver, 2002; Telezhenko et al., 2012; Charlton et al., 2014).

Providing more space to cows may also be considered a more socially acceptable way of housing livestock and given that consumer confidence in food production is essential, this may be an additional incentive to increase space provision for dairy cattle.

There are several study limitations worthy of consideration. It is uncertain how generalisable these findings from a single herd will be to other commercial dairy herds in the UK or worldwide. However, given that the high space group represented the living space provided to the top 5% of UK commercial dairy farms and the control space the median space provided (Thompson et al., 2020), it is entirely possible that an even greater positive effect would be seen in those herds where space is more restricted. The Holstein dairy cows on this trial were very high yielding animals, and additional work is required to test the effect of living space for different breeds and levels of production.

Further research is therefore warranted on the provision of living space to dairy cows. This should include the types of space available, the impact on reproduction, behavioural welfare and economic returns. The research investigations should also include long term changes to cows' physiology and behaviour, for different amounts of living space. Therefore, the requirement for substantially more research to be conducted on the basic characteristics of housing to improve these environments for dairy cows is clear.

In conclusion, our preliminary findings indicate that increased living space has an important impact on dairy cows' productivity and physiology.

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DEVELOPMENT OF A LOG-NORMAL ACCELERATED FAILURE TIME MODEL FOR SURVIVAL OF DUTCH DAIRY COWS UNDER ALTERING AGRICULTURAL POLICY

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SUMMARY

Changes in agricultural policy such as milk-quota abolishment impact herd size and might influence the culling decisions of dairy farmers. The purpose of this study was to develop a modelling strategy for studying the relevant cow-level risk factors for survival of Dutch dairy cows representing production, reproduction and metabolic health performances under perturbations due to agricultural policy changes. An interval-censored Accelerated Failure Time (AFT) model was developed using the data from a large national level database including observations on time-varying relevant risk factors under three different dairy policy periods between 2009 and 2019. Results show that the productive lifetime survival of dairy cows reduced over time and changed under the influence of different policies. However, the relevance of associated cow-level risk factors remained the same.

INTRODUCTION

Replacement of dairy cows is a fundamental part of dairy farm management. The replacement decisions involve culling of underperforming dairy cows and subsequent replacement by suitable heifers. On average, 25-30% of Dutch dairy cows are replaced annually (Nor et al., 2014; CRV, 2018) indicating a cow-longevity of 6 to 7 years (Nor et al., 2014) which is much lower than the natural biological longevity. Culling on individual cow level has been shown to be associated with older parity/age, older age at first calving, calving complications, longer calving intervals, lower relative production level, health indicators like high somatic cell count in milk, and very high or very low fat-protein ratios in early lactation (Schukken et al., 2003; Huijps et al., 2008; Nielsen et al., 2010; Pritchard et al., 2013; Gussmann et al., 2019; Rilanto et al., 2020). These factors can be termed as associated cow-level risk factors for culling.

Changes in national agricultural policies can influence farmers' culling decisions. Dairy farmers might change their strategy either in anticipation or as a result of changes in agricultural policies of the country. Failure to respond to such policy changes might negatively affect the

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future profitability of the dairy farms (McDonald et al., 2013). So, in combination with the changing policy climate, the culling strategy of dairy farms operates in a dynamic environment where the relevance of associated risk factors and replacement criteria might change periodically. Literature on risk factors influencing culling decisions and their trade-offs representing the changed Dutch farming policy climate is, however, lacking. There is a need for a study of effects of policy and associated risk factors related to the voluntary culling of dairy cows.

Risk of culling and its relevant factors can be studied epidemiologically by looking at the survival of individual producing cows on dairy farms. Survival in terms of productive longevity can be studied with survival analysis models such as Cox-proportional hazards, semi-parametric models or parametric Accelerated Failure Time models (AFT) depending on the nature of data and interpretation (Kleinbaum and Klein, 2010). Time-varying effects or hazards of associated risk factors can be analysed with censored longitudinal survival data with appropriate survival models (Klein and Moeschberger, 2006). A large-scale survival analysis of such a nature in the Dutch dairy farming system is pertinent.

The purpose of this study was therefore to develop a modelling strategy for studying the relevant cow-level risk factors for survival of Dutch dairy cows representing production, reproduction and health performances under perturbations due to agricultural policy changes.

MATERIALS AND METHODS

Data

Anonymized production data on individual Dutch dairy cow-level were obtained from the Cattle Improvement Cooperative – CRV (CRV Holding BV, the Netherlands). This data comprised of 4 subsets, containing 1) Milk Production Registration (MPR) test records, 2) cow removal/exit data records, 3) lactation records and 4) insemination records (see Table 1 for details). The data spanned from the years 2009 to 2019 and included information on approximately 80% of all the milk-producing cows in the Netherlands. The raw data files included repeated measures of 6,033,922 dairy cows from 19,885 farms.

Table 4. Overview original data (sub)sets

No.	Names of data (sub)sets	Contents
1	Milk Production Registration (MPR data)	Test-day records of producing cows on milk, fat%, protein%, somatic cell count, number of lactations, parity, etc.
2	Animal removal/ exit from herd records (Exit data)	Exit date of animals, code of exit (dead, alive/no exit, slaughter, export)
3	Lactation records data (Lactation data)	Lactation records on 305-milk, 305-fat, 305-protein, calving date, etc.
4	Insemination records (Insemination data)	Records of inseminations per parity, total inseminations, type of insemination, etc

Only data from commercial farms were selected. A commercial Dutch dairy farm was defined as a farm having (a) records (being active) for at least 5 years between 2009 and 2019,

(b) an average of at least 30 producing cows (with a minimum of 25 in any given year) and (c) an average of 4 test-day observations per year for all cows (with a minimum of 3 observations in any given year). Furthermore, for farms that ended their farming operations, the records from the year of closure were omitted. Cow-level records containing missing birth dates, missing test records etc., as well as records containing unrealistic values, were omitted. Records on cows which changed farms more than twice in their production lifetime were also excluded. Moreover, cows which were exported to other countries were excluded completely from the data as the information on their survival was not available. The four data (sub)sets were merged per cow into a single final dataset, consisting of repeated records on 4,779,676 dairy cows from 13,936 commercial farms.

Based on the literature, variables representing the associated risk factors for culling were selected from the merged data. The final factors and their levels are presented in Table 2. Parity was categorised into 4 levels and Lactation value (LV), which denotes the relative milk production level of a cow in a herd, into 3 levels. Details of how LV is calculated can be found at CRV (2020). Fat and protein percentages in the first 100 days of lactation were converted to fat-protein ratios (FPR). The proportion of test-days with ratios above 1.5 and below 0.9 were determined in each parity per cow, representing very high and very low FPR, respectively. The two factors representing very high and very low FPR were split into two levels representing small proportion (less than 50%) and large proportion (more than/ equal to 50%) of low/high-FPR values in first 100 lactation days. Individual somatic cell counts in test-day milk were classified in 4 levels, whereas the rolling average number of inseminations per parity was factorized in 3 levels. A factor for policy periods was generated based on calendar year with three levels representing the targeted policy periods, namely the Milk Quota period 2009-2013 (MQ), the Post-Milk Quota period 2014-2016 (PMQ) and the Phosphate regulation period 2017-2019 (PH).

The data were transformed into survival data with start time, stop time and event variables (removal/exit) representing left-truncated (initial start time was the difference between the test date and birth date of the cows), and interval-censored repeated measures data according to Klein and Moeschberger (2006). Each interval represented the time period between two test-days of MPR recording. Start and stop times for the intervals were represented in weeks of survival. Event "1" represented removal of cows from MPR records as where the cow can be considered as "culled (voluntarily)" or "sold alive to another herd" and the event of "0" represented cows which were still producing, censored, or those which died naturally during the period between 2009 and 2019. Factors were time-varying variables with observations per interval.

Table 2. Selected risk factors and their levels with number of observations

Factor	Abbrev.	Explanation	Levels	No. of test-day records
Lactation value	LV	Relative milk production level on test-day in comparison to the herd average of 100. Three levels represent less than 90, between 91 and 110, more than 110	below average	26,023,175
			average	65,532,040
			above average	22,950,930
Parity	-	Parity number of cows	1st parity	34,517,660
			2nd parity	28,438,152
			3-4th parities	35,092,020
			> 4 parities	16,458,313
Very high-fat protein ratio	highFPR	Indicator for subclinical ketosis, reflected by the proportion of tests in first 100 days of lactation resulting in FPR>1.5	< 50%	112,705,286
			$\geq$ 50%	1,800,859
Very low-fat protein ratio	lowFPR	Indicator for Sub-acute Ruminal Acidosis reflected by the proportion of tests in first 100 days of lactation resulting in FPR < 0.9	< 50%	114,436,777
			$\geq$ 50%	69,368
Test day-Somatic cell count (x 1,000)	SCC	Somatic cell count in thousands per millilitre of milk on test-day	< 200	91,912,692
			$\geq$ 200 and < 600	15,257,987
			$\geq$ 600 and < 1000	3,202,567
			$\geq$ 1,000	4,132,899
Insemination	Insem	Rolling average of total number of inseminations per parity	< 2	63,753,449
			$\geq$ 2 and < 5	47,408,119
			$\geq$ 5	3,344,577
Policy Periods	Period	Time periods of test-day records MQ (Milk quota): 2009-2013, PMQ (Post-milk quota): 2014-2016, PH (Phosphate regulation): 2017-2019	MQ	49,613,372
			PMQ	33,652,664
			PH	31,240,109

<u>Statistical analysis</u>

Time-varying effects or hazards of associated risk factors can be analysed with censored longitudinal survival data with appropriate survival models (Klein and Moeschberger, 2006). Given the nature of the data, a parametric survival model with appropriate distribution for survival time was to be chosen. An Accelerated Failure Time (AFT) model with an underlying theoretical distribution assumes a specific distribution for time-to-event or survival time that is analysed log-linearly against covariates or, in this case, factors with distinct levels. Interval censored data of animals can be utilized in such a model along with time-dependent factor levels (Klein and Moeschberger, 2006; Kleinbaum and Klein, 2010).

Several parametric survival models with different underlying distributions for time-to-event (the dependent variable) were tested graphically. To check the validity of the assumptions of the parametric models, residuals from the models were plotted. This was done to visually appreciate the conformity of residuals to the expected error distribution. However, since the residuals were estimated from censored data, they were censored as well. To circumvent this issue, the Kaplan-Meier (KM) estimates of the residuals were computed and then plotted against the survival probability. The expected error distribution curves were superimposed on this graph to visually validate the conformity of the parametric models to the assumptions. The expected error distributions corresponding to the distributions of the dependent variable are presented in Table 3.

Table 3. Underlying error distributions for the parametric models that were tested

No.	AFT model distribution	Expected error distribution
1	Weibull	Extreme value distribution
2	Exponential	Extreme value distribution
3	Log-Logistic	Logistic distribution
4	Logistic	Logistic distribution
5	Log-normal	Normal distribution

Out of these tests, the lognormal AFT model was selected based on a visual conformation of the residuals and expected residual distribution as well as lowest Akaike-Information Criterion (AIC) (see Results description). Logarithm of time-to-event was linearly regressed against these associated time-dependent factor-levels which were assumed to linearly increase or decrease time-to-event based on their effect.

Selected relevant factors (Table 2) were added to the model as fixed time-varying effects along with a random (shared variance) term of farm corrected by clustering (Eq. 1),

$$\log T_{ij} = X_{ij}\beta + \varepsilon_{ij} \qquad (1)$$

where, $\log T_{ij}$ are log 'time-to-event', β is a vector of time ratio (TR) estimates, X_{ij} is a matrix of factor levels with "i" clusters and "j" observations per cluster and ε_{ij} are random errors within cluster (not independent inside cluster). This structure represents correcting for cluster dependence by marginalizing the TR estimates similar to the method used in Fan and Datta (2011).

Consequently, the model was refined by using the AIC-based stepwise backward selection protocol. The final model was defined as follows (Eq.2):

$$Y => \log(Ftime) \sim \mu + LV + Parity + SCC + lowFPR + highFPR + Insem + Period$$

$$+ Period:LV + Period:Parity + Period:SCC + Period:highFPR +$$

$$cluster(Farm) + e \qquad (2)$$

where, '*Ftime*' represents time-to-event, $'LV, Parity, SCC, lowFPR\ highFPR, Insem, Period$' represent the factors as denoted Table 3 and '$cluster(Farm)$' denotes the cluster effects of the farms in which cows are producing and *e* represents the random error. In this model, the '*Ftime*' is representative of survival intervals between previous and next testing date in the MPR records. The interaction terms represent the proportion of effect of the factors under different policy periods. Interaction terms '*Period:lowFPR*' and '*Period:Insem*' were removed from the final model by AIC-backwards selection. Estimates of the factor levels were calculated 'inside' the levels of the period term with their standard errors.

RESULTS

Descriptive statistics

The data spanned the years 2009 to 2019, with a maximum of 13,590 farms and minimum of 11,737 farms per year (Table 4). However, the majority of the selected farms (~78%) continued production for the entire span of 11 years. Producing cows from the selected herds in the MPR data were tested on an average of 10 times per year.

Table 4. Recorded number of commercial farms and producing cows between 2009 and 2019

Year	Cows	Farms
2009	1,308,083	13,375
2010	1,371,412	13,450
2011	1,405,444	13,531
2012	1,443,133	13,590
2013	1,492,813	13,453
2014	1,536,476	13,407
2015	1,600,403	13,355
2016	1,695,173	13,176
2017	1,634,629	12,732
2018	1,529,185	12,244
2019	1,388,810	11,737

Between 2010 and 2019, 337,754 new primiparous cows were introduced to the farms with a maximum of 396,909 cows in year 2016 and a minimum of 253,251 cows in year 2019 (Fig. 1). Similarly, on average 268,206 cows had an event i.e., they were voluntarily culled or sold alive with a maximum of 338,076 and a minimum of 230,002 cows in years 2017 and 2015, respectively (Fig. 1).
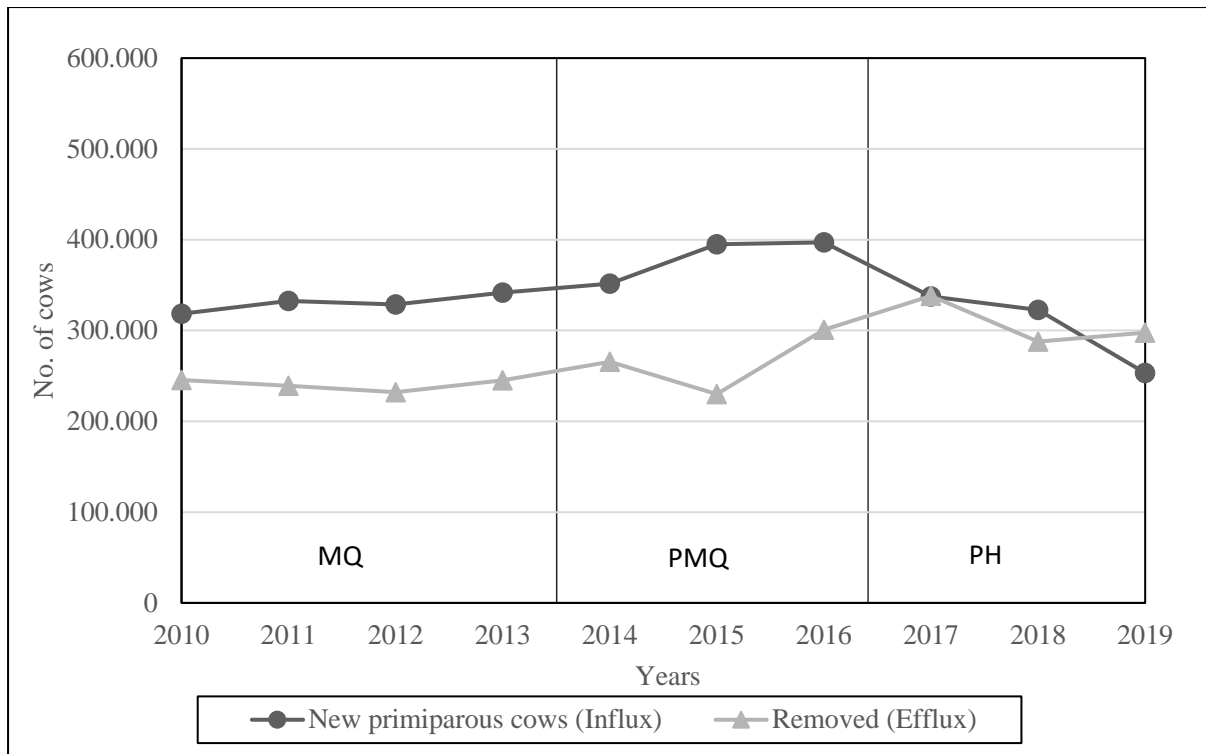
Fig. 1 Total Influx and Efflux of cows in years 2010 to 2019.

Note: Influx-efflux figures for year 2009 are not displayed as they were biased due to left-truncation of cows that were already producing. X- axis divided in three policy periods viz., Milk Quota (MQ, 210-2013), Post-Milk Quota (PMQ, 2014-2016) and Phosphate regulation (PH, 2017-2019)

Model selection

In order to select a parametric model, a graphical procedure for testing the residuals was employed. The same model was fitted with different parametric variations of distributions for log-survival time such as Weibull, Loglogistic, Logistic and exponential. It was shown that although none of the Kaplan-Meier (KM) estimates of residuals follow the exact expected error distribution, the KM estimates of the lognormal AFT model and log-logistic AFT model reasonably follow the expected error distribution (Fig. 2). This showed that the AFT model assumptions of lognormal distribution for the dependent variable (time-to-event) were sound. Out of all the variations, lognormal distribution had the lowest AIC thus validating the choice of the model assumptions (Table 5). The graphical procedure and AIC estimation showed that the AFT model assumptions of lognormal distribution for the dependent variable (time-to-event) were sound. Lowest AIC also meant that the selected model had the least out-of-sample variance.
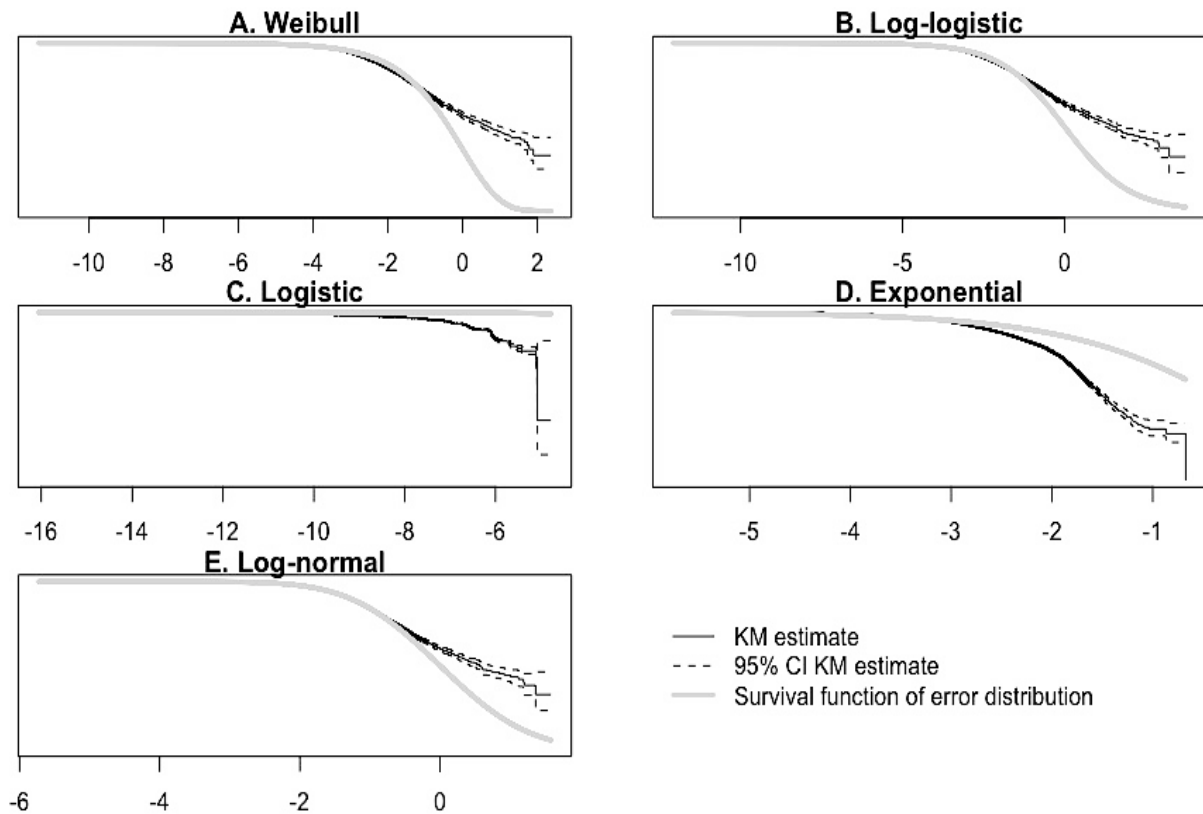
Figure 2. Graphical test for Kaplan-Meier (KM) estimates of residuals from parametric survival models against five different expected error distributions

Note: On x-axes, residuals from respective model distributions; on y-axes, survival probabilities from 0% →100% are plotted

Table 5. Comparison of different parametric survival models and their AIC scores

Model distribution type	Degrees of Freedom	Akaike-Information Criterion (AIC)
Lognormal AFT[±]	34	46881680
LogLogistic AFT	34	47397611
Weibull	34	47832641
Logistic	34	50139935
Exponential	33	57346377

[±]AFT: Accelerated Failure Time (Model)

Survival analysis using AFT model

Table 6 shows the effects of associated risk factors in the final model under the levels of policy periods in terms of survival time in weeks. All main effects of the associated factors were significant based on 95% confidence intervals. In terms of difference between the policy period, the median survival of the cows decreased by 2.7 weeks and 15.3 weeks in PMQ and PH, respectively, compared to MQ (Table 6). Hence, the lowest median survival for cows under policy period was found in PH period. Based on the results of the AFT model (Table 6), it was shown that estimated survival increased with higher parities, above average LV, higher

proportion of HighFPR and higher Insem and lower SCC and lower proportion of lowFPR within all three policy periods.

Table 6. Estimates of survival time based on lognormal accelerated failure time model

Factor [a]	Survival time in weeks	95% CI	
	exp(ß)	LOW	UP
Intercept	246.7	244.7	248.6
Log(scale)	+0.3		
Policy Periods			
MQ [a]	Ref		
PMQ	-2.7	-2.7	-1.2
PH	-15.3	-15.3	-11.4
	MQ	PMQ	PH
Factor	Survival time in weeks[b]		
Parity			
1st parity	Ref	Ref	Ref
2nd parity	+85.0	+83.3	+87.0
3-4 parities	+217.4	+215.1	+222.1
> 4 parities	+459.6	+441.5	+451.2
LV			
below average	-29.8	-30.3	-29.5
average	Ref	Ref	Ref
above average	+25.1	+25.2	+20.6
SCC (X 1,000)			
< 200	Ref	Ref	Ref
≥ 200 and < 600	-11.8	-9.7	-9.2
≥ 600 and < 1000	-18.5	-14.8	-12.3
≥ 1,000	-32.4	-29.2	-24.4
highFPR			
< 50%	Ref	Ref	Ref
≥ 50%	+9.8	+9.6	+6.0
lowFPR			
< 50%	Ref	Ref	Ref
≥ 50%	-12.0	-10.9	-5.1
Insem			
< 2	Ref	Ref	Ref
≥2 and < 5	+18.9	+17.4	+15.3
≥ 5	+29.2	+24.6	+24.6

[a] Abbreviations in the table: Ref (reference level of factor), MQ (milk quota), PMQ (post-milk quota), PH (phosphate regulation), LV (lactation value), SCC (test-day somatic cell count), highFPR (very high test-day fat-protein ratio), lowFPR (very low test-day fat-protein ratio), Insem (rolling avg. of inseminations per parity)
[b] 95% confidence intervals for Parity, LV, SCC, low/highFPR and Insem were too small to report (< ±1 week)

DISCUSSION

The purpose of this study was to develop a modelling strategy for studying the relevant cow-level risk factors for survival of Dutch dairy cows representing production, reproduction and health performances under perturbations due to agricultural policy changes. In this study, it was shown that lognormal AFT model was appropriate for determining the associations between the risk factors and the survival of dairy cows under changing agricultural policy. The survival analysis of the cows was utilized to gain insight in the culling/ sale pattern of the dairy cows in the different policy periods.

Survival in terms of productive longevity can be studied with survival analysis models such as Cox-proportional hazards, semi-parametric models or parametric AFT models (Wei, 1992) depending on the nature of data and interpretation (Kleinbaum and Klein, 2010). Frequently, survival analysis of dairy cows has been done on the basis of age-longevity in herds (Gröhn and Rajala-Schultz, 2000; Gussmann et al., 2019; Rilanto et al., 2020) or on the basis of stages of lactation per parity (Roxström et al., 2003; Rocha et al., 2018). The distribution of dependent variable assumed under AFT models depend on the nature of data. Log-normal and log-logistic distribution AFTs are the most commonly utilized models in biological studies (Klein and Moeschberger, 2006).

In this study, the AFT model was developed per policy over calendar years which signified major changes to agricultural policy. Unlike the non-parametric survival methods, use of an AFT model involved the assumption of an underlying log-normal distribution of "time-to-event" variable which was reasonable given the nature of the data. Due to the parametric nature of the model, AFT models are more robust for incorporating multiple covariates, fixed factors and random components (Keiding et al., 1997; Lambert et al., 2004). In this study, the shared random variance of farm effect was controlled for using clusters from R package 'Survival' (R Core Team, 2020). The collinearity between repeated records for the dairy cows was also addressed due to the design of interval censoring of survival times. Moreover, the estimates of the associated factor levels in the AFT model are in the form of log-time ratios which are easy to interpret in terms of 'acceleration' in log-time towards event (Wei, 1992). Besides that, AFT models are flexible towards neglected covariates and factors (Hougaard, 1999) such as disease indicators for mastitis, lameness, etc. in dairy cattle which influence culling decisions and were not recorded in the data.

In this study, a large-scale national level database was utilized for the analysis. This enabled very precise estimation of associated effects of the relevant factors with small 95% confidence intervals. However, based on the results of the AFT model, no large-scale differences in the survival of cows were found between the different policy periods. Based on these results, it was speculated that the adjustments made by the farmers under the changing policy climates might not be strictly within the bounds of particular periods. Some farmers make early changes while others have delayed reactions.

The factors selected in this model encompassed production, reproduction and metabolic heath performances based on the literature. Based on the results, there were no changes in the 'pattern' of estimated survival under the levels of associated risk factors within different policy periods. This showed that there were no differences in the relevancy of associated risk factors between the three policy periods. Hence, conducting a survival analysis of dairy cows under changing policy was not straightforward. Under a continuously changing policy climate the perturbations caused in culling patterns of the farms could be treated as a continuum rather than

discrete changes per year or per period in future research. In conclusion, this study demonstrated that a parametric AFT model can be used to analyse the survival of dairy cows in association with risk factors.

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THE EFFECT OF A NEW INTRODUCTION OF BOVINE VIRAL DIARRHOEA VIRUS ON SOMATIC CELL COUNT AND CALF MORTALITY BASED ON 10 YEARS OF DUTCH FIELD DATA

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SUMMARY

Bovine viral diarrhoea virus (BVDV) infections reduce the performance of dairy herds, therefore BVDV control programmes have been implemented in many cattle-raising countries. A voluntary BVDV-free programme has been implemented in the Netherlands since 1997. Few studies have determined the effects of a new BVDV introduction on performance in herds participating in a BVDV control programme. The aim of this study is to investigate the effect of a new BVDV introduction in BVDV-free herds participating in a BVDV control programme on somatic cell count (SCC) and calf mortality (CM). Ten-year longitudinal field data were used. Results showed that for BVDV-free herds, a new BVDV introduction has negative effects on SCC and CM. In the year post BVDV breakdown, the SCC and CM in herds that lost BVDV-free certificate were 1,357 cells/ml and 0.24 percentage points higher than in the year prior breakdown, respectively.

INTRODUCTION

Bovine viral diarrhoea (BVD) is an infectious cattle disease that has a negative effect on the production and economy of dairy herds (Houe, 1995, 2003). Existing research suggests an association between bovine viral diarrhoea virus (BVDV) infection and poor herd performance, such as decreased milk yield, udder health problems, and increased mortality among cows and calves (Gunn et al., 2005; Richter et al., 2017). To improve herd performance and reduce losses, many countries have implemented BVDV control programmes (Beaudeau et al., 2005; Scharnböck et al., 2018; Richter et al., 2019). A voluntary BVDV-free control programme was implemented in the Netherlands in 1997 (Mars and Van Maanen, 2005). The BVDV-free programme proved to be successful, as evidenced by an increasing number of BVDV-free herds (Van Duijn et al., 2019). Meanwhile, there are still herds that lose their BVDV-free certificate. The percentage of the dairy herds that had recent BVDV circulation (thus a new introduction of BVDV) was 19.6% in 2009 and 2010, and dropped to 8.7% in 2015 and 2016 (GD, 2011, 2018). Although the negative effects of BVDV infections have been studied and published (Baker, 1995; Houe, 1999; Burgstaller et al., 2016; Pinior et al., 2019), few studies

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have evaluated the effects of a new introduction of BVDV on performance in herds participating in a BVDV control programme (Yue et al., 2020). The aim of this study is to investigate the effect of a new BVDV introduction in BVDV-free herds participating in a BVDV control programme on somatic cell count (SCC) and calf mortality (CM).

MATERIALS AND METHODS

Data

This study included BVDV surveillance data, milk production registration data (including SCC data) and CM data. The BVDV surveillance data is previously described in detail by Veldhuis et al. (2018) and consists of longitudinal herd level data of 4,334 dairy herds participating in the Dutch BVDV-free programme from 4 September 2006 to 15 June 2016. The BVDV-free control programme consists of three phases. Phase I is the test and cull phase. In phase I, the whole herd is first tested for BVDV, and if persistently infected (PI) animals are detected, they will be removed. Then, all new-born calves are tested for virus during the following ten consecutive months. When no new-born calves are virus positive in the ten-month testing, the herd obtains the BVDV-free certificate and enters the monitoring phase (Phase II). If BVDV is detected during Phase II, the dairy herd loses its BVDV-free certificate and enters the removal phase for PI animals (Phase III). The BVDV surveillance data includes two types of herds, i.e., 'free-herd' and 'breakdown-herd'. A free-herd is a herd that obtained the BVDV-free certificate and stayed free in Phase II until the end of the study period. The date of obtaining the BVDV-free certificate was defined as the "free-date". A breakdown-herd is a herd that lost its BVDV-free certificate and entered Phase III during the study period. The date of losing the BVDV-free certificate was defined as the 'breakdown-date'. The milk production registration data, including SCC data, was obtained from CRV (Cattle Improvement Cooperative, Arnhem, the Netherlands) and contained monthly test-day SCC records at herd level from 1 January 2007 to 31 December 2016. The test-day SCC was the average SCC of all cows in the herd sampled on the test day. Data for CM originated from the identification and registration (I&R) data from 1 January 2011 to 30 June 2016, which reports the quarterly CM. In the I&R data, the CM was calculated by dividing the number of ear-tagged calves up to 1-year old that are reported 'dead' in a quarter by the average number of calves present in that age category that quarter.

Data editing

To estimate the effect of a new introduction of BVDV on SCC and CM, two datasets were developed by merging SCC and CM with the BVDV surveillance data, based on anonymous unique herd number. The SCC and CM datasets were further edited in five steps: (i) remove herds that left the BVDV-free programme before the end of the study period; (ii) remove herds with breakdown-date before January 1, 2008 in the SCC dataset (before January 1, 2012 in the CM dataset) and remove herds with breakdown-date after December 31, 2014, to ensure sufficient pre or post-breakdown-date records; (iii) remove herds with meaningless values (e.g. SCC=0, CM<0) or records with missing data; (iv) remove herds with a herd size less than 20 as these were not considered commercial herds; (v) remove records earlier than 10 months before the free-date to ensure that the study herds are indeed free of BVDV before its defined new introduction. The final SCC and CM dataset included 3,332 and 3,167 Dutch dairy herds, respectively. The SCC dataset comprised information of 234,367 monthly test-day records in 703 breakdown-herds and 2,629 free-herds from 1 January 2007 to 31 December 2016. The CM dataset consisted of 62,721 quarterly records in 327 breakdown-herds and 2,840 free-herds

from 1 January 2011 to 30 June 2016. Data editing and further data analysis were performed in R version 3.5.0 software (R Core Team, 2018).

Data analysis

The effect of a new introduction of BVDV on SCC and CM in BVDV-free herds was estimated by comparing SCC and CM before and after the new introduction of BVDV. To correct for fluctuation in SCC and CM over time, a random breakdown-date was artificially generated for free-herds by a sampling procedure based on the distribution of the breakdown-dates of the breakdown-herds. Consequently, the SCC and CM in relation to the new introduction of BVDV could be determined by comparing the SCC and CM before and after the breakdown-date in the breakdown-herds, taking into account the free-herds.

Somatic cell count: The effects of new introduction of BVDV on SCC were analysed annually and quarterly. For the annual analysis, the two years prior and three years post new introduction of BVDV were considered. A linear mixed-effects model was conducted as follows (Eq. 1):

$$lnSCC_{ij} = \beta_0 + \beta_1 Herdtype_i + \beta_2 BVDstatus_{ij} + \hspace{2cm} (1)$$
$$\beta_3 (Herdtype_i \times BVDstatus_{ij}) + \beta_4 N\_animal_{ij} + \beta_5 Year_{ij} +$$
$$\beta_6 Season_{ij} + \mu_{herd(i)} + \varepsilon_{ij}$$

For herd $i \in \{1,..., 3{,}332\}$ and test-day $j \in \{01/01/2007,...,12/31/2016\}$, where SCC_{ij} is the SCC on test-day j of herd i, in units of 1,000 cells/ml. Due to the right skewed distribution of the SCC value, it was natural log-transformed. The intercept β_0 is the SCC for the reference level of each of the explanatory/fixed variables, β_i ($i= 1,...,6$) is the difference between the mean SCC of a specified class of the fixed factor compared to the mean SCC in the reference class. $Herdtype_i$ is a dummy variable that represents herd type (breakdown-herd or free-herd). $BVDstatus_{ij}$ is a defined categorical variable, which indicates the BVDV infection status of herd i on test-day j. $BVDstatus_{ij}$ was defined based on the breakdown-date (real and artificial) and consists of five categories: 2 years prior BVDV, 1 year prior BVDV, 1 year post BVDV, 2 years post BVDV, and 3 years post BVDV. The category '1 year prior BVDV' was used as the reference category. The effect of new introduction of BVDV on SCC within the breakdown-herd can be explained by the coefficients of the interaction term $Herdtype \times BVDstatus$ in the model results. N_animal_{ij} is the number of cows sampled for SCC on the test-day j of herd i. $Year_{ij}$ (2007–2016) is a categorical variable that corrects for variation in SCC across different calendar years, with 2007 as the reference category. $Season_{ij}$ is a categorical variable defined as Spring (March–May), Summer (June–August, reference category), Autumn (September–November) and Winter (December to next February). $\mu_{herd(i)}$ refers to the random herd-effect in the ith herd that takes into account repeated measures within one herd (Dohoo and Martin, 2003). Further, the errors $\varepsilon_{1j,...,}\varepsilon_{3,332j}$ are assumed to be independent with $\sim N(0, \sigma^2)$. Maximum likelihood estimates of the parameters in the linear mixed model were determined using the 'lmer' function in the 'lme4' package for R (Bates et al., 2015).

The model estimating the quarterly effect of new introduction of BVDV on SCC was similar to the annual analysis model in Eq.(1), the only difference was the definition of the $BVDstatus$ variable. The $BVDstatus$ variable in the quarterly analysis consists of five categories including the reference category "1 year prior BVDV", and the first four quarters (Q1–Q4) in the year after BVDV breakdown-date.

The annual and quarterly linear mixed-effects models were iterated 200 times. In each iteration, the random breakdown date was randomly generated for the free-herds. In this way, the uncertainty of randomization can be included. The coefficients for each of the 200 iterations were summarised to show the final outcome, and 2.5th to 97.5th percentile of the natural exponential transformed coefficients indicated the significance of the model results instead of the P-value. The 2.5th to 97.5th percentile interval was considered as the range of estimated coefficients that could be used to judge the hypothesis about a systematic increase or decrease of SCC. The model quality was assessed using the R package 'performance' (Lüdecke et al., 2019).

Calf mortality: The association between the new introduction of BVDV and CM was also modelled on an annual and quarterly basis. For the CM annual analysis, a generalized linear mixed-effects model was applied. The model is written (Eq. 2):

$$QCM_{ik} = \beta_0 + \beta_1 Herdtype_i + \beta_2 BVDstatus_{ik} + \\ \beta_3 (Herdtype_i \times BVDstatus_{ik}) + \beta_4 Herdsize_{ik} + \\ \beta_5 Year_{ik} + \beta_6 Season_{ij} + Zu_{ik} \tag{2}$$

For herd $i \in \{1, \dots, 3{,}167\}$ and year $k \in \{2011, \dots, 2016\}$, where QCM_{ik} is the CM on year k of herd i. The definition of explanatory variables $Herdtype_i$, $Year_{ik}$ and $Season_{ik}$ was same as the SCC model. $Herdsize_{ik}$ is the number of lactating cows on year k of herd i. $BVDstatus_{ik}$ in the CM model consists of six categories: 2 years prior BVDV, 1 year prior BVDV, year of breakdown, 1 year post BVDV, 2 years post BVDV, and 3 years post BVDV. The category '1 year prior BVDV' was used as the reference category. The random herd effect is u_{ik} and Z is the design matrix for the random part of the model (Dohoo and Martin, 2003). Maximum likelihood estimates of the parameters in the generalized linear mixed model were determined using the 'glmmTMB' function in the 'glmmTMB' package for R (Magnusson et al., 2017). The number of iterations and model fit checking procedures are consistent with the SCC model. The intercept term β_0 was transformed back to the probability, and the modelling results of fixed variables were summarized in the same way as the SCC model.

The model estimating the quarterly effect of the new introduction of BVDV on CM was similar to the CM annual analysis model in Eq.(2). The only change in the CM quarterly analysis model is the definition of the *BVDstatus* variable. The *BVDstatus* variable in the CM quarterly analysis consists of five categories including the reference category "1 year prior BVDV", and the first four quarters (Q1–Q4) in the year after BVDV breakdown-date.

RESULTS

Descriptive results

The descriptive results presented in Table 1 show that the SCC and CM in breakdown-herds was higher than in free-herds. The SCC in breakdown-herds was 11,000 cells/ml higher than in free-herds, and the CM in breakdown-herds was on average 0.44 percentage points higher than in free-herds.

Table 1. The descriptive statistics of the herd level SCC (somatic cell count) and CM (calf mortality) in dairy herds participating in the Dutch BVDV-free programme

Variables	Breakdown-herd Mean (SD)	Free-herd Mean (SD)
SCC		
Number of herds in the analysis	703	2,629
Number of cows sampled for SCC per herd	82 (38)	80 (37)
SCC[a], 1,000 cells/ml	198 (87)	187 (87)
CM		
Number of herds in the analysis	327	2,840
Number of dead calves, n/herd/quarter	1 (2)	1 (2)
Number of present calves, n/herd/quarter	43 (25)	41 (22)
CM[b], %	3.33 (4.76)	2.89 (4.26)

[a] SCC (1,000 cells/ml) is the average SCC of all cows sampled on the test day.

[b] CM (%) is defined as the number of ear-tagged calves (age < 1 year) that are reported 'dead' in a quarter, over the average number of calves present in that age category that quarter.

Model results

Somatic cell count: The annual and quarterly effects of new introduction of BVDV on SCC are presented in Fig.1a and Fig. 2a. The figures show the SCC for breakdown-herd and free-herd in years prior and after BVDV breakdown, and illustrate that the SCC in breakdown-herds was higher than in the free-herds. Within the breakdown-herds, the annual analysis showed that the SCC in breakdown-herds in the year post breakdown was on average 1,357 cells/ml higher than in the year prior breakdown (Fig.1a). In the second year post breakdown, the SCC was not significantly affected by BVDV introduction. In the quarterly analysis, the SCC mainly increased in the second quarter post breakdown, which was 1,925 cells/ml higher than in the year prior BVDV breakdown (Fig.2a).
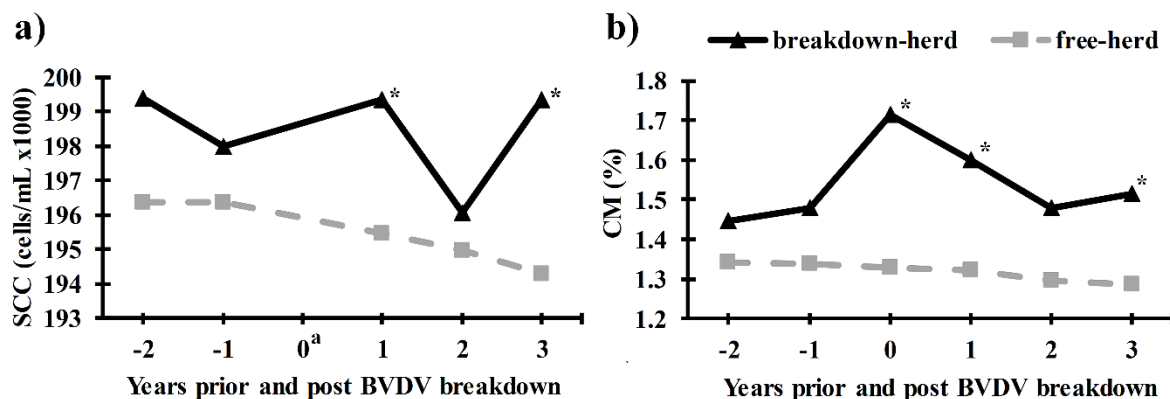
Figure 1. Annual effects of new introduction of BVDV on SCC (somatic cell count) and CM (calf mortality) in breakdown-herds and free-herds participating in Dutch BVDV-free programme. *indicates significance with 2.5th and 97.5th percentiles of the natural exponential transformed coefficients of the interaction term *Herdtype × BVDstatus* in 200 iterations both >1 or <1). ([a] Year 0 was not defined in the SCC analysis, and therefore there is no Y value. For SCC analysis, the BVD status corresponding to the monthly SCC records was defined according to the breakdown-date.
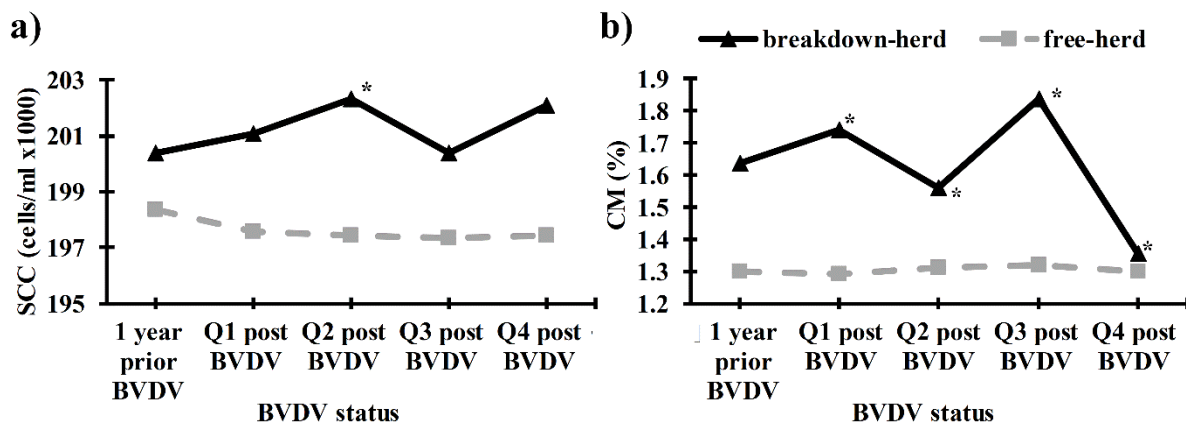
Fig. 2. Quarterly effects of new introduction of BVDV on SCC (somatic cell count) and CM (calf mortality) in breakdown-herds and free-herds participating in Dutch BVDV-free programme. (* indicates significance with 2.5th and 97.5th percentiles of the natural exponential transformed coefficients of the interaction term *Herdtype* × *BVDstatus* in 200 iterations both >1 or <1).

Calf mortality: Figure 1b and Fig. 2b compare the CM between breakdown-herds and free-herds in years prior and post BVDV breakdown in the annual and quarterly analysis, respectively. The results showed that CM was higher in breakdown-herds than in free-herds. Within the breakdown-herds, a new introduction of BVDV had significant effects on CM in the year of the breakdown and the year post breakdown in the annual analysis, which were respectively 0.24 and 0.12 percentage points higher than in the year prior breakdown (Fig.1b). The CM increased in the first and third quarter after BVDV breakdown, which was on average 0.10 and 0.20 percentage points higher than in the year prior breakdown, respectively (Fig.2b).

DISCUSSION

This study showed that the SCC and CM in breakdown-herds were higher than in free-herds in the Dutch BVDV-free programme. Within the breakdown-herds, the new introduction of BVDV has a negative, but on average relatively small, effect on SCC and CM.

The negative effects of the new introduction of BVDV on SCC observed in this study were smaller than in previous studies. Previous studies reported that the bulk (tank) milk SCC in BVDV infected herds was 6,000 to 31,400 cells/ml higher than uninfected herds (Lindberg and Emanuelson, 1997; Beaudeau et al., 2005; Laureyns et al., 2013). One of the main reasons for the difference in results between the current study and previous studies is that the studied herds participated in the BVDV-free programme. This enables farmers to more quickly detect the new introduction of BVDV and take measures to control further losses. Another possible explanation for this difference may be that previous studies tend to compare the differences in SCC between herds with different BVDV infection status and may not be able to eliminate the inherent differences in SCC levels between herds. In the current study, the SCC was not only analysed between the breakdown-herds and free-herds, but also evaluated within breakdown-herd before and after the new introduction of BVDV. In this way, the impact of individual differences on the results can be reduced.

The results of CM in this study are in accordance with previous studies indicating that BVDV infection attributes to mortality in young stock (Houe and Meyling, 1991; Viet et al., 2004; Presi et al., 2011; Thomann et al., 2017). Previous studies showed that the calf mortality in BVDV infected herds was 3.05 to 7.3 percentage points higher than in uninfected herds (Ersbøll et al., 2003; Gates et al., 2013). The negative effects of BVDV infection on calf mortality in previous studies is greater than in the current study; differences in data and study design may partly explain this. For instance, Ersbøll et al. (2003) defined a case herd as a herd with a PI animal. PI animals continuously shed large amounts of BVDV that spreads to other susceptible calves and cows in the herd, which will then increase the calf mortality. Moreover, 50% of PI animals themselves will die in the first year of life (Khodakaram-Tafti and Farjanikish, 2017), and thus contribute to a higher calf mortality in the herd. In the BVDV-free programme, the PI animal was not detected in all breakdown-herds. Van Duijn et al. (2019) reported that 49% of breakdown-herds detected antigen positive animals. The reason why in only about half of the herds PI animals were detected is that all bull calves and part of the heifer calves were moved to veal herds at 14 days of age. Thus, about half of the PI animals had already left the herd before investigations started. Mortality in veal herds was not attributed to the dairy herd of origin. Therefore, the effect of the new BVDV introduction on CM is probably underestimated in the current study.

The relatively small effects of the new introduction of BVDV could be explained by study herds participating in the BVDV-free programme more quickly intervening when an introduction is detected. In the monitoring phase (Phase II) of the programme, the BVDV-free herd is monitored by testing BVDV antibodies in five young stock every 6 months or testing BVDV antigen in all new-born calves in the herd (Van Duijn et al., 2019). When the BVDV circulation is detected in the herd, the herd has a BVDV breakdown and loses its BVDV-free certificate (Veldhuis et al., 2018). The herd can be re-certified as BVDV-free only if further tests show that there is no PI animal in the herd, or the herd removes the detected PI animal and no PI animals have been detected for 10 consecutive months thereafter (Van Duijn et al., 2019). The control measures in the BVDV-free programme enable participating herds to detect and remove the PI animals more quickly, so as to avoid the new introduction of BVDV from having a greater negative effect on the herd performance.

The risk period for SCC and CM increase due to the new introduction of BVDV is mostly in the year post breakdown. In the annual analysis of SCC and CM, no significant effect of BVDV introduction was found in the second year post BVDV breakdown. In the third year after the introduction of BVDV, the results showed a significant impact on SCC and CM. However, combined with the field experience from experts and the control measures of the BVDV-free programme (e.g., removing PI animals after they are detected), the third year post breakdown was not considered as a high-risk period affected by the introduction of BVDV. On the other hand, the significant results in the third year post breakdown also showed the possibility of a long-term effect of the introduction of BVDV on SCC and QCR in a BVDV control programme.

In this study, the breakdown-date was assumed to be the moment of new introduction of BVDV. The exact moment of introduction of BVDV may lie somewhere before the breakdown-date. Yue et al. (2020) studied four different scenarios to determine which period seemed closest to the true period of BVDV outbreak in the herd. In the default scenario, BVDV introduction date is BVDV breakdown-date, and in the other scenarios, BVDV introduction date is 3 months, 6 months, and 9 months prior to the BVDV breakdown-date. Yue et al. (2020) showed that the default scenario seemed closest to the true period of BVDV infection. In the

current study, the same scenarios were modelled. However, the model results and conclusion in different scenarios were almost the same, thus these results are not presented.

Overall, in the Dutch BVDV-free control programme, a new introduction of BVDV had a negative, but on average relatively small, effect on SCC and CM in BVDV-free herds. The effect is relatively small because herds in the BVDV control programme were able to detect the virus circulation and cull PI animals more quickly.

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POLICY SUPPORT

CONFIDENCE OF FREEDOM FROM INFECTION WITH BVDV IN THE EU:

APPLICATION OF A BAYESIAN HIDDEN MARKOV MODEL ON FIELD DATA

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SUMMARY

The STOC free model, a Bayesian Hidden Markov model, was applied to field data from bovine viral diarrhoea virus (BVDV) control or eradication programmes (CPs) in six different countries, to estimate monthly herd-level probabilities of freedom from infection. The latent status regarding infection that was modelled was the presence of at least one persistently infected animal (PI) in the herd. By applying the model to field data from different countries, we have learned about the possibilities and challenges of the model for very different CPs. This output-based method is promising, but not yet fully applicable to estimate and compare freedom from infection between countries or regions with different CPs. The model appears most useful in endemic situations with infected as well as free herds. In the Dutch situation it was challenging to include antibody and virus test results in one model with a latent class of PI presence.

INTRODUCTION

For endemic infections in cattle that are not regulated at European Union (EU) level, such as infection by the bovine viral diarrhoea virus, European Member States (MS) have implemented CPs tailored to their specific situations. This means that control measures are implemented based on factors such as herd infection prevalence, cattle density, farm management practices or other risk factors. Within Europe, these heterogeneous approaches have led to differences in disease status and in the way the disease free status is assigned (van Roon et al., 2020). Therefore, herd-level disease free status is not comparable between countries and problems may arise when cattle are traded between them. As yet, there is a lack of agreed methodologies to assess and compare confidence of freedom from infection of cattle from areas with different CPs (Martin et al., 2007; More et al., 2009; Cameron, 2012).

Currently, scenario tree modelling is the most frequently used method to assess the confidence of freedom from infection (Martin et al., 2007). This is a tool to calculate the probability of freedom at a given design prevalence. This method is based on the assumption that the country or region is already free of infection and that the specificity of the surveillance

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system is 100% (Martin et al., 2007), and is therefore considered most valuable for countries that wish to confirm their free status (Norström et al. 2014). However, few countries meet this criterion for BVDV. There is a need for a method to estimate the probability of freedom for herds classified as being free that are located within non-free countries or regions. Modelling freedom from infection was recently investigated using Bayesian and latent class methods (Heisey et al., 2014). As part of the STOC free project (van Roon et al., 2019) a Bayesian Hidden Markov model to estimate the confidence of freedom from infection has been developed, called the STOC free model (Madouasse et al., 2020). The aim of this model is to generate outputs that are comparable, from heterogeneous input from different CPs. The model represents infection at the herd-level as a latent class (called the latent status) with monthly Markovian dynamics. The impact of risk factors on the transition from latent status negative to latent status positive is modelled using logistic regression. The latent status determines test results through test sensitivity and specificity. Therefore, the model uses longitudinal test result from CPs, risk factor data and prior information about test characteristics and infection dynamics to model the latent status regarding infection at the herd-month level. In our study, the latent status of interest was the presence of a persistently infected (PI) animal.

To test the STOC free model, a number of case studies based on actual data from BVDV CPs in six different countries (Germany, France, Ireland, the Netherlands, Sweden, Scotland) were performed. The current paper illustrates application of the model in more detail. The differences in CPs between the six countries are described and the challenges that were encountered when applying the model on field data and possible solutions are discussed. The model results of the Dutch case study are presented in full as an example.

MATERIALS AND METHODS

BVDV control programmes in six different countries

Germany: In Germany, a nationwide BVDV CP was implemented, which came into force on 1 January 2011 (Wernike et al., 2017). The main objective of this CP is fast and efficient reduction in the prevalence of PI animals, and the establishment of farms with a status certified as keeping only "BVDV-unsuspicious" (i.e. virus free) animals. The CP includes mandatory testing of all newborn calves by testing ear notch samples for BVDV. In addition to ear notch samples, blood samples are investigated for BVDV, primarily for confirmatory testing. All cattle within the country must have a negative BVDV status before moving off farm.

France (Loire-Atlantique): In France, BVDV control is organised at regional rather than national level, and managed by organisations called Groupements de Défense Sanitaire (GDS) which deal with cattle health at farm level. In this study, data are included from Loire-Atlantique in which all dairy herds are tested twice a year with an antibody ELISA performed on bulk tank milk (Madouasse et al., 2020). The aim of the CP is to gain knowledge about the BVDV herd status.

Ireland: The BVDV CP is implemented nationwide and testing is performed at animal-level (Graham et al., 2014). All cattle within the country born after the start of the CP (1st January 2013) must have a negative BVDV status before moving off farm. The CP includes testing ear notch samples of newborn calves and serum testing of imported cattle for BVDV. After a positive ear notch test, confirmatory virus tests may be conducted, supplemented by serum sampling of the dam and offspring of a PI for BVDV.

The Netherlands: In the Netherlands, a voluntary CP was in place between 1998 and 2017 (van Duijn et al., 2019). Following slight adaptation, an industry-led CP became mandatory for dairy herds in 2018. The aim of the CP was to eliminate BVDV from herds by detecting and removing PIs and monitoring the subsequent BVDV free status. Until 2018, herds participated in either the bulk milk unsuspected programme or the BVDV free CP. The former was based on quarterly bulk milk testing for BVDV antibodies and negative test results led to the BVDV bulk milk unsuspected status. The BVDV free CP is more complicated as farmers can choose different test options to obtain a BVDV free status i.e. testing for virus or antibodies and different matrices such as blood, ear notch or milk.

Sweden: The Swedish cattle population is considered free from BVDV since 2014, after a voluntary CP on BVDV that commenced in 1993 and subsequently evolved into a mandatory CP from 2001. The surveillance is risk-based and herds are categorised in different risk categories according to their trading pattern in the past 12 months. The CP is designed to detect the presence of infection at an overall herd design prevalence of 0.2% with 99% confidence, with a within-herd design prevalence of 30% seropositive animals. The status is monitored using bulk milk sampling in dairy herds and serum sampling at slaughter for beef herds. The CP only includes antibody testing. In case of a positive test-result, follow-up testing is performed in the test-positive herd to confirm or rule out infection (National Veterinary Institute, 2019).

UK (Scotland): In Scotland, a mandatory industry-led CP is in place, which has had five stages to date and is aiming to eradicate BVDV from Scotland (Scottish government, 2016). Breeding herds are required to update their herd status annually using one of the three testing options currently available – check-test, calf screening and whole herd screening. Check tests are serum antibody tests of young cattle that indicate if the herd was recently exposed to BVDV. Calf screening entails individual testing of all calves born in the herd for BVDV by blood or ear notch samples. During whole herd screening, all animals in the herd are individually tested for BVDV by serum or ear notch samples.

Model requirements

The STOC free model is described in more detail by Madouasse et al. (2020). This Bayesian latent class model estimates a probability of infection at the herd-month level. The latent disease status in the model was the presence of a PI animal. To run the STOC free model, quantitative data were included on test results from the CP. It is also possible to include risk factor information on introduction of infection. The model further requires prior information for all model parameters.

Latent status: In this study, the aim was to estimate the confidence of freedom from BVDV infection for herds that are deemed free according to the CP in place. Because PI animals are the most important source for BVDV transmission, and CPs consider herds free when no PIs are present in the herd, the latent status was defined as presence of at least one PI animal in the herd.

Test results of the CP: All six countries had test results for at least 2017. The required input data are a herd ID, test dates, a test ID when different tests are used and the test results. The latter could be either positive or negative. The model distinguishes between animal- and herd-level data. For the CPs with animal-level testing, all individual animal test results are entered into the model. The model subsequently aggregates this into a number of positives out of a number of animals tested at the herd-month-level. For herd-level CPs, all test results per herd

per month are entered as input information. If herds perform different tests in one month, e.g. bulk milk testing and serological youngstock screening, this will be included as two test results that month for that herd.

Priors: The model requires prior distributions for the sensitivity (Se) and specificity (Sp) of the different diagnostic tests with respect to the latent status of interest, for the herd-level probability of being status positive at the first time step ($\pi1$), for the probability of becoming status positive between consecutive months ($\tau1$) and for the probability of remaining status positive between consecutive months ($\tau2$) (Table 1). These priors are specified with beta distributions by including alpha and beta parameters. The latent status that was modelled was the presence of a PI animal, therefore the test characteristics of the antibody tests were adapted accordingly. These sensitivities and specificities had to account for the laboratory test sensitivity and specificity but also sampling errors and misclassification due to epidemiological factors such as the probability of not detecting the virus because bulk milk was tested but the infection was present in the youngstock. Each of the countries estimated the priors with historical data or by expert opinion.

Table 1. Model parameters for which prior information is needed

Model parameters	Definition
Se	The probability of a positive test result in a herd with at least one PI
Sp	The probability of a negative test result in a herd with no PI
$\pi1$	Probability of being latent status positive at the first test
$\tau1$	Probability of becoming latent status positive between two months
$\tau2$	Probability of remaining latent status positive between two months

Sensitivity analysis: To test the influence of changes in priors on the posterior results, all countries tried different priors in their case studies.

RESULTS

General results

Six countries tested the STOC free model (September 2020) on their own CP data. While preparing data for the model, challenges were encountered given that the BVDV CPs were very complicated with different types of testing i.e. different age groups, detecting virus or antibodies etc., and/or data that were not readily available. Therefore, some countries decided to only include part of their test results. For example, in Scotland only serum and ear notch virus test results were used to test the model and results of the youngstock antibody screening in serum were omitted. The specific test characteristics that were relevant for the model are shown in Table 2. As a consequence, no conclusions can be drawn yet about the model parameters and an estimation of confidence of freedom from infection. Rather, we describe the full results based on analysis of the Dutch CP. The experiences of the other countries are further elaborated in the discussion.

Table 2. Diagnostic results of BVDV CPs included in the STOC free model per country

Country	Type of sample	Target population of testing	Test for virus or antibodies	Data restrictions
Germany	Ear notch	Calves	Virus	One district in North Rhine-Westphalia.[a]
	Serum	Confirmatory testing, adult cattle, imported cattle	Virus	
France	Bulk milk	Dairy cattle	Antibodies[b]	One department, Loire-Atlantique.[c]
Ireland	Ear notch	Calves	Virus	
	Serum	Imported cattle, adult cattle, confirmatory testing	Virus	
The Netherlands	Serum	Youngstock	Antibodies	Only herds that started with a negative first test month.[d] Only five of the seven tests were included.[e]
	Bulk milk	Dairy cattle	Antibodies	
	Serum	Purchase, confirmatory test	Virus	
	Bulk milk	Dairy cattle	Virus	
	Ear notch	Calves	Virus	
Sweden	Bulk milk	Dairy cattle	Antibodies	
Scotland	Ear notch	Calves, adult cattle	Virus	Scotland. Only beef cattle. Only virus test results.[f]
	Serum	Calves, adult cattle, confirmatory test	Virus	

[a] As most of the districts in Germany did not have any BVD cases for years we focused on one district with (some) BVDV cases. [b] Two different antibody ELISAs were performed on bulk milk, with one test being more sensitive than the other. [c] In France, BVD control is not organised at the national level, therefore we focused on one department. [d] For the Netherlands all test types regardless of the test detecting virus or antibodies were included, but because the latent status that is modelled is virus, only herds that started with a negative test month were included. [e] It was decided to exclude the Virus blood PCR and Post mortem tissue ELISA as both tests were only performed 11 and 12 times in 2017, respectively. [f] For Scotland data from check tests (serum antibody tests), although part of the CP, are currently not included in the present model.

The Dutch case study

Data from the BVDV CP that was in place in the Netherlands in 2017 were included in the model. Table 3 shows the number of monthly test results of all participating herds per type of test and the number and percentage of these results that were BVD positive.

Table 3. The type, number and results of BVDV tests performed in the Netherlands in 2017

Test	Number of herd-month level test results[a]	Positive herd result[b]
Antibody serum ELISA	11,094	683 (6.2%)
Antibody bulk milk ELISA	8,351	1,374 (16.5%)
Virus serum ELISA	20,125	288 (1.4%)
Virus bulk milk PCR	1018	16 (1.6%)
Virus ear notch ELISA	5,353	75 (1.4%)

[a] Because the herd-level model is used, these data show one test per type of test per month, regardless of the number of animals tested e.g. 5 youngstock tested for antibodies in 1 month is included as 1 Ab blood ELISA result. [b] A positive result means that at least 1 animal tested positive in the herd in one month. In case of herd-level testing, this means that at least 1 test (e.g. one group of youngstock in the spot test, or 1 bulk milk test) returned a positive result in one month.

Prior information: Prior information was included for test characteristics (Fig.1 and Fig.2) and infection parameters (Fig.3). The prior for herd infection prevalence is not shown, but was estimated at around 10% with Beta (17, 150). For the Netherlands, priors were estimated based on experience of previous years of BVDV control.

Convergence: The model was run with 10,000 iterations and three chains. Convergence was evaluated with trace plots. The trace plots showed good mixing for most parameters indicating convergence of the model. For the parameter "the probability of remaining infected ($\tau2$)" convergence was considered acceptable.

Parameter estimation: The posterior results are shown in Fig.1, Fig.2 and Fig.3. The posterior estimates for the sensitivity of most virus tests are similar (99–100%), only the virus serum ELISA test was predicted to have a lower sensitivity (82%). The posterior estimates for the specificity of the virus tests were all 100%. The sensitivities of the antibody tests for PI detection were predicted to be low: 39% for the serum test and 7% for the bulk milk test. The specificities for the antibody serum and bulk milk tests were estimated at 95% and 82% respectively. The probability of new infection in the predicted month ($\tau1$) was 1.2% and the probability of remaining infected between two months ($\tau2$) was 48%.

Predicted probability of freedom in herds in the Netherlands in December 2017 (model output): According to the STOC free model, the median probability of infection in all herds participating in the Dutch CP was 0.012, corresponding to a probability of freedom of 0.988 (1-0.012).
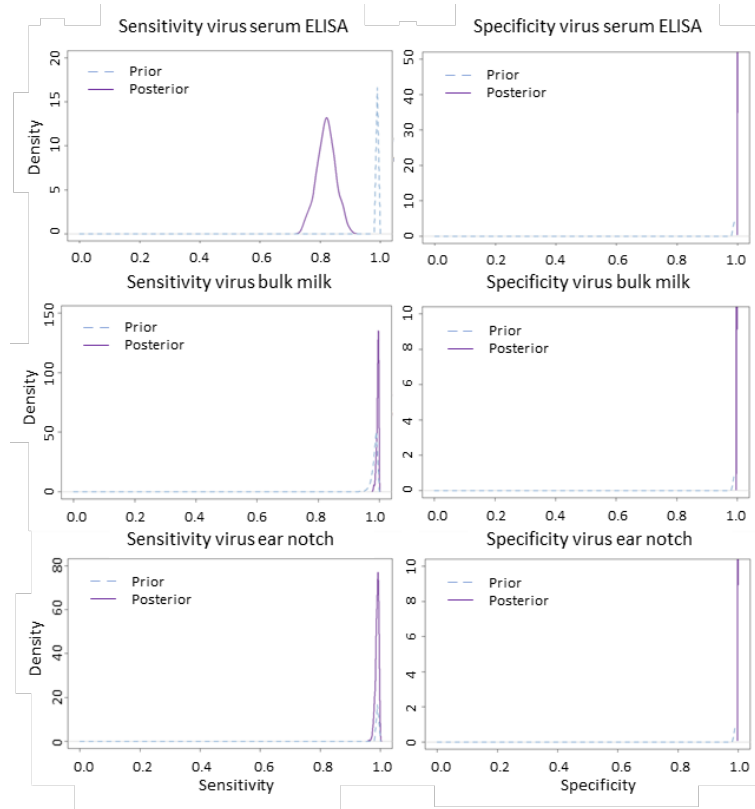
Figure 1. Priors and posterior model outputs for BVD antibody detecting diagnostic tests performed in the Netherlands. The alpha and beta prior estimates (α, β) for sensitivity and specificity of all three tests were (500, 2) and (500, 0.5) respectively.
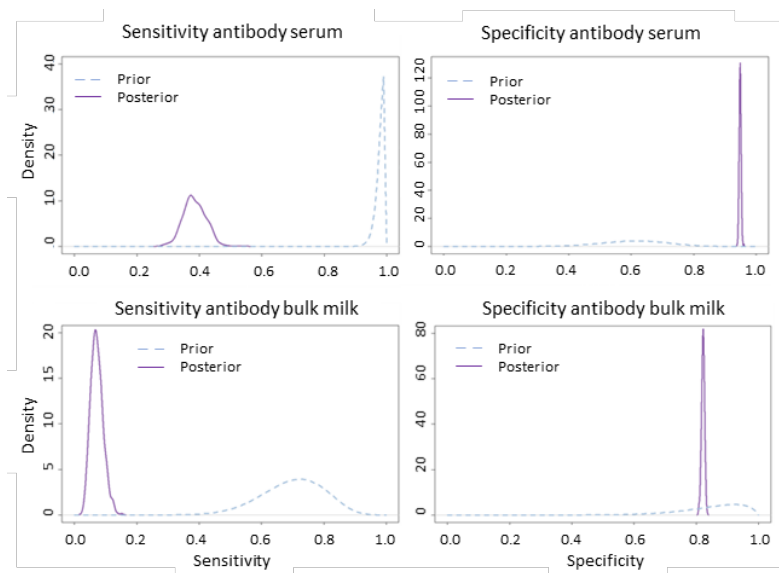
Figure 2. Priors and posterior model outputs for BVD antibody detecting diagnostic tests performed in the Netherlands. The alpha and beta prior estimates (α, β) for sensitivity and specificity of the antibody serum ELISA were (100, 2) and (14, 9) respectively and for the antibody bulk milk ELISA (14, 6) and (10, 1.75) respectively.
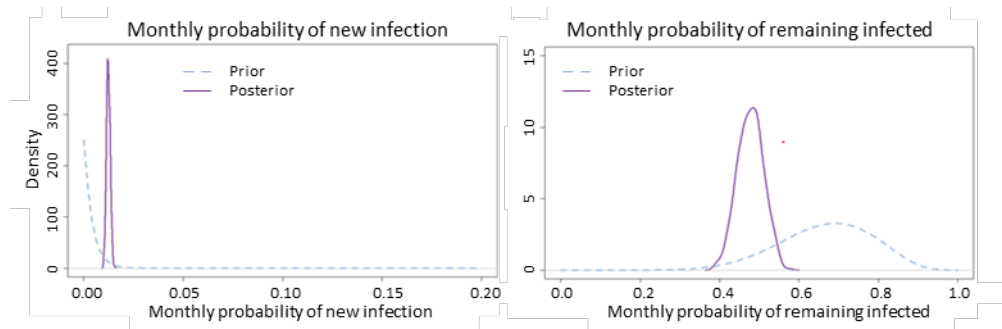
Figure 3. Priors and posterior model outputs for the probability of new infection (τ1) and the probability of remaining infected (τ2). The alpha and beta prior estimates (α, β) for τ1 and τ2 were (1, 250) and (10, 5), respectively.

Sensitivity analysis: In the first set of priors, the probability of being latent status positive at the first test was set at 10%, i.e. the herd-level prevalence of BVDV in 2017 in the Netherlands. This is probably on the high side as this is a yearly rather than a monthly figure. Therefore, it was decided to run the model again with a lower prior estimate for the prevalence of infection (4%) at the first test ($\alpha = 0.92$, $\beta = 22.08$), to see how the results would change. All other priors for infection dynamics and test characteristic were unchanged.

The change in the median values of all predicted model parameters are shown in Table 4 and Table 5. The median predicted probability of infection in December 2017 increased from 0.012 to 0.015, which was unexpected given that the prior probability of infection was decreased.

Table 4. Comparison of estimated median sensitivities and specificities for a model(1) with initial priors and a model (2) with a lower prior for the probability of being latent status positive at the first test

	Antibody serum	Antibody bulk milk	Virus serum	Virus bulk milk	Virus ear notch
Median sensitivity 1	0.39	0.07	0.82	1.00	0.99
Median sensitivity 2	0.96	0.98	0.72	0.99	0.98
Median specificity 1	0.95	0.82	1.00	1.00	1.00
Median specificity 2	0.98	0.92	1.00	1.00	1.00

Table 5. Comparison of estimated infection dynamics for a model(1) with initial priors and a model (2) with a lower prior for the probability of being latent status positive at the first test

Infection dynamics	Probability of new infection	Probability of remaining infected
Median model 1	0.01	0.48
Median model 2	0.02	0.72

Main challenges per country

Many lessons were learned from the application of the STOC free model on data from six different CPs. Table 6 shows the main challenge of each case study.

Table 6. Main challenges encountered in the application of the STOC free model on CP data of six European countries.

Country	Key learnings and challenges in application of the model on field data
Germany	A small number of births per month and a high number of negative tests complicates convergence of the animal-level model because there is only little information available.
France	The accessibility of data is difficult because of regional BVDV control. Only antibody test results were available, making it impossible to model the latent status of PI presence.
Ireland	In the Irish model confirmatory test results were included, which may have influenced the results as these animals had two results, possibly in two different months. For comparison between countries it should be clear how to include such data in the model.
the Netherlands	The combination of a latent status of PI presence and different test types in one model i.e. antibody detection and virus detection complicates estimation of priors and model output.
Sweden	Sweden is considered free of BVDV. Without any positive test results the model cannot estimate the parameters.
UK	Combining data in one model was very complex i.e. animal-level testing and herd-level testing, antibody tests and virus tests, dairy cattle herds and beef cattle herds.

DISCUSSION

The STOC free model, a Bayesian Hidden Markov model for output-based comparison of confidence of freedom, was tested by applying it to CP data from six different European countries. In the current paper application of the model is illustrated with results from the Dutch case study and the main challenges encountered in all case studies are discussed, and form the basis for further optimisation of the STOC free model. The cases highlight the challenges of output-based modelling of BVDV due to complexity of the infection and the high level of heterogeneity between CPs. Examples of the differences between CPs include the combinations of different tests representing different types of outcomes i.e. direct (diagnosing virus) or indirect (diagnosing antibodies) indication of BVDV in the herd. Also differences such as confirmatory testing after a positive result, delayed removal of PI animals, application of biosecurity measures etc. are further examples of real life scenarios that are difficult to include in the model and compare between countries.

In the Dutch case study, it was especially challenging to combine antibody and virus detecting tests in one model. Estimating the priors for sensitivity and specificity of both test types is very complex as they had to be adapted to the latent status of PI presence. Therefore, these prior distributions had to include the sensitivity of the entire diagnostic chain i.e. not only the laboratory values for sensitivity and specificity, but also corrected for mistakes that can occur during the sampling process that may result in false-positive or false-negative outcomes. Furthermore, the animals tested are usually not a random sample of the herd, but are drawn from age groups with varying probabilities of PI presence. For example, when testing the lactating herd for antibodies, it is expected that adult cows from infected herds will have seroconverted by having been in contact with infected animals. In this case, the laboratory sensitivity and specificity are poor measures of the sensitivity and specificity of interest. The predicted sensitivities and specificities for the virus tests were high according to expectations but the sensitivities of the antibody tests in serum and bulk milk were expected to be higher

than 0.39 and 0.07, respectively. Different priors were tested, but without the narrow and high priors for the sensitivities and specificities of the virus tests, the model had difficulties converging. As a result, in the further application of the model we split the data from different test types and run them separately.

The challenge of finding the right priors was also seen in the other case studies. In the current study, each country was free to decide whether to base the priors on expert opinion, historical data or literature. However, it appeared that more guidance may be needed to make sure priors are correctly used in the model, with correct definitions and that conclusions about model outputs are correctly drawn. To enable comparison between countries and secure reliable results it is important that priors are estimated in a similar, scientific and transparent way.

In the veterinary field, latent class modelling is recommended because diagnostic tests are known to be imperfect. However, in these models, the latent class is defined by the prior distributions put on the test characteristics. If the test characteristics in relation to the latent class of interest are not known or if there are inconsistencies resulting from the different prior distributions (e.g. two perfect tests that give conflicting results), the model will return inconsistent results or will fail to converge. It could be questioned whether a latent class model is the most suitable method for modelling freedom of disease for different CPs when test characteristics in relation to the latent status of interest are unknown. In this study, the latent status of PI presence was modelled, but this could be problematic when the PI animal happened not to be tested e.g. in case of bulk milk monitoring and a PI calf is present that is not in contact with the cows. This is difficult to incorporate in a generic latent class model. However, the other commonly used method, scenario tree modelling, requires a design prevalence, which may sometimes be difficult to determine. Unlike the STOC free model, the scenario tree method will return a result, regardless of the validity of the model inputs. In addition, the advantage of a latent class model is that posterior estimates for all parameters are estimated rather than only the probability of freedom that also results from a scenario tree model.

In several case studies, convergence issues were encountered. It could be questioned whether a model with monthly time steps is feasible for all different types of BVDV CPs. For example, in herds that test for antibodies in bulk milk or youngstock, it is common that test results are available once every three or six months. This data could be too sparse to provide sufficient information to the model to predict the monthly model parameters, leading to convergence issues. The same problem may arise in seasonal-calving herds and small herds that perform ear notch testing of calves. When no calves are born in a given month, there is no information for the model to learn from. A possible solution could be to include results for all animals in the herd with a negative test result each month, independent of the month of testing. For BVDV this could be valid because confirmed non-PI animals cannot subsequently become PI. This was explored in the Irish and German case studies and shows more stable results.

In the current study the beta version of the STOC free model was tested. The feedback resulting from the case studies forms the basis for further optimisation of the model. To meet the overall purpose, i.e. to analyse data and estimate the probability that infection is truly absent in a herd which is considered BVD-free according to the CP in place, a standardised and user-friendly way to summarise the model output is needed.

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A LARGE-SCALE EPIDEMIOLOGICAL MODEL OF THE IRISH CATTLE POPULATION TO SUPPORT DECISION-MAKING IN THE CONTEXT OF BOHV-1 CONTROL

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SUMMARY

We present a new modelling framework to address the evaluation of national control/surveillance programmes planned in line with the Animal Health Law (AHL) for livestock diseases. Our modelling framework is applied to the cattle sector in Ireland where there is need for policy support to design an optimal programme to achieve BoHV-1 free status under the AHL. In this contribution, we show how our framework results in a regional model that is able to mechanistically reproduce the demography, management practices and transport patterns of an entire cattle population without being dependent on continuous livestock registry data. Finally, we apply our model to support current discussions regarding the structure and implementation of a potential national BoHV-1 eradication programme in Ireland.

INTRODUCTION

The new Animal Health Law (AHL) and its implementation principles present new opportunities for the management of livestock diseases (European Commission, 2016). Especially for the bovine sector, this regulation allows the use of modern technologies for the monitoring of animal health activities. With the implementation principles laid down in the AHL, the herd management type has become an important criterion for shaping the sampling strategy of national surveillance and control programs. This requires not only a rethinking by policy and decision makers, but also an update of epidemiological models which are tailored to support the decision-making process.

The use of epidemiological models representing the spread of infectious diseases in cattle populations does assist with animal health decision-making and policy support. In recent years the development of these models has increased (Brock et al., 2020). In particular, regional models have been proposed because they allow possible consequences of decisions on the success or costings of a surveillance/control programme to be simulated across an entire region.

Most regional cattle disease models developed so far are data-driven, with birth, death and transport-related events directly simulated from observed data. Data-driven modelling provides

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a realistic representation of interacting farms at a regional scale, and allows precise evaluation of the mechanisms involved in the spread of pathogens. However, the simulation period is constrained to the range of observed data, which makes it difficult to provide long-term predictions about the success of a potential eradication program. This limitation could be overcome if it was possible to generate animal movement dynamics for yet unobserved time horizons. If a generic transportation network could be incorporated into regional models of pathogen spread, this would allow for testing trade-related interventions, e.g. restricting animal transports to farms of equal epidemiological status.

It has also been shown that the majority of regional cattle disease models are tailored to specific production systems, e.g. Beaunée et al. (2015) for the dairy sector in Brittany, France. Because other production systems are not included, it is not possible to evaluate the impact of national programmes across all participating production sectors. Furthermore, less detail in representation of cattle herd management may complicate effective model-based decision-support. Hence, it would be valuable to have a more detailed representation of management specific to herd type to assess animal disease control and surveillance activities under the AHL.

In this contribution, we present a new modelling framework to overcome the recognised limitations of national control/surveillance planning in line with the AHL. We apply our modelling framework to the cattle sector in Ireland to design an optimal programme to achieve BoHV-1 free status under the AHL. Here, we show how our framework results in a regional model that is able to mechanistically reproduce the demography, management practices and transport patterns of an entire cattle population without being dependent on continuous livestock registry data. For simplicity and run time reasons, we constrained our model to County Kerry, Ireland, which has been identified in previous analyses as a representative subset of the total Irish cattle population. Finally, we apply the model to support decision-making in the context of current discussions regarding the structure and implementation of a potential Irish BoHV-1 eradication programme.

MATERIALS AND METHODS

Datasets

Several datasets were analysed to gain an understanding of the dynamics of the Irish cattle population (Table 1). All data listed in Table 1 was obtained from the Animal Identification and Movement (AIM) database maintained by the Department of Agriculture, Food and the Marine (DAFM) in Ireland. In line with EU requirements, the AIM database includes records of all births, movements and disposals (i.e. origin, destination and date) and tracks every bovine animal in Ireland from birth or import to death or export (DAFM, 2017). In the database, each animal is recorded with its own unique identity (ID) or tag number, along with its sex, breed and birth date, as well as the IDs of its herd and dam.

From this database, three different datasets were generated that were considered important for describing the characteristics of the Irish cattle population. Taking into account the patterns of calving in Ireland outlined by Tratalos et al. (2017), we calculated herd-level demographic data for all cattle registered on three dates in 2017 (1st January, 1st May and 1st September). Birth data were drawn from 2016 to 2018 to get an understanding of the calving characteristics. Movement data was extracted for each day in 2017 (1st January – 31st December 2017).

Table 1. Data used to describe the Irish cattle system

Dataset	Description	Source
Herd population in 2017	List of all cattle registered in Ireland on three different dates (1st Jan, 1st May and 1st Sep)	AIM database
All births from 2016 to 2018	List of all births from 2016 to 2018	AIM database
All animal transports in 2017	List of all animal movement in 2017	AIM database

The cattle population in Kerry

The data shown in Table 1 has been used to classify each individual herd in Kerry into one of 16 different herd types. A herd type was considered as a population of farms with broadly similar farming and management practices. The herd types were identified from a previous analysis where expert knowledge was coupled with a machine-learning algorithm called self-organising-maps (SOMs). Detailed documentation of the classification procedure and a description of the identified herd types is described by Brock et al. (2021). Overall, the cattle population in Kerry was classified into 5 main herd types, some of which can be further divided into subgroups (Fig. 1). The main herd types present in Kerry are dairy, beef, store, mixed and fattening herds, with beef herds accounting for the largest proportion (~ 45%) of all of the approx. 5000 registered herds.
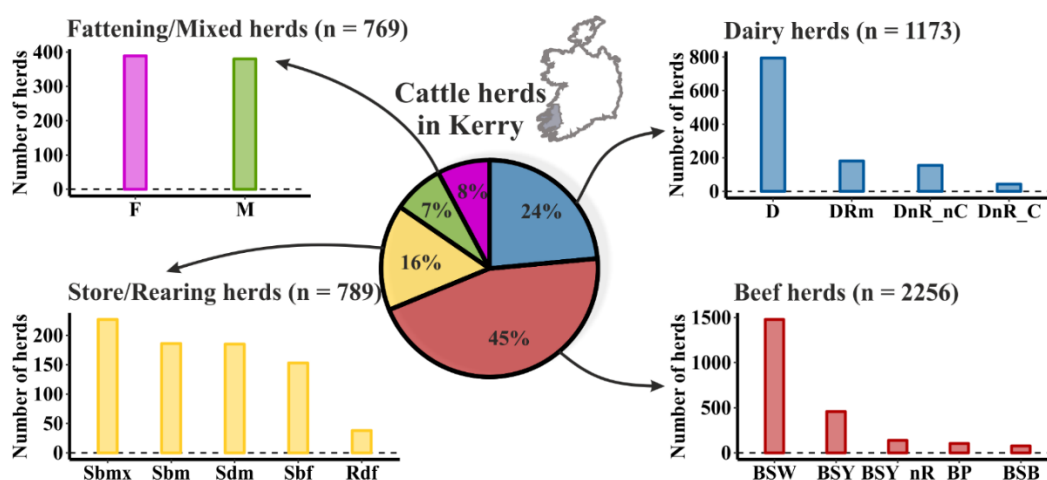
Figure 1. Herd types and their distribution in Kerry. See Fig.2 for herd type abbreviations.

Description of the model

A stochastic regional, spatially explicit, individual-based model was developed to simulate the entire cattle population in Kerry. The model called FarmECS-IBR represents each of the ~5000 cattle herds (Fig.1) and ~360,000 bovines along with its herd type-specific management. Following Brock et al. (2020), the model is composed of three main components representing [1] biological processes, [2] management-related processes and [3] pathogen-related processes. The simulation model runs in weekly time-steps and is implemented in Scala (https://www.scala-lang.org/). A complete technical documentation of the model according to

the ODD protocol (overview, design concepts, and details) (Grimm et al., 2006) is provided at https://ecoepi.eu/CattleDisMM/#IBR-svepm21.

Biological processes

In the model each bovine is represented with its biological processes. At each simulation step the age of the animals increases by one week. Ageing triggers other biological and management-related processes, e.g. transfer between management units, breeding of cows or an increased mortality.

Herd management processes

For each of the 16 herd types shown in Fig. 1, a rule-based management scheme was implemented which is followed by all herds of the respective type. This management scheme determines, for example, how long herds of a certain type keep youngstock, at what age they sell them and whether herds maintain themselves through breeding of their own replacement females or by purchase. An overview of the implemented management rules for all 16 herd types is provided at https://ecoepi.eu/CattleDisMM/#IBR-svepm21.

All self-maintaining herds are subject to a breeding process. In the model, breeding is done by the farmer whenever a herd is in its breeding season, with timing influenced by the desired calving interval. For simplicity and in accordance with the findings by Tratalos et al. (2017), we represent each breeding herd in Kerry as spring calving because that was the general pattern for most herds in Kerry. Breeding is applied to all heifers if their age exceeds a minimum age threshold (57 weeks) and to all cows that have exceeded a predetermined waiting time (4 weeks) after their last calving and only if herds are in their breeding season.

After successful breeding, each farmer estimates whether the number of inseminated animals is sufficient to meet his/her annual production target. If the number of inseminated animals is higher than desired, the farmer has a surplus and sells unneeded bred animals. Due to unpredictable biological processes (e.g. mortality, infertility, abortion) the number of animals inseminated may not be sufficient. In this case, this is typically compensated for by purchasing animals from herds with a surplus.

What happens to new-born calves depends on the management rules that we have defined for each herd type. Some herds sell most of the calves that they do not need for replacement soon after birth to store herds where calves are reared. Other herds keep most of their new-borns to sell at a later age as youngstock for fattening. Except for the beef-suckling-to-beef (BSB) herds, all self-calving herds sell all their male offspring. The timing of these animal sales varies between herd types. As an example, dairy herds (D) sell all their male calves at between two and 13 weeks of age. In contrast, dairy rearing male (DRm) herds keep their male offspring up to 104 weeks of age.

Animals not needed for replacement are predominantly moved to store or fattening herds. In the model, these herds are non-breeding and non-calving, and therefore completely dependent on the purchase of animals. Store herds rear their purchased animals until they reach a certain age at which point they are sold as youngstock for fattening to fattening herds. The current number of animals in each individual herd and the actual number of traded animals emerges from the herd-management rules.

Animal movements

In the model, animal movements emerge from the herd type-specific management rules and stochastic processes, e.g. mortality or infertility, and are driven by supply and demand.

Categories of animal transport cohorts between farms were determined based on the herd types identified (Fig.2). The transport network in the model was derived from these cohorts. Figure 2 shows the predominant transport routes with the corresponding animal transport types. Time and volume of these transports is determined by the transport algorithm according to supply and demand in the respective time step of the simulation.

The sex of an animal, as well as the time (age) when the animal is to be sold, determines to a large extent the trading cohort to which animal is assigned. For example, male calves sold from dairy (D, DnR_C and Dnr_nR) herds between 2 and 13 weeks of age are assigned to the calf dairy male (CDM) cohort. In the model, certain herd types, such as store-dairy-males (Sdm) herds in this example, have a demand for exactly this trading cohort according to the availability of space within their premises (Fig.3).
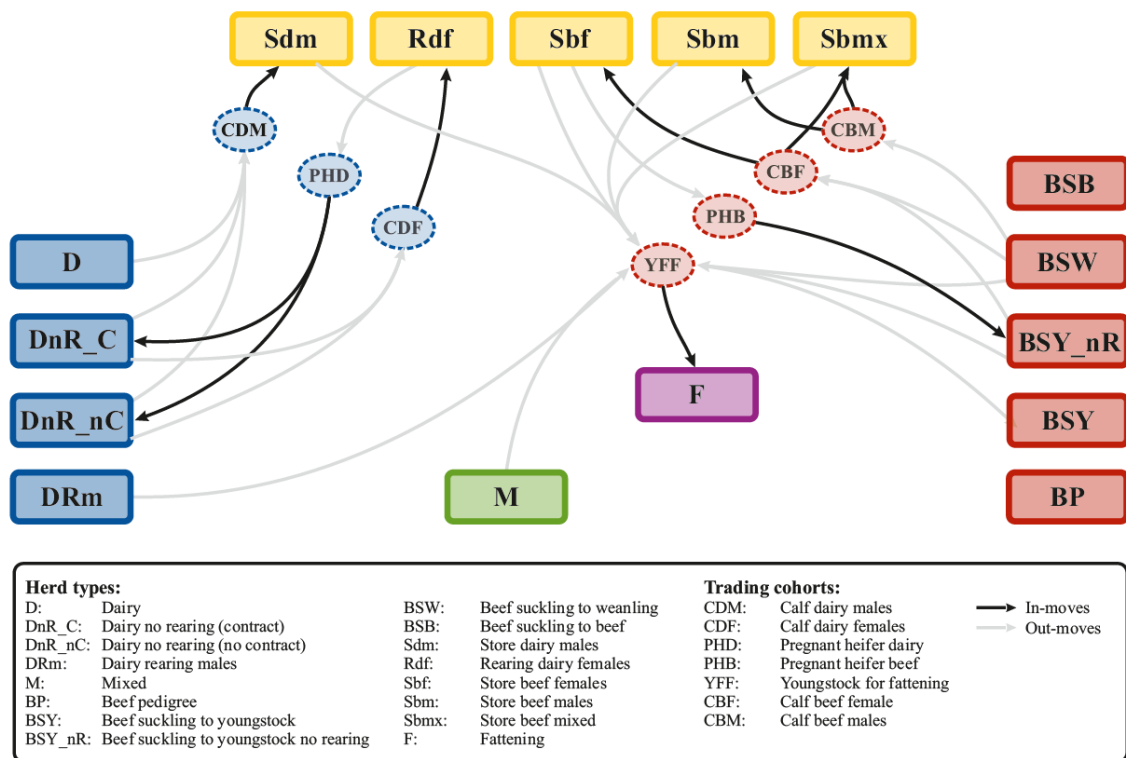
Figure 2. Typical animal transports under the cattle management system in Kerry. To assist with clarity in presentation, only primary movement cohorts are shown. Out-moves (supply) from herd types are indicated by grey lines while in-moves (demand) are indicated with black lines.

Epidemiological processes

In the model, each animal is assigned to one of four BoHV-1 epidemiological states. Seronegative animals that have never been exposed to BoHV-1 are susceptible. If a susceptible animal becomes infected, it enters the primary infection state and will be a lifelong carrier of the virus. In the model, the infectious period of a primary infected animal is two weeks. During this period, the animal is highly contagious and poses a risk of infection to other susceptible animals. Following primary infection, hosts recover and become latently infected. These animals become seropositive but do not shed virus. The virus can be reactivated in latently infected animals, leading to secondary infection and viral shedding. In the model, secondary infection lasts for one week, with affected individuals again posing a risk of infection to

susceptible animals, albeit a lower risk than from primary infected animals. After recovery from secondary infection, animals return to the latently infected state.

The event of getting infected with BoHV-1 is modelled stochastically by means of a frequency-dependent transmission rate that is converted into an individual infection probability. Latently infected animals have the potential to reactivate the virus which causes a secondary infection. Virus reactivation is simulated as a binomial random process, with a constant weekly reactivation probability. If an animal is transported in the model the reactivation rate is increased at the time point when the moved animal enters the new herd. This is intended to reflect the increased stress associated with animal transport and thus an increased probability for virus reactivation.

Model initialization, simulation and output

The model was initialized from a table listing the position, herd type, target size, breeding and outdoor period for each of the ~5,000 cattle herds in Kerry. Then, for each herd in the table, animals were created and added to the simulation. Afterwards, a 20-year pre-simulation was computed, to ensure a proper initial population structure and valid animal components and state variable values.

After the pre-simulation, and for the sake of model validation, the cattle population in Kerry was simulated for a period of 30 years. A simulation year has 52 weeks starting on 1st January and ending on 31st December, corresponding to weeks 1 and 52, respectively. In simulation year 30, we extracted the population composition of all herds in the model for the 3 points in time for which we have demographic data available (Table 1). Furthermore, we extracted the transport network generated by the model for simulation year 30, which makes it possible to calculate from and to farm transport volumes from the represented herd types.

Model validation

The behaviour of the model has been qualitatively validated using a technique called pattern-oriented modelling (POM). POM requires the model developer to define multiple patterns that can be observed in real world systems to reproduce these patterns accurately in the outputs of the model (Grimm et al., 1996).

In consultation with veterinarians and livestock experts from Ireland, we have identified two different data patterns. The first pattern we expect the model to replicate is the age structure of the cattle population in Kerry. Notice that the age structure was not used as an input to the model. Rather, we have derived the "field-based" version of this pattern from the animal data listed in Table 1. The age distribution for each herd type in Kerry was recorded from the data based on 1st September in 2017 (Table 1) as follows: based on the date of birth of each tested animal, we calculated the age in days at the time of sampling. Animals were then grouped into age cohorts by year from 0 to 9. Animals older than 9 years were assigned to cohort >9. Animals were then aggregated according to the age cohort and herd type.

The second pattern the model is expected to replicate relates to the animal transport behaviour in Kerry. Again, trading volumes and relationships were not inputs to the model algorithm but herd-type specific management rules created demand and supply at the level of the individual herd. For the field-based pattern, we extracted the total annual transport volume between the five main herd types from the daily resolved animal transport data (dairy, beef, mixed, storage and fattening) for the year 2017.

After running the model for validation purposes, we analysed the model output in the same way as herd register and movement data. At simulation week 34 (equivalent to September 1st) of year 30, we read the number of animals per herd type and age cohort grouped by years from 0 to >9. To achieve comparison between field and model regarding transport volume and network connectedness between herd types, we have read the total number of animals transported between the five main herd types.

Modelling BoHV-1 intervention strategies

Finally, the validated FarmECS-IBR model was applied to support the current discussions regarding the structure and implementation of a potential national BoHV-1 eradication programme in Ireland. Simulation experiments were conducted to compare alternative intervention strategies by their impact on herd-level prevalence. Each model run was started with a 30 year burn-in period following BoHV-1 introduction, to result in an endemic situation as described by e.g. Barrett et al. (2018). From the 31st year onwards, the following disease management scenarios were applied:

- S1-"base line scenario": In the base line scenario, no BoHV-1 intervention strategies are simulated. All processes continued as before.

- S2-"transport restriction scenario": In the transport restriction scenario, only seronegative animals are traded. Each animal in the model intended for trade is serologically tested for BoHV-1 and moved only if the test result is seronegative (dependent on diagnostic specifications of the test, here assumed as perfect).

- S3-"eradication programme scenario": The last scenario is a combination of three separate interventions. As in S2, transport restrictions are introduced at the start of the program. In addition, each herd gets fully tested once a year at a random date (census test). Depending on the resulting within-herd prevalence, further interventions are applied to the herd. If within-herd prevalence is above 15%, all animals of the herd are (re-)vaccinated that year. If within-herd prevalence is less than 15%, all sero-positives animals remaining in the herd are culled and vaccination is stopped.

For each scenario, 20 repetitions were performed to account for variability induced by the stochastic processes in the model. For each scenario the proportion of sero-positive herds (containing one or more sero-positive animals) in the model is recorded on a weekly basis. Model outputs are compared between the scenarios.

It is important to note that these simulations are preliminary in terms of the scenarios modelled. We want to make clear that the scenarios do not replicate an AHL model, but are used to gain a theoretical understanding of how specific intervention strategies can be used to reduce the overall prevalence of BoHV-1. The scenarios show the utility of the model to explore this further, with the potential to refine the scenarios further to reduce potential program costs.

RESULTS

Model validation

Herd demography: Figure 3 shows the comparison between observed age structure data of the cattle population in Kerry (solid lines) and our output data extracted from model population

(dashed lines). For each herd management type herd type, the observed and simulated age structure is presented by sex, i.e. once for male (grey) and once for female (black) animals. We explicitly mention that the simulations are performed completely independently of the age structure data. Rather, the age structure of each herd in the model emerges from the herd type specific management characteristics we implemented.

In these age/sex distributions there is a high degree of similarity between observed and model data for those herd types that comprise the largest proportions of the total population (e.g. BSW and D). In all breeding herds, a right-skewed age profile can be seen. The high number of very young animals between 0-1 years of age is due to the number of calves retained in the herds. Logically, non-calving herds such as Fattener or Store herds have almost no animals in this age cohort because they only buy older animals. It is important to realise that age distributions differ substantially between the herd types, and these differences are reproduced by the herd-type specific rules used to manage the individual bovines of the herds.
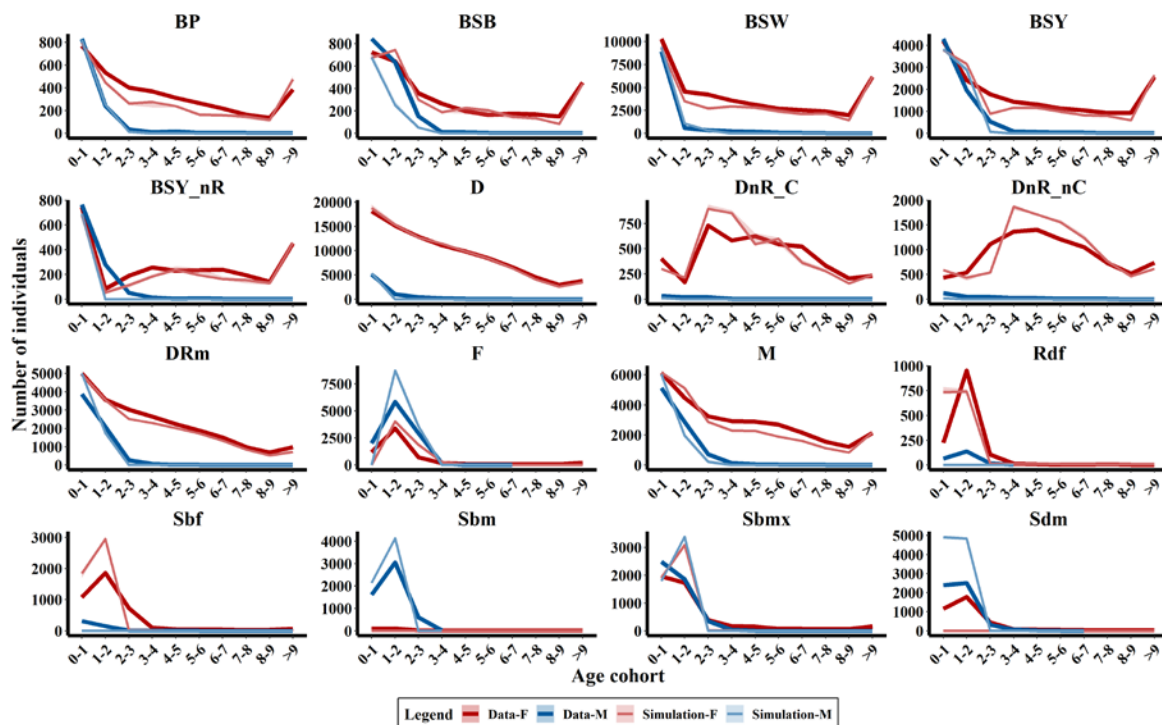
Figure 3. Comparison of observed (solid lines) and simulated (dashed lines) age structure per herd type and sex. For the simulation, the median number of animals per age cohort is shown, calculated from a total of 10 model runs with identical parameter settings. An uncertainty band is also illustrated for the simulation runs (0.95 and 0.05 percentile), but this only becomes visible in herd types where the total number of animals is small, e.g. DnR_C or BP herds.

Transport patterns: We have created centred alluvial plots to show the transport flows between herd types, as described by the transport network (Fig.4). The thickness of the arrows is a measure of the transport volume. It can be seen that the ratio of the transport flows between observed data and our emergent simulation results is consistent. It can be seen that the transport streams in the data are more erratic than in the simulation. Furthermore, there are differences between the data and our simulation, especially in the transport behaviour of Stores herds.
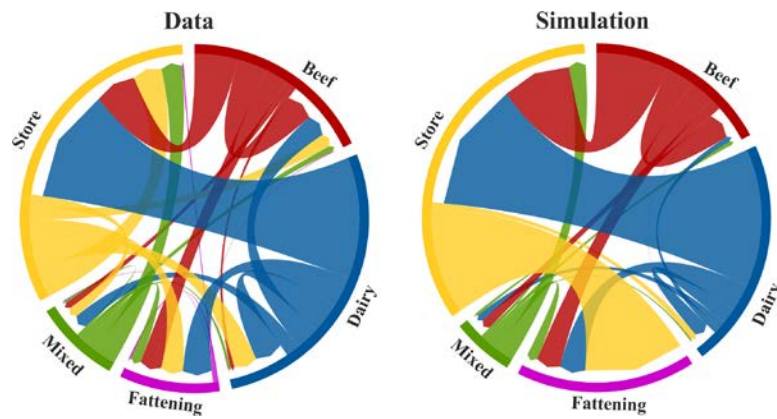
Figure 4. Comparison of observed (left) and simulated (right) transport flows per herd type. The thickness of the flows indicates the transport volume.

When validating the model we have paid attention to replicate further transport patterns which, according to our experts, have an influence on the epidemiological properties of the pathogen to be simulated. For example, we analysed the distribution of distances moved in trades between herds as well as the number of trading partners a herd had per year and. These additional comparisons are shown at https://ecoepi.eu/CattleDisMM/#IBR-svepm21.

BoHV-1 control strategies

The herd-level sero-prevalence of the BoHV-1 for scenarios S1, S2 and S3 is shown in Fig. 5. In the base-line scenario S1 (solid line), no intervention strategies were applied and the herd-level prevalence remained at a constant high level over the simulated years.
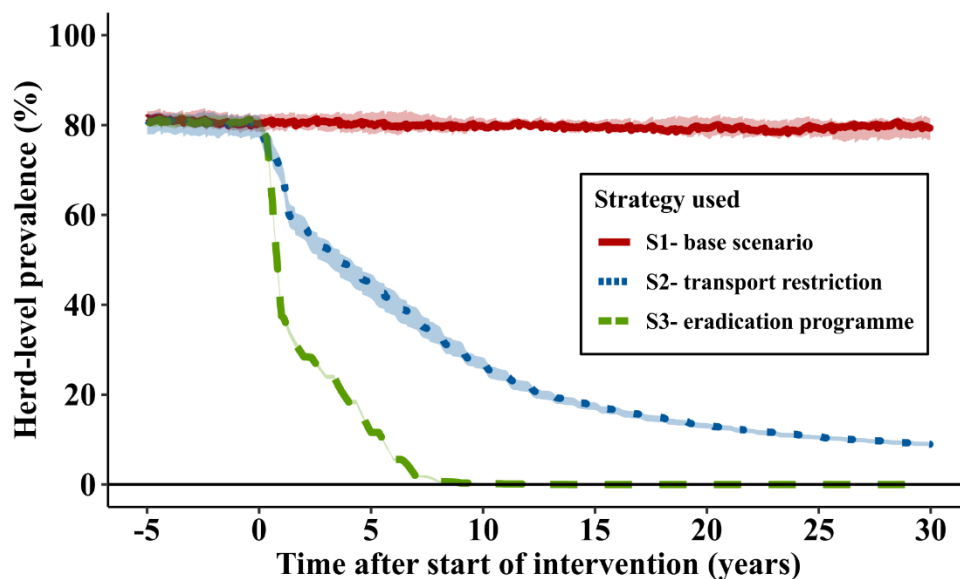
Figure 5. Comparison of the simulated strategies to reduce the total herd-level prevalence in Kerry (all herds in Kerry). Each scenario was simulated for 30 years (subsequent to 30 years burn-in). For each scenario the coloured/shaped lines indicate the median herd-level prevalence in Kerry calculated for each model step over 20 repetitions. In addition for each scenario the 95th and the 5th percentile is plotted.

This scenario indicates that without intervention there is no downward trend in the long-term herd-level sero-prevalence predicted by the model. Hence, any difference between the graphs of S1 and the intervention scenarios S2 or S3 can be attributed to the intervention measures applied.

The transport restriction strategy (S2, dotted line) resulted in a decrease of the herd-level prevalence. If 100% of herds participated, a herd-level prevalence of 9% would be reached after 30 years. Based on this scenario, BoHV-1 will only be eradicated within the practical time horizon of an eradication program with additional interventions.

In contrast, as outlined in S3 (dashed line), transport restrictions combined with BoHV-1 vaccination and targeted culling in herds where prevalence is low (< 15%) did eradicate BoHV-1 from the cattle population in Kerry. As a median value, the additional measures are effective in reducing the overall herd-level prevalence from 80% to 0% within 12 years (after 13 years in 90% of the simulations).

DISCUSSION

The FarmECS-IBR model in the context of regional cattle disease models

Mechanistic simulation models are being increasingly used as tools to assist with animal health decision-making in the cattle sector. While the vast majority of models developed so far represent virus dynamics and intervention within single herds, only a few models have been developed to simulate the spread and control of diseases within entire regions (Brock et al., 2020b).

We propose a new and innovative spatially explicit, individual-based, regional cattle disease model. The innovative feature of our model comes with the inclusion of herd typing, thereby extending the basis of dairy, beef or mixed herds usually considered in other modelling studies (e.g. Beaunée et al., 2015). We learned that herd features relevant for epidemiological predictions may differ systematically between the herd types. In our model sixteen different herd types were needed, to represent existing management schemes of the cattle sector in Ireland. This detailed representation of herd type-specific management is an advantage to other approaches if it comes to the evaluation of detailed policies in the context of the AHL. With regard to BoHV-1, there are several pathways possible how a herd or country may achieve the status of disease freedom. The explicit regulations and their implementation interact with specifics of the management regime in different herds. For example, bulk tank milk testing on its own can only applied to herds that have a minimum proportion of lactating cows. Contract rearing dairy herds (Rdf) would never fall into this category. However, bulk milk samples could be used in non-rearing dairy herds (DnR_C & DnR_nC). Such details become even more important when models are used to estimate program costs.

The herd specific management and animal transport rules alone generate animal numbers and age distributions in the individual herds that are consistent with the demography and transport data available from animal databases (see Fig.3 and Fig.4). Nevertheless, the model itself remains independent of direct data input. In this respect, it is possible to simulate trade structures that do not statically or randomly repeat observed data extracted from an animal movement tracing database (e.g. Beaunée et al., 2015; Thulke et al., 2018). The generic management and transport scheduling facilitates to actively intervene with the modelled population, e.g. when specific animals must be removed according to a detailed treatment

protocol. With our generic model implementation such off-management demands are easily satisfied without destroying the overall similarity of the model with real world data of a regional cattle population.

Flexible interventions on regional animal transports are required when it comes to evaluating the efficiency of restrictions of individual moves for virus eradication purposes (scenario S3). A different generic animal transport algorithm has been described with a model implantation by (Gethmann et al., 2019). However, the authors had to balance demand and supply through randomly generated animal movements whereas we use rules based on herd and cohort type to do this. Trading partners are also grouped together on the basis of physical proximity and specific herd type when deriving trading batches. This reduced the number of trading partners leading to more realistic picture of pathogen spread within regional systems.

The limitations of our approach are mainly that it is challenging and time consuming to develop a management specific regional cattle disease model. An intensive participatory approach was followed during the conception and development of the simulation model. For this purpose, each model rule was discussed and agreed between the project partners from the start of the project. In addition, the developed model rules were verified with inventory and transportation data from only a single year (2017). This could lead to the model being overfitted to the current management situation and not reflecting changes to the overall Irish cattle system over time.

Model application in the context of BoHV-1 control

Based on the results shown, implications for future BoHV-1 eradication programs are discussed. The development of herd-level prevalence under the transport restriction scenario (S2) shows the importance of a having a more closed farming system or implementing quarantine measures for purchased animals. Without further measures, the prevalence at herd level decreased by about 90% (overall prevalence of 80% to 9% after 30 years of intervention; Fig.5). Farmers therefore become aware of the risks concerning introduction of BoHV-1 through introduction of animals. Indeed several epidemiological studies report frequent animal purchases as a major risk factor for a herd testing positive for BoHV-1 antibodies (e.g. Boelaert et al., 2005). Measures at a national level may include compulsory testing of purchased animals for BoHV-1, or more frequent surveillance of certified herds, to reduce the risk of moving the virus from herd to herd.

However, the model has shown that BoHV-1 eradication will not result from transport restrictions used in isolation. More stringent interventions were required to guarantee prevention of reintroduction of infection into free herds and to remove the final pockets of infection in older animals (see Fig.5). These gaps were closed by protective vaccination and targeted culling, dependent on a herd's BoHV-1 sero-prevalence level (Fig.5). It is well acknowledged that culling of seropositive animals has been the most successful method for BoHV-1 eradication (Ackermann and Engels, 2006). However, this is not economically feasible in herds with medium to high sero-prevalence. Many studies have shown that vaccination can effectively reduce virus circulation and thereby decrease sero-prevalence in a herd or population (Bosch et al., 1998; Mars et al., 2001). Hence, our scenario S3 does effectively reduce herd- and animal-level prevalence through vaccination before the herd is finally cleaned-up.

Understanding the temporal dynamics of herd-level prevalence during an eradication program is an important component in our evaluation of the usefulness of individual

interventions and their combinations. Our study, which accurately applies these intervention protocols to detailed herd management structures and trading behaviour in a cattle population, will effectively contribute to the eradication planning of BoHV-1 in cattle and beyond.

We present the first regional epidemiological model representing an Irish cattle population along with its specific herd type categories. The herd type specific management parameters allow a generic simulation of realistic age and sex distributions across all production types. The model allows a realistic simulation of disease transmission and control/surveillance in the overall population. In difference to existing cattle disease model, our tool facilitates comparative assessment of BoHV-1 eradication strategies targeting different production types with individual strategy protocols.

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INVESTIGATING OPPURTUNITIES FOR AND CHALLENGES TOWARDS

ESTABLISHING COMMUNITY-BASED SURVEILLANCE OF EQUINES IN

SWITZERLAND

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AND S. DÜRR

SUMMARY

In the present study, a community-based surveillance (CBS) approach was tested among the equine community in Switzerland by revealing the intentions of equine owners towards reporting clinical signs, operationalizing an online CBS tool, and investigating potential reasons for the lack of its use via qualitative telephone interviews. Three main issues were identified that have led to the failure of the CBS approach: 1) for successfully implementing CBS, the need for surveillance within the community of interest must exist; 2) for the target audience, here the equine owners, to remember the presence and use of such a CBS tool, the population under surveillance, here the equine, need to present enough clinical signs, and 3) targeted and extensive communication and management of the system is key for its success. With this study, both the potential and challenges of CSB in animal health was showcased, which may be useful for similar future initiatives.

INTRODUCTION

Animal health surveillance has been developing continuously over the past decades providing new concepts, approaches and methods for the improvement of animal health (Hoinville et al., 2013; Dórea and Vial, 2016; Bisdorff et al., 2017). Health professionals, such as veterinarians, play a crucial role in surveillance. They are involved in routinely collecting animal health and production data, in active surveillance programs or in providing necropsy reports and diagnostic laboratory data (Gibbens et al., 2008; Faverjon et al., 2016; Muellner et al., 2016; Bisdorff et al., 2017; Küker et al., 2018; Tapprest et al., 2019). Passive surveillance data predominantly depend on clinical cases being presented to health professionals (Doherr and Audigé, 2001). Yet, not all diseased humans or animals seek – or are brought to receive – medical care, for reasons such as concern about health care costs or personal perception of a clinical case severity (Carrat et al., 2002; Marquet et al., 2006; Mörk et al., 2009; Espetvedt et al., 2013). Meanwhile, animal owners' potential to observe clinical signs, as the persons with the closest contact to their animals, is often neglected. Engaging animal owners in animal

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health surveillance, in the frame of community-based surveillance (CBS), could complement and strengthen existing surveillance efforts.

Up to date, the term CBS has mainly been used in the context of public health and One Health surveillance, predominantly in low and lower-income countries (Guerra et al., 2019a). The World Health Organization (WHO) defined the term community-based surveillance as follows: "CBS is the systematic detection and reporting of events of public health significance within a community by community members" (Guerra et al., 2019b). While an official definition and uniform use of the term CBS in animal health is lacking, there are various approaches and initiatives making use of community members' involvement and engagement in animal health surveillance. Such approaches are embedded in participatory surveillance, participatory epidemiology, citizen science and owner-based reporting (Mariner et al., 2002; Catley et al., 2012; Smolinski et al., 2017; Pollard et al., 2019). Within the field of equine health, multiple studies have described the use of owner-based reports to limit knowledge gaps of certain health disorders, such as in Australia (Cole et al., 2005) or in Great Britain (Ireland et al., 2013). While such studies made use of equine owners' capability to observe and report clinical signs, they have in common that reporting was limited in time, and thus did not provide continuous surveillance. As an exception, the French epidemiological network for equine diseases (Réseau d'Epidémio-Surveillance en Pathologie Equine, "RESPE") announced the implementation of the project VigiRespe, which was created to complement the existing veterinary surveillance system RESPEand increase its sensitivity with the help of observations by equine owners (Valon et al., 2012; Legrand et al., 2013; VigiRESPE, 2020). Yet, up until now no peer-reviewed publication about their experiences with the system is available. Thus, to the best of our knowledge, attempts to include non-sentinel and voluntary equine owners in disease surveillance have not been investigated yet.

The surveillance of equine health in Switzerland currently includes the mandatory reporting of 17 notifiable diseases according to the Swiss animal health law (Federal Council of Switzerland, 1995). In addition, Equinella (www.equinella.ch), a veterinary-based voluntary surveillance system for clinical signs and equine diseases not notifiable by Swiss law, is in place (Struchen et al., 2016; Wohlfender-Remy et al., 2016; Özçelik et al., 2020). During a presentation on Equinella at the conference of the Netzwerk Pferdeforschung Schweiz (translates to Swiss annual conference of the network of equine research) in 2016, equine practitioners as well as various stakeholders from the Swiss equine industry raised the question whether equine owners could also contribute to Equinella. This input from the equine community showed their interest to participate in surveillance systems and was one of the drivers to develop the CBS approach presented here.

In the present study we first aimed to reveal the perception, attitude and intention of Swiss equine owners towards CBS and reporting clinical signs of their equine, using a cross-sectional online questionnaire. Second, we set up and implemented an online CBS tool, named Equi-Commun. By implementing Equi-Commun our aim was to collect data on clinical signs reported by equine owners. Herewith we aimed to assess the benefits of such surveillance data in terms of timeliness, as well as data quality and quantitiy, in addition to data of existing surveillance system, in terms of timeliness. Finally, we investigated potential reasons for the lack of use of Equi-Commun by applying qualitative telephone interviews among equine owners.

MATERIALS AND METHODS

Online survey

The questionnaire contained 31 questions embedded in four main parts: 1) information on equine owned and/or kept on the own premises, 2) clinical signs observed amongst their equine and/or the equine kept on their own premises within the last year, 3) intention to use and attitudes towards a proposed CBS tool, and 4) demographic data of the respondent. To enable participants to reflect on the questions regarding their attitudes toward a CBS tool, a description of the not yet launched Equi-Commun was provided within the questionnaire. A sample of 7,500 equine owners was randomly retrieved from the national registry of equine owners and keepers. This number of equine owners contacted was calculated using a design prevalence of 50%, a confidence level of 95%, a precision of 5% and an estimated response rate of 20%. The questionnaire was sent out per e-mail in July 2018. Participants were invited to provide their e-mail address at the end of the questionnaire to be informed about future steps regarding the project; otherwise the survey was anonymous. A reminder was sent to all recipients after one week. The survey was accessible for two weeks in total.

Data analysis

Only fully completed questionnaires were taken into account for the statistical analysis. Descriptive statistics on the study population were performed. We also reported the number of clinical cases observed by the equine owners during the last 12 months. One question from the survey was used to evaluate the self-reported intention to contribute to the CBS tool. This question asked the extent to which respondents could imagine themselves reporting to a CBS tool, on a 5-point Likert-scale ranging from 1 = "not at all" to 5 = "certainly yes". Data analysis was conducted using R software (R Core Team, 2019).

Community-based surveillance tool development and testing

The CBS tool Equi-Commun was conceptually designed following the structure of the veterinary-based voluntary surveillance system Equinella (Struchen et al., 2016; Wohlfender-Remy et al., 2016; Özçelik et al., 2020). The publicly accessible online tool was technically implemented by a professional IT company (https://www.4eyes.ch/#start) and went live in October 2018. Equi-Commun was addressed to equine owners (further referred to as reporting person(s)) to report observation on clinical signs of their equine as soon as they are observed. For each record, the following data had to be registered on Equi-Commun: identification of the affected equine, location and postal code of the equine's holding, number of equines on the same premises, observed clinical sign (at least one has to be selected from a provided radio-button list), date of onset of the observed sign(s), and duration of the observed sign(s). In addition, whether or not a veterinarian was contacted, identification of the veterinarian, when the visit took place, and the diagnosis made, were requested as optional data. A reporting person was given the option to register and profit from a user-protected database of own records, or it can report a clinical sign without registration. When a record was submitted, an automatic response was generated on the website stating that the report was successfully submitted and the Equi-Commun team received an automated e-mail notification.

In multiple rounds, Equi-Commun was tested by the authors, the project supporting team of the Swiss Federal Food Safety and Veterinary Office and equine owners for practicability, logic, user-friendliness, and correct automatic responses. Equi-Commun was communicated and promoted through diverse communication strategies and multiple media channels between July 2017 and June 2019. They consisted of presentations at scientific conferences, print media

articles in equine magazines, distribution of flyers in equine clinics, e-mails sent to participants of the online survey to inform about the launch of Equi-Commun, to the Swiss veterinary faculty staff, and to veterinarians via the Equinella newsletter, and regular social media performance via the Equi-Commun facebook page.

Qualitative interviews

To assess potential reasons for the equine owners' lack of compliance towards Equi-Commun, we conducted semi-structured qualitative telephone interviews. An interview guideline was drafted according to the recommendations of Cornelia Helfferich (Helfferich, 2016) and based on previous knowledge collected through the online questionnaire. Interview questions focused on capturing the knowledge and understanding of equine owners in regards to CBS and the tool Equi-Commun, reasons why they did not use Equi-Commun and reasons they thought why other equine owners did not use Equi-Commun. Interviewees were selected from a pool of equine owners that had previously provided their e-mail addresses voluntarily when completing the online questionnaire. The phone interviews were conducted in November 2019 and were recorded digitally with prior oral consent from the interviewees. The interviews lasted on average 15 minutes. After ten interviews, there was no variation in the responses, and thus the interviews were terminated. A verbatim transcription of the recordings was made. The transcripts were analyzed using the qualitative data analyzing software MAXQDA2020 Analytics Pro (VERBI Software, Berlin, Germany) applying an inductive open coding approach.

RESULTS

Online survey
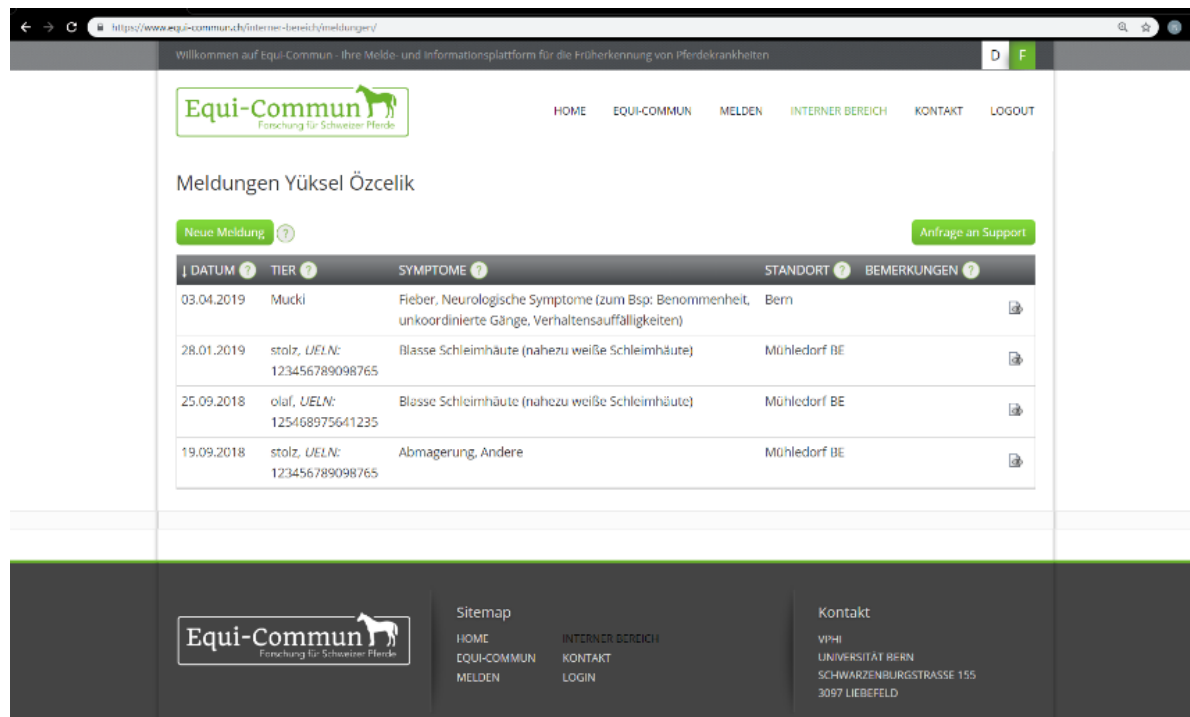
We received 1,078 completed questionnaires, corresponding to a response rate of 14.4%. All 1,078 respondents reported to have observed in total 17,016 clinical signs among their own equine and/or the equine on their premises during the last 12 months. The median number of clinical signs observed per respondent was 4 (range 0 – 340, IQR: 1 – 11). The most common observed clinical signs were pruritus (29.3%, 4,988/17,016), respiratory signs (23.5%, 4,006/17,016), lameness (19.1%, 3,248/17,016) and diarrhea (14.5%, 2,462/17,016). Overall, respondents contacted a veterinarian in 14.2% (2,420/17,016) of the cases after observing clinical signs (median = 1, range 0 – 60, IQR: 0 – 3).

Intentions to contribute to CBS

The majority of respondents (65.5%, 707/1,078) answered that they could certainly (12.5%, 135/1,078, "certainly yes"), presumably (26.4%, 285/1,078, "presumably yes") or potentially (26.6%, 287/1,078, "maybe") report clinical observations of their equine in a CBS tool such as described in the questionnaire. Approximately a third (34.5, %, 371/1,078) of all respondents answered they would not (8.4%, 91/1,078) or rather not (26.0%, 280/1,078) report clinical signs they observed.

Community-based surveillance tool Equi-Commun

Equi-Commun was technically functioning without issues after its launch on the 22nd of October 2018. Print-screens of the platform are presented in Figure 1. Until the 31st of December 2019, Equi-Commun received four reports from three unique users. These consisted of two cases of lameness, one case of colic and one case of mallenders. None of the reports was explicitly related to suspicious or confirmed infectious diseases. Due to lack of use, the Equi-Commun reporting tool website was inactivated by the end of December 2019.

Figure 1. Screen-print of the user interface of Equi-Commun. List of submitted reports (dummy data) of one user in the internal space.

Qualitative phone interviews

Among the ten interviewed participants, all stated to have a positive attitude, i.e. that they believe in its benefit for surveillance, towards the CBS tool Equi-Commun. Further statements deriving from the respondents included the mentioning of their limited interest in the tool and the statement they previously felt well informed about Equi-Commun. Yet from the course of the interview it became apparent that respondents could not correctly remember the aim and use of Equi-Commun. Respondents further mentioned that they perceived active and repetitive information as necessary to improve compliance with the platform. The following reasons for the non-use of Equi-Commun were mentioned by the respondents: a) lack of awareness about Equi-Commun, b) possible hesitation about creating a negative impact for the equine premise of the own equine, if clinical signs were reported, c) missing clinical knowledge among the equine owners regarding general issues about equine and medical understanding, and d) that their equine were healthy, and thus they were not able to report health issues.

DISCUSSION

The present study is the first attempt at determining the potential and challenges of CBS within the Swiss equine community. Additionally, this is the first study describing the process of establishing and disseminating a CBS tool for equine surveillance. Although the aim of Equi-Commun was to assess the benefit of surveillance data derived from a CBS approach compared to already existing equine health surveillance data, this aim was not achieved in the current project, as Equi-Commun received only four reports during the period it was online.

The success of a CBS system is dependent on the perceived need of the community towards generating surveillance information. El Allaki et al. (2012) argued in their work on health surveillance theory that the initiation of a surveillance process requires three steps: i) a dissatisfaction regarding the current (health) situation, ii) a need for knowledge and/or time dependent information, and iii) some level of motivation to eliminate the dissatisfaction and to approach the information need on the population health status (El Allaki et al., 2012). Applying this concept to the equine CBS tool we have strived to implement, equine owners must recognize and perceive a certain dissatisfaction regarding their equine's health and/or their surveillance in order to show compliance to a CBS approach. In other words: only if there is a perceived need for CBS in the Swiss equine community, such a system would likely be successful.

While our study has not directly assessed the dissatisfaction of equine owners nor the perceived need for CBS, we assessed the intentions of Swiss equine owners towards CBS. The majority of respondents (65.5%, 707/1,078) answered they could see themselves reporting clinical signs. In addition, at the conference of the Netzwerk Pferdeforschung Schweiz in 2016, stakeholders from the Swiss equine industry clearly stated their interest in being actively involved in the surveillance system. These aspects were regarded as a promising prerequisite for the success of a CBS approach and we therefore expected to receive more interest in and reports submitted to Equi-Commun. However this was not the case.

Possible explanations for this non-use of Equi-Commun can be found in disciplines investigating the complexity of human behavior. According to the Theory of Planned Behavior a certain human behavior is a product of humans' "intention" to carry out certain planned actions, their "attitudes" (i.e. values, priorities) towards this behavior, "social norms" about the behavior (i.e. external expectations placed upon them), and their "perceived behavioral control" over the behavior (i.e. their perceived ability to put actions of their choice into effect) (Ajzen, 1991; Alarcon et al., 2013). In our study, we investigated the intention to report to Equi-Commun and the behavior of people by observing the reports submitted to Equi-Commun. The majority (65.5%) stated that they intent to report or at least maybe report to a tool such as Equi-Commun. However, respondents have possibly reflected on their role in disease surveillance as more positive than they actually do. In addition, we assessed the intention to use Equi-Commun via a description of the yet to be established Equi-Commun. Hence, respondents were not able to reflect on previous experiences directly, yet only on their reflection on a hypothetical case scenario description of a CBS tool. In the qualitative telephone interviews, after it was apparent that Equi-Commun failed, we again observed a positive perception towards the CBS tool, with all interviewees responding to clearly see a benefit in the tool. However, selection bias is expected, as participants of the telephone interviews were selected from the pool of online survey respondents that left their e-mail address voluntarily at the end of the questionnaire, in order to be updated in regards to Equi-Commun developments. Thus, it is likely that telephone interviewees represent the more motivated and interested equine owners with a positive attitude towards CBS.

This study enables to investigate the intention of the equine owners in regards to CBS, as well as their actual behavior. With the majority of respondents having stated positive intention towards CBS and only four reports recorded in Equi-Commun, we clearly observed a so-called intention-behavior gap. The concept describes the discrepancies between human intention to perform a certain behavior and them acting accordingly and has found particular interest in the research of medical and life-style behavior of patients within the health sector (Sheeran, 2002; Faries, 2016; Sheeran and Webb, 2016). Obstacles influencing the intention-behavior gap can be divided into three main categories: getting a new tool started (e.g. in our case, setting the intention to report clinical signs), keeping it ongoing (e.g. keeping informed about Equi-Commun), and reach the goal (e.g. the actual act of reporting to Equi-Commun) (Sheeran and Webb, 2016). Elaborating on the complexity of equine owners' intentions and how these translate it into their behavior is of critical importance. These topics should be object of future research.

One of the most relevant shortcomings of successfully implementing a CBS system may lie in the project dissemination, communication and marketing. An effective CBS approach requires staff dedicated to manage the project, who continuously contact, inform and support community members in collecting data, maintain a database, analyze and visualize data, and disseminate analysis outcomes (Bonney et al., 2009). Within the scope of our study, we have adapted several strategies to disseminate Equi-Commun, such as via print media, social media, on conferences or through personal communication at clinics and private veterinarians. However, through the qualitative interviews we revealed that although information provided by the project management team during the communication phases was perceived as clear and understandable, it was not efficient and persistent enough to be remembered after one year's time. This demands for consistent, targeted and more frequent information campaigns. Nevertheless, when planning more resource demanding interventions for setting up a CBS system, the benefit should be weighted in comparison to the necessary resources, such as personnel, finances and time. Even though CBS systems can be less material demanding compared to active surveillance system (for example continuous serological surveillance) other resources (for example to consistently promote the system) needed to set up and maintain the system have to be accounted for.

Furthermore, the relatively high level of equine health among the Swiss population was a potential reason for the equine owners' non-compliance to Equi-Commun. This was confirmed by interviewees of the qualitative survey mentioning their equine's good health as a reason for non-reporting of clinical signs. Although census studies on the health or diseases of the Swiss equine population are lacking, the low number of official reports on notifiable infectious diseases supports the argument that at least critical equine infectious diseases are rare (InfoSM, 2020). Similarly, although a voluntary reporting system of non-notifiable diseases, and therefore not expected to be thoroughly representative of each disease event in the equine population, reports submitted to Equinella have also been rather low in number (Özçelik et al., 2020). On the other hand, respondents of the online questionnaire reported as a median to have observed four times clinical signs among their equine within a year. Therefore, in case Equi-Commun would be more present in the equine-owners mind, there is potential for reports submitted to a CBS tool. Additionally, respondents stated to have contacted a veterinarian in only 14.2% of all observed clinical cases. This highlights that the large majority of clinical signs observed by equine owners were not communicated to veterinarians and therefore could point towards a high potential of observation capacity that might only be captured by CBS. Nonetheless, the currently known good health status of equine in Switzerland does not urge the

requirement of a CBS system as an addition to existing surveillance systems, particularly in terms of covering infectious disease surveillance.

To conclude, this study contributed to the little explored potential of CBS in equine health. The intention of contributing to CBS was found to be given by equine owners, and equine owners reported to observe health issues of their animals four times a year on average. However, a clear intention-behavior gap was observed, as the implemented CBS tool was not used amongst the equine owners. We here identified three critical, interlinked issues that may have led to the failure of the CBS approach: 1) for successfully implementing a CBS system, the need for surveillance within the community of interest must be present; 2) for the target audience, here the equine owners, to remember the presence and use of such a CBS tool, the population under surveillance, here the equine, need to present enough clinical signs , and 3) targeted and extensive communication and management of the system are key for its success. While the potential of CBS as a complementary tool to existing surveillance systems might be given, it is questionable whether the added value forsurveillance is in balance with the efforts necessary to successfully implement the system. With this study, we showcased both the potential and challenges of CBS in animal health; this study can provide guidance for similar future initiatives.

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AMR SURVEILLANCE

THE IMPORTANCE OF FARM MANAGEMENT PRACTICES FOR ANTIMICROBIAL RESISTANCE IN DAIRY HERDS

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SUMMARY

There is increasing emphasis on the need to reduce antimicrobial use on dairy farms to slow the emergence of resistant bacteria which could compromise animal health and impact human medicine. In addition to antimicrobial usage, the role of farm management is an area of growing interest and represents a route for possible intervention alongside decreasing antimicrobial use.

In this study, sentinel bacteria species (*Enterococcus spp.* and *E.coli*) were isolated from bulk tank milk on dairy farms to monitor antimicrobial susceptibilities by means of minimum inhibitory concentrations (MICs). Data relating to farm management were captured using a questionnaire. Subsequent modelling identified a number of management areas that were found to be contributing to increased MICs and that therefore may be important with respect to antimicrobial resistance on dairy farms.

INTRODUCTION

Antimicrobial resistance (AMR) is a major concern in human medicine and is also a growing concern in veterinary medicine (Stevens et al., 2018). Antimicrobials play an important role in the rearing of food producing animals due to the improvements in health, welfare and efficiency of growth. Healthier, more productive animals are likely to provide high quality produce for human consumption at a lower cost (Oliver et al., 2011). The use of antimicrobials within agriculture, however, places a selective pressure on bacteria, both pathogenic and commensal, within the host and poses a risk for the emergence and propagation of antimicrobial resistant bacteria as well as the transfer of resistance related genes between bacterial species and populations (Palma et al., 2020). These may be passed on down the food chain and may compromise human health (Paphitou, 2013).

The potential risk for the emergence and dissemination of AMR related genes between bacteria and amongst hosts warrants judicious monitoring of antimicrobial susceptibilities. Knowledge of trends in antimicrobial susceptibilities in bacterial populations is crucial. When studying or monitoring AMR in the dairy environment, there are a number of options available in terms of sampling strategy. It has been suggested that bacteria isolated from milk samples

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taken from the bulk tank may be of value (Berge et al., 2007). Many studies into AMR on dairy farms report using bacteria isolated from bulk tank milk, across a range of bacterial species (Kreausukon et al., 2012; Del Collo et al., 2017). The bulk tank represents a convenient reservoir for sampling which is accepted to be representative of the herd population and its environment. In addition, resistant strains of bacteria and antibiotic residues in bulk milk may pose a risk for human consumption (Del Collo et al., 2017) making the testing of bulk tank milk important.

One approach for surveillance of antimicrobial susceptibilities is through the use of indicator bacteria. For example, the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) use *Enterococcus* and *Escherichia Coli* as indicator bacteria. The former allows monitoring of resistance in Gram positive bacteria, using Enterococci as a proxy, with *E.coli* allowing monitoring of resistance in Gram negative bacteria. These bacterial species are used as indicators for a number of reasons. Firstly, they are ubiquitous in the environment of livestock. Secondly, they form part of the natural makeup of the host microbiota in the gastrointestinal tract in both animals and humans. Finally, they can rapidly acquire and develop resistances to a range of antimicrobial agents and therefore have the ability to disseminate them across the bacterial population to members of the same species but also to others as a result of bacterial gene transfer (Borck Høg & Korsgaard, 2016). Therefore, these indicator bacterial species are commonly used for research into AMR, and were selected to be studied as part of this research.

When considering farm based AMR, patterns of antibiotic use are commonly considered but an additional area of potential importance, which receives less attention, is the impact of specific farm management practices. This area is of considerable interest as it represents a practical route through which farmers may be able to lower bacterial antimicrobial susceptibilities on farm as well as reducing antimicrobial use.

When modelling data in veterinary epidemiology research, there are a range of techniques and methods available to researchers. For handling datasets that contain many predictor variables in relation to observations, conventional approaches may lead to poor estimation of model parameters as a result of high variance (over fitting of the data). One method to counter this is regularised regression in which over fitting is limited by the penalisation of regression coefficients. Regularised regression techniques that introduce penalty terms have been shown to give an improved model performance in this situation (Zou & Hastie, 2005). The Elastic Net, as described by Zou and Hastie (2005) is a regularisation technique used to build robust models incorporating Ridge and Lasso regression. The former, or L_2 penalisation, decreases model complexity whilst keeping all of the variables in the model while the latter, or L_1 shrinks some of the coefficients towards zero but has the added effect of variable selection. The reliability of Elastic Net modelling can be improved by introducing a bootstrapping procedure (Austin & Tu, 2004; Lima et al., 2020). The use of bootstrapped Elastic Net regression was the method chosen for analysing the effect of farm management factors on antimicrobial susceptibilities in sentinel bacterial species on dairy farms.

The aim of this study was to identify farm management practices associated with differences in minimum inhibitory concentrations (MICs), of sentinel bacterial species isolated from farm bulk tank milk samples. Farm data were collected using face-to-face questionnaires and patterns of MICs inferred from isolates retrieved from bulk milk.

MATERIALS AND METHODS

The data for this study were sourced from two, geographically distinct regions within the British Isles. Study group 1 consisted of 16 herds in an island location. Study group 2 consisted of 125 dairy farms located across England.

Farm Management Data Capture

For both study groups, data were collected by means of a questionnaire carried out face to face with farmers during dedicated farm visits. Data from study group 1 were collected from January – April 2019. For study group 2, farm visits were carried out by five members of a dairy consultancy organisation between December 2014 and March 2015 with each farm being visited once by one consultant. The questionnaires addressed areas relating to farm management, such as milking routines, milk yields, cubicle bedding and housing management. All data were entered into a spreadsheet (Microsoft Excel, Microsoft Corporation, 2016). Data were checked for outlying or implausible values. Questions that were multiple choice but could have multiple answers were given numeric codes for the purpose of analysis.

Recovery of Bacteria from Bulk Tank Milk

For study group 1, frozen samples from the study herds (approximately 500 ml each) were received and processed on the day of delivery. For study group 2, a 500ml milk sample was collected on the day of the farm visit. Samples were taken either from the top of the bulk tank or from the outlet following some drainage of milk. Samples were placed in insulated boxes with icepacks and sent to the lab for bacterial culturing.

For all samples, *Enterococcus* and *E.coli* were cultured from processed milk samples using selective media (Slanetz & Bartley and Tryptone Bile X-Glucuronide agar respectively). Isolated organisms were identified by MALDI-TOF MS (MALDI Biotyper, Bruker Daltonics). For study group 1, a minimum of 6 colonies of *E.coli* were isolated from each sample across five sampling dates between August 2018 and November 2019. For *Enterococcus spp.* a minimum of 6 colonies of were isolated across four sampling dates between August 2018 and August 2019. For study group 2, a minimum of three isolates of both bacterial species were isolated from the single 500ml sample for each farm.

Antimicrobial Susceptibility Testing

Bacterial colonies that were identified as *E.coli* or Enterococci subspecies *E.faecalis*, *E.faecium* or *E.durans* were selected for susceptibility testing. Susceptibilities for isolates obtained from study group 1 were determined by Thermofisher's Sensititre system (Thermo Scientific; Massachusetts, USA) according to manufacturer's instructions. Sensititre COMPGN1F (antibiotic susceptibility testing) AST cards were used to test both E.coli and Enterococcus spp. antibiotic susceptibilities. Following inoculation, AST cards were incubated at 35°C for 18-20 hours. Following incubation, the plate was read using the Sensititre Vizion and SWINTM software. Susceptibility data, given as minimum inhibitory concentrations (MICs) were exported and collated in a master file for data handling and analysis. Further susceptibility testing using Micronaut-S Mastitis 3 (Merlin; Bornheim-Hersel, Germany) AST plates was carried out to provide a broader range of susceptibility profiles. Preparation of pure culture was as for the Sensititre procedure, with MIC results being read and entered into the database. Antibiotics selected for further analysis were **amoxicillin, ampicillin, penicillin, gentamicin, enrofloxacin, marbofloxacin**, **cefquinome, doxycycline** and **erythromycin**

(*Enterococci*) and **amoxicillin, ampicillin, gentamicin, enrofloxacin, cefquinome** and **doxycycline** for *E.coli*.

For study group 2, antimicrobial susceptibilities were determined using a VITEK® 2 (Biomerieux; Basingstoke UK) according to manufacturer's instructions. VITEK® 2 AST GN65 and GP76 cards were used for determining *E.coli* and *Enterococci* MICs respectively. MIC data were entered into Excel for data handling.

Antimicrobial Susceptibility Scoring

MIC data for both groups was used to generate an overall "score" for each farm. The mean MIC for all antibiotics tested across the six samples for each sample delivery (group 1) was used to provide an overall MIC score. For study group 2, a mean of all MIC values for antibiotics tested was taken for the single sampling period carried out on each farm. For both groups, this overall farm mean MIC was used as the outcome variable to evaluate the impact of farm management.

Data Analysis

Due to the large number of potential explanatory variables compared to the number of observations (herds) regularised regression with stability selection was conducted for inference. Regularisation was carried out using Elastic Net regression, which combines the effects of ridge and lasso regression (Zou & Hastie, 2005) using the "glmnet" and "caret" packages (Friedman et al., 2010; Kuhn et al., 2018) within the R statistical software platform (R Core team, 2014).

Explanatory variables were coded according to whether they were numeric e.g. milk yield, or factorial (categorical) responses. Numeric covariates were standardised to a common scale as previously reported (Gelman, 2008). Elastic Net model parameters were optimised using five-fold cross validation repeated 20 times to minimise the mean absolute error (MAE). To estimate covariate stability and P-values, a bootstrapping procedure was undertaken following methods previously reported (Lima et al., 2020). Model inference was based on two model outputs; parameter stability and a bootstrapped P-value (Lima et al., 2020). Parameter stability refers to the percentage of times that a particular variable was selected in the model across 100 bootstrap samples; the higher the percentage, the more consistent the effect of the explanatory variable is across the study farms. Model terms with high stabilities and bootstrap P value <0.05 were selected as the 'final model'.

RESULTS

For the sixteen farms comprising study group 1, herd size ranged from 10-280 dairy cows, with a mean dairy herd size of 151 and a median of 183. For the *Enterococcus* sampling period (August 2018 – August 2019), 407 bacterial samples were isolated (*E.faecalis;* n=279, *E.faecium; n=109, E.durans; n=19*). Between August 2018 and November 2019, 305 *E.coli* samples were isolated. Final analysis of study group 2 data included 171 *E.coli* isolates and 293 *Enterococcus* isolates (*E.faecalis;* n=93, *E.faecium; n=107, E.durans; n=93).* Herd size ranged from 110-1550 dairy cows, with a mean dairy herd size of 358 and a median of 290.

Three Elastic Net regression models were built to identify key management areas of importance in relation to bacterial MICs. For study group 1, one model was produced for

Enterococci while two were produced for study group 2 (one for *Enterococci* and one for *E.coli*). *E.coli* MIC data for study group 1 displayed exceptionally low variation between farms, therefore, differentiation of farms was not possible.

Study group 1; Enterococci MIC model

Results of the final model for management factors associated with *Enterococci* MICs for study group 1 are presented in Table 1. The presence of a slurry store on farm was found to be important; farms without slurry stores had lower MICs than those with a store. Farmers who purchased antimicrobials online had *Enterococci* isolated from bulk milk with higher MICs than those who purchased medicines from their vet only. Several factors relating to cubicle management were found to be important. Farms where hydrated lime was used on cubicles as an antibacterial product resulted in a higher MIC than those which did not use any antibacterial products. Where mattresses were used on cubicles rather than rubber mats, MICs were higher. For farms where bulls used for breeding were reared on farm rather than being borrowed or purchased, lower mean MICs were identified. Farms that did not practice 'natural' drying off (always used either antibiotic therapy or teat sealants) had a higher mean MIC than those farms where natural drying off was practiced.

Table 1. Final Elastic Net regression model for Enterococcus spp. (mean MIC from bulk tank milk samples) for study group 1. Covariate stabilities are expressed as a percentage alongside the effect size of the covariate.

Variable	Reference category	Covariate stability (%)	Coefficient	Bootstrap P-value
No slurry store present on farm	Slurry store on farm	81	-0.031	<0.001
Medicine purchase from vet & online	Medicine purchase from vet only	71	0.072	<0.001
Breeding bulls reared on farm	Some or all breeding bulls brought into herd	65	-0.027	<0.001
No natural drying off of cows	Natural drying off occurs	59	0.022	<0.001
Mattresses on cubicles	Rubber mats on cubicles	58	0.032	<0.001
Hydrated lime used on bedding	No antibacterial used	57	0.031	<0.001

Study group 2; Enterococci MIC model

Results of the final model for management factors associated with *Enterococci* MICs for study group 2 are presented in Table 2. The size of the milking parlour, in terms of number of milking units, was found to be important. Farms with parlours of between 13-24 units had higher MICs than those with fewer units (twelve or less). MICs were also higher again on farms with parlours of between 25 and 36 units. Geographic location was identified as important; farms in the north west of England had higher *Enterococci* MICs when compared to farms elsewhere in the country. Farms with automated milking systems in use were associated with higher MICs than those where cows were milked conventionally. Practices associated with cubicle bedding were found to be important with farms using recycled manure solids (RMS) having increased MICs compared to those who used sawdust. A decreased frequency of cubicle bedding was associated with lower MICs in *Enterococci*.

Table 2. Final Elastic Net regression model for *Enterococcus spp.* (mean MIC from bulk tank milk samples) for study group 2. Covariate stabilities are expressed as a percentage alongside the effect size of the covariate.

Variable	Reference category	Covariate stability (%)	Coefficient	Bootstrap P-value
No. of parlour units 13-24	No. of parlour units ≤ 12	97	0.166	0.033
No. of parlour units 25-36	No. of parlour units ≤ 12	88	0.265	<0.001
Farm location – North West England	Farm location – Mid East England	92	0.177	0.033
Automated milking	Conventional parlour milking	87	0.345	0.023
Bedding material – RMS	Bedding material – sawdust	84	0.206	0.012
Cubicles bedded once per week or less frequently	Bedding cubicles twice per day	83	-0.318	0.036

Study group 2; *E.coli* MIC model

Results of the final model for management factors associated with *E.coli* MICs for study group 2 are presented in Table 3. It was found that bedding of cubicles once daily resulted in lower MICs in *E.coli* compared to farms that bedded cubicles twice per day. Lower MICs were seen on farms that did not use bedding conditioning materials on cubicles compared with farms that did. Milk yield was found to be important; increasing yields (litres produced per cow per year) were associated with increased MICs. Milking preparation procedures involving teat brushing resulted in increased MICs, whereas the wiping of teats with dry clothes or towels results in reduced MICs compared to the use of pre-milking teat disinfection. Milking system was again found to be important, with farms using automated milking systems being associated with lower MICs than those where cows were milked in a conventional parlour.

Table 3. Final Elastic Net regression model for *E.coli* (mean MIC from bulk tank milk samples) for study group 2. Covariate stabilities are expressed as a percentage alongside the effect size of the covariate.

Variable	Reference category	Covariate stability (%)	Coefficient	Bootstrap P-value
Bedding cubicles once daily	Bedding cubicles twice per day	90	-0.064	0.011
No use of bedding conditioners on cubicles	Bedding conditioners used on cubicles	87	-0.057	0.023
Milk sales (litres/cow/year)[a]	-	84	0.059	0.000
Teats brushed before milking	Teat preparation with pre milking disinfection	84	0.131	<0.001
Teats wiped with dry cloth before milking	Teat preparation with pre milking disinfection	80	-0.105	<0.001
Automatic milking	Conventional parlour milking	78	-0.064	0.013

[a] Standardised variable; coefficient relates to change of one unit on a standardised scale.

DISCUSSION

The contribution of antibiotic use to the emergence of AMR is important and widely recognised (Hommerich et al., 2019). In the context of livestock agriculture, as well as antimicrobial use, other factors may be of important for the emergence of AMR and should be considered, including the contribution of farm management practices (Murphy et al., 2018). The aim of this study was to identify farm management factors that most influence MICs in sentinel bacterial species isolated from farm bulk tank milk samples. These factors may provide a basis for potential on-farm interventions to help limit increases in MICs of important bacterial species within the farm environment (Murphy et al., 2018).

A number of management factors were identified to be associated with a net increase or decrease in MICs in Enterococcus spp. and E.coli across study farms. These factors covered a range of specific areas, such as the influence of slurry management, cubicle bedding, teat management at milking as well as frequency of milking, dry cow management and entry of animals onto farm from elsewhere.

Enterococci Susceptibility Models

The importance of slurry in the context of antimicrobial susceptibilities was identified for study group 1. Slurry storage is noteworthy due to the mixing of bacteria, antibiotic residues as well as the presence of other antimicrobial substances (Baker et al., 2016). In this study we found that on farms where there were no slurry stores there were lower MICs compared to farms where stores were in use. Previous work evaluating the role of slurry storage and resistance reported that the proportion of bacteria showing AMR characteristics increased throughout the storage period as a result of horizontal gene transfer and by selection of resistant

genes (Baker et al., 2016). Results from our study are in agreement with these previous findings and suggest the role of slurry storage may be important in contributing to increased MICs on farm. Importantly, the spreading of stored slurry onto land used for grazing and silage may represent a potential route for transmission of resistant organisms to dairy cows and perpetuate their existence in the farm environment.

Results from study group 1 indicated that the use of antibacterial materials on cubicle bedding to be important to *Enterococcus* MICs. The use of hydrated lime was associated with increased MICs, whereas the converse was seen on farms that did not use any antibacterial products on cubicles. It has been reported that the use of antibacterial materials, such as lime based products, significantly reduce bacterial counts in bedding and on cow teats (Janzen et al., 1982; Paduch et al., 2013). The association found in this study, between the use of antibacterial products on bedding and increased MICs, may be a result of an increased selection pressure on the bacterial populations present in cubicle bedding. This may inadvertently encourage selection for genes giving rise to higher antimicrobial susceptibilities. However, the mechanisms for such co-selection are unclear and warrants further investigation.

Practices relating to the management of cubicles and bedding were associated with MICs in *Enterococcus* isolates in study group 2. Here, the practice of less frequent bedding application on cubicles was associated with lower MICs. An underlying mechanism for this association is not clear and the relationship is contrary to perceived 'best practice' which is to apply clean bedding frequently. The relationship between bedding frequency and AMR may not be causal and this warrants further consideration in future work. Additionally, there were higher MICs seen on farms that used recycled manure solids as a bedding material. Bradley et al., (2018) reported that there were significantly higher bacterial counts in RMS bedding when compared with sawdust or sand. Within a larger population of bacteria, there may be more variation of genes (as well as potential for gene transfer) and an increased chance for mutations to appear in the population. This may be a possible explanation for increased MICs and also warrants further investigation.

E.coli Susceptibility Models

As identified for Enterococci, the use of antibacterial bedding conditioners was associated with increased MICs in *E coli*. Teat management practices prior to milking were associated with changes in MIC. MICs were reduced when teats were wiped with a dry cloth when compared with pre-dipping with a teat disinfectant, while MICs were higher when teats were brushed compared with pre-milking teat disinfection. In a previous study evaluating resistance in bacteria isolated from bulk tank milk, farms that practiced dry wiping at milking were more likely to have lower MICs than farms that didn't practice dry wiping (Kirk et al., 2005). It was postulated that milking cows with wet teats is associated with an increased incidence of mastitis, which had the potential to increase antibiotic use and therefore increased bacterial susceptibilities. The effect of brushing teats is more difficult to interpret and warrants further investigation.

Automated milking systems were shown to be important in both E.coli and Enterococci MICs. In the E.coli model, farms where cows that were milked in an automated system rather than in a conventional milking parlour were shown to have lower MICs. However, the converse of this effect was seen in Enterococci. These contrary findings are difficult to interpret and provide a point of interest for consideration in future research.

Study limitations & conclusion

Many of the effect sizes were small for the impact of management practices on bacterial MICs. Therefore it appears that multiple management changes would be needed to influence the MIC of these sentinel organisms in bulk milk. The small sample size of study group 1 means that important management practices may have been missed in the analysis due to a lack of power. Study group 2 data were sourced from farms that had been recruited for previous work evaluating bacterial loads in different bedding materials. Farms had been selected with the aim of recruiting at least 40 that used either sawdust, sand or recycled manure solids. Due to this sample selection, it is uncertain how representative these farms may be of farms across Britain. Additional research with the use of true random sampling, should be considered in future to further explore the impact of farm management on patterns of bacterial resistance.

In conclusion, it has been established that a variety of routine farm management practices are associated with MICs of sentinel bacteria in bulk milk. Although causal relationships are unclear, this provides evidence that changes in farm management may play a role reducing bacterial resistance. Further work to establish to establish causality and identify the most important practices would be of value.

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ASSESSING THE DIAGNOSTIC ACCURACY AND BENEFITS OF WHOLE GENOME SEQUENCING TO MONITOR ANTIMICROBIAL RESISTANCE IN LIVESTOCK

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SUMMARY

Antimicrobial resistance (AMR) monitoring in animals is performed in commensal *Escherichia coli.* It is expected that whole genome sequencing (WGS) will mostly replace culture-based phenotypic typing. So far, no studies have been performed without culture-based susceptibility testing as gold standard. Our aim was to use Bayesian latent class analysis to evaluate the accuracy of susceptibility testing of commensal *E. coli* by WGS versus broth microdilution. OpenBUGS was used to model two independent tests in three animal populations (N=150, 50 bacterial isolates per population): veal calves, pigs and broilers. This resulted in the first estimation of sensitivity and specificity of WGS versus broth microdilution without gold standard. Both methods had high sensitivity (>0.92, lowest limit probability interval: 0.76) and specificity was generally high for both WGS and MIC, for all antimicrobial classes except for aminoglycosides and macrolides. We recommend repeating this analysis with more data to further investigate sensitivity and specificity of WGS, especially for low prevalent resistance.

INTRODUCTION

As part of global action plans to restrict antimicrobial resistance (AMR)(O'Neill, 2016), monitoring AMR in animals is performed in sentinel organisms like commensal *Escherichia coli* (Frimodt-Moller, 2004; Munk et al., 2017; Hesp et al., 2019). In 2020, this is mostly done with the culture-based method broth microdilution, determining minimum inhibitory concentrations (MIC) for pre-defined panels of antimicrobials. Epidemiological cut-off values (ECOFFs) or clinical breakpoints are used to determine if bacterial isolates have reduced susceptibility or resistance, respectively. Recently, whole-genome sequencing (WGS) is becoming more widely available for routine AMR monitoring, and it is the expectation that WGS will mostly replace culture-based phenotypic typing in the near future (Ellington et al., 2017). In the European Union, AMR monitoring in food-borne pathogens and indicator organisms from food-animals is mandatory by EU legislation (2013/652/EU), and prescribed by guidelines of the European Food Safety Authority (EFSA, 2012). As part of the recently revised EFSA guidelines (EFSA, 2019), WGS is allowed in the monitoring of Extended Spectrum Beta-Lactamase producing *E. coli* in European member states from 2021 onwards as a first step towards the transfer to WGS-based AMR monitoring. Many studies have shown

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that WGS performs well in identifying acquired resistance genes and point mutations that lead to phenotypic resistance (McDermott et al., 2016; Shelburne et al., 2017; Hendriksen et al., 2019; Bortolaia et al., 2020; Mahfouz et al., 2020). Next to information on AMR mechanisms, WGS provides additional information, which is considered to enhance AMR monitoring (McDermott et al., 2016; Hendriksen et al., 2019). WGS elucidates the genetic relatedness of resistant strains, as well as providing information on virulence factors, and if AMR genes are genetically linked to mobile genetic elements, which enable resistance genes to spread among bacteria. These aspects are relevant from a public health perspective, for zoonotic potential, and for (veterinary) clinical interest. For veterinary practitioners, it is relevant to know whether AMR genes in the commensal organism *E. coli* are likely to spread to veterinary pathogens. Furthermore, WGS has other advantages over culture-based antimicrobial susceptibility typing: the potential to store sequence data indefinitely, sequence data is easier to share with other laboratories and stakeholders, and it solves the lack of reproducibility across different laboratories described for broth microdilution (Bortolaia et al., 2020).

So far, no studies have been performed without culture-based susceptibility testing as gold standard (Mahfouz et al., 2020). Most existing studies focus on estimating the concordance of WGS to culture-based, phenotypic testing (Hendriksen et al., 2019), in which an objectivity bias may exist when comparing sensitivity and specificity of WGS to these other methods. Bayesian latent class analysis enables the estimation of sensitivity and specificity of diagnostic tests without a gold standard (Johnson et al., 2019). The purpose of this work is to use Bayesian latent class analysis to evaluate the accuracy of susceptibility testing of commensal *E. coli* by WGS and broth microdilution.

MATERIALS AND METHODS

Included in the analysis were 150 commensal *E. coli* isolates collected on broiler-, pig- and veal calf farms in the Netherlands in the EFFORT project (EFFORT, 2020) from October 2014 to December 2015, 10 isolates from five farms for each animal population. To sample a representation of the Dutch livestock sector, the farms in EFFORT were selected by different levels of antimicrobial use on farms (low to high). Faecal isolates from individual animals were randomly collected on these farms. It was part of the EFFORT sampling protocols that all animals should be sampled as close to slaughter age as possible. The EFFORT sampling protocols are described extensively in the Supplementary material of Munk et al. (2018).

From the 150 randomly isolated *E. coli* strains, bacterial DNA was isolated using Pure Gene kits and consequently sequenced with Illumina HiSeq. High-quality filtered reads were assembled using Unicycler (version 0.4.5) and screening for resistance genes using ResFinder 3.0 and PointFinder locally (April 2020). Prevalence of resistance genes for each antimicrobial class was compared with prevalence of phenotypic resistance determined by MIC (Sensititre, EUVSEC antimicrobial panel) with epidemiological cut-off values (ECOFF) (EUCAST, 2019) for the three animal populations. In this latent class analysis, we used the results of the ResFinder default setting of length/identity being 60/90 and cross-classified these test results with the MIC results.

Isolates were considered resistant by WGS conform, the ResFinder 3.0 and Pointfinder database of resistance genes for antimicrobial classes. In the EUVSEC panel for broth microdilution, aminoglycosides were represented by gentamicin, although it is known that isolates resistant to gentamicin are not resistant to all aminoglycosides. The genes coding for gentamicin resistance are *aac(3')-IId* and *aac(3')-IV* (Bortolaia et al., 2020), these genes do not

encode resistance to all aminoglycosides. We compared results for all resistance genes belonging to the class aminoglycosides as positive for WGS, versus only the two genes that encode gentamicin resistance, to show what effect this had on sensitivity and specificity. Similarly, only the gene *mph*(A) encodes for azithromycin resistance in the class macrolides (according to ResFinder), and these results were also compared with results for all resistance genes in the class of macrolides.

Counts of positive and negative isolates for resistance by WGS and MIC were cross-classified in tables, per antimicrobial class (Supplementary Table S1). Latent class analysis was performed in OpenBUGS software, as described by Johnson et al. (2019). Based on the difference between the two test methodologies, we assumed the two tests to be conditionally independent. MIC detects expression of resistance genes by culturing on plates, while WGS detects resistance genes in the bacterial genome. Therefore, we used a model comparing two independent tests in three animal populations (50 isolates for each animal population) that differed in expected prevalence of AMR resistance. We based the prior probability distributions of resistance prevalence in the three animal populations on data from the Dutch National monitoring program (MARAN) in which broth microdilution is used (Veldman, 2016). The dataset of MARAN is large (300 commensal *E. coli* isolates per animal species/year) and consists of random samples of animals at slaughter. Our data were a relatively small sample (50 per animal species) obtained on the farm, although of animals close to slaughter age. Therefore, we used weak-informative priors for resistance prevalence, based on the MARAN data of 2015 (Veldman, 2016) for each antimicrobial class in broilers, veal calves and pigs. Beta distributions were calculated with Betabuster 1.0 freely available software (Table 1). As prior information for the sensitivity and specificity of both tests, we used weak-informative priors for *E. coli*, similar to the methodology of Benedict et al. (2014). That study is a latent class analysis of broth microdilution versus disk diffusion in *E. coli* and other microorganisms, and although it does not provide prior information on WGS, it is one of few studies that resembles our approach. In order to compare both methods as objectively as possible, we assumed both tests to perform just as well, and included the same weak informative priors of that study for both WGS and MIC. In a sensitivity analysis, model results of the priors by Benedict et al. (2014) were compared with a non-informative, uniform prior distribution Beta(1,1) for sensitivity and specificity of both tests. All prior probability distributions used in the model are presented in Table 1. Code for the OpenBUGS model was adapted from previous publications with two independent tests in multiple animal populations (Johnson et al., 2019). In all models, 1000 iterations were used as burn-in and discarded, and summary statistics were based on the next 10,000 iterations. Convergence of each model was assessed by standard diagnostic procedure for latent class analysis, similar to procedure in Benedict et al. (2014).

Table 1. Prior probability distributions for prevalence of antimicrobial resistance and for sensitivity and specificity of whole genome sequencing (WGS) and broth microdilution (MIC) to detect antimicrobial resistance

Antimicrobial class		Beta distribution parameters (a, b)	Mode (%)	95% PI[a]
Gentamicin / Aminoglycosides	Veal calves	(1.2, 25.7)	1.0	0.2-15
	Pigs	(1.2, 25.7)	1.0	0.2-15
	Broilers	(3.0, 50.1)	4.0	1.2-13
Beta-lactams (ampicillin)	Veal calves	(14.0, 60.0)	19.0	11-30
	Pigs	(22.6, 53.8)	29.0	20-40
	Broilers	(2.8, 2.6)	53.0	15-88
Phenicols	Veal calves	(5.9, 38.4)	11.5	5-24.5
	Pigs	(3.7, 26.6)	9.4	3-26
	Broilers	(5.2, 34.9)	11.0	4.6-25
Trimethoprim	Veal calves	(3.4, 17.7)	12.7	4-34
	Pigs	(5.4, 8.9)	35.9	16-63
	Broilers	(3.5, 4.5)	41.5	14-76
Azithromycin / Macrolides	Veal calves	(1.0, 21.85)	0.0	0-15
	Pigs	(1.25, 25.7)	1.0	0.2-15
	Broilers	(1.85, 34.5)	2.5	0.5-14
Quinolones	Veal calves	(2.9, 27.8)	6.7	2-22
	Pigs	(1.1, 13.0)	0.7	0.3-25
	Broilers	(47.3, 60.0)	44.0	34-54
Sulfonamides	Veal calves	(19.0, 60.2)	23.3	15-34
	Pigs	(8.0, 11.3)	40.3	21-63
	Broilers	(4.5,4.9)	47.0	19-77
Tetracyclines	Veal calves	(10.2, 14.2)	41.0	23-61
	Pigs	(6.4, 7.5)	45.3	22-71
	Broilers	(27.4, 48.4)	35.8	26-47
Broth microdilution	Sensitivity	(4.8, 1.2)	83.3	43.1–99.0
	Specificity	(4.8, 1.2)	83.3	43.1–99.0
Whole-genome sequencing	Sensitivity	(4.8, 1.2)	83.3	43.1–99.0
	Specificity	(4.8, 1.2)	83.3	43.1–99.0

[a] Probability interval

RESULTS

Model convergence was good, based on history plots and auto-correlation plots. Results are presented in Table 2. For an overview of the cross-classified tables per antimicrobial class of MIC and WGS test outcomes, see Supplementary Table S1. Discordant test results are presented in Supplementary Table S2, with only isolates with genes coding for gentamicin and azithromycin reported for aminoglycosides and macrolides respectively. Relatively few

differences were found in the cross-classified test outcomes of WGS and MIC (Supplementary Table S1). For tetracyclines, test results were identical in all animal populations. For the other antimicrobial classes, only a small number of isolates was found to be discordant between the two tests (Table S1, Table S2). As expected, discordance was much higher for the complete classes of aminoglycosides and macrolides, when all genes which encode any aminoglycoside or macrolide were considered as positive for resistance (Table S1).

Table 2. Latent class analysis estimates (median and 95% probability interval) for sensitivity and specificity of broth microdilution (MIC) versus whole-genome sequencing (WGS) to detect antimicrobial resistance in veal calves (n=50), pigs (n=50) and broilers (n=50)

Antimicrobial class	Prevalence[a]			Sensitivity[b]		Specificity[c]	
	Veal calves	Pigs	Broilers	MIC	WGS	MIC	WGS
Gentamicin	0.01	0.02	0.03	0.77 (0.36-0.98)	0.76 (0.35-1.00)	0.98 (0.95-1.00)	0.99 (0.97-1.00)
Amino-glycosides	0.02	0.03	0.03	0.73 (0.29-0.98)	0.79 (0.40-0.98)	0.99 (0.96-1.00)	0.46 (0.38-0.54)
Beta-lactams (ampicillin)	0.34	0.29	0.67	0.99 (0.94-1.00)	0.99 (0.95-1.00)	0.99 (0.95-1.00)	0.98 (0.93-1.00)
Phenicols	0.45	0.24	0.53	0.92 (0.76-0.99)	0.92 (0.76-0.99)	0.99 (0.95-1.00)	0.99 (0.95-1.00)
Trimethoprim	0.08	0.03	0.41	0.97 (0.90-1.00)	0.99 (0.94-1.00)	0.99 (0.95-1.00)	0.97 (0.92-1.00)
Azithromycin	0.03	0.04	0.04	0.84 (0.43-0.99)	0.83 (0.44-0.99)	0.83 (0.43-0.99)	0.83 (0.43-1.00)
Macrolides	0.03	0.02	0.02	0.77 (0.36-0.99)	0.88 (0.54-0.99)	0.99 (0.95-1.00)	0.07 (0.04-0.12)
Quinolones	0.38	0.34	0.55	0.96 (0.84-1.00)	0.97 (0.86-1.00)	0.99 (0.97-1.00)	0.99 (0.96-1.00)
Sulfonamides	0.25	0.08	0.08	0.98 (0.94-1.00)	0.99 (0.95-1.00)	0.99 (0.95-0.99)	0.99 (0.94-1.00)
Tetracyclines	0.70	0.55	0.40	0.99 (0.96-1.00)	0.99 (0.96-1.00)	0.98 (0.93-1.00)	0.98 (0.93-1.00)

[a] Median for estimated prevalence
[b] Median for sensitivity, the 95% probability intervals are listed in parenthesis.
[c] Median for specificity, the 95% probability intervals are listed in parenthesis.

Estimated prevalence was very low for gentamicin (veal calves 0.01, pigs 0.02, broilers 0.02) and azithromycin resistance (veal calves 0.05, pigs 0.02, broilers 0.02). For sulfonamides prevalence was moderate in veal calves (0.26) and low in pigs (0.06) and broilers (0.04), Table 2. For both MIC and WGS, sensitivity and specificity for most antimicrobial classes was high, with exception of sensitivity of the complete class of aminoglycosides and gentamicin and the complete class of macrolides and azithromycin. When WGS detection was limited to isolates with gentamicin encoding genes *aac(3')-IId* and *aac(3')-IV* as positive isolates, sensitivity of WGS was 0.76 (probability interval, PI: 0.35-1.00) and specificity of WGS was 0.99 (0.97-1.00) while the sensitivity of MIC was 0.77 (0.36-0.98), and specificity was 0.98 (0.95-1.00). For azithromycin encoding resistance gene *mph*(A), sensitivity of WGS was 0.83 (0.43-0.99) and specificity 0.83 (0.43-0.99). The WGS results for all genes in the class of aminoglycosides

versus only gentamicin encoding genes were similar for the sensitivity of WGS (0.79 versus 0.76), but were much lower for specificity of WGS (0.46 versus 0.99). For the class of macrolides this was even more prominent: sensitivity was 0.88, specificity of WGS was 0.07. For all other antimicrobial classes, sensitivity was >0.92 (lowest probability interval limit: 0.76) and specificity was generally high for both WGS and MIC.

DISCUSSION

The purpose of this study was to evaluate sensitivity and specificity of WGS versus broth microdilution (MIC) to monitor AMR in livestock, without gold standard, by means of latent class analysis. Results indicate that both WGS and MIC have high sensitivity and specificity to detect AMR in livestock, and that sensitivity and specificity of WGS and MIC are similar for the different antimicrobial classes. Latent class analysis resulted in a more objective evaluation of test accuracy of WGS versus MICs, showing that both methods perform similarly in detecting resistance and revealing some interesting aspects for the different antimicrobial classes, which we will discuss below. This objective comparison can enhance the interpretation of WGS results when WGS is applied to monitor AMR in livestock.

For some antimicrobial classes, sensitivity of WGS was slightly higher than of MIC, although probability intervals overlapped (Table 2), the set of isolates that was examined in our study was not large enough to consider our results definitive. Also, there was some indication that overall specificity of WGS was lower than of MIC, but again probability intervals overlapped. Our latent class analysis should be repeated with more data to check whether overall sensitivity of WGS is higher, and/or specificity is lower than MIC, or that both methods perform similarly.

If sensitivity of WGS were higher than MIC, this could be because resistance genes may be detected before the MIC is above the cut-off value for resistance. However, false-positives may appear (and therefore a lower specificity of WGS) when the isolate in question has a resistance gene but this gene is not expressed, and the isolate is genuinely susceptible. This is one of the critical aspects of determining the 'true prevalence' of resistance in bacterial isolates from a specific animal population with WGS. In contrast, the phenotype is clearly resistant when the MIC is above the cut-off value and hence broth microdilution is considered the standard for determining resistance. Another advantage of MIC is that in theory unknown resistance genes, which have not been added to the ResFinder database yet, could be detected. For *E. coli*, it is the consensus that most resistance genes are known and new genes will mostly be variations of known genes, which can still be detected by decreasing identity or length settings when searching genetic databases. Apart from potentially unknown genes, there is still the issue of the expression of the phenotype, where other genes play a role. For example, genes encoding efflux pumps lead to resistance to multiple antimicrobial classes (Swick et al., 2011). However, although culture-based susceptibility testing is considered the standard for both appropriate clinical use of antimicrobial agents as well as for AMR monitoring, it has known diagnostic limitations (Bortolaia et al., 2020; Mahfouz et al., 2020). Differences in MIC reading by different persons, as well as interpretation of MIC reading software, may lead to false-negatives or false-positives. In analyses with MIC as the gold standard, it is impossible to find if WGS performs better than MIC (i.e. is more sensitive or specific), underlining the added value of using latent class analysis to compare the two methods.

In summary, five bacterial isolates were found phenotypically resistant, without detection of specific resistance mechanisms (Supplementary Table S2). The two isolates that were

discordant for gentamicin resistance between WGS and MIC were close to the MIC cut-off value, being 4 and 8 respectively (ECOFF = 2 mg/L)(Table S2). At least one of the isolates was borderline resistant, this may have been an example of the limitation of broth microdilution. In testing susceptibility against aminoglycosides (including gentamicin) with broth microdilution, end-point-reading is sometimes hampered by so called skips (unexpected lack of growth in a certain well), which can result in lower reproducibility of the measured MIC-values. In these isolates resistant by MIC, no known resistance genes were found. The model defined MIC equally sensitive for gentamicin resistance as WGS, looking at the median estimates (0.77 versus 0.76 respectively, Table 2). However, the prevalence was very low with 2/50 isolates MIC positive in pigs and 1/50 WGS positive isolates in broilers (Supplementary Table S1), which makes a precise sensitivity estimate difficult. For azithromycin resistance, in pigs one isolate was found resistant by MIC without detection of known resistance genes by WGS. Similarly, for phenicols, two isolates in veal calves were (borderline) resistant by MIC (MIC 32 mg/L, ECOFF = 16 mg/L) and negative by WGS. It would be interesting to repeat the MIC, to explore whether these isolates were truly resistant or not, and if they were, why were no resistance genes detected. Naturally, this would not change the estimation of sensitivity and specificity in this analysis, as these were based on singular test results.

Conversely, 10 bacterial isolates were tested susceptible by MIC despite the presence of specific resistance mechanisms (except for the presumably false-positive isolates for the complete classes of aminoglycosides and macrolides) (Table S1, S2). For beta-lactams, no *E. coli* with resistance genes encoding Extended Spectrum Beta-Lactamases (ESBL) were detected by MIC or WGS. Since 2009, ESBL prevalence in all three animal production sectors has decreased (Hesp et al., 2019), which presumably resulted in absence of ESBL resistant *E. coli* in this collection of isolates. Regarding resistance to other beta-lactams, one isolate from veal calves was found to have a *bla*TEM-1C resistance gene, but for ampicillin the MIC indicated susceptibility (MIC 2 mg/L, ECOFF = 8mg/L) (Table S2). Similarly, for trimethoprim resistance, three isolates in veal calves were found to have resistance genes (two with the *dfrA1* and one with the *dfrA7* gene, Table S2), but these were susceptible by MIC. Other examples were: one isolate in veal calves having two genes for sulfonamide resistance (*sul1* and *sul2*), but being susceptible by MIC, and another isolate in veal calves, having two genes for macrolide resistance (*mph*(A), *mph*(B)), but being susceptible by MIC (Table S2). For phenicols, two isolates were found in veal calves with resistance genes (*catA1* and *floR*) with susceptibility indicated by MIC. And for gentamicin resistance, an *aac(3')IId* gene was found, also without phenotypic resistance. For quinolones, a susceptible isolate was predicted to be resistant, based on the detection of a single a point mutation in the *parC* gene (Table S2). However, it is known that resistance to quinolones requires a mutation in a specific region of the *gyrA* gene. Apart from this last example, all these isolates could be candidates to repeat MIC and re-evaluate DNA sequences to find possible explanations. It could be that the genes have mutations, and therefore are not expressed as phenotypical resistance. Again, this would not change the sensitivity and specificity outcomes of our model. Nonetheless, it could extend knowledge on phenotypical expression of resistance genes and thereby enhance interpretation of WGS outcomes in AMR monitoring.

The comparison we made for the antimicrobial classes of aminoglycosides and macrolides illustrates the importance of the definition of genes encoding certain resistance phenotypes in the interpretation of WGS. This influences specificity: when all aminoglycoside genes are counted as positive, instead of only gentamicin encoding resistance genes, this leads to a high number of false-positives for gentamicin resistance (specificity of WGS: 0.46, Table 2). These monitored resistance genes encode resistance to other relevant antimicrobials, but these should

be reported as separate categories, for example streptomycin resistance. It is a known issue of culture-based susceptibility testing that the limited size of the plates leads to pragmatic choices in antimicrobials as representants of antimicrobial classes. That is another important advantage of WGS: it detects all known resistance genes of an antimicrobial class. The other comparison we made for macrolides and azithromycin shows an example of an efflux pump encoding gene. This gene, *mdf*A, is listed by ResFinder 3.0 as belonging to the antimicrobial class macrolides, but leads to resistance to many other antimicrobials as well. This is a known issue with efflux pump encoding genes. As we see in our data, it does not (or not always) lead to phenotypic azithromycin resistance, resulting in a high number of false positives (specificity of WGS: 0.07, Table 2).

A latent class model is not aware of the biases in the diagnostic methods, but estimates the true resistance prevalence by combining the data with the prior information (from prior probability distributions), and consequently estimates how well both tests perform in identifying the true resistance prevalence in the different animal populations. Therefore, the prevalence of resistance will influence the precision of the estimated sensitivity and specificity. As we can see from Table 2, for gentamicin and azithromycin the sensitivity was lower for both WGS and MIC. These two antimicrobials both have a similar and low prevalence in all three animal populations (Table S1) and in the prior information (Table 1). This may result in lower estimates (with wider probability intervals) for sensitivity for those two antimicrobial classes. Similarly, the estimated sensitivity of both MIC and WGS for macrolides was relatively low and had wide probability intervals (0.77, PI 0.36-0.98 and 0.88, PI 0.54-0.99 respectively). Test validity should be further investigated in populations where resistance prevalence is higher, or with more sequence data, should these become available. Interestingly, in another study with MIC as gold standard for WGS, also a lower sensitivity of WGS for gentamicin resistance (0.93) than for other antimicrobial classes was found (McDermott et al., 2016). In our latent class model, an assumption was made that resistance prevalence should differ in the different animal populations. However, for some antimicrobials the prevalence was almost equal in the three populations, which can potentially affect the accuracy of the sensitivity and specificity estimates. Others investigated the impact of breaching the prevalence assumption and found that with tests with a fairly high sensitivity or specificity this was of little influence (Toft et al., 2005).

The relatively small dataset per animal population (n=50), and the fact that these isolates come from five farms per population, is a limitation of this study. Resistance may be clustered within farms, and this dataset may not have the same variation as in regular AMR monitoring. We know from parallel analysis of the phylogenetic relatedness of these bacterial isolates that within farms identical Sequence Types were occasionally found (results not shown), indicating a clonal spread of these bacterial strains on farms. On the other hand, our analysis shows that, even with a limited number of samples, it is possible to determine the sensitivity and specificity of WGS and MIC in these data. The fact that the farms were selected based on different levels of selective pressure by AMU may have helped to create variability in this resistance data. Another important requirement for test validation was met: that all results were generated in the same laboratory with high standardisation. However, in order to improve external validity, it is advisable to repeat this analysis with more data, and preferably less clustered data.

To conclude, latent class analysis has led to fairly precise estimates for sensitivity and specificity of both methods, and the first evaluation without culture-based susceptibility as the gold standard. This can be of aid in future interpretation of AMR monitoring data from livestock, when WGS will be further implemented in routine AMR monitoring. This analysis

has highlighted some critical aspects of comparing WGS data to traditional monitoring data generated by culture-based methods. Since the dataset was relatively small, and some resistance prevalence was low, we recommend repeating this analysis with more data, from more farms, as well as in different countries and different laboratories, to validate our findings.

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Supplementary Table S1. Cross-classified test results of broth microdilution (MIC) versus whole-genome sequencing (WGS) to detect antimicrobial resistance in livestock (N=150)

Antimicrobial class		Veal calves (n=50)		Pigs (n=50)		Broilers (n=50)	
		WGS+	WGS-	WGS+	WGS-	WGS+	WGS-
Gentamicin	MIC+	0	0	0	2	0	0
	MIC-	0	50	0	48	1	49
Aminoglycosides	MIC+	0	0	1	1	0	0
	MIC-	36	14	32	16	14	36
Beta-lactams (ampicillin)	MIC+	28	0	14	0	34	0
	MIC-	1	21	0	36	0	16
Phenicols	MIC+	15	2	3	0	2	0
	MIC-	2	31	0	47	0	48
Trimethoprim	MIC+	27	0	10	0	27	0
	MIC-	3	20	0	40	0	23
Azithromycin	MIC+	2	0	0	1	0	0
	MIC-	1	47	0	49	0	50
Macrolides	MIC+	2	0	1	0	0	0
	MIC-	46	2	46	3	49	1
Quinolones	MIC+	4	0	1	0	17	0
	MIC-	0	46	1	48	0	33
Sulfonamides	MIC+	30	0	16	0	28	0
	MIC-	1	19	0	34	0	22
Tetracyclines	MIC+	42	0	29	0	23	0
	MIC-	0	8	0	21	0	27

Supplementary Table S2. Discordant isolates in results of broth microdilution (MIC) versus whole-genome sequencing (WGS) to detect antimicrobial resistance in livestock (N=150)

Antimicrobial class	Antimicrobial	Isolate ID	MIC	ECOFF	Resistance gene
Aminoglycosides	Gentamicin	100302010	4	2	None for gentamicin
		101702014	8		None for gentamicin
		103003004	1		$aac(3')$-IId
Beta-lactams	Ampicillin	110704022	2	8	bla_{TEM-1C}
Phenicols	Chloramphenicol	110004010	32	16	None for phenicols
		110004014	32		None for phenicols
		110504004	8		$catA1$
		111604014	8		$floR$
Trimethoprim	Trimethoprim	110504004	0.5	2	$dfrA1$
		111604014	0.25		$dfrA1$
		111804010	0.25		$dfrA7$
Macrolides	Azithromycin	110504020	8	16	mph(A), mph(B)
		102702012	128		None for azithromycin
Quinolones	Ciprofloxacin	102302012	0.015	2	$parC$ p.A56T
Sulfonamides	Sulfamethoxazole	111604014	8	16	$sul1$, $sul2$

DRIVERS OF DISEASE SPREAD

MOVEMENT REWIRING AMONG RELEVANT HERD STATUSES TO CONTROL

PARATUBERCULOSIS AT A REGIONAL SCALE

P. EZANNO*, S. ARNOUX, A. JOLY AND R. VERMESSE

SUMMARY

Paratuberculosis is a worldwide disease mainly introduced through trade. Protecting herds from purchasing infected animals is not straightforward due to the low sensitivity of diagnostic tests. Our objective was to assess if promoting risk-based movements contributes to control the regional spread of *Mycobacterium avium* subsp. *paratuberculosis* (MAP). A stochastic individual-based between-herd epidemiological model was developed and adapted to the dairy cattle farming system in western France. MAP regional spread was compared when using the observed trade movements versus simulating risk-based movements using a rewiring algorithm. Herds having a high probability of being MAP-free were identified to assess the effect of decreasing their risk of purchasing infected animals. Movement rewiring to prevent selling animals from high- to low-prevalence herds reduced MAP regional spread, but only if herds with a high probability of not being infected were targeted and if their purchased animals could be guaranteed free with a high probability.

INTRODUCTION

Paratuberculosis is a worldwide endemic disease mainly introduced through trade (McAloon et al., 2019). Movements are sufficiently numerous among dairy cattle herds to maintain the regional circulation of the pathogen, *Mycobacterium avium* subsp. *paratuberculosis* (MAP) (Beaunée et al., 2015). In addition, once introduced into a herd, MAP has a high probability of persisting over several years (Marcé et al., 2011) resulting for infected herds in milk losses, early culling of animals, and increased mortality (Garcia and Shalloo, 2015).

MAP spread is hard to control in such endemic regional situations (Beaunée et al., 2017). Susceptibility decreases with age (Windsor and Whittington, 2010), thus mainly young animals are newly infected without showing early clinical signs. Clinical signs usually appear after the first calving but can in some cases never be observed (Mitchell et al., 2015). Shedding starts before clinical signs appears, at a level which is highly heterogeneous among animals and over the life course of an infected animal. However, MAP shedding animals are difficult to detect due to the low sensitivity of diagnostic tests (Magombedze et al., 2013; Barkema et al., 2018),

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impairing test-and-cull strategies as well as tests performed when purchasing animals (More et al., 2015; Camanes et al., 2018).

Protecting herds from purchasing infected animals is thus not straightforward, whereas it could both prevent MAP-free herds from being infected and recently MAP-infected herds from getting worse. A complementary strategy to protect MAP-free herds and herds with a low prevalence is to promote trade towards risk-based movements (Gates et al., 2013; Gates and Woolhouse, 2015; Hidano et al., 2016). With such a strategy, animals sold by herds with a poor sanitary status (e.g. due to their geographical location, their location in the trade network or their infection history), and who thus have a high risk of being infected, should not be sold to herds with a good sanitary status.

Our objective was to assess if trade movement rewiring to promote risk-based movements could reduce MAP spread between dairy cattle herds at a regional scale. We used a stochastic individual-based and between-herd epidemiological model adapted to the dairy cattle farming system from Brittany, western France. The regional spread of MAP was compared when using observed trade movements versus using a rewiring algorithm simulating risk-based trade movements. Herds having a very high probability of being MAP-free were also identified to assess the effect of decreasing their risk of purchasing infected animals on MAP regional spread.

MATERIALS AND METHODS

A regional epidemiological model of MAP spread between dairy cattle herds was combined with a trade movement rewiring algorithm to manipulate observed trade movements between herds and simulate risk-based movements.

Epidemiological model

At the within-herd scale, the epidemiological model is a stochastic individual-based model of MAP spread in a structured dairy cattle herd with year-long calving. This model is fully described in Camanes et al. (2018). In brief, animals can be of six mutually exclusive age groups: newborns, unweaned calves, weaned calves, young heifers, bred heifers, and cows, each of these groups being reared in a specific environment of the farm. Animals change group at a given age or time of the year. Animals are also defined by their age or parity for cows, as well as by their health status: susceptible (S), resistant (R), transiently infectious (I_T), latently infected (I_L), moderately infectious (I_M), and highly infectious and possibly clinically affected (I_H). Susceptibility decreases exponentially with age, and the rare infection events that may occur after one year of age are neglected. Shedders belong to health statuses I_T, I_M, and I_H. They can shed MAP in colostrum/milk (if they have calved) and in faeces. The quantity of MAP shed is heterogeneous and depends on animal health status. Five transmission routes are considered: *in utero*, via ingestion of contaminated colostrum, via ingestion of contaminated milk, indirectly via a contact with the local environment where susceptible animals are raised and where infected animals of the same age group shed MAP, and finally indirectly via a contact with the farm environment which level of contamination is influenced by all shedders of the herd. MAP survives in the environment and thus indirect transmission is due to infected animals held in the same farm but not necessarily at the same time. Transmission due to the farm environment is only considered indoors at a lower probability than transmission due to the local environment. During the pasture season (from April to mid-November), animals older than 6 months of age are raised outdoors. They are only exposed to MAP shed by infected

animals from their own age group. We assumed farmers manage calves so that – if the herd is infected – the true prevalence in adult females (older than 2 years and a half) remains below 60%, as mostly observed in the field.

At the between-herd scale, herd size was calibrated as well as demographic parameters (calf mortality, culling rate per age group and per parity for cows, and births) for each herd-year using a comprehensive database of cattle detention and trade movements (French cattle identification database). Trade movements connect herds in a data-driven manner. When an animal is sold in the observed database, an animal of the same age is picked at random in the selling herd and sent to the purchasing herd. Hence, only the health status of the traded animal is random, but the date and type of movement is data-driven. The epidemiological model was applied to the population and infection dynamics in each of the 12,857 dairy cattle herds of Brittany (western France) from 2005 to 2013 (919,304 trade movements).

Around 54% of the animals purchased come from outside the modelled metapopulation. These incoming movements can also be a source of MAP. Thus, they were assumed to be associated with a similar risk as movements occurring among herds of the modelled metapopulation, assuming this metapopulation to belong to a larger production area implementing similar control options. To do so, the probability of purchasing an infected animal from outside was assumed to be similar to the average proportion of infected animals in the age group of the purchased animal in the modelled metapopulation.

Trade movement rewiring algorithm accounting for MAP herd statuses

The trade movement rewiring algorithm accounts for MAP herd statuses for connecting herds through trade, and thus modifies observed movements. Herds can purchase animals only from herds of similar or better infection statuses (e.g. with three statuses: Fig. 1).
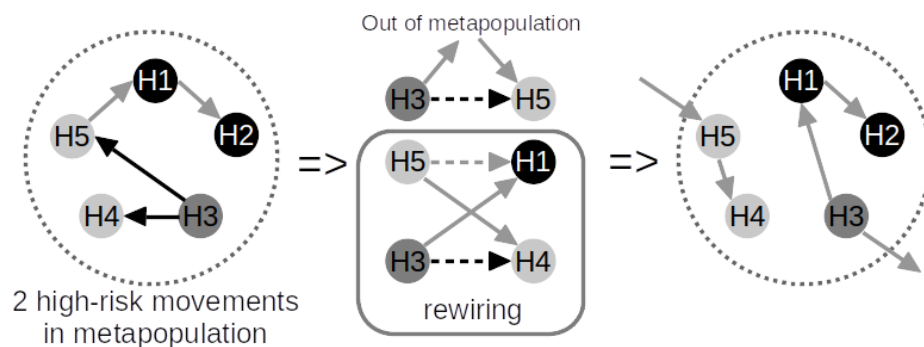
Figure 1. Principle of the rewiring algorithm (light grey: MAP-free or lowly infected herds (status A), grey: moderately infected herds (status B), black: highly infected herd (status C), black arrows: unauthorised movements).

To do so, first movements occurring over a 7-day time window (the model time step) in a given age group were listed: less than 10 weeks, 11-26, 27-52, 53-91, 92-101, 102-111, 112-121, 122-130, and then per parity. In Brittany, dairy animals are mostly Holstein, thus breed was not considered. Second, these movements were distributed according to the infection statuses of the selling and the purchasing herds. Third, unauthorised movements were identified and switched as much as possible with a relevant alternative. Fourth, for movements that could not be switched, the sold animal was culled and an animal was purchased from outside the metapopulation with the same risk of being infected as in other movements coming from outside in the observed database. These movements coming from outside were assumed to be

also controlled. The probability to purchase an infected animal from outside equals the average proportion of infected animals in the age group of the purchased animal in herds of similar or better infection statuses than the purchasing herd.

Three herd statuses were defined according to the capacity of diagnostic tests to detect infected herds. Herds with a true prevalence (proportion of infected animals among females older than 2 years and a half) below 7% are in group A (can barely be detected as infected), herds with a true prevalence between 7 and 21% are in group B, and herds with a true prevalence above 21% are in group C (often have already had at least one animal detected as highly positive).

First, to mimic a best case situation, the herd statuses were defined based on the true and instantaneous prevalence in females older than 2 years and a half at the time of trade, and used to modulate movements.

Second, to mimic a more realistic situation, herd statuses were defined once a year based on their apparent prevalence. A serum ELISA was performed on all females older than 2 years and a half. The specificity was assumed to be perfect. An average sensitivity of 0.2 was assumed for detecting infected animals irrespective of their infection status, thus neglecting the variation of the test sensitivity with the infection status (Nielsen and Toft, 2008). The test was performed at a random date for each herd between January and April each year, i.e. before the period of grazing.

Scenarios, model settings, initial conditions and model outputs

First, the impact of movement rewiring on MAP regional spread was assessed under two scenarios: herd statuses A, B and C are defined using the true and instantaneous prevalence *versus* the yearly apparent prevalence.

Second, herds with three consecutive yearly favourable apparent statuses (herds labelled AAA) were targeted by the rewiring algorithm. These herds have a low probability to be infected. The effect of guaranteeing purchases in AAA herds as MAP-free with probability p (3 scenarios: 0.5, 0.75, 1) was assessed. This means that, in addition to preferentially purchase animals from AAA herds, each purchased animal is for sure not infected with probability p, and is picked at random among animals of the relevant age group in the source herd attributed by the rewiring algorithm with probability 1-p. Movements among other herds are not modified in this scenario, thus focusing the management effort at a regional scale on much fewer herds.

The model was developed in C++ Standard 11. Ten stochastic repetitions were simulated for each scenario. Initially, herds were realistically distributed among the three herd statuses, based on observations in Brittany: 33% in herd status A, 40% in herd status B, and the remaining 27% in herd status C.

The model predicts over the nine years: the proportion of infected herds in the metapopulation each week, the number of newly infected herds each week, the proportion of infected females older than 2 years and a half per herd per year, and the proportion of AAA herds the 1st of January of each year.

RESULTS

Starting from an endemic situation as observed in Brittany, 87% of the herds were consistently infected when no additional control was implemented (Fig. 2b). New herds were regularly infected (Fig. 2a), indicating a regional circulation of MAP with fade-out / recolonization events. Irrespective of the initial herd status, the proportion of infected females older than 2.5 years progressively deteriorated when no control is implemented (Fig. 3).
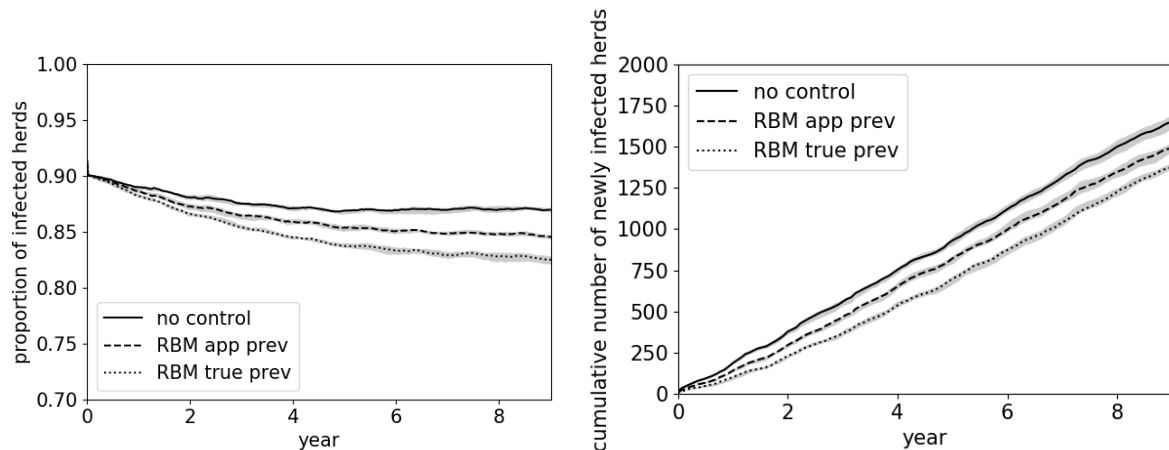
Figure 2. Regional MAP spread over time in Brittany, western France, according to the rewiring scenario. The proportion of infected herds (upper graph) and cumulative number of newly infected herds (lower graph) are compared in a scenario with no movement control and two scenarios with risk-based movements (RBM), MAP herd statuses being defined either based on the true and instantaneous prevalence at the movement date or based on the yearly apparent prevalence (test sensitivity = 0.2). The prevalence is the proportion of infected females among females of more than 2.5 years.
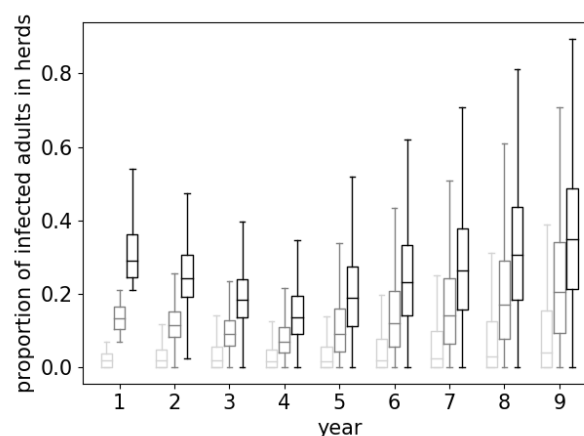
Figure 3. Yearly proportions over time of infected females of more than 2.5 years, according to the initial true prevalence in that age group when no control is implemented (light grey: initial herd status A (0-7% true prevalence), grey: initial herd status B (7-21%), black: initial herd status C (>21%)).

Movement rewiring reduced herd prevalence and incidence when herd statuses were based on the true and instantaneous prevalence the week the movement occurred. However, when

herd statuses were defined more realistically once a year based on apparent prevalence, movement rewiring barely impacted the proportion of infected herds (Fig. 2a) and MAP spread between herds (Fig. 2b).

When herd statuses were defined yearly on apparent prevalence but using three consecutive favourable statuses (AAA), the proportion of infected herds then decreased over time (Fig. 4a). When the probability for AAA herds to be sure not to purchase infected animals was kept as high as 0.75, the same results were obtained as when using the true and instantaneous prevalence to modulate movements to all of the herds of the metapopulation, thus counter-balancing the low test sensitivity. The herd incidence became even very low when animals purchased by AAA herds were guaranteed as not infected (Fig. 4b, *p*=1), while AAA herds represented a moderate fraction (from 25 to 30%) of the herds of the modelled metapopulation (Fig. 5).
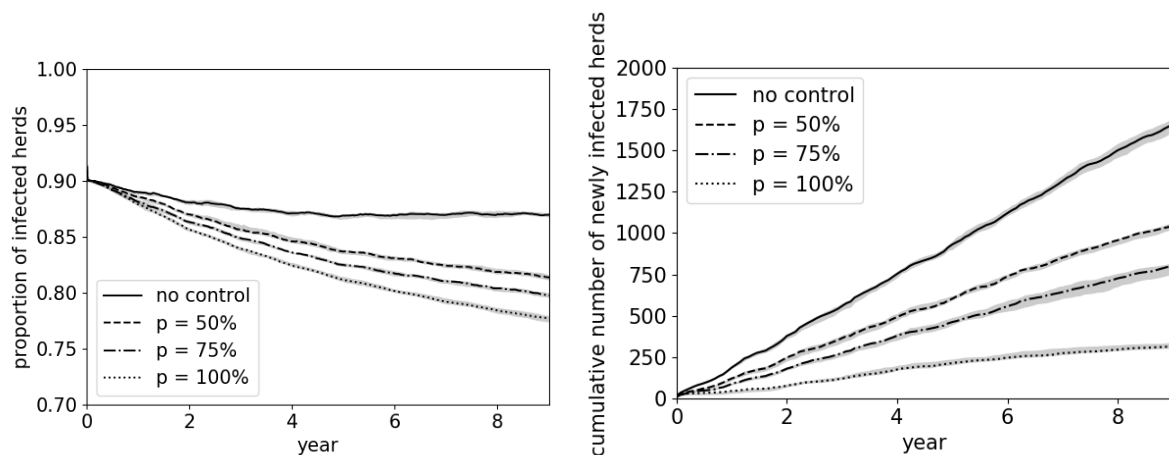
Figure 4. Regional MAP spread over time in Brittany, western France, when AAA herds are targeted for movement control proportion of infected herds (left graph) and cumulative number of newly infected herds (right graph) are compared in a scenario with no movement control and three scenarios targeting the MAP herd status AAA, defined by three successive yearly statuses using apparent prevalence (test sensitivity = 0.2). The apparent prevalence is the proportion of infected females detected among females of more than 2.5 years.

DISCUSSION

MAP is mainly transmitted between cattle herds by trade. However, manipulating trade movements to promote risk-based movements between herds of similar MAP statuses does not significantly reduce the prevalence of infected herds nor the number of newly infected herds. This is especially due to the low sensitivity of diagnostic tests. Here, an average sensitivity of 0.2 was considered for detecting infected animals. The analyses should be further extended to consider a higher sensitivity for higher shedders and older animals (Nielsen and Toft, 2008). It is expected that herds with a higher prevalence also have a higher number of high shedders (Marcé et al., 2011), and thus could be more easily detected as such.
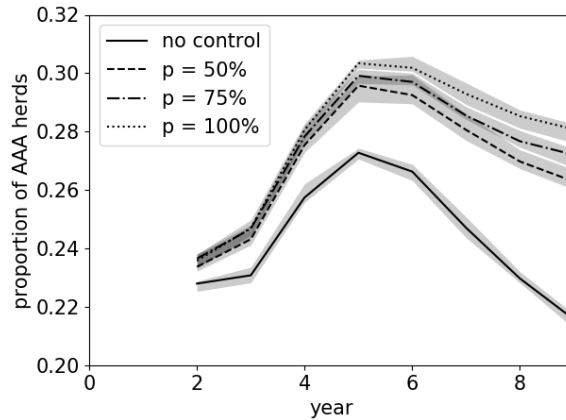
Figure 5. Proportion over time of herds with a high probability to be MAP-free (AAA herds), according to their risk of not purchasing infected animals.

Preventing at least half the purchases to be at-risk in herds having a high probability of being MAP-free (herd status AAA) should help reducing the regional herd prevalence and the number of newly infected herds. The practical implementation of trade rewiring would also be eased as AAA herds represent around a third of the herds in Brittany but less than 10% of the purchases. Nevertheless, such a guarantee at purchase is difficult to achieve. Further analyses are required to estimate more precisely the distribution of the risk of selling infected animals per herd status, in order to identify the largest population of herds as possible which could serve as sources of animals for AAA herds.

Complementary control actions could also be implemented in these AAA herds. For example, hygiene and calf management could be further improved as they are known to impact MAP persistence in newly infected herds (Marcé et al., 2011). Here, we assumed calf management is such in all of the herds of the metapopulation as to prevent too high within-herd prevalence (only a few herds with a true prevalence higher than 60% are observed in the field). However, calf management was considered similar among herds (similar exposure of calves to the farm environment possibly contaminated by shedding adults). The hygiene level of a given farm is hard to quantify and to explicitly relate to the exposure of its calves to MAP contamination of its farm environment. More observational data are required to gain knowledge on what can be achieved on farm to reduce calf exposure to adults. In addition, models such as the one used in this paper could help assessing which highest level of risk can be accepted for purchases in AAA herds as a function of on-farm levels of calf exposure to the farm environment as contaminated by shedding adults.

Control measures could also target infected herds to decrease their prevalence, and thus the risk to spread MAP to other herds (Whittington et al., 2019). However, control options may vary a lot over time at farm scale (Nielsen and Toft, 2011) as well as among farms according to their characteristics (Donat et al., 2016), which is difficult to represent in epidemiological models. Also, some available options cannot be easily implemented in a large number of herds. For example, improving calf management often requires changing farm practices. While calf management has been highlighted as an important way to control MAP in infected herds (Doré et al., 2012, Camanes et al., 2018), it is sometimes considered as inducing too high costs to be used in practice (Sorge et al., 2010). Decreasing the duration before culling highly positive animals could also be an option. However, more analyses are needed to quantify the expected

impact of this measure (More et al., 2015), especially because animal testing is performed generally only once a year for practical reasons.

In conclusion, targeting herds having successively three favourable yearly statuses has been shown to be a good solution to minimize the effort to decrease MAP spread at a regional scale. These herds have a high probability of being MAP-free. However, animals purchased in these herds should be guaranteed as MAP-free with a probability higher than 0.75.

ACKNOWLEDGMENTS

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SIMILARITIES AND HETEROGENEITY OF FREE-ROAMING DOMESTIC DOGS'

SOCIAL NETWORKS AND ITS PREDICTING FACTORS

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SUMMARY

Free roaming domestic dogs are the main vectors for rabies transmission to humans. By targeting specific dogs, such as those with high connectedness to their counterparts, vaccination strategies to combat rabies could become more efficient. In this study, social networks analysis in eleven dog populations in four countries was conducted. Small-world network structure and right-skewed degree distribution were found in all study sites, suggesting that targeting highly connected dogs would make vaccination campaigns more effective than random vaccination. Attempts to identify dog characteristics associated with individual connectedness revealed that factors predicting individual dog's centrality varied across networks. We hypothesize that the varying influence of potential predictors for contacts depends on the social, cultural and economic contexts of the study sites. Investigation into the sociocultural structures impacting dog ownership and driving dog ecology is thus a requirement to assess the potential of targeted vaccination in dog populations.

INTRODUCTION

Free-roaming domestic dogs (FRDD) are known to be the main source of human rabies, a disease which is responsible for an estimated 60,000 yearly human deaths worldwide (Hampson et al., 2015). It has been repeatedly shown that mass vaccination of dogs can eliminate rabies from dog populations and therefore prevent human deaths (Kumarapeli and Awerbuch-Friedlander, 2009; Zinsstag et al., 2009; Mindekem et al., 2017; Zinsstag et al., 2017). The World Health Organization (WHO) currently recommends a random vaccination of at least 70% of dogs in susceptible dog populations. However, in its third Expert Consultation on Rabies, WHO stated that vaccination programs should be tailored to local characteristics of those populations (WHO, 2018). Sustainable vaccination campaigns require considerable resources (Hampson et al., 2007) and refining vaccination recommendations, for example by targeting specific dog populations to reduce the overall vaccination coverage needed to prevent outbreaks, would increase vaccination strategies' feasibility and effectiveness (Vial et al., 2006; Laager et al., 2018).

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Rabies is transmitted by direct contact from infected to susceptible individuals. The understanding of the potential impact of contact networks on the spread of rabies within dog populations could help in tailoring vaccination strategies to the local contexts. Recent studies suggest that dog contact rates influence disease spread in FRDD populations (Hudson et al., 2019a) and that highly connected dogs might play a critical role in rabies transmission (Laager et al., 2018). Combining network analysis and disease modelling, Laager et al. (2018) highlighted that targeting vaccination on a small number of the most central dogs in the contact network would reduce the vaccination coverage required for rabies elimination, and therefore increase its likelihood of success. However, assessing the structure of a contact network in a dog population is labour-intensive, and would be inefficient to study in each dog population prior to vaccination campaigns. Instead, if characteristics associated with the dog's position in the network were identified, they could inform the design of risk-based vaccination strategies, allowing vaccinators to predict central dogs for which vaccination should be prioritized (Brookes et al., 2018; Laager et al., 2018).

The aim of the current study was to identify such factors predicting the network centrality of the dog within a population. The study assessed contact patterns between FRDDs at eleven different sites in four countries, namely Guatemala, Chad, Uganda and Indonesia, on three continents where canine rabies is endemic. The structures of these networks were compared, and it was assessed whether tangible dog characteristics are associated with the dogs' positions in those networks. Such information could be of high value to inform targeted, more effective vaccination campaigns for canine rabies control.

MATERIALS AND METHODS

Data collection

The study was conducted in Chad, Guatemala, Indonesia and Uganda. In each country, three study sites were selected – an urban or semi-urban and two rural - except from Chad, where two rural sites were selected. Data were collected between January 2018 and January 2019. In each site, an area of 1 km^2 was defined using Google Earth®. All domestic dogs whose owner's household was located in these areas and that can roaming freely for at least part of the time were aimed at being included in the study. Dogs less than four months old, dogs that were too small to wear the collar, sick dogs and pregnant bitches (to avoid miscarriage due to stress) were excluded from the study. The study was presented to the head of the household or an adult person living in the same house and written or oral consent was requested. Recruited dogs were offered to be vaccinated against rabies and/or dewormed. Ethical approval was requested in each country according to local regulations.

In total, 714 dogs were collared for 3-5 days (restricted by the battery capacity) with a geo-located contact sensor (GCS, developed by www.bonsai-systems.com) that have also been used in an earlier study on FRDD (Laager et al., 2018). The GCS included a global positioning system (GPS) device and a contact sensor, functioning by ultra-high frequency technology. The contact sensor recorded proximity to other contact sensors by broadcasting beacons every minute and constantly scanning for other sensors' beacons. Structured questionnaires, adapted for each country, were administered in each household to collect information on the collared dogs. These included sex, age, body condition score (BCS) of the dog, purpose for keeping the dog (watch dog, shepherd dog, hunting dog, pet or meat production) and the period the dog was allowed to roam freely (permanently, all day, all night, a few hours, never). The interviews

were performed in local language by trained team members. The data were electronically recorded using KoboCollect Android application (KoBoToolbox, 2020). The GPS location of each participating household was recorded.

Statistical analysis

Social network analysis was applied to characterize the 11 networks (one per study site) in an undirected and unweighted way. A contact between two dogs was defined as a proximity event recorded by at least one of the two contact sensors. Within the social network, dogs represented the nodes and contacts represented the edges between dogs. The following network metrics were calculated: Network size (number of dogs with at least one connection to another dog), largest component size (number of dogs within the largest group of connected dogs), network density (number of edges observed over the maximal possible number of edges), average shortest path length (low mean number of edges on the shortest path between all pairs of nodes), clustering coefficient (probability that adjacent nodes of a node are also connected), and the degree for each dog (the number of individual dogs being in contact with each dog). In order to compare degree distributions across networks, relative degree (i.e. degree divided by the maximum possible degree (i.e. network size minus one)) was also computed. Wilcoxon rank sum tests were used to assess if the distribution of the relative degree differed between any two networks. In addition, the small-world index was computed as the ratio of the observed clustering coefficient to the mean clustering coefficient of the simulated random networks, divided by the ratio of the observed average shortest path to the mean average shortest path of the simulated random networks (Humphries and Gurney, 2008; Brookes et al., 2019).

To identify predictors of dogs' centrality in a network, permutation-based multivariate linear regression models (PBLM) were applied for each network. This model type was selected because the assumption of independency between observations of standard linear regression models is violated with network data, increasing the risk of type I error (Croft et al., 2011). The p-values associated with each coefficient were computed based on a permutation of the regression residuals. Log-transformed degree was used as the dependent outcome variable for the PBLM. This metric was chosen because of its relevance for disease spread throughout the network. Dogs with a high degree are likely to infect a high number of dogs and vaccinating such dogs may slow down disease transmission over-proportionally (Wey et al., 2008). Independent variables (directly retrieved from the questionnaire study) included sex, age, BCS, and purpose of the dog. In addition, three covariates were forced into each regression model. These were the length of time the dog is allowed roaming freely (FRT), the number of dogs collared in the same household (NDC) and the distance from the household to the centroid of the study area (distance). The first two were included because of the high likelihood that they influence the degree of a dog. The distance was included to correct for potential edge effect, in case contacts with non-collared dogs were missed for dogs living close to the edge of the area. The distance of the dog's home from the centroid of the study area, i.e. the centroid of the minimum convex polygon including all the household locations, was calculated using QGIS. All possible combinations of independent variables were tested in the regression models and the best fitting models were selected based on the Akaike information criterion (AIC) using the formula presented by Gordon (2015). The set of best models were identified for each network by including the model with the lowest AIC and all models with ΔAIC < 2 compared to the lowest AIC. The variables' coefficients, their p-values associated, and deviance explained by each explanatory variable included in these models were extracted for each model.

RESULTS

The structure of two of the 11 unweighted and undirected networks is presented in Fig. 1. One network in Uganda was too small to be further analysed (number of nodes = 4). The size of the 10 remaining networks ranged from 15 to 121 dogs (or nodes), representing 67% to 100% of the dogs for which their contact sensor could be retrieved at the end of the data collection period (Table 1). In nine of the 10 networks ≥90% of dogs (with the network size as denominator) were directly or indirectly connected within a single component, highlighting the high connectedness within the observed dog populations. Within each country, network density and relative degree were significantly lower in semi-urban and urban study sites compared to rural sites (Wilcoxon rank sum tests p-values < 0.001, Table 1). The degree distributions were over-dispersed (coefficients of variation ranging from 54% to 80%) and right-skewed (8 out 10 networks had skewness index above 0.5). The small-world index of all networks was above one. These findings are characteristics of small-world networks.
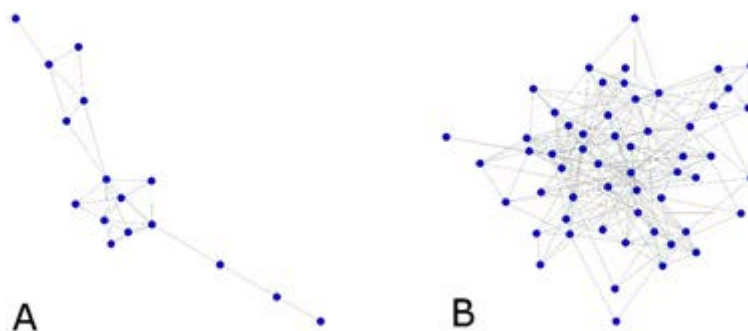
Figure 1. Two examples of the 11 undirected and unweighted dog contact networks. Nodes represent dogs and edges represent contacts between two dogs. A: Chad - rural 1 (network size = 16), B: Guatemala - rural 1 (n = 56)

Results from the PBLM displayed high heterogeneity between study sites (Table 2). The set of predictors selected for the best PBLM varied from one site to another and some predictors were found to be positively associated in one site, and negatively associated in another site. For example, female dogs were associated with lower degree than males in a rural site in Guatemala (rural 2), but higher degree in a rural site in Uganda (rural 1) (Table 2). Dogs with ideal BCS tended to have a higher degree than dogs with BCS under or above the ideal score, but not in all studied populations. Being a shepherd dog was negatively associated with degree in Uganda, whereas this variable did not impact the degree in the two other networks where this variable was investigated. Other purposes of the dog and the dog's age did not significantly influence the dogs' degree in any network.

Table 1. Size and network statistics of six dog contact networks in Guatemala, Indonesia, Chad and Uganda. IQR= Interquartile range

Metric	Guatemala			Indonesia			Chad		Uganda		
	Rural 1	Rural 2	Urban/ semiurban	Rural 1	Rural 2	Urban/ semiurban	Rural 1	Rural 2	Rural 1	Rural 2	Urban/ semiurban
Number of dogs collared	61	125	117	52	65	100	21	25	17	8	124
Number of collars retrieved	57	123	113	52	65	99	21	25	15	8	122
Network size	56	121	102	37	61	81	16	24	15	4	82
(% of the collars retrieved)	(98)	(98)	(90.3)	(71)	(94)	(82)	(76)	(96)	(100)	(50)	(67)
Size of the largest component	56	116	93	35	59	81	16	24	11	2	77
(% of the network size)	(100)	(96)	(91)	(95)	(97)	(100)	(100)	(100)	(73)	(50)	(94)
Network density	0.17	0.06	0.04	0.12	0.12	0.05	0.25	0.17	0.25	-	0.08
Small world index	2.2	5.1	6.4	3.3	3.5	6.5	2.0	2.3	2.9	-	4.5
Degree											
median (IQR)	8 (6 - 13)	7 (4 – 10)	3 (2 - 5)	4 (2 - 6)	6 (4 - 9)	4 (2 - 5)	3.5 (2 - 4.25)	4 (2 - 5)	3 (2 - 5)	-	6 (3 - 10)
coefficient of variation	0.59	0.59	0.77	0.71	0.8	0.68	0.58	0.54	0.59	-	0.71
skewness coefficient	1.1	1.4	2.6	0.8	2.1	1.3	0.8	0.4	0.3	-	0.6
Relative degree, median (IQR)	0.15 (0.11-0.24)	0.06 (0.04-0.08)	0.03 (0.02-0.05)	0.11 (0.06-0.17)	0.10 (0.07-0.15)	0.05 (0.02-0.06)	0.24 (0.13-0.29)	0.17 (0.09-0.22)	0.21 (0.14-0.36)	-	0.07 (0.04-0.12)

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Table 2. Regression coefficients and p-values of the permutation based linear regression models using log-transformed degree as output. FRT = Free roaming time, NCD = number of dogs collared in the same household, Distance = distance from the household to the centroid of the study area. Blank fields indicate that the factor was not selected within the set of best regression models for the specific study population.

Factor	Guatemala Rural 1	Guatemala Rural 2	Guatemala Urban/ semiurban	Indonesia Rural 1	Indonesia Rural 2	Indonesia Urban/ semiurban	Chad Rural 1	Chad Rural 2	Uganda Rural 1	Uganda Urban/ semiurban
Sex	-0.13, 0.50	-0.31, 0.01	0.06, 0.66	0.14, 0.62	-0.05, 0.82	0.08, 0.64	-0.45, 0.12		0.52, 0.10	0.11, 0.60
Age				0.01, 0.25	0.00, 0.65	0.00, 0.90		0.00, 0.58		0.00, 0.57
Low BCS			-0.10, 0.46		-0.43, 0.09	-0.36, 0.06			-0.19, 0.48	
High BCS			0.24, 0.29			-0.31, 0.34			-0.93, 0.01	
Guardian		-0.27, 0.28	0.22, 0.22			0.16, 0.69				
Shepherd			0.22, 0.72				0.54, 0.26			
Hunting	0.04, 0.84	0.01, 0.94		-0.24, 0.66	-0.30, 0.24	-0.10, 0.63	-0.20, 0.74		-0.54, 0.07	-1.16, 0.00
Source of meat						0.02, 0.05				
FRT	0.01, 0.22	-0.01, 0.16	0.02, 0.00	-0.03, 0.36					-0.01, 0.50	-0.02, 0.55
NDC	0.01, 0.82	0.01, 0.80	0.15, 0.02	0.59, 0.01	-0.14, 0.31	0.22, 0.01		0.25, 0.10	0.03, 0.92	0.14, 0.01
Distance	-0.08, 0.31	-0.03, 0.27	0.05, 0.42	-0.13, 0.21	-0.17, 0.18	0.00, 0.98		-0.16, 0.53	-0.18, 0.04	-0.20, 0.02

In contrast to this heterogeneity identified, the daily duration a dog was allowed to freely roam and the number of dogs collared in the same household were generally positively associated with the degree in most, but not all, networks. The distance from the centroid of the study area to the dog's home was negatively associated with the degree in most sites, with a significance found in both Ugandan sites. This justifies the inclusion of this control variable to adjust for the edge effect. The proportion of deviance explained by the variables included in the best models ranged from 0.001% to 23.3% (median = 1.5%), with the number of dogs collared, distance to the centroid, but also BCS and sex in single networks, substantially contributing to explaining the deviance of the models.

DISCUSSION

Understanding the ecology of the host population is essential for effective disease control. For rabies, several studies call for improved knowledge on dog ecology to plan vaccination campaigns more effectively (Flores-Ibarra and Estrella-Valenzuela, 2004; Ratsitorahina et al., 2009; Davlin and VonVille, 2012; Atuman et al., 2014; Dürr et al., 2017; Schildecker et al., 2017; Hudson et al., 2019a,b). This study contributes to the understanding of the structure of FRDD contact networks and assessment of the factors influencing those networks. The inclusion of a larger number of dogs than in other studies (Brookes et al., 2018; Laager et al., 2018; Hudson et al., 2019a,b) and from different countries enabled to directly compare dogs from varying settings, which is a clear strength of this study.

The 10 dog contact networks investigated here displayed strong similarities in terms of their structure, but were heterogeneous as regards factors associated with dogs' centrality. The similarity of the networks structures included the high direct or indirect connectedness of most dogs with one another. In addition, all investigated networks showed small-world properties. Small world networks are widespread in social, biological, technological and information networks (Watts and Strogatz, 1998; Humphries and Gurney, 2008; Telesford et al., 2011). A study on Torres Strait Islands, Australia, identified small-world networks in three FRDD populations (Brookes et al, 2018). Small-world networks are characterized by rapid spread of diffusion processes within the network, including diseases (Watts and Strogatz, 1998; Christley and French, 2010). We also observed in the studied networks that they presented right-skewed degree distributions. Within such networks, targeting crucial nodes – i.e. those that hold the network together – interrupts the connectedness very effectively (Kiss et al., 2006; Büttner et al., 2013). Therefore, in the case of a rabies outbreak, targeting highly connected dogs would make vaccination campaigns more effective than random vaccination.

Another similarity detected between the study sites was that dogs in rural sites tended to be more connected (higher relative degree and higher network density) than dogs in urban and semi-urban sites. One potential reason for this is that dogs in urban settings are more likely to be confined, as identified by previous studies on FRDD management performed in various regions of the world (Ortega-Pacheco et al., 2007; Acosta-Jamett et al., 2010; Mustiana et al., 2015; Wallace et al., 2017). One of these studies highlighted that households in urban areas were better equipped with fences to prevent dogs from unsupervised roaming (Ortega-Pacheco et al., 2007). Responsible dog ownership, which refers to confinement practices but also identification, neutering and vaccination of dogs, is a critical point for rabies control (Davlin and VonVille, 2012; Atuman et al., 2014; Mustiana et al., 2015), but often impossible to be implemented by dog owners in rabies endemic countries by their own means. Therefore, measures supporting dog owners to reduce contacts of their dogs with counterparts, such as

improving fenced building constructions, promoting feeding dogs at home and offering neutering campaigns are essential aspects of holistic rabies control programs.

Apart from these detected similarities, the PBLM analysis revealed heterogeneity in the set of predictors for dogs' centrality. What makes a dog central in the network, i.e. having a higher degree than other dogs, differs from one study site to another. This suggests that the local context matters. Male or female dogs, shepherd or hunting dogs might be managed differently from one country to another. For example, in the study site rural 2 in Chad, some interviewees reported that having a female dog was perceived as a potential source of problem because many people keep intact males as guardian dogs and the presence of a female in oestrus would result in dog fights. This has not been reported in the other study sites. Despite the absence of dog management data to support this hypothesis, it can be assume that if dogs are perceived differently across regions based on their sex, males and females might also be managed differently across regions. Similarly, dogs used for the same purpose may be managed differently from one country to another. For example in Australia, hunting dogs from several communities are gathered for pig hunting and therefore come into close contact to each other (Hudson et al., 2016) – a practice that has not been reported in our study sites.

The small proportion of the deviance of the model explained by the investigated variables suggests that factors not measured in the current study highly influence the network metrics. Factors that are not presented in this article but which may influence the dog's connectedness within a population include owner-dog relationship and perception of the dog by the owner. Some people might walk with their dogs around the village and take them for daily trips, others prefer to keep them at home regardless of the dog's purpose. Perception of dogs varies between cultural groups (Turner et al., 2013), and might impact the dog contact network in a way that has not been captured by the current study. Research would be need to understand the human-dog bound across various cultures and how it influences the social behaviour of dogs. In particular, studies on short- and long-range human mediated transportation networks might be of high value. Dog are impacted by human-induced movements (Villatoro et al., 2016) and it might be easier to identify highly connected owners than highly connected dogs. Studies on dog transportation conducted in Africa identified long-range movements as a key factor for rabies persistence (Colombi et al, 2020). Another driver of the dogs' centrality in the network may be the personality of individual dogs, with more friendly dogs being more often in contact with others. Dog personality and individuality have been studied for decades using approaches similar to human personality studies (Kubinyi et al., 2009; Ley et al., 2009; Siniscalchi et al., 2013; Miklósi et al., 2014; Eken Asp et al., 2015).

In conclusion, we hypothesize that investigating the importance of social and cultural structures impacting owners and therefore shaping dog ecology would be needed to inform the potential use of targeted vaccination.

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EXPLORING ENVIRONMENTAL DRIVERS FOR CANINE LEPTOSPIROSIS IN GREAT BRITAIN

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SUMMARY

Environmental drivers for canine leptospirosis are poorly understood. Spatial scanning statistics and ecological niche modelling (ENM) were used to explore spatial clustering of leptospirosis cases and environmental associations at a bacterial species and serogroup level. A high relative risk (RR) spatial cluster of cases was identified in the West Midlands of England (RR=2.16) and a high RR seasonal cluster was identified in October-January (RR=1.54). Leptospira serogroups appear to have distinct ecological preferences. Increasing annual average temperature was the most important environmental variable for all models, whereas the retention of and relationships with other environmental variables differed between models. Overall, it appears that leptospira survival and transmission is enhanced in areas with warmer annual temperatures and urban/suburban areas. Additionally, individual serogroup models indicated that there are serogroup specific associations with different livestock species. ENMs have increased our understanding of this bacteria's biology and can help inform control strategies, such as vaccination.

INTRODUCTION

Leptospirosis is an important zoonotic disease with a global distribution. It is a disease of greatest importance in tropical and developing areas of the world. Leptospira spp. are a complex family comprising 250 serovars which are grouped into 24 serogroups on the basis of antigenic similarity (Adler and de la Peña Moctezuma, 2010). Leptospirosis infection occurs largely through indirect transmission, typically through contact with urine contamination of the environment (Levett, 2001). Survival of leptospires in the environment is influenced by factors such as temperature, precipitation, soil pH, soil dampness and water quality (Lau et al., 2010; Azócar-Aedo and Monti, 2016; Bierque et al., 2020). Additionally, leptospiral shedding in the environment is dependent on presence of reservoir hosts such as rodents, livestock and dogs (Adler and de la Peña Moctezuma, 2010; Barragan et al., 2017). The specificity of different leptospira serovars to different maintenance hosts appears to vary, although the precise mechanisms for this are not clear (Adler and de la Peña Moctezuma, 2010).

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It is apparent that there are numerous potential associations between leptospirosis cases and environmental factors, with these reports largely coming from tropical areas and human studies. Some of these associations include temperature, rainfall (Ward, 2002; Zhao et al., 2016), flooding (Raghavan et al., 2012a), proximity to livestock (Mwachui et al., 2015) and urban/rural land use (Raghavan et al., 2012b; Mwachui et al., 2015). However, the ecological preferences of leptospires have not been explored in-depth, and at the individual serovar or serogroup level little is known (Jara et al., 2019). Furthermore, there appears to be limited exploration of environmental factors in more temperate climates such as northern Europe. Therefore it is unclear if associations identified in previous, tropics focussed work remain relevant in other climates.

Ecological niche modelling (ENM) is used to determine the relationship of ecological variables to the presence of a species at a particular site and then to identify its potential suitable range in a wider environment (Elith et al., 2011). Different species will occupy distinct niches across a wider environment depending on their ecological preferences. There are numerous methodologies available for ecological niche modelling (Stevens and Pfeiffer, 2011). One widely used algorithm is MaxEnt (Phillips et al., 2006). MaxEnt was originally used to explore ecological associations and predicted distributions of invasive or threatened species (Elith et al., 2011). However, more recently it has been applied to infectious disease ecology for a wide range of diseases such as canine leishmaniasis (Chamaillé et al., 2010) and also disease vectors, such as the Culicoides midge (Ciss et al., 2019). MaxEnt has been used to explore the distribution of leptospirosis in horses in Brazil and humans in China (Zhao et al., 2016; Jara et al., 2019). However, to date, it has not been used to explore canine leptospirosis.

MATERIALS AND METHODS

Study population

The dataset consisted of positive test submissions, either from the microscopic agglutination test (MAT) or polymerase chain reaction (PCR) from IDEXX laboratories between January 1st 2009 and December 31st 2018. PCR testing was performed on blood and/or urine and MATs were performed on blood. A positive submission was defined as either a single MAT titre >1:800, paired titres with a four-fold increase in serology or a PCR result reported as positive; this classification was in accordance with ECVIM guidelines (Schuller et al., 2015). From these submissions test results were extracted together with submitting clinic postcode and submission date. The PCR and MAT results were recorded as either positive/negative with MAT results additionally recording antibody titres to each serovar represented in the panel. Although MAT results are often reported as titre to serovars, the test has poor sensitivity due to cross-reactivity between serovars. Serogroup level reporting has much higher sensitivity (Blanco et al., 2016; Public Health England, 2017). Therefore, positive MAT test dogs were recorded as the serogroup demonstrating the highest titre, rather than the serovar (where multiple serovars had been reported as positive). Serogroups included in the study were: Australis, Canicola, Icterohaemorrhagiae, Grippotyphosa and Sejroe. Clinic postcodes were geocoded to generate British National Grid cartesian coordinates (Bell, 2000). Submission date was categorised into seasons: Autumn (September, October, November), Winter (December, January, February), Spring (March, April, May) and Summer (June, July, August). Negative submissions were retained as controls for use with the spatial scan statistic.

Spatial and temporal cluster detection

Spatial scan statistics (SaTScan v9.6 (Kulldorff, 1997)) were used to identify clusters of high or low risk for leptospirosis. Analysis was performed using three different models: purely spatial Bernoulli model, purely spatial multinomial model and the seasonal scan statistic (Kulldorff, 2015).

To explore any potential differences in the location of clusters, the spatial scan statistic was applied to three different datasets: all tests submissions (n=4137), only PCR submissions (n=2273) and only MAT submissions (n=2080). High or low rate clusters were identified. Additionally, since different leptospira serogroups may have varied geographical distributions the multinomial model was then used to differentiate by serogroups. In order to ensure adequate power and validity of the clusters detected, Monte Carlo replication of data sets was used. For each analysis the default parameters were retained ie. Maximal cluster size <50% of population, circular shape scanning window and 999 Monte Carlo replications were performed (Kulldorff, 1997, 2015). Shapefiles were generated from cluster output and exported into ArcGISv10.6 for visualisation.

The seasonal scan statistic was used to identify temporal clusters of leptospirosis cases, using month as the temporal unit. The seasonal scan statistic ignores year of sampling and just looks at months/day clustering. This was important due to a relatively small number of cases and controls to examine within-year clustering.

Agro-ecological data

Environmental variables examined in this study were chosen on the basis of plausible biological mechanisms of the leptospira bacterium, previous research and the availability of open-source datasets. A combination of categorical and continuous variables was used (Table 1).

For variables such as livestock density and land classification it was deemed appropriate to use a single value for the study time period. However, due to climatic variables having large variation between years and seasons a single time point value was not appropriate. Therefore, annual and monthly temperature and rainfall from the Met Office was aggregated to generate annual average rainfall and temperature values for the entire study period at 5km resolution (Meterological Office, 2018). Rasters of different projections or cell sizes were all reprojected to British National Grid (BNG) and interpolated using inverse distance weighting (IDW) to a 5km grid size. All rasters were generated and manipulated in QGIS v3.40 (Open Source Geospatial Foundation Project, 2020).

Ecological niche modelling with MaxEnt

An ecological niche modelling (ENM) approach was used to explore in greater depth the relationship of environmental variables on the probability of presence of leptospirosis in Great Britain and to compare the ecological niches of different leptospiral serogroups.

Table 1. Environmental variables included in a MaxEnt model of spatial distribution of canine leptospirosis in Great Britain (GB), together with variable type and source of data

Parameter examined	Source	Description	Format
Livestock density- cattle	FAO gridded livestock of the world (2010) (FAO, 2018)	Density of cattle(heads/km^2)	Continuous
Livestock density- sheep		Density of sheep (heads/km^2)	Continuous
Livestock density-pigs		Density of pigs (heads/km^2)	Continuous
Livestock density- horses		Density of horses (heads/km^2)	Continuous
Rural urban classification	Office of National Statistics (2012)	Rural urban classification of LSOA of GB (10 classes)	Categorical
Land coverage classification	Centre for Ecology and Hydrology (2017)	Land coverage of the UK (21 classes)	Categorical
Temperature	Met Office Had-UK^{TM} (Meterological Office, 2018)	Average of monthly mean air temperature minimum and maximum between 2009-18	Continuous
Rainfall		Average of total annual precipitation (mm) between 2009-18	Continuous
Flooding	European and Global Flood Awareness Systems (EFAS and GloFAS) (Dottori et al., 2016)	Flood hazard map of maximum flood depth based on streamflow from past 10 years (metres) (2006-16)	Continuous
Soil pH	International Soil Reference and Information Centre, (2020)	Soil pH in H$_2$O at 0cm depth	Continuous

MaxEnt uses recorded locations of a species and the values of environmental areas where a species has been recorded to create a probability distribution of the environment a species has been found in. It then creates a probability distribution of the whole study area using background points which characterises the environment of the study region. Ratios between these two probability distributions are calculated and are used to build models which quantify a specific area's relative environmental suitability for species presence (Elith et al., 2011).

Since MaxEnt relies on presence-only data, multiple cases at the same raster cell were removed, leaving 242 unique sites remaining. For individual serogroup models only samples with a titre >1:800 to a single serogroup were retained. Many cases had titres >1:800 to multiple serogroups but since the relationship between highest titre and being the infecting serogroup is unclear these were not retained. In total, four distinct models were built: an All Cases model and separate models for each of the Australis, Icterohaemorrhagiae and Sejroe serogroup cases.

Variables were automatically transformed in the MaxEnt programme into different features. For each model, background points (n=10,000) were randomly generated and the model was trained on a random 25% of presence records. Optimised models were generated using the jack-knife method, which determines the predictive performance of the model with and without inclusion of a variable. If exclusion of the variable improves model predictive performance

then the variable is removed from the final model. Final model performance was assessed through several evaluation methods used previously with MaxEnt: AUC$_{TEST}$ (maximum Area under Curve of Test data value), the True Skill Statistic (TSS) and Kappa Statistic. TSS and Kappa Statistic were calculated using the prevalence threshold (Allouche et al., 2006; Elith et al., 2011).

RESULTS

Description of study population

There were 315 positive submissions (7.8%) and 3671 negative submissions (92.2%) between 2009-18. Submissions came from all 12 regions of the UK, with the South East (n=693, 17.4%) and the South West (n=563,14.1%) reporting the most submissions. Northern Ireland had both the least cases (n=4, 1.3%) and overall submissions (n=61, 1.6%), so was removed from further analyses. Prevalence was highest in the West Midlands (12.5%) and East Midlands (11.5%).

Spatial and temporal clustering of cases

The purely spatial scan statistic identified three significant clusters amongst PCR submissions. One cluster was high relative risk (RR=2.16, p=0.005, Fig. 1 red circle A) and two were low risk (RR=0, p=0.01 and 0.03 respectively, Fig. 1 blue circles B and C). The high risk clustered was centered in Shropshire. There were no significant MAT clusters. In the multinomial spatial scan, one significant cluster was identified (p=0.013). This cluster had significantly increased RR for Canicola (RR=10.19), Grippotyphosa (RR=3.82) and Australis (RR=2.29). The cluster was centered over southern Wales and Devon/Somerset counties (Fig. 1, shape D dotted red circle). The seasonal scan statistic identified a significant temporal cluster of increased leptospirosis risk from October to January (RR=1.54, p=0.004).

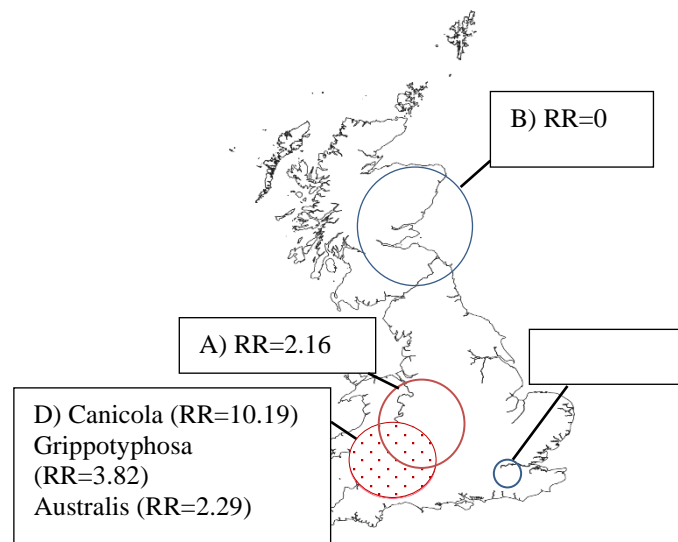Figure 1. Map showing the location of four spatial clusters. Cluster A) exhibits a significantly higher proportion of cases, Clusters B) and C) exhibit significantly lower proportions of cases and Cluster D) indicates significantly higher proportions of Canicola and Grippotyphosa serogroup cases when compared to the area outside of the clusters. Clusters were identified through Kuldorff's spatial scan statistic.

Ecological niche modelling

Model performance and selection: The All Cases model was built using all positive tests at unique geographic locations (n=242) and the Australis, Icterohaemorrhagiae and Sejroe models were built using positive MAT tests submissions which reported a single highest titre (of ≥1:800) to the respective serogroups (n=23,24 and 25 unique sites respectively). The predictive performance of all final models was evaluated using several assessment methods (Table 2). From these assessments the Sejroe model had both the lowest AUC (AUC= 0.80) and the lowest TSS (True Skill Statistic) value (TSS= 0.44). The Australis model appeared to have the best predictive ability, with the highest AUC, high sensitivity/specificity and highest TSS value. Sensitivity was lower in the individual serogroup models rather than the All Cases model. The reverse was true for specificity. All models had a moderate TSS (>0.4), using Landis & Koch's (1977) categories.

Table 2. Assessment of the performance of each ecological niche model built in MaxEnt to explore canine leptospirosis in GB using Area Under the Curve (AUC), Kappa statistic, sensitivity, specificity and the True Skill Statistic (TSS).

Model performance index	All Cases	Australis	Icterohaemorrhagiae	Sejroe
AUC	0.81	0.92	0.84	0.80
Threshold value (prevalence)	0.24	0.14	0.17	0.28
Kappa statistic max	0.29	0.34	0.31	0.27
Sensitivity	0.91	0.89	0.86	0.86
Specificity	0.53	0.68	0.61	0.58
TSS (True skill statistic)	0.45	0.56	0.47	0.44

Variables included in models

Variable retention and importance varied between the serogroups. Annual average temperature was the most important variable for all models. Temperature accounted for 63% of variation in the All Cases model but importance varied widely between the serogroup models from only 42.5% in the Icterohaemorrhagiae model to 75.2% in the Australis model. As temperature increased, probability of presence increased up to maximum probability between 9°-12°C, with a lower maximum probability threshold of 9°C in the Sejroe model and a higher threshold of 12°C in the Australis and Icterohaemorrhagiae models. Urban/suburban land use was the most important class of land use and was retained as an important variable in the All Cases, Australis and Icterohaemorrhagiae models. The importance of other variables varied between models. For the All Cases model, urban-suburban land use and rainfall less than 1000mm a year were the next most important. In the Australis model increasing density of horses and increasing annual rainfall were important associations with Australis probability of presence. For the Sejroe model, acidic soil pH (5.5-6.5) and decreasing cattle density were important contributors to probability of presence. Finally, in the Icterohaemorrhagiae model annual rainfall between 750-1000mm, followed by low densities of pigs were the most important predictors of presence.

Probability of presence of leptospirosis in Great Britain

Maps were generated to indicate the relative probability of presence according to each model (Fig. 2). Probability of presence was heterogenous across GB, although predicted probabilities in each model followed a general pattern of lower probability (blue/green colours) in northern England/Scotland and higher probability in the south east of England (red/orange). Probability

predictions varied between different serogroups, namely that the Australis model appeared to have more areas of high probability in coastal areas and the Sejroe model appeared to have small areas of high probability in Scotland, which were not seen with the other serogroup models.

DISCUSSION

This study has identified regional variation for canine leptospirosis cases and also identified variation in the ecological preferences and distribution of leptospira serogroups across Great Britain. Understanding associations between environmental factors and leptospirosis occurrence is important since direct dog-to-dog transmission is rare, and environment-acquired infections are more significant.
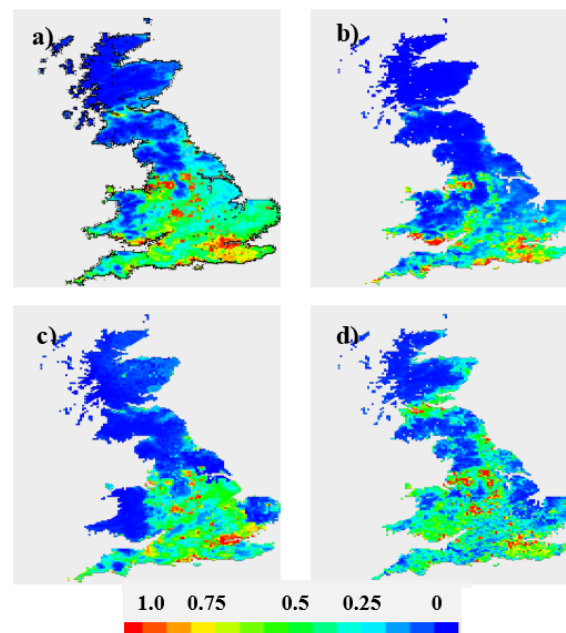
Figure 2. Maps indicating gridded probability of presence of leptospirosis in each model for Great Britain. A) All Cases model B) Australis model C) Icterrohaemorrhagiae model and D) Sejroe model. Red/orange areas have high probability of presence, blue areas have low probability of presence.

In general, the ENMs suggests that leptospira transmission favours locations with warmer annual average temperatures and urban/suburban land types, with serogroup specific relationships to livestock densities and soil pH. A preference for warmer annual temperatures likely reflects increased survival time in warmer weather but additionally higher temperatures can lead to increased water-based activities, thereby facilitating increased transmission (Lau et al., 2010). Increased probability in urban/suburban areas has been reported in some previous dog studies and could reflect increased contact with rodents or other reservoir hosts and increased usage of same shared walking spaces (Lau et al., 2010; Raghavan et al., 2011). It appears that individual serogroups associate with distinct large animal species. The Australis model found increased horse density to be associated with higher probability of Australis presence. L. Bratislava, part of the Australis serogroup, is the serovar most frequently identified in horses and is reportedly host-adapted (Verma et al., 2013). As it is a clinically important serovar for dogs, identified in a 2015 canine leptospirosis outbreak in Somerset (Wilson et al., 2015), the role of horses in transmission of leptospirosis in Great Britain merits further

exploration. Low pig densities (up to 100 heads/km2) in the Icterohaemorrhagiae model were associated with high probability of presence. Lower pig densities may reflect areas with small holdings or smaller commercial farms predominating or areas with fewer pig farms. Biosecurity and vaccination may be less commonplace in smaller-scale production and therefore increased environmental shedding of leptospires may occur. Indeed, a recent outbreak on a pig farm in England was attributed to L. Icterohaemorrhagiae (Animal and Plant Health Agency, 2018). Consistent with other studies, increased prevalence of cases in Autumn was reported (Ward, 2002; Raghavan et al., 2011; Lee et al., 2014; Major et al., 2014).

MaxEnt offers advantages over more traditional risk factor exploration methods such as logistic regression. Relationships between diseases and environments are rarely linear and MaxEnt is able to perform highly complex transformations of variables to identify these relationships and also identify the interactions between variables in different transformations. Few studies, with exception of work by Jara et al., (2019), have explored environmental associations at a serovar or serogroup level. Given the distinct preferences between serogroups noted here and by Jara et al. (2019) it seems appropriate to do this. Knowledge of serovar or serogroup specific niches may improve understanding of local disease transmission and implementation of control strategies.

Retrospective laboratory submission datasets have key limitations for these analyses. Firstly, the case locations are the submitting clinic's postcodes and not the owners' home postcode. Since clients will travel varying distances to seek veterinary treatment this may have impacted our ability to assess for highly localised risk factors, although our study is performed at 5km^2 resolution so some of this variability will be accounted for. Other studies have used IDEXX leptospirosis test submissions (in America) and therefore used location information at the clinic postcode level only (Lee et al., 2014; White et al., 2017; Smith et al., 2019). Given the long asymptomatic period for leptospirosis, even a home postcode would be unlikely to encompass all potential environmental exposures. As leptospires have numerous wildlife reservoirs, with rodents being most notable, exploring the relationship between environment suitability and wildlife density, particularly rodents, would have been interesting to evaluate.

A limitation with MAT result interpretation is the acknowledged poor serovar predictivity. Levett (2003) found serovar was predicted correctly in only 44-46% of single samples. Higher sensitivity (94%) at the serogroup level for humans is reported (Blanco et al., 2016). Since there are no studies at present assessing the serovar/ serogroup predictive sensitivity in dogs we elected to only use the serogroup level result. Although this means we can be much more confident that the serogroup identified is truly correct it does mean that if serovars within a serogroup have very distinct, varied ecological niches then details of these niches may be lost.

Ecological niche modelling is a useful tool to explore complex associations between environment and disease. Areas in GB with a warmer annual average temperature have more favourable conditions for leptospira presence. The ENMs evidence that ecological preferences vary between serogroups, namely that there are serogroup specific associations with distinct livestock species, rainfall and soil pH. These preferences impact the geographical distribution of leptospirosis in GB. ENM has identified areas of higher risk for leptospirosis and this information could be utilised in more targeted control strategies in certain areas, such as ensuring vaccine usage at areas of higher probability of leptospirosis.

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DECISION SUPPORT FOR DAIRY HERDS

DATA IN SUPPORT OF CALF REARING TO DECREASE CALF MORTALITY IN DUTCH DAIRY HERDS

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SUMMARY

The Dutch dairy industry initiated data-driven tools to support calf rearing and decrease mortality that were implemented in 2018. The aim of this study was to evaluate the association between calf mortality and the implementation of the tools next to other influential factors. Mortality indicators for perinatal calves, postnatal calves, preweaned calves and weaned calves were available for 98% of Dutch dairy herds in 2014-2019. Data were aggregated to herd and monthly level and were analysed with multivariable population-averaged models. After implementation of the tools, all calf mortality indicators significantly decreased. Other explanatory variables associated with calf mortality were e.g. extreme weather, antimicrobial and antiparasitic treatments and vaccinations. Given the study design, we could not prove that implementation of the data-driven tools caused the reduction in calf mortality. Nevertheless, all actions and communication to improve calf rearing may have led to an increased focus on calf rearing with a reduced calf mortality on national level.

INTRODUCTION

An important indicator of calf health is calf mortality (Kelly et al., 2013). Between 2009 and 2017, calf mortality in the Dutch dairy sector showed a slight but steady increase. Factors associated with the increase were investigated by Santman-Berends et al. (2014). They concluded that there was no clear disease related cause for the increased mortality rates but that there appeared to be an association between calf mortality and the mindset of the farmers. Based on these results, the dairy industry decided to take action and supported the development of several data-driven tools that would match the mindset of different groups of farmers.

The data-driven tools that were developed consisted of a management tool named KalfOK in which routinely collected herd data were combined into a number of key indicators for quality of young stock rearing at herd level (Santman-Berends et al., 2018), obligatory surveillance of calf mortality (Santman-Berends et al., 2019) and implementation of a calf track system for veal calves. The aim of these tools were to benchmark calf rearing performance, stimulate dairy farmers to improve quality of young stock rearing and to reduce calf mortality. The three tools were either implemented on a voluntary basis (KalfOK) or obligatory basis (calf mortality surveillance and the calf track system) from 2018 on. At the end of 2018, more

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than 90 percent of the dairy farmers voluntarily participated in KalfOK and all dairy farmers participated in the mandatory tools. The extra attention on young stock rearing and increased insight in the herds' performance also resulted in other initiatives to support calf rearing.

The Trend Analysis Surveillance Component (TASC) of the Dutch Cattle Health Surveillance System (CHSS) (Santman-Berends et al., 2016) provided the possibility to evaluate the effect of all these efforts on calf mortality 1.5 years after the implementation. The aim of this study was to evaluate the association between calf mortality and i) all the actions that were taken by the Dutch dairy industry to improve the quality of calf rearing and ii) other potential management or environmental factors associated with mortality in Dutch dairy herds.

MATERIALS AND METHODS

Study population and available data

For this study, anonymized census data were available from Dutch dairy herds that participate in the CHSS. In total, approximately 98 percent of the Dutch dairy farmers gave consent to use their anonymized routinely collected data for monitoring of cattle health and therefore participate in the CHSS (approximately 15,500 dairy farmers).

For these herds census data were available from six different data sources from July 2014 until June 2019. Registrations on cattle movements, including registrations of births, purchase, slaughter, and mortality were available from the national identification and registration database (RVO, Assen, the Netherlands). Cattle mortality data were obtained from the rendering plant and included the number of rendered cattle per herd and date (Rendac, Son, the Netherlands). Milk recording records were available from approximately 75 percent of the herds that participate in the CHSS and were available from the Royal Dutch Cattle Syndicate (CRV, Arnhem, the Netherlands). Herd health statuses were available from Royal GD (Deventer, the Netherlands) and Qlip laboratories (Zutphen, the Netherlands) and included the status for Bovine Viral Diarrhea Virus (BVDV), Bovine Herpes Virus type 1 (BHV-1), salmonellosis, leptospirosis and paratuberculosis. Data of veterinary registrations on deliveries of antimicrobials, vaccines and other medicines were available per age category of cattle at herd level and date (MediRund, Zuivel NL, the Hague, the Netherlands). Finally, data on the regional weather conditions i.e. relative humidity and temperatures in the Netherlands on a daily basis were available from the Royal Dutch Meteorology Institute (KNMI, the Bilt, the Netherlands).

Definitions

For this study four different calf mortality indicators were distinguished according to the definitions that are generally used in the CHSS (Santman-Berends et al., 2019).

Perinatal calf mortality risk (Perinatal_CMR): was defined as the number of deceased perinatal calves per herd h and time period t relative to all calves born (irrespective whether aborted, dead or alive) and was calculated according to Eq. (1). Deceased perinatal calves included late abortions (>6 months pregnancy), still births and calves that died before the moment of ear tagging (within at maximum 3 days after birth).

$$Perinatal\_CMR_{ht} = \frac{(\sum abortions, stillbirts, deceased\ new\ born\ calves)_{ht}}{n\ calvings_{ht}} * 100\% \qquad (1)$$

Postnatal calf mortality risk (Postnatal_CMR): was defined as the number of deceased calves from the moment of ear-tagging, within three days after birth according to Dutch legislation, until 14 days (14 d) of age (the minimum age after which calves are allowed to move off-farm in the Netherlands) relative to the number of calves that were ear tagged in the same herd and time period as described by Eq. (2).

$$Postnatal\_CMR_{ht} = \frac{\text{n deceased calves}_{(ear-tagging \leq 14d)ht}}{\text{n ear-tagged calves}_{ht}} * 100\% \qquad (2)$$

Preweaned calf mortality rate (preweaned_CMRA): was calculated by dividing the number of deceased calves from 15 until 55 days of age by the number of calf days at risk which resulted in the calf mortality rate per day (Eq. (3)). The number of calf days at risk was defined as the number of calves from 15 until 55 days old that were present in herd h and in time period t corrected for the time that they were present in the herd. For example, a calf that was in the correct age category and that was present during a whole month was included for a DAR of 30.4 days (average number of days per month). Calves that were only in the correct age category during part of time period t or that were moved on- or off-farm during the time period of interest were included for the number of days that they were in the correct age category and present in the herd.

$$Preweaned\_CMRA\_d_{ht} = \frac{\text{n deceased calves 15-55d}_{ht}}{DAR_{ht}} \qquad (3)$$

Subsequently the preweaning calf mortality rate per day $Preweaned\_CMRA\_d$ was multiplied by the number of days in the period t of interest and presented as percentage.

Weaned calf mortality rate (weaned_CMRA): was calculated similarly to the preweaned_CMRA (formula 3) and included another age category of calves i.e. 56 days until one year of age in both the numerator and the denominator.

Evaluated data tools and other explanatory variables

Data-driven tools: The association between implementation of the different data-driven tools and mortality was evaluated by adding a categorical variable that indicated whether the tool was implemented or not. The first evaluated tool was KalfOK, a scoring system in which farmers are graded points on 12 different young stock rearing indicators. The sum of the scores of the individual points add to a total score between 0 and 100 points. This score is communicated to the farmer together with the value of each indicator, a bench mark and the strengths and weaknesses in the young stock management (Santman-Berends et al., 2018). The second tool was the obligatory calf mortality surveillance, which provides the calf mortality risk for perinatal and postnatal calves ≤14 days on herd and quarterly level together with a benchmark. Farmers of herds with the five to ten percent highest mortality risk according to this tool are obliged to make a plan to improve their rearing management with their herd veterinarian. The third tool is the Calf track system (CTS) in which the trader has to check whether a calf is healthy, more than 14 days old and has complete and correct registration in the identification and registration database. Only calves that meet all requirements are allowed to leave the dairy herd to the veal herd.

For the different tools, three periods were distinguished (Table 1). Given that the moment of implementation of KalfOK and the obligatory surveillance of calf mortality overlapped completely, it was not possible to distinguish the tools in a statistical analysis and they were included as a one parameter in the statistical models.

Table 1. Description of the three different data-driven tools that were developed to support young stock rearing in Dutch dairy herds and the period a possible association with calf mortality could be expected.

Tool	Voluntary/ mandatory	Implementation date	Defined periods
KalfOK	Voluntary	01-01-'18	<2018: no tools Jan 2018- Jan 2019: start period >Jan 2019: fully implemented
Surveillance of calf mortality	Mandatory	01-01-'18	<2018: no tool Jan 2018-Jan 2019: start period >Jan 2019: fully implemented
Calf track system	Mandatory	01-04-2017	<Apr 2017: no tool Apr 2017-Jan 2018: start period >Jan 2018: fully implemented

Other evaluated explanatory variables: In the TASC of the CHSS there are always a number of potential explanatory variables that are considered to be associated with calf mortality that were therefore included in this study. These parameters include herd size, growth in herd size, replacement rate, location represented by province, milk production level, season, milk price, value of postnatal calves, purchase of cattle, status (free vs. non free) for endemic diseases such as salmonellosis, leptospirosis, BVDV, BHV-1 and paratuberculosis, milking parlour (regular vs. automated milking system), an indication whether young stock rearing is outsourced or not and a variable representing the trend in time. The continuous variables were categorised into four categories (10% smallest, 40% smaller, 40% larger and 10% largest) and the mean of the whole population was included as reference category. For this study additional data enabled us to include a number of additional parameters that described whether there was an association between calf mortality:

1. Influence of transition cow problems: To evaluate whether calf mortality was associated with transition cow problems, information on occurrence of ketosis during the start of lactation i.e. first 60 days (Van der Drift et al. 2012) was available (CRV). Additionally, cow-mortality rates during the start of lactation were calculated and included as proxy for issues during calving.

2. Medicine supplies: In the Netherlands, all medicines supplied to farmers by veterinary practices are centrally recorded in the national database MediRund (ZuivelNL). Based on these data several parameters that were potentially associated with calf mortality were considered as additional explanatory variables such as Antimicrobial usage (AMU) for respiratory treatments (AMU$_{res}$) and AMU for treatment of diarrhea (AMU$_{dia}$), treatments for coccidiosis or cryptosporidiosis and vaccination.

3. Weather conditions: These were included by means of the temperature-humidity index (THI) (Crescio et al. 2010) per month and for each 2-digit postal code.

Analyses

All data validation was executed using SAS® version 9.4 (SAS, 2019). After the final dataset was generated, descriptive analysis were performed in which the mortality in the most recent

period after implementation of the data tools i.e. July 2018 until June 2019 was compared to the mortality in the year before implementation of the data tools i.e. June 2016 until July 2017.

Multivariable population-averaged models (PA GEE) with a poisson distribution and a log link function were used for analyses in Stata® 15 (Stata, 2018). The calf mortality indicators were included as dependent variables and the potentially associated parameters were included as independent variables. The data were analysed on a herd and monthly level between July 2014 until June 2019 and the model corrected for repeated measures within herds. A *P*-value below 0.01 was considered significant because of the large numbers of observations in our model. Model fit was evaluated using the quasi-likelihood under the independent model criterion (QIC) (Cui, 2007) and the amount of variance explained by the model (R^2).

RESULTS

Descriptive results

Between July 2018 and June 2019, the perinatal calf mortality risk in the 15,500 dairy herds was 7.6 percent compared to 8.5 percent between July 2016 and June 2017. The postnatal calf mortality risk slightly improved from 3.6% to 3.5%. The preweaned calf mortality reduced from on average of 5.4 percent from July 2016 until June 2017 to 4.4 percent from July 2018 until June 2019. The weaned calf mortality reduced from on average 3.4 percent to 2.4 percent in the two evaluated periods. Besides a reduction in average calf mortality, we observed that there were more herds without mortality in the different age groups of calves and fewer herds with high mortality between July 2018 and June 2019.

Multivariable results

A selection of the model results, which were of particular interest and described in material and methods are presented in Table 2. A significant association was found between the periods in which the data-driven tools in support of young stock rearing were implemented and all four calf mortality indicators. The period in which KalfOK and the calf mortality indicator were fully implemented was associated with an 1.2 to 1.5 times lower mortality (IRR=0.8 to 0.67) relative to the period before implementation (Table 2 and 3). Implementation of the Calf track system was associated with a 1.05 to 1.2 times lower mortality (IRR= 0.81-0.95). Vaccinating dams to prevent diarrhea in their newborn calves, was associated with a lower postnatal calf mortality (IRR=0.91), but a higher mortality rate in preweaned calves (IRR=1.07). Vaccination of calves for respiratory infections was associated with a significantly lower preweaned calf mortality rate (Table 2 and 3). Herds with more health issues during the transition period of cows were associated with higher calf mortality. Occurrence of mortality in transition cows during the first 60 days in lactation was associated with a significantly higher perinatal calf mortality. Having cows with ketosis during the start of lactation was associated with higher mortality in perinatal, postnatal and preweaned calves.

Other factors that were associated with higher mortality included extreme temperatures, purchase, low production levels, autumn, high replacement percentages and larger herd sizes. A free or unsuspected herd status for infectious diseases such as BHV-1, BVDV, salmonellosis or paratuberculosis was associated with significantly lower mortality in all four analyzed calf mortality indicators (Table 2 and 3).

Table 2. Selection of multivariable results of the population averaged poisson regression models to evaluate factors associated with perinatal and postnatal calf mortality in approximately 15,500 Dutch dairy herds from July 2014 until June 2019.*

Parameter	Description	Perinatal calf mortality (IRR & 95%CI)*	Postnatal calf mortality (IRR & 95%CI)*
KalfOK/ Calf mortality			
Before implementation	<2018	Reference	Reference
Start implementation	Jan '18-Jan '19	0.94 (0.91-0.97)	0.97 (0.94-1.01)[ns]
Full implementation	>Jan '19	0.84 (0.81-0.87)	0.75 (0.71-0.79)
Calf track system			
Before implementation	<Apr '17	Reference	Reference
Start implementation	Apr '17-Jan '18	1.00 (0.98-1.02)[ns]	0.77 (0.73-0.80)
Full implementation	>Jan '18	0.95 (0.92-0.98)	0.81 (0.76-0.86)
Vaccination (vs. no vcc.)			
Against diarrhea	17% herds	X	0.91 (0.88-0.93)
Antimicrobial use (vs. no use)			
For diarrhea (3 mo)	9% herds	X	1.46 (1.42-1.51)
For respiratory infections (3 mo)	14% herds	X	1.13 (1.10-1.16)
Treatments (vs. no treatment)			
For cryptosporidiosis (yr)	38% herds	X	1.25 (1.22-1.28)
Ketosis <60d in lactation			
0%	27% herds	Reference	Reference
>0 and <12.1% of cows	36% herds	1.07 (1.05-1.08)	1.05 (1.03-1.08)
≥12.1% of cows	36% herds	1.05 (1.03-1.06)	1.05 (1.02-1.09)
Mortality <60d in lactation			
0%	57% herds	Reference	
>0 and <7.1% of cows	22% herds	1.16 (1.15-1.18)	X
≥7.1% of cows	21% herds	1.26 (1.24-1.27)	X
Temperature humidity index			
Normal	≥3.6ºC<18.2ºC	Reference	Reference
Very cold	<1.6ºC	1.09 (1.05-1.13)	1.01 (0.96-1.07)[ns]
Cold	≥1.6ºC<3.6ºC	1.05 (1.03-1.06)	1.07 (1.04-1.10)
Warm	≥18.2ºC<19.3ºC	1.02 (1.00-1.04)	1.18 (1.14-1.22)
Hot	≥19.3ºC	1.09 (1.05-1.13)	1.44 (1.36-1.52)
Herd health status			
BHV-1-free (vs. non-free)	39%	0.97 (0.95-0.99)	0.98 (0.95-1.01)[ns]
BVD-free (vs. non-free)	45%	0.98 (0.96-0.99)	0.96 (0.93-0.99)[ns]
Salmonella-unsuspected (vs. test positive)	93%	0.97 (0.95-1.00)[ns]	0.88 (0.84-0.91)
Paratbc unsuspected (vs. test positive)	80%	0.99 (0.97-1.00)[ns]	0.91 (0.88-0.94)

*IRR= Incidence Rate Ratio, 95% CI=95% Confidence Interval; X=not evaluated; ns= not significant at $P<0.01$

Table 3. Selection of multivariable results of the population averaged poisson regression models to evaluate factors associated with preweaned and weaned calf mortality in approximately 15,500 Dutch dairy herds from July 2014 until June 2019.*

Parameter	Description	Preweaned calf mortality (IRR and 95% CI)*	Weaned calf mortality (IRR and 95% CI)*
KalfOK/ Calf mortality			
Before implementation	<2018	Reference	Reference
Start implementation	Jan '18-Jan '19	0.85 (0.80-0.90)	0.83 (0.79-0.87)
Full implementation	>Jan '19	0.70 (0.65-0.75)	0.67 (0.62-0.72)
Calf track system			
Before implementation	<Apr '17	Reference	X
Start implementation	Apr '17-Jan '18	0.80 (0.75-0.84)	X
Full implementation	>Jan '18	0.82 (0.75-0.89)	X
Vaccination (vs. no vcc.)			
Against diarrhea	17% herds	1.07 (1.03-1.11)	X
Against respiratory infections	14% herds	0.90 (0.87-0.94)	1.03 (0.99-1.07)^{ns}
Against lungworm infections	2% herds	X	0.86 (0.79-0.94)
Antimicrobial use (vs. no use)			
For diarrhea (3 mo)	9% herds	1.36 (1.31-1.42)	X
For respiratory infections (3 mo)	14% herds	1.26 (1.22-1.30)	X
Total use in calves 56d-1yr (yr)	30% herds	X	1.31 (1.26-1.35)
Treatments (vs. no treatment)			
For coccidiosis (yr)	32% herds	0.86 (0.83-0.89)	1.00 (0.96-1.03)^{ns}
For cryptosporidiosis (yr)	38% herds	1.26 (1.22-1.30)	
Ketosis <60d in lactation			
0%	27% herds	Reference	
>0 and <12.1% of cows	36% herds	1.07 (1.04-1.11)	X
≥12.1% of cows	36% herds	1.02 (0.99-1.06)^{ns}	X
Temperature humidity index			
Normal	≥3.6ºC<18.2ºC	Reference	Reference
Very cold	<1.6ºC	1.14 (1.06-1.23)	1.14 (1.04-1.26)
Cold	≥1.6ºC<3.6ºC	1.17 (1.12-1.22)	1.16 (1.10-1.23)
Warm	≥18.2ºC<19.3ºC	0.98 (0.92-1.04)^{ns}	0.99 (0.93-1.06)^{ns}
Hot	≥19.3ºC	1.23 (1.11-1.35)	1.04 (0.92-1.17)^{ns}
Herd health status			
BHV-1-free (vs. non-free)	39%	0.95 (0.92-0.99)^{ns}	0.91 (0.87-0.94)
BVD-free (vs. non-free)	45%	0.90 (0.87-0.93)	0.86 (0.83-0.89)
Salmonella-unsuspected (vs. test positive)	93%	0.79 (0.76-0.83)	0.85 (0.81-0.90)
Paratbc unsuspected (vs. test positive)	80%	0.89 (0.85-0.93)	0.87 (0.84-0.91)

*IRR= Incidence Rate Ratio, 95% CI=95% Confidence Interval; X=not evaluated; ns= not significant at

DISCUSSION

The aim of this study was to evaluate the association between calf mortality in Dutch dairy herds and i) the efforts of the cattle industry to improve the quality of calf rearing and ii) other potential management and environmental factors.

Descriptively, a decreased mortality was observed in perinatal, preweaned and weaned calves. The mortality risk in postnatal calves however, was similar when the periods before and after implementation of the data-driven tools were compared. The fact that the mortality in this age group of calves (from moment of ear tagging until 14 days of age) was not reduced could be because there might have been a change in the moment of ear-tagging. A rule in the Calf track system is that calves are only allowed to leave the dairy herd for the veal industry, 14 days after ear-tagging and registration in the identification and registration system. Given that farmers want to sell the bull calves and part of the heifer calves as soon as possible, they may ear-tag these calves earlier than before this regulation in the calf track system existed. Given that the probability for calves to die decreases with increasing age, and that ear-tagged calves may have become a bit younger, one would expect an increase in postnatal calf mortality. However, we observed a decrease.

To study whether all efforts to improve the rearing quality and to reduce calf mortality paid off, multivariable models were used. The period in which the tools were implemented was associated with a decrease in mortality. Given the observational study design, we could however not prove that the implementation of the data-driven tools resulted in this decrease. All Dutch dairy herds were exposed to the tools given that it was mandatory to participate in two out of three tools that were implemented i.e. the calf mortality indicator and the calf track system. This resulted in lack of a control group that could have provided more insight in the added value of implementing the tools. Participation in KalfOK was voluntary and during the study period five to ten percent of the herds did not participate in this programme. In theory, we could have used these herds as a control group. However, no herd-level data was available whether or not herds participated and what the starting date of participation was. One could further question the true value of this small control group. The herds that decided not to participate in the KalfOK programme are not random herds will not be representative for the population of Dutch dairy herds. Nevertheless, given the timing of the introduction of the data-driven tools and the subsequent change in trend of the calf mortality indicators, we believe that the implementation of the data-driven tools has supported the reduction in calf mortality. Providing information alone does not improve young stock mortality. Awareness of the situation and possibilities to improve management can result in changed behaviour. From our previous study (Santman-Berends et al. 2014) we concluded that part of the farmers had a lack of knowledge about calf mortality in their herds relative to other herds. Information about the calf mortality levels in their herds relative to a benchmark could possibly initiate a change in mindset, resulting in changed behaviour i.e. management practices that would help to reduce calf mortality. Development and implementation of the data-driven tools provided this information and subsequently resulted in all sorts of initiatives to improve. For example a blueprint for good calf management was introduced (Blueprint Calf Rearing project group, 2018) and the Dutch veterinary board developed guidelines to develop a plan to improve young stock rearing management (KNMvD, 2018) that could be used by the farmer and the herd veterinarian when the calf mortality indicator detected a high calf mortality in the herd. Additionally, many initiatives tailored to the needs of individual farmers were initiated by veterinary practices and feed suppliers and environmental factors such as extreme temperatures were taken into account more often. Another change was that a phosphate regulation was

introduced in the beginning of 2017. On average, this resulted in a decrease in the number of replacement calves in dairy herds. A smaller number of calves may be associated with a lower infection pressure and may also have helped to reduce morbidity and subsequent mortality in Dutch dairy herds. In conclusion, the incidence rate ratios of the data-driven tools provide the association of implementation of the combination of all initiated actions rather than implementation of the tools alone.

In our study, calf mortality in herds that were assumed to be protected by vaccination was compared to herds that were not protected by vaccination i.e. herds that did not vaccinate or herds that did apply vaccination but outside the period in which protection could be expected. Vaccination was associated with a higher preweaned calf mortality risk which can be explained by the fact that farmers often start vaccination after problems occur. When we conducted an analysis on a subset of vaccinating herds and compared calf mortality after vaccination with mortality in the year prior to vaccination, vaccination was always associated with a reduction in calf mortality (results not shown). However, vaccination is very often part of a strategy to improve young stock rearing and reduce calf mortality. In this study, only centrally registered data were available and only supplies of medicines and vaccinations could be included as proxy of such an improvement strategy. Our results therefore provide an indication of the association between mortality and implementing a young stock rearing improvement strategy rather than application of vaccination alone. Larger herd sizes were associated with higher calf mortality rates in postnatal, preweaned and weaned calves. These findings are in accordance with previous findings of Reimus et al. (2020) who also found higher mortality in larger herds. A possible reason for higher mortality in larger herds is a higher infection pressure, but herd size is also a known proxy for factors that were not included in this study such as time spend per calf per day, amount of foreign labour, number of visitors, etc. A noteworthy finding was that herds with more transition cow problems had higher mortality rates in calves. This may indicate that dry-cow management also plays a role in preventing calf mortality and warrants further research. To our knowledge there is no literature that studied the association between transition problems in cows and calf mortality, although other studies did find that general herd problems such as presence of pathogenic infections or higher mortality rates in other age groups were a risk factor for calf mortality (Reimus et al., 2020). Also extreme outside temperatures appeared to be associated with higher mortality rates. This finding was supported by Egberts et al. (2019) who found an association between mortality and extreme temperatures and the results of Hyde et al. (2020) who found that the temperature in the month of birth played an important role in neonatal on-farm mortality rates. When we analysed mortality in veal calf herds we did not observe an effect of extreme outside temperatures on calf mortality, probably due to the mechanic ventilation systems and better isolation of the barn in veal herds (results not shown). Such results indicate that mortality related to extreme temperature could be avoided by tailored management.

In this study, we presented calf mortality figures in Dutch dairy herds according to the definitions agreed upon in the Netherlands. Comparison between the Dutch mortality risk and rates and those found in other countries is complicated because of differences in definitions, different age categories and differences in calculation methods (Compton et al., 2017). Ideally, the definitions and calculation methods would be similar between countries as recommended by Fetrow et al. (2006). However, definitions and age categories that are distinguished when calculating calf mortality are often related to data availability and the aim for which the figures are calculated. Therefore, one has to be very careful when comparing mortality figures between countries.

This study showed that increased awareness of calf health and calf mortality provided by data-driven tools and all subsequent actions of Dutch dairy farmers, resulted in a nation-wide decrease in calf mortality rates. The use of data can have an impact on cattle health, by providing more insight into the key performance indicators and the influence of several factors.

ACKNOWLEDGEMENTS

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INVESTIGATING THE ADVISOR AND FARMER RELATIONSHIPS THAT
INFLUENCE DAIRY COW HEALTH AND WELFARE IN THE TRANSITION PERIOD
BEFORE AND AFTER CALVING

E.A. REDFERN*, L.A. SINCLAIR AND P.A. ROBINSON

SUMMARY

An understanding of advisor and farmer opinions and practices, and the challenges they face in managing transition dairy cows in the weeks before and after calving, may lead to more effective and practical disease prevention and control. The study reported here, based on qualitative interview research, investigated the opinions of dairy farmers and their veterinary and non-veterinary advisors on transition cow management and nutrition, the individual complexities and barriers to managing cows during this crucial time, and working with their respective advisors/clients to help improve transition cow success. The findings demonstrate the varied influences of 'people factors' such as the nature of the advisor-farmer relationship, advisor-farmer communication and herd-level collaboration between advisors for transition cow health and management.

INTRODUCTION

During the transition from the dry period to lactation the dairy cow undergoes a period of physiological, metabolic and immunological change, and is at greater risk of developing disease, to the detriment of health, welfare and production (Drackley, 1999). These associated metabolic diseases include hypocalcaemia, ketosis, fatty liver syndrome, metritis, mastitis and retained fetal membranes (LeBlanc, 2010). How the transition cow is managed during this time is strongly associated with the incidence of metabolic diseases, milk yield and fertility in early lactation (Roche et al., 2018). During the last two decades extensive research has been conducted which has refined nutrient requirements and strategies for managing transition cows (Horst et al., 1997; Huzzey et al., 2007; Van Saun and Sniffen, 2014). Yet according to Mulligan and Doherty (2008) and Mills et al., (2020), dairy herds experience high rates of metabolic diseases, with rates in well-managed herds remaining similar to those published decades ago indicating it is a perennial problem.

Farmers are exposed to multiple sources of veterinary and nutritional advice to manage transition cow health, to which they attribute varying levels of influence and credibility (Atkinson, 2010). Advisors influence farmers to adopt behavioural changes and implement

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practical husbandry improvements at farm level. A review paper on transition dairy cow management highlighted the need for social science approaches to better understand the attitudes and drivers affecting the management of transition cows (Mulligan and Doherty, 2008). There has however been a lack of qualitative research to date on this research theme, despite the significance of the problem in commercial dairy herds around the world. Although one interview-based study has been conducted in Canada investigating farmer and veterinarian opinions and barriers to transition cow management (Mills et al., 2020), there have been no similar qualitative studies involving non-veterinarian advisors on this topic. The aim of this study was to investigate the opinions and behaviours of dairy farmers and their advisors concerning transition cow management in England using an interview methodology. The data presented here focuses particularly on the opinions and experiences of the nutritional and veterinary advisors who advise farmers on transition cow management.

MATERIALS AND METHODS

The study was conducted in the North West and Midlands of England, and involved in-depth semi-structured interviews carried out face-to-face and on the telephone with 22 farmers from all-year-round (AYR) calving herds, 10 farmers from block calving herds (3 spring calving herds, 2 autumn calving herds, and 5 combined spring and autumn herds), 12 veterinarians (6 dairy specific and 6 mixed veterinary practice) and 12 non-veterinary advisors (3 independent nutritionists, 7 compound feed company representatives and 2 mineral supplement representatives). Participants were recruited using non-random purposive sampling (Given, 2008), providing access to a range of farmers with various herd sizes and transition cow management systems, and a range of advisors with different roles and experiences. Ethical approval was obtained from the Research Ethics Committee of Harper Adams University.

For logistical reasons, interviews with farmers were conducted face-to-face in a geographical radius within travelling distance, and the interview was followed up by a farm audit of the cattle and their nutrition and housing. Because of Covid-19 restrictions 16/24 of the advisor interviews were conducted by telephone. The semi-structured interviews followed four separate topic guides: one each for all-year-round herds, block calving herds, veterinary advisors and non-veterinary advisors, that were designed to use open-ended questions, ensuring the interviews were free-flowing and flexible. This allowed the participants' responses to guide the direction of the conversation, whilst remaining relevant to the topic of transition cow management. All of the interviews were conducted by the first author. Interviews were audio-recorded with consent, manually transcribed in full and analysed thematically using *NVivo 12* software (QSR International Ltd, Australia).When the interviewer determined that data saturation had occurred and no new themes were being identified, data collection ceased (Guest et al., 2006). Transcripts were coded in *NVivo 12* into common themes which accurately reflected the attitudes and beliefs of the participants (Guest et al., 2011).

RESULTS

All the farmers in this study employed the services of different ruminant nutritionists and used different primary veterinarians across 10 different veterinary practices in the two regions. The average interview length was 83 min, which included introductory questions about their farm or respective role (range 22 – 140 min). All-year-round farmer participants milked an average of 376 cows (range 80-1500) and had an average age of 41 years. Key themes emerging from the qualitative data represent some of the complexities of managing the

transition period from the perspectives of farmers and their advisors. This paper focusses specifically on the perceived lack of focussed transition management advice provided by veterinary and non-veterinary advisors. The following subsections present extracts of the transcripts of the interviews.

Time pressures

Transition cow management was perceived to be a confusing area of dairy cow management by farmers and advisors, which, according to nutritionists required more time spent on farm and more training. Many nutritionists spoke of being under time pressure, particularly feed-sales representatives who were under pressure to meet sales targets and visit as many farms as possible:

'When you're selling feed, you've got to get around as many farms as possible. And meet targets. And sometimes, if you don't meet those targets, you don't keep your job. So, you can't blame reps for not wanting to stand around chatting about transition when its complicated, and the commission is small' (A4 - feed sales representative).

'I've operated independently for 22 years… I'm not answerable to anyone else other than the client, I don't have a sales manager over me asking me why my sales are down this month. [Feed sales representatives] are not there to stand around talking about transition cows, they're expected to go on farms selling them a tonne of milk powder or get the dairy cake [concentrate] order' (A17 - independent nutritionist).

Financial disincentives

The financial rewards for feed sales representatives to gain commission on dry cow feed is minimal in comparison to that gained when feeding the main milking herd. For this reason, advisors speculated that feed sales representatives were less eager to advise farmers on transition cow management and gain financial gain from the sale of a product:

'From a commercial point of view…it is minimal tonnage really isn't it? Most people will focus on the milking herd to get the tonnage… I think the consultants and the other people that aren't paid per tonne probably look at dry cow management more, but I know full well that the guys I work with will go after the dairy cake (concentrate) long before going near the dry cows.' (A20 - mineral supplement representative)

'I think a lot of advisors on farm are nervous about tampering with the dry cow system and I think commercially as well, when we look at commercial businesses, they do tend to focus on the lactating dairy business because of the volume of food to consume, and miss out a lot on the dry cow element. So, I think there is a commercial element to it which I don't like to say but we've got to be realistic and honest about it and I think that is the case. I'm not saying it's right, but I think that it does occur.' (A16 - feed sales representative)

'Transition is a really complicated topic, it's a very short time where an awful lot happens, and so much has the potential to go wrong. And I can understand from a sales perspective it's not really where the tonnes are is it? To be really frank about it.' (A16 - mineral supplement representative)

Some nutritionists and feed sales representatives were reluctant to ask questions relating to current performance of transition cows and to get involved in advising that area of dairy cow health for fear that it would highlight the need to make changes or recommendations, which may not result in a positive outcome for their business:

'Why would you risk your commercial involvement of the farm for trying to take on one last bit of area of management that isn't going to work? You know he's going to have cows that calve within a week of being dried off, you know he's going to have cows that never calve, so he could see what you have done as perhaps...failing. That's why you would keep away from being proactive, and just getting involved where there is a problem, but otherwise just keeping away.' (A1 - feed sales representative)

'If you're supplying product in there, you're not inclined to go looking for trouble. If the farmer hasn't mentioned it, they're not going to go and open a can of worms by saying actually something could be better, when they are feeding your fancy dry cow roll (concentrate feed)… with a customer I think human nature means that nutritionists don't want to ask certain things' (A17 - independent nutritionist).

Responsibility

Providing transition cow management advice on farm was perceived to be high-risk, with a fear of receiving blame if the advice provided did not result in a positive outcome. This was perceived to be more of a problem for nutritionists than veterinarians, as highlighted in the following quotes:

'I thought I was under pressure as a vet but in a way, it is much more pressure as a nutritionist I think' (A5 - feed sales representative who previously practiced as a veterinarian)

'The minute you take on some responsibility for dry cow feeding your head is on the block isn't it? Sometimes it's safer to just not enter that arena. The more prescriptive you get, the more responsibility you carry. And the more the problem lands with you if it doesn't work' (A1- feed sales representative).

'When things are going well, nutritionists get no credit, then if things go badly everyone is pointing fingers at you, so I can see it's a terribly difficult job and... you don't want to bring up a problem do you? If you're in that situation... Whereas with vets, it's a bit easier because it doesn't necessarily reflect directly on us' (A14 - dairy specialist veterinarian).

'This is where the commercial side comes in, if the nutritionist is on a farm and he thinks the farmer is not listening, he should really tell the farmer and go. Because if they aren't listening and they get inspected by a vet, who's going to get the finger pointed at them? So, it is a difficult one. There are commercial pressures on a lot of people.' (A3 – independent nutritionist).

This nervousness about getting more deeply involved in transition cow nutrition was due to the perceived high risk of farmers suffering losses to metabolic diseases:

'I think quite a few people know how to ration a dairy cow, but dry cows can really freak people out. I think if you get dry cows wrong you can have quite big impact on subsequent

lactations, fertility and all sorts of things. I think people shy away from it.' (A20 - mineral supplement representative).

In the current study, farmers held both their vets and nutritionists accountable for the success of their transition cows. This was highlighted in the following quote from a farmer speaking of both his vet and nutritionist:

'I feel like they are bullying me a bit [be]cause they'll say 'You need to do this, you need to do that', so then I think to myself, 'I'm going to show you now, and I'll do what you say and then if that fails it's all back on them then', You know what I mean? So, I sort of take the approach with [my nutritionist] that there's no point in me paying him if I don't listen to him. Because once you listen to him and implement it, it's on his head then, isn't it?' (F2 - AYR, herd size: 340).

Lack of confidence

The majority of nutritionists in this study spoke of having a lack of confidence when advising dairy farmers on transition management, particularly due to conflicting management strategies, and the issue that one nutritional strategy could work on one farm but not on another:

'I do think there is a lot of conflicting advice from a nutrition point of view definitely. There're no hard and fast rules at the moment, because there's not a huge amount of evidence… so many people have different systems and different things suit different systems. Farmer A could do something that if Farmer B tried could be a complete disaster, even though Farmer A had no problems' (A17 - mineral supplement representative).

 [Speaking of working previously at a firm specialising in transition management] 'I didn't feel like I had enough training in the [transition] feeds they were selling, I didn't feel confident advising farmers which dry cow feed to go on that much because I didn't know them well enough.' (A15 - feed sales representative).

'It takes a lot of trust, its nerve wracking. I still get that funny feeling when you make a big ration change, and they say they will try it and you think oh [*swears*]! And you know in your own head on paper it will work, but this is the problem with everything on farm, there are so many components, with management and health.' (A19, mineral supplement representative).

This lack of confidence was exacerbated by the high number of environmental and management factors influencing the effectiveness of transition management strategies, which are beyond the advisors' control:

'There's a confidence issue too because it is a very complicated matter, it is complex. A simple dairy cow ration is straightforward, but with transition, every dairy farmer has different limitations, environment, stocking density, climate, cow history - they've all got huge parts to play. You can have a transition programme on one farm that works like a dream, you could replicate it on another but it won't work because there are other variables in the background that are just screwing it up. So, I do think it's a confidence issue' (A18, feed sales representative).

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'The metabolic issues are coming from not managing them properly, whether its heat and space, or overstocking and high cell counts. They build these massive new sheds for their milkers and increase their milking cow numbers and not realise that in 9 months' time there will be an extra 20 cows calving down into a shed that is the same size!' (A15, feed sales representative).

Despite advisors understanding the external factors that influence transition success, both veterinarians and nutritionists reported feeling blamed for when it went wrong, despite their best efforts, and even when the fault was due to the farmer not implementing the management strategy properly, or making changes to the ration without notifying the nutritionist:

'A farmer rang me up, absolutely raging that none of these cows were cleansing. He was shouting 'We do everything you say, we do everything the nutritionist says, NOTHING works'. Absolutely raging, saying he was moving vets, moving nutritionists… it turned out that he'd ran out of the bespoke dry cow mineral and had been buying something off the shelf that had completely altered, so we corrected that and suddenly everything went better. But his immediate response was to blame everybody apart from him. But it took loads of drilling down, because we asked him had everything stayed the same and he said yes, it was all the same, we asked about the mineral he said it had all stayed the same! And fortunately, me and the nutritionist work well together so there was never any shift in blame between us two, it just took the communication between us to tie it down. I think that's how the relationship should work' (A2 - dairy specialist veterinarian).

Advisors not feeling valued

Both veterinarians and feed-sales representatives were discouraged from giving proactive transition advice to farmers when they did not feel valued or listened to by their farming clients. The competitive nature of the feed industry and the vulnerability of the nutritionist's position is high, and farmers are not always loyal to their feed advisors. In particular, feed advisor representatives felt disinclined to provide advice to farmers when they thought there was a possibility of losing that customer to another firm, based on price per tonne of feed, because they perceived that the farmer did not value the feed representative who provides advice alongside selling a product. Therefore, the farmer was considered undeserving of that advice. This was often backed up by an anecdote with a negative experience of losing a farm client, despite the nutritional input and advice from the sales representative:

'You can lose a customer to £3 per tonne, so you think why should I break my neck investing a lot of time and giving them a lot of free transition advice when they go and leave you? If you don't feel valued, you don't want to stick your neck out for them all the time.' (A4 - feed sales representative).

'I think farmers give people a try and when they feel like they've learnt something from them, they shift. They're not very loyal sometimes' (A13, mixed practice veterinarian).

'So, I gave the farmer a free bit of advice based on sensible observation of his cubicles, that worked. That will have earned him lots of money for the rest of his farming career. And a delivery goes wrong [snaps fingers], and like that I am sacked. And that's another reason why sometimes you can understand the cynical salesperson who just sells' (A1 - feed sales representative).

Veterinarians however, felt discouraged to give proactive transition advice for different reasons. Rather than commercial competition affecting this, veterinarians felt frustrated when they couldn't make a positive difference on their clients' farms because some farmers did not adopt the advice provided:

'I can think of a farmer I went to on Tuesday, 1000 cows and I've worked with him for 12 years, and I can't think of ANYTHING I have managed to change there, from a transition cow perspective, that's stuck. We have done specific transition visits, loads of reports, data analysis. So that's quite depressing. You think, why do you bother?' (A2 - dairy specialist veterinarian).

'When I was younger I would be going in investigating all these problems and saying let's do a transition review, and lets sort all this and lets measure your water trough and all the rest of it, and now with those farms I am just aware that it is literally like banging your head against a wall! Because you put all that effort in, they don't do anything that you've suggested and three months later they say to you, 'Do you know why we might be getting a few milk fevers?' And you literally stare at them with your jaw on the floor and that's so frustrating because you just feel like everyone's laughing at you, because you go above and beyond putting a lot of effort in and it's just thrown back in your face. And you've not charged for it appropriately either.' (A11- dairy specialist veterinarian).

However, farmers with independent nutritionists appeared to trust their nutritionists more, spoke positively of them and were more likely to adopt the advice provided, and spoke negatively of feed sales representatives. This was reported to be due to the sales motive of the feed representatives, and perceived lack of genuine interest in their herd:

'The old corn rep job is finished, isn't it? That job is dead and buried. They would finish it, they would. I think they are absolute rogues…I just don't trust feed reps at all' (F2 - AYR, herd size: 340).

'Half the time someone's trying to sell you something so they're not really... I wouldn't say they're not bothered if it works or not, but they're more interested in selling it than if it works or not' (F1 - AYR, herd size: 200).

'My nutritionist is independent; I trust what he tells me. I buy what he tells me to buy. And also, if I have a rep[resentative] and he's pushing something I say, "Yeah that's fine, leave me your details and literature, and I'll pass it on to my nutritionist." And he says, "No no no - you don't want to buy that!"' (F9 – AYR, herd size: 560).

DISCUSSION

It is important to understand the context within which farmers and their advisors operate, as demonstrated by Palczynski et al. (2020) and Robinson (2020). The themes explored in this study, many of which are interconnected, demonstrate a diverse group of farmers and advisors whose individual experiences, perspectives, and contexts impact their management of transition cows, their farmer-advisor relationships, and the advisor's willingness to provide focussed transition-advice.

A lack of time during farm visits was a factor that impacted the amount of focussed transition management advice provided. This is likely to be due to commercial pressures to

visit as many farms as possible, in order to increase their chances of selling more products and feed and meeting sales targets. As transition cow management was perceived to be a complicated area with multiple farm-specific limitations, it was also considered to be an area of farm management that required considerable time and discussion. Time pressure has similarly been shown to influence veterinary behaviour in companion animal care, with veterinary surgeons feeling that they had to rush and keep discussions minimal to ensure that consultations were within their allocated time (Belshaw et al., 2018), and in farm animal practice where veterinarians felt that time constraints impacted their ability to collect and analyse herd-fertility data (Mee 2007).

This study outlined that most nutritionists and feed sales representatives are paid a commission when they sell a compound feed or product. For this reason, nutritionists and feed representatives who were paid per tonne prioritised the feed sold to the main milking herd. The commission gained from advising farmers and selling a dry cow product was perceived to be less of a financial incentive. To the authors' knowledge, no research has been conducted to specifically investigate the commercial incentives influencing veterinary and non-veterinary farm advisor behaviour. However, Mee (2007) suggested that veterinarians may not be 'hungry enough' to provide fertility management services when there is less of a financial incentive, and because the opportunity cost of their time is high, they see less of a competitive return on investment when upskilling and providing additional services. Likewise, Charlton and Robinson (2019) suggested that veterinarians were disincentivised to provide anthelmintic advice for cattle to their clients when they were unlikely to sell significant volumes of the product.

When the dialogue moved specifically to transition cow management, nutritionists were more likely to 'shy away' from providing focussed transition advice due to a lack of confidence in this area. This lack of confidence was due to the perceived high risk of the outcome being unfavourable - a risk partly attributed to farmers not properly implementing the management strategy, or external factors beyond the control of the advisor. Both veterinarians and nutritionists reported feeling blamed for a lack of transition success, even when the fault was due to the farmer, which could further increase the 'high-risk' perception of advising in this area of dairy cow health. Mills et al. (2020) investigated farmer perception and barriers to transition cow management and presented similar findings, where farmers felt that nutritionists were largely responsible for the success or failure of their transition cows, and that they would be "held accountable" for the outcome. The lack of confidence by nutritionists and feed representatives was partially attributed to the complexities of managing transition cows with farm-specific limitations, and the conflicting strategies that are published in scientific literature. Mills et al. (2020) also illustrated disagreement in the application of scientific research, with some farm advisors finding scientific research motivating and others finding it difficult to apply at farm level, due to conflicting results from different studies. Nutritionists and feed representatives stated that they cannot afford to risk untested practices in such a competitive industry. The risk of losing credibility with farmers governs the actions of some advisors, as shown by Ingram (2008) when investigating knowledge exchange between farmers and their agronomists.

As there is generally less focus on transition cow management due to commercial factors and financial incentives, advisors may not prioritise learning new knowledge relating to this area of dairy cow management, and this may contribute to lower confidence levels. Roberts and Murray (2013) investigated perceptions of equine veterinarians and established that they were less confident advising on areas that they had received less professional training in, and

were less confident when their role covered multiple species rather than being equine-specific. Repeated and frequent use of transition cow knowledge may increase advisor confidence, as found by Heath (2004), where mixed practice veterinarians who had limited equine clientele were concerned about staying current on equine-related information. Lower advisor confidence levels in the current study may be due to relatively little education and training on transition management during professional training, or perhaps these participants had encountered fewer transition related cases in their practices. Similarly, advisors covering other species in general practice (mixed practice veterinarians, or nutritionists that cover beef and sheep nutrition) may be less confident and knowledgeable providing transition information compared to dairy-specific advisors, as demonstrated by Roberts and Murray (2013) for equine nutrition advice. Additionally, because nutritionists are not regulated, some nutritionists and feed representatives may have had no formal training. Veterinarians have shown to be more confident in topics that are perhaps discussed more by their clients, as seen in equine practice (Parker et al. 2018). In the current study, farmers were reluctant to discuss transition cow management with their advisors because they found it complicated and confusing, and often prioritised discussing issues pertaining to the main milking herd first.

Interestingly, while the farming participants would hold their nutritionists accountable for their transition cow success or failure, several farmers and veterinarians spoke of proactive veterinary advice not being valued highly. Veterinarians in this study were keen to engage and educate their farming clients on managing their transition cows effectively, a finding similar to Robinson (2020) when investigating farmer and veterinarian opinions of managing Johne's disease in dairy cattle. However, in the current study veterinarians reported challenges in evoking change and persuading farmers to take on new or improved practices that would improve the health and welfare of transition cows, despite having a long-term trusting relationship with the farming client. Farmers the current study had strong relationships with their nutritionists, and in particular thought highly of their independent nutritional advisors. This is in contrast with Mills et al. (2020) who reported that Canadian nutritionists had "limited" relationships with dairy farmers. Farmer adoption of advice was examined in detail by Ritter et al. (2019) who determined that farmer preparedness to adopt veterinary advice was negatively associated with the dominance of the veterinarian during the farm visit, and positively associated with farmer satisfaction.

The agricultural feed industry is competitive, and in cases where nutritionists and feed sales representatives were paid commission on the tonnes of concentrate they had sold, some did not feel their advice was valued, and farmers were considered undeserving of focussed transition advice based on the lack of farmer loyalty to their nutritionists and feed suppliers. This is in contrast to the farmer-veterinarian relationship, where Ruston et al. (2015) explained that although trust must be earned, farmers are very loyal to their veterinarians. Farmer loyalty appears to be influenced by their trust in the advisor, and farmers in the current study were reluctant to trust feed sales representatives, due to their sales motive. Farmer loyalty and respect has been shown to influence the farmer-advisor relationship in the field of agronomy, with farmers shifting their loyalty to "more switched on" agronomists if they lost confidence in their advice (Ingram 2008). Veterinarians in the current study were frustrated when they were unable to instigate a behaviour change, or when farmers would not adopt advice, particularly when they had a long-standing relationship with their client.

The current study found that while transition cow management and the control of metabolic diseases was strongly influenced by farmers' advisors, there are specific barriers to providing advice to farmers, such as commercial competition and the complexity of the subject or lack

of confidence discussing transition management. This left farmers feeling frustrated and confused about which approach is most appropriate for their herd, to the detriment of cow welfare and farm profitability. It is evident that commercial and financial factors influence all advisors and their willingness to provide advice on this complex area of the dairy cow health and nutrition. The current study therefore found that transition cow health and management is influenced by more than the individual farm characteristics and farmer attitudes, rather it is heavily affected also by the advisors who influence farmer behaviour, and their individual barriers to communicating effectively to provide focussed transition advice. The nature of the farmer-advisor relationship and the advisor's own financial gain all interconnect to create complex barriers to providing farmers with advice and motivating them to make changes or improvements relating to their transition cows. The opinions of non-veterinary advisors such as feed sales representatives and nutritionists rarely feature in dairy cow health and welfare literature. Including nutritionists in this study has provided a wealth of perspectives ascertaining to the confusion and frustration that many farmers feel towards transition cow health, management and the (lack of) advice provided.

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OPTIMIZING REPRODUCTION MANAGEMENT STRATEGY IN DAIRY PRODUCTION: INSIGHTS FROM BIOECONOMIC MODELLING

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SUMMARY

Reproduction management decisions are complex and central in milk production systems. Assessing the optimal decisions is difficult due to multiple criteria to be accounted for since it is linked to diseases prevalence, milk production and cows' longevity. This call for trade-off between objectives and multicriteria consideration. To determine the marginal costs and benefits of reproduction management strategies and optimal strategy maximizing farmer's revenue under labour constraint, an innovative bioeconomic optimisation model (DairyHeatlhSim™) is used. When the breeder is gradually constrained on his reproduction related workload, optimal solutions suggest a change in strategy for the detection rate (DR). When higher constrained on longevity, optimum results suggest a change in the voluntary waiting period (VMP) strategy for high level of constraints on labour. Farmer's medium annual gross margin is highly sensitive to DR. The average marginal gain per additional hour devoted to oestrus detection range from €166 to €450.

INTRODUCTION

Dairy farming aims at producing milk as a primary output and meat as a secondary output. Diseases such as mastitis, lameness and reproductive disorders, or poor nutrition and health management decrease the quantity and the quality of outputs (Gröhn et al., 2004; Barbano and Lynch, 2006; Bar et al., 2007, 2008; Hertl et al., 2010, 2011; Schukken et al., 2009; Cha et al., 2013).

Reproduction management decisions (RMD) are complex and central in dairy production. Reproduction management is recognised as a key parameter for dairy herd economic equilibrium, since it is directly linked to milk production. After calving, a delay in conception leads to reproduction inefficiency and indirectly milk production decrease. Deteriorated reproductive performances is associated with important economic losses and risk in involuntary culling (Bourdeau et al., 1995). Calving interval (CI) is a raw indicator commonly used to evaluate reproductive performance in dairy herds. Decreasing CI improves milk production and rate of genetic progress, but improve the frequency, at the herd level, of the stressful period of *peripartum* where diseases, culling and even mortality is increased. This calls for trade-off between objectives and multicriteria consideration. The reproduction management efficiency is highly influenced by the farmer's decision-making, who makes

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decisions under structural, financial and time constraints. In addition, many actors are involved in the dairy herd management, such as inseminators, nutritionists and veterinarians (Gaines, 1989; Fetrow et al., 1990; Upham, 1991). In addition to CI, other indicators are used in the field to analyse and manage herds performances. The voluntary waiting period (VWP) -time between calving and a cow is said to be eligible for insemination- represents a key management decision made by the farmer that influences CI value. The conception rate (CR) -success rate of artificial insemination- varies according to the cow's day in milk (DIM) and cow's characteristics, are also directly influencing CI (Barr, 1975; Esslemont, 1992; Pecsok et al., 1994). Conception success is influenced by the ability of farmers to detect heats (DR), as well as other risks factors such as nutrition, housing, infectious diseases. Mastitis and lameness have also been shown to affect reproductive performance (Fourichon et al., 2000).

Combining all these factors is required to define the optimal strategy for reproduction management, i.e. the relative costs and benefits of various interventions and decisions, some of them being highly time consuming or expensive. Several researchers have investigated the economics of reproductive disorders in dairy cattle, and most of recent studies use dynamic programming and simulations models to evaluate the optimal strategy to be implemented on farms, in order to maximize farmers' revenue (or Net Present Value). Most yet fail to combine the system approach that defined dairy production and produce analysis that are too partial. With the recent progress in the information technology sector, computational barriers fall, and it has become easier to build complex models encompassing multiple parameters. Literature in the dairy sector is extensive and allow a precise calibration of complex models, with expert opinion when required to complete or adapt to local situation.

Additionally, coupling biological models and economic optimization models is not a common practice in economics research applied to animal health, in spite this enables to assess realistic scenarios, really accounting for constraints that are facing farmers in routine. This helps to better consider farmer behaviours and the complexity above mentioned. The present study aims at determine the marginal costs and benefits associated with a set of strategies of reproduction management in French dairy production, to define optimal strategy maximizing farmer's revenue under labour constraint.

MATERIALS AND METHODS

An integrated bio economic modelling approach was used; this model consists of a biological simulation model coupled to an economic optimization model. The biological model is defined on a cow-week basis and on the weekly probabilities for all cow events, including milk production, reproduction and diseases (Figure 1). It aims at a dynamic representation of a dairy herd. In brief, from birth to death, each animal was characterized weekly by his/her physiological and production status (e.g., male calf, female calf, pregnant, in-milk cow, and dry cow). This framework was applied to three main interconnected types of functions, namely, production (e.g., growth and milk production and reproduction), diseases (as damage to production) and treatment (as one type of damage control). Cow's reproduction simulation was based on a transition state machine between reproduction states. The transition from one status to another (cyclicity, heat expression, heat detection, insemination, pregnancy and calving) is conditioned by animal characteristics such as i) age and bodyweight conditions for heifer's puberty and heifer eligibility to first AI (HeifAgePub, HeifWeiPub, HeifAgeElig, HeifWeiElig, see Table 1) and ii) heat expression (P_{heat}), heat detection (P_{detect}) and successful insemination probabilities (P_{insem}) for mature cows to start a pregnancy period (Table 1).
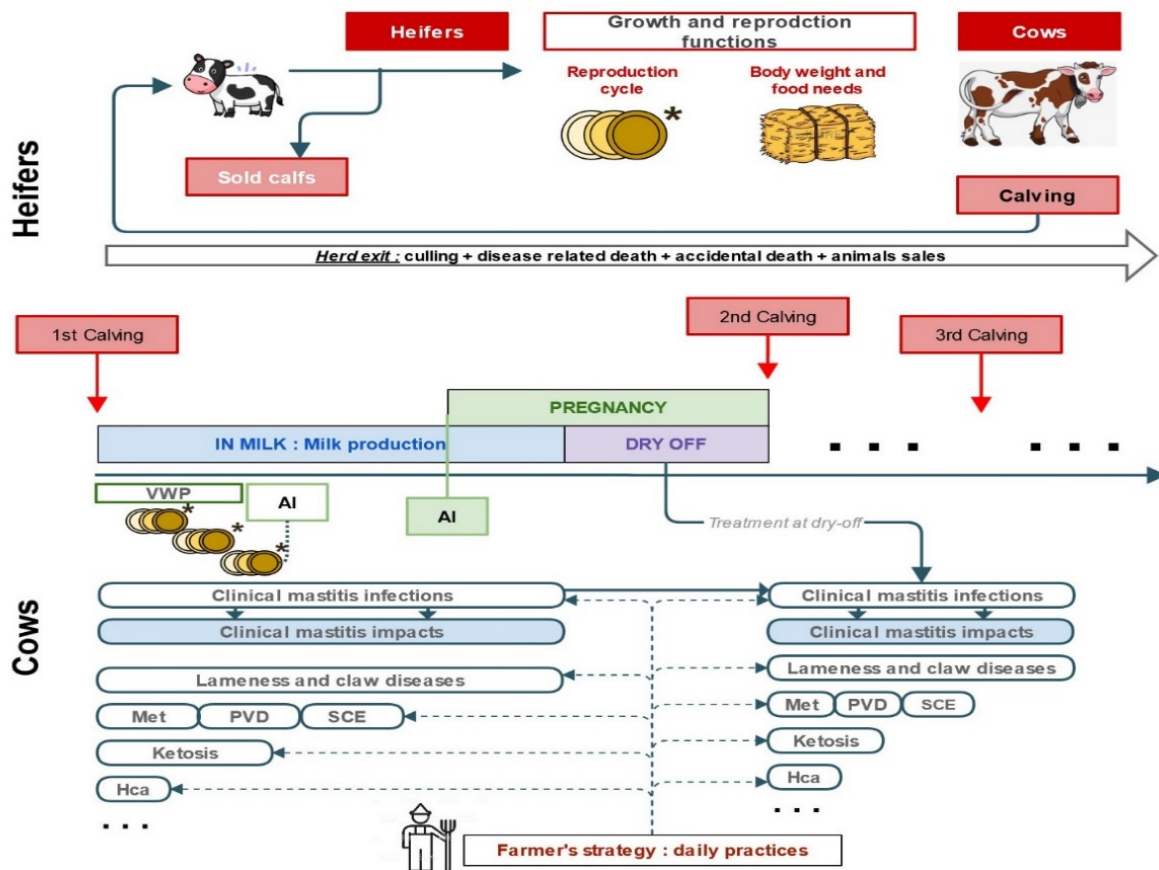
Figure 1. DHS biological model overview. VWP: Farmer's voluntary waiting period before insemination, IA: Artificial insemination, SCE: Subclinical endometritis, PVD: Purulent vaginal discharge, Met: Metritis, Ketosis: Clinical and subclinical ketosis, Hca: Hypocalcaemia and milk fever. (*) Cow reproduction simulation as a state machine with atypical cycles simulation.

During pregnancy period, abortion probabilities are applied (Pabort). New born gender is determined according to a GenderRatio. After calving, cow's ovarian cycle could start again after a 15-day anoestrus period and a 28-day voluntary waiting period (VWP), depending on cycle probability (Pcycle) and atypical cycles occurrence. Three main atypical cycles are considered: delayed cycle, prolonged luteal phase cycle and interrupted cycle (Table 2). Subclinical and clinical ketosis occurrences are risk factors for cycle delay, and endometritis systematically delays the start of a new cycle by 3 days. Metritis, endometritis, early resumption of cyclicity, and parity are considered to be risk factors for the persistence of the corpus luteum and therefore the prolongation of the luteal phase. In the model, the persistence of the corpus luteum causes lengthening of the cycle duration of: 1 week in 45% of cases, 2 weeks in 45% of cases or 3 weeks in 10% of cases. Subclinical ketosis is the risk factor for interrupted cycles. In the model, multiple atypical cycles occurrence is possible for one open animal and each of these atypical cycles can revert to a normal cycle.

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Table 1. Reproduction simulation parameters calibration.

Label	Definition	Unit	Value	Reference
$PREG_{Length}$	Pregnancy length	weeks	40 ± 0.86	(De Vries, 2006)
P_{heat}	Probability of heat	%	1st ovulation: 10 2sd ovulation: 50 3rd ovulation : 70	(Inchaisri et al., 2011b)
P_{detect}	Heat detection rate for heifers, prob for cows is scenarized (DR)	%	63	(Wathes et al., 2014)
P_{insem}		%	65	(Wathes et al., 2014)
P_{abort} [**]	Probability of abortion, per month, for months 2 to 8	%	3.5; 2.5; 1.5; 0.5; 0.25; 0.1; 0.1	(Santos et al., 2004)
P_{cycle} [***]	Probability of cycling for WIM 1 to 8	%	PP[a]: 0; 10; 10; 10; 50; 50; 50; 100 MP[a]: 0; 10; 50; 50; 50; 100; 100; 100	Authors
Stillbirth	Stillbirth risk	%	5	Authors
$HeifAgePub$	Heifers age condition to puberty	weeks	U(32, 58)	(Wathes et al., 2014)
$HeifWeiPub$	Heifers weight condition to puberty	kg	274	(Wathes et al., 2014)
$HeifAgeElig$	Heifers age condition to AI eligibility	weeks	59	(Wathes et al., 2014)
$HeifWeiElig$	Heifers weight condition to AI eligibility	$\% BW_{MAT}$	U(55, 65)	(Margerison and Downey, 2005)
$GenderRatio$	AI gender ratio (non sexed)	%	50%	Authors

[a] : PM : primiparous ; MP : multiparous

Table 2. Atypical cycles simulation parameters calibration

Atypical cycle	Parameter	Definition	Value
Delayed Cycle	OR_Ck and OR_Sck	OR on cycle start probability	1,5
ProlongedLuthPhase_Cycle	R_Base	Base risk of occurrence	0.117
	RR_Pvd	OR purulent vaginal discharge on	4,6
	RR_Met		3.03
	RR_EarlyCycle		2.8
	RR_Parity		3.06
Interrupted_Cycle	R_Base	Base risk of occurrence	0.119

Economic optimization modelling considering technical constraints and farmer behaviour

The economic model developed is a recursive mean-variance optimization framework. It dynamically represents the farmer's input allocation decisions while maximizing his/her utility under constraints.

Dairy farmers' decision-making processes under business uncertainty were simulated using an expected utility framework (Von Neumann and Morgenstern, 1947). It implies that rational decision-makers maximize their expected utility with respect to a set of constraints. They choose between risky alternatives by comparing their expected utility values. Here, farmers are assumed to be risk minimizers. They are willing to sacrifice a portion of their income to avoid facing business risk. In a typical French dairy farm, milk sales represent more than 80% of income, and feeding costs represent 40% to 60% of a farm's variable costs. The uncertainty of milk and feed prices is the major source of dairy farm business risk. The risk considered in the model is a market risk, related to the volatility in milk and feed prices (based on prices over the last 10 years). A Markowitz-Freund mean-variance objective function was used to incorporate risk-averse behaviour in farmer decision-making (Freund, 1956; Hardaker et al., 2004; Markowitz, 1959). The decision-maker's expected utility (F) can be represented as defined in Eq. (1):

$$\max \mathbf{F} = E[Z_{k,t}] - \phi \sigma(Z_{k,t}) \tag{1}$$

where F is the objective function of farmers, $\mathbf{E}$ denotes the expected values, $\mathbf{k}$ represents the state of nature (defined here as the possible price level), $\boldsymbol{Z_{k,t}}$ is the equivalent-gross margin generated per state of nature $\mathbf{k}$ in year $\mathbf{t}$, $\boldsymbol{\phi}$ is the risk aversion coefficient, and $\boldsymbol{\sigma(Z_{k,t})}$ is the standard deviation of income. According to Anderson and Dillon (1992), the risk aversion level of individuals may be represented by a relative risk aversion coefficient as follows: this coefficient is less than or equal to 0.5 for hardly risk-averse to risk-neutral individuals and greater than or equal to 4 for extremely risk-averse individuals. However, most authors consider values above 5 to be very unlikely (Kocherlakota, 1996). The risk aversion coefficient was set to 1, and a sensitivity analysis was conducted for values from 0 to 5, as these values represent different farmers' attitudes towards risk.

The equivalent-gross margin $\boldsymbol{Z_{k,t}}$ generated per state of nature $\mathbf{k}$ in year $\mathbf{t}$ is equal to the difference between revenue $\boldsymbol{R_{k,t}}$ and expenditures $\boldsymbol{Ex_{k,t}}$ per state of nature $\mathbf{k}$ in year $\mathbf{t}$ (Eq. (2)):

$$Z_{K,T} = R_{K,T} - EX_{K,T} \qquad (2)$$

Expenditures are the sum of health and veterinary expenses (e.g., purchased medicines including antibiotics, veterinary consultations/interventions and surgery) ($Ex\_Vet_{k,t}$), food expenses (e.g., purchases of concentrate), ($Ex\_Feed_{k,t}$) and other expenses ($Ex\_Oth_{k,t}$), including related expense surcharges for housing and milking hygiene, insemination and other practices (Eq. (3)). Dairy revenues (Eq. (4)) are the sum of the revenues from each product sold, namely, milk ($R\_Milk_{k,t}$), one-month-old calves, heifers ready for calving R_Ani_t and cull meat (R_Cull_t):

$$EX_{K,T} = EX\_VET_{K,T} + EX\_FEED_{K,T} + EX\_OTH_{K,T} \qquad (3)$$

$$R_{K,T} = \sum_L R\_MILK_{K,T} + \sum_A R\_ANI_T + R\_CULL_T \qquad (4)$$

Where L denotes the cytological qualities of milk and A denotes the types of animals sold (e.g., heifers or male calves).

The weekly milk quantities produced and sold by the farm are recorded, and the mean weekly milk cytological and biochemical (fat and protein) qualities are considered to determine the monthly milk price paid to the farmer according to the usual payment criteria. Cytological quality refers to the milk somatic cell count (SCC), which is a proxy for udder health and mastitis occurrence.

Four main categories of constraints are used during the optimization. First, the structural constraint of the barn is accounted for through a defined barn capacity $Capacity_t$ and a simulated number of occupied places $X_{t,s}$ for year **t** and management strategy **s**. This constraint is independent of herd size and can vary somewhat around the barn capacity (Eq. (5)):

$$\sum_{T,S} X_{T,S} \leq CAPACITY_T \qquad (5)$$

Second, the workload is considered a management constraint on dairy cattle farms. Because the daily labour flow is difficult to capture and describe, changes in labour if there are changes in practices or new treatments for a given strategy are considered here. The additional labour time $W_{t,s}$ the farmer has to bear in year **t** for management strategy **s** is limited to a threshold $W_{Threshold}$ that corresponds to the additional workload that farmer **f** is willing to bear, as indicated in Eq. (6):

$$\sum_{T,S} W_{T,S} * X_{T,S} \leq W\_THRESHOLD_F \qquad (6)$$

Nine levels of farmer's workload constraints were considered, ranging from a free-of-constraint situation "GTIME_Free" to a most restrictive situation where no more than 5 hours a month additional workload are accepted "GTIME_5".

Third, the model is assumed to feed dairy cows with corn silage produced at the farm level and with a market supply of concentrated feed (e.g., wheat and soybean meal). The dietary composition is based on corn silage at 61% ± 10% of the dry matter requirement, on hay for 10% of dry matter, and on wheat concentrate and soybean meal at 29% ± 10%. The dietary composition must also meet the needs of cows for energy and crude protein. Risk applied to corn silage quantity and quality leads to changes in concentrate quantities (for compensation),

which are considered to be purchased. Based on how food is included in the model, $Z_{k,t}$ must be called the equivalent-gross margin instead of the gross margin.

Fourth, sustainable dairy production is accounted for by a dairy cow longevity criterion (cow's adult medium age). Guaranteeing that the optimum condition is not obtained through deteriorated animals welfare is a key point.

The cow's AMA $AMA_{t,s}$ in year **t** for management strategy **s** is limited to a threshold $AMA_{Threshold}$ that corresponds to the less acceptable threshold by the farmer as indicated in Eq. (7):

$$\sum_{T,S} AMA_{T,S} * X_{T,S} \leq AMA_{THRESHOLD} \tag{7}$$

Five levels of accepted medium cow's longevity constraints were considered, ranging from a free-of-constraint situation "ADULTAGE_ Free" to a most restrictive situation where cows' medium adult age longer than 130 weeks is accepted.

Model resolution was carried out in two stages. First, a resolution for all the management scenarios to identify the quantity of concentrate to buy on the market in order to cover the herd nutritional needs in a context of market prices volatility. This step allowed the calculation of the breeder's gross margin for all reproduction management strategies. Then, the choice of the reproduction management scenario that maximizes the utility of the breeder under constraints of work and longevity. For each level of constraint, the model identifies the optimal scenario as well as the inherent technical and health indicators.

The detection rate represents a determining criterion for the income of the dairy farmer, this axis of reproduction management requires a significant investment in working time for the farmer for the observation of heat. From the differences in workloads between each detection scenario and the DR25 baseline (detection level with no dedicated oestrus detection time), we calculated the average marginal gain per additional hour devoted to oestrus detection.

Scenarios formulation

We first formulated reproductive management strategies as scenarios combining two decision criteria at the herd level and at the animal level, and two farmer behaviour criteria for heat detection. The combined reproduction management scenarios (540) were simulated in 3 contextual situations related to the clinical mastitis prevalence's (See Table 3). The results of the scenario simulations are the production inputs (food requirements, workload, drugs, including antimicrobials, veterinary interventions, dose of semen, etc.), production outputs (milk, meat and animals sold) and indicators health and technical performance of the herd (disease prevalence, conception rate, calving interval, average adult age of cows, average lactation duration, turnover rate, culling rate and percentage of barn occupancy). For each simulated scenario, we defined an engineer production function that represent the relationship of inputs to outputs (Koopmans, 1951). This concept implies that each production process will be defined as an activity by technical coefficients that represent the use of the inputs needed to produce different outputs, including a joint production. This representation allows a representation of all outputs produced by any dairy production activity as well as the different ways to produce a single product through the use of an engineering production function approach, which constitutes the main link between the biological and economic parts of BEM.

Table 3. Herd management scenarios description

Lab	Scenarios description
Farmer's behaviour : detection rate (DR)	
DR25	Farmer's detection rate = 25% ; Time dedicated to heat detection = 0 mn/day
DR35	Farmer's detection rate = 35% ; Time dedicated to heat detection = 15 mn/day
DR45	Farmer's detection rate = 45% ; Time dedicated to heat detection = 30 mn/day
DR55	Farmer's detection rate = 55% ; Time dedicated to heat detection = 40 mn/day
DR75	Farmer's detection rate = 75% ; Time dedicated to heat detection = 2 x 30 mn/day
Farmer's behaviour : Fake heats detection	
FH0	0.4% risk per cow per week of insemination before heat expression
FH1	0.8% risk per cow per week of insemination before heat expression
Populational decision criteria : Voluntary waiting period	
VWP5	Voluntary waiting periode = 5 weeks
VWP6	Voluntary waiting periode = 6 weeks
VWP7	Voluntary waiting periode = 7 weeks
VWP8	Voluntary waiting periode = 8 weeks
VWP9	Voluntary waiting periode = 9 weeks
VWP10	Voluntary waiting periode = 10 weeks
VWP11	Voluntary waiting periode = 11 weeks
VWP12	Voluntary waiting periode = 12 weeks
VWP13	Voluntary waiting periode = 13 weeks
Individual decision criteria : Insemination	
IA0	Farmer always try to inseminate after a heat detection
IA1	Farmer does not inseminate a cow ; before the 13th week in milk in case of subclinical ketosis (SCK) occurrence and/or 3 weeks after purulent vaginal discharge (PVD) occurrence.
Contextual scenario : clinical mastitis incidence	
H0	1 clinical mastitis infection per cow per year
H2	2.5 clinical mastitis infection per cow per year
H4	4.5 clinical mastitis infection per cow per year

RESULTS

The 540 reproduction management scenarios were simulated from a common stabilized herd (data not shown) over a period of 728 weeks (14 years). We have assumed that the first 4 years of simulation correspond to a transitory state and only the last 520 weeks (10 years) of simulation have been considered in the results. Annual indicators were calculated downstream of the simulations to assess herd reproductive performance, milk production, the prevalence of disorders and herd dynamics.

The model has been validated for each main output parameter. An increase in oestrus detection is associated with additional AI trials, better occupation of barn because of less turnover and a better longevity (+18 weeks on cow's medium adult age for DR75 compared to DR25), better milk production and a shorter calving interval. A longer voluntary waiting period was associated with a better conception rate on cows (CRC), in accordance with calibration parameters) and a higher first service conception rate (FSCRC) and a higher calving interval. Less false oestrus detection was associated with a better CRC (+2.46 points for FH0 compared

to FH1). Selecting cows to be breeded on cow's characteristics compare to blinded insemination is associated on average on slightly longer calving interval (+1.1 days for IA1 compared to IA0) and a slightly better CRC (+0.65 points, IA1 vs IA0). Simulation results shows a non-linear trend in the change for main indicators when DR or VWP increase, with a gap for DR 25 compared to other. This gap is associated with lower occupation of the farm due to extra culling, in accordance to culling rules applied.

The optimization model was run separately on the 3 contextual scenarios related to hygiene and mastitis situation of the farm (H0, H2 and H4 - Table 3). The optimal management scenarios for each combination of constraints was defined (results not shown). It appears that when the breeder is gradually constrained on his reproduction related workload, optimal solutions suggests a change in strategy for the detection rate. When higher constrained on longevity is applied, optimum results suggest a change in the voluntary waiting period strategy only for high level of constraints on labour. For all constrained combinations, results suggest high VWP associated to a low DR. Few changes were observed for contexts H. Figure 2 illustrates these results: whatever the scenario H, the more the farmer is constrained by his working time for reproductive management, the longer is the optimum calving interval.
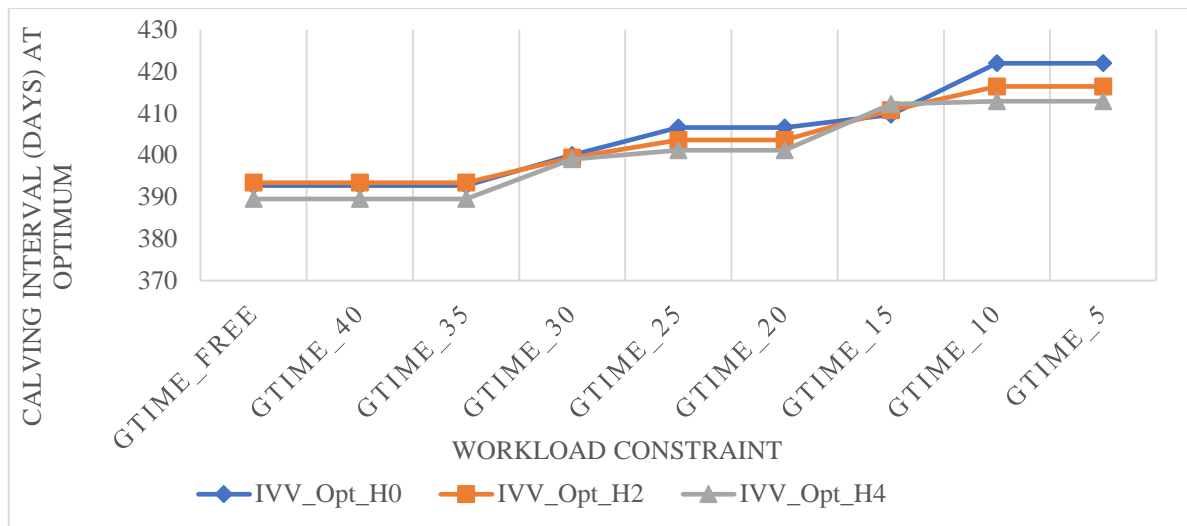
Figure 2. Optimal calving interval (days) of the optimal scenario under different farmer's workload constraint

The results also show that farmer's medium annual gross margin is highly sensitivity to the DR scenario, especially for DR25 but also for other DR (Fig. 3). A slight interaction between DR and WVP for the gross margin is observed: the VWP associated with the best gross margin for a given DR was decreasing when DR was increasing. These results are the illustration of the optimization, since DR is a key driver (but not the only one) of farmer's labor in the present simulation.

The average marginal gain per additional hour devoted to oestrus detection was calculated. From a DR25 situation, the first efforts are the most profitable with an average marginal gain of 450 €/ hour of observation. For an effort from DR25 to DR75, the hourly marginal gain is 166 €
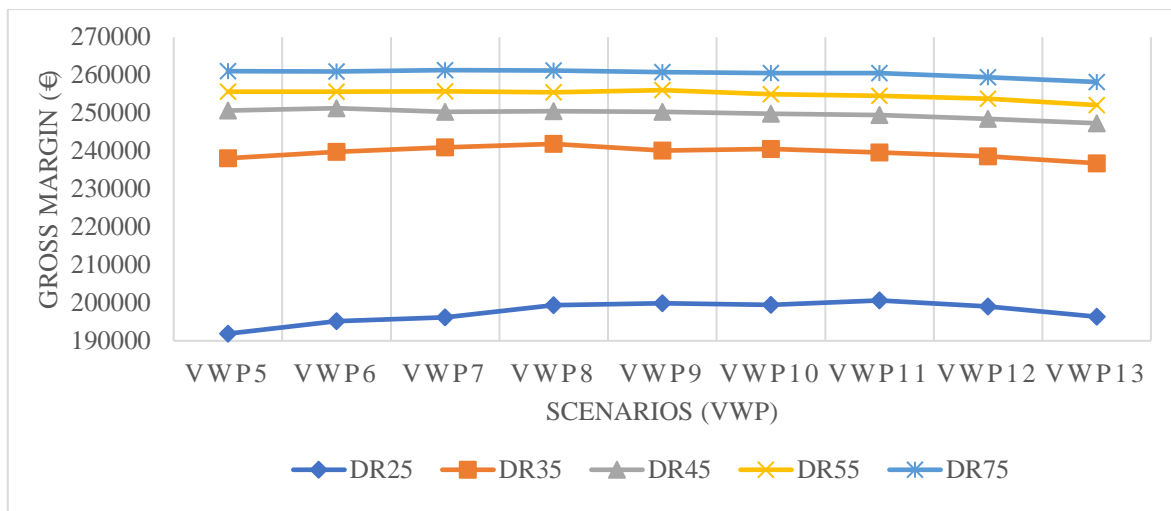
Figure 3. Medium gross margin per detection rate and voluntary waiting period scenarios (€)

DISCUSSION

The present study combines innovative biologic simulation modelling and economic multicriteria optimisation, as provided by DHS™ tool. This original combination helps to improve methodological standards in economics applied to animal health, at two level (Ferchiou et al., 2021). First, the biological part has been developed so as to limit bias due an explained part of the model (black box), as often seen in dairy herd dynamic modelling (Bruijnis et al., 2010; Gussmann et al., 2018). Here, only accidental mortality remained unexplained, all other event behind explained even if out of the scope of the analysis. For instance, herd size can change depending on culling rules that varied depending on barn occupation rate. This place the farmer's strategic farm management at the heart of the model. This systemic and holistic approach of the dairy herds permit a better consideration of disease interactions and limit a priori that may lead in biased estimation of output. The economic optimisation model coupled to the biologic part is new. Except few recent studies, economics applied to dairy health management is limited to monetary estimation of epidemiologic impact, far away from realistic farm running and farmer's behaviour. The present approach improves the realistic miming of the field, in spite improvement are still necessary. Multicriteria decision and labour consideration are the two key items improve compared to current and previous literature. Daily labour constraint of farmers cannot be limited to market price of labour in most of EU farms, due to their size and characteristics. Last but not least, the present study does not juxtapose the biologic and economic part but propose a close integration, with scenarisation involves both parts and constraints combines both technical and behavioural criteria.

The present results are in agreement with previous studies showing increasing marginal gain of extended VWP (Ma et al., 2020). Here we show that marginal cost of VWP is interacting with DR, and is not always increasing with VWP. More importantly, the present results clearly show that VWP is not a key performance indicator for dairy herd management, since the marginal cost of VWP is low, whatever the DR and all the other technical parameters associated with this strategic approach of reproduction management. On the opposition, the present results clearly show that gross margin is highly dependent on DR that should be considered as key performance indicator. Considering DR yet let to focus on labour and overall farmer time allocation, as done in the present study. The scenario considered here show that DR was associated with the most labour change observed, even in situation with other good or average

practices (H0 to H4). Multicriteria optimisation including labour is required to try to represent farmers' behaviours. First, the results show that DR could lead to very large amounts of extra time spent in the farm, based on monthly extra work. Second, including costs of hired employees has limited relevance since only one extra hour per day is required. The use of the marginal gain per hour allows to fix this issue, and to express the opportunity cost of labour that help the farmer for resources allocation.

The optimal situations demonstrated in the present study were associated with a value of CI around 400 days with a replacement rate of 35 %. This can be seen as a technical situation associated with economic performances in farms with situation closes to the present simulations.

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SMALL RUMINANTS

FINANCIAL IMPACT OF SHEEP AND GOAT POX AND ECONOMIC VIABILITY OF VACCINATION FOR SUBSISTENCE FARMERS IN SELECTED STATES OF NORTHERN NIGERIA

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SUMMARY

Production and economic stochastic models were developed to assess financial impact of sheep and goat pox (SGP) and the effect of vaccination to sedentary and transhumance farmers in Northern Nigeria. Models were parameterised using survey data from 300 herds, expert opinion and literature estimates. Annual SGP cost to farmers ranged between £5-£30 per reproductive female, depending on severity. Vaccination increased farmers' profits up to 138% in severely affected farms. Economic viability of regional vaccination programmes, with an average 3-year cumulative SGP regional incidence rate of 16% and 67.5% vaccination coverage, was found cost-effective over 3 years, with benefit-cost ratios (BCR) between 14 - 252 depending on the level of vaccine subsidies. Vaccination was also cost-effective over 3 years in high SGP-incidence (23.9%) areas and low-SGP incidence (0.7%) areas with government subsidies at 50-100% (BCR: 2-889). Results can be utilised to advocate for implementation of a vaccination programme in the region.

INTRODUCTION

Nigeria's small ruminant population is estimated at 35.4 million goats and 22.1 million sheep which typically exist in either transhumance (pastoral) or sedentary (backyard) systems (The World Bank, 2018). About 70% of the small ruminants population is found in the Northern region, with total estimates for Bauchi, Kaduna and Plateau states estimated at 8.5 million, 1.82 million, and 3 million respectively (KDSG, 2008; BSMANR, 2017) Small ruminants contribute to people's livelihoods within Nigeria in a variety of ways, including improving food access and availability, contributing to income through direct sale of animals and by-products, generation of employment opportunities through production and associated value chains, and their use as banks and insurance (Dominguez-Salas et al., 2019). However, small ruminant production within Nigeria is not fully optimised, due to constraints including poor market access, shocks such as outbreaks and natural disasters, and endemic diseases (Aphunu and Okoedo-Okojie, 2011).

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Sheep and goat pox (SGP) is a transboundary small ruminant disease caused by sheep and goat pox virus (SGPV) of the *Capripoxvirus* genus. SGPV is considered endemic in Nigeria, based on empirical evidence and observational studies, however true prevalence is currently unknown (Gelaye and Lamien, 2019). Viral transmission primarily occurs through direct contact, aerosol inhalation or contamination of skin abrasions (Bhanuprakash et al., 2006). Clinical manifestations include cutaneous lesions, abortion, weight loss and mortality (Bhanuprakash et al., 2011). Morbidity and case-fatality rate during outbreaks are on average 20% and 40% respectively (Limon et al., 2020), although vary depending on severity.

SGP causes short and long-term economic impacts. Despite acknowledgement of SGP causing substantial hardship on subsistence farmers in low and middle-income countries, few studies have quantified its economic impact. Existing studies in Northern Nigeria suggest that mortality losses and reduction in value of infected animals are the most significant contributors to economic loss due to SGP in herds (Bolajoko et al., 2019; Limon et al., 2020). Quantifying SGP economic losses provides a valuable baseline for evaluating economic viability of interventions, such as vaccination. Indeed, vaccination is an effective control measure in endemic countries, with successes observed in Morocco and Tunisia following 75-80% vaccination coverage with a live attenuated SGP vaccine (Ben Chehida et al., 2018). However, no official vaccination control programme exists in Nigeria, and, to the authors knowledge, no studies have explored the potential economic benefits of vaccination at the herd and regional level in the country.

Our objectives of this study were to (1) develop herd production models for transhumance and sedentary small ruminant production systems in selected states of Northern Nigeria, (Bauchi, Kaduna and Plateau states), (2) estimate herd-level financial impact of SGP, and (3) assess the economic viability of SGP vaccination with different levels of subsidies.

MATERIALS AND METHODS

Analysis comprised of seven stages (1) Developing herd production models without SGP (baseline) and with SGP for slightly and severely affected herds, (2) calculating herd level gross margin (GM) from both models, (3) using partial budget analysis (PBA) to estimate the net SGP cost at the herd and (4) regional level, (5) assess economic viability of vaccination at the herd level, (6) cost-benefit analysis (CBA) to assess economic viability of a 3-year vaccination program at regional level, and (7) conducting sensitivity analyses. The developed herd production and economic models are accessible upon request to the corresponding author.

Study area

Bauchi, Kaduna and Plateau States are located in Northern Nigeria. They are sub-divided into Local Government Areas (LGA's) of which there are 20, 23 and 17 in Bauchi, Kaduna and Plateau, respectively. A large proportion of their populations are subsistence farmers (KDSG, 2008). These states were selected because they have some of the largest small ruminant populations in Nigeria, SGP outbreaks have been reported in these states and previously collected empirical data (Adedeji et al., 2019; Bolajoko et al., 2019; Limon et al., 2020).

Model parametrisation

Data used for the model were based on (1) expert consultation, (2) published literature, (3) primary data from a sero-survey conducted in 300 herds within the study area, and (4) a focus group with small ruminant farmers. Experts consulted were from the National Veterinary Research Institute (NVRI), Nigeria and The Pirbright Institute and had a range of knowledge and experience within virology, vaccinology, SGP epidemiology, small ruminant husbandry methods and animal health programmes within Northern Nigeria. The survey provided data on SGP incidence, with incidence obtained from the number of households reporting SGP clinical signs. Hundred randomly selected households participated from each state (Georgina Limon, unpublished). Focus group discussion with farmers were conducted to get some economic parameters for the models. Participants in these were identified via convenience sampling.

Herd production models

Herd production models were developed for transhumance and sedentary small ruminant systems. Production models were created in Microsoft Excel, simulating herd population dynamics over an annual production cycle. Herd inputs and outputs were modelled (e.g. number of animals offtaken, or number of deaths per year), and recorded at the beginning and end of the cycle. Four sub-populations were considered within these models: male and female reproductive and non-reproductive animals. Any animal greater than 1-year old was considered to be reproductive, thus, classifying all non-reproductive animals remaining at the end of the production cycle as reproductive. The models were developed based on common herd management practices within the area.

Initially, herds were modelled without SGP to create baseline models. Further production models were created for unvaccinated and vaccinated herds with SGP, considering two theoretical scenarios: (1) An SGP severely affected herd, and (2) An SGP slightly affected herd. These two scenarios accounted for variability in morbidity and mortality. Previous studies in Northern Nigeria represent severely affected herds, given study designs focused on farmers whom had experienced outbreaks (Bolajoko et al., 2019; Limon et al., 2020). Slightly affected scenarios reflect those previously exposed herds which have developed some immunity, and hence may experience reduced signs of disease (this scenario is currently theoretical). Epidemiological and vaccination parameters were incorporated to simulate disease and vaccination impact. Key epidemiological parameters included were SGP morbidity rate, case-fatality rate and abortion rate. SGP infection was assumed to occur at the beginning of the production cycle. In total, ten herd production models were created, five representing sedentary herds and five transhumance herds: (1) model without disease, (2) slightly SGP affected herd, (3) severely SGP affected herd, (4) slightly SGP affected herd vaccinated, and (5) severely SGP affected herd vaccinated.

Herd-level economic models

Annual GM were estimated for each of the production models created. Outputs included in the GM analysis were revenue from sale of animals, revenue from sale of dead animal carcass and change in herd value. Variable costs included were replacement costs, veterinary and medicine and labour and marketing costs. SGP effects were incorporated into GM analysis, for example by increasing veterinary and medicine costs. GM was calculated as shown in Eq.(1):

$$Gross\ margin = Ouputs - Variable\ Costs \qquad (1)$$

Subsequently, the results of the GM with disease and without disease were used in a PBA to estimate herd-level SGP financial disease impact in both severely and slightly affected scenarios for a one-year cycle, as shown in Eq.(2):

(2)

$$
\begin{aligned}
SGP\ cost\ at\ herd\ level \\
&= SGP\ treatment\ costs + Costs\ of\ replacement\ ewes\ and\ does \\
&+ Reduction\ in\ herd\ value + SGP\ mortalities \\
&+ Reduced\ revenue\ from\ offtake\ of\ non\ reproductive\ animals \\
&- Additonal\ carcass\ sold - Reduced\ marketing\ costs
\end{aligned}
$$

Impact of SGP on farmers GM were calculated by comparing differences in baseline GM's to GM with SGP disease, as shown in Eq.(3):

$$Profit\ loss\ due\ to\ SGP = \frac{Net\ SGP\ cost}{GM\ without\ SGP}\ X\ 100 \qquad (3)$$

To assess the economic efficiency of vaccination at herd-level, GM of models with disease and unvaccinated and with disease and vaccinated were compared using a PBA. Vaccine efficacy of 90% was utilised, obtained from vaccination challenge studies for RM65 and Gorgan strains (EFSA, 2014). For this, it was assumed that 50% of the cost of the vaccine was paid by the farmers. Profit increase from vaccination was calculated by comparing differences in GM of vaccinated and unvaccinated affected herds, as shown in Eq.(4):

$$Profit\ increase\ with\ vaccination = \frac{Net\ benefit\ of\ SGP\ vaccination}{GM\ with\ SGP\ and\ no\ vaccination}\ X\ 100 \qquad (4)$$

Economic viability of regional SGP vaccination strategies

An expert consultation was undertaken to assess the cost of implementing a vaccination program at the regional level and the potential vaccination strategies which could be implemented within the region. For this, experts participating in the delivery of Peste de Petits Ruminants (PPR) vaccination campaign within Northern Nigeria were also involved. The vaccination strategies modelled were: (1) SGP vaccination only and (2) SGP vaccination incorporated into the annual PPR vaccination programme. In both cases encompassing all LGA's in Bauchi, Kaduna and Plateau States. In addition, the economic viability of an SGP only programme for an LGA with a high (26.7%) and low (0.7%) incidence rate were also calculated. In all strategies it was agreed with experts that an overall vaccination coverage of 67.5% could be achieved, with 75% of sedentary herds and 60% of transhumance herds vaccinated.

To assess the profitability of a regional vaccination program, a CBA was conducted. For this, the proportion of transhumance and sedentary herds within Bauchi, Plateau and Kaduna states were provided during expert consultation. State-level SGP incidences were then used to estimate the number of sedentary and transhumance herds that would experience an SGP outbreak over an annual production cycle. These were considered to be severely affected herds, since, no data exists on the likelihood of herds to experience an SGP slightly affected scenario. The estimated number of affected farms were then multiplied with net cost of SGP at herd-level to obtain an estimation of the overall regional financial disease impact.

The benefits of the intervention were estimated by calculating the total number of sedentary and transhumance herds that will be vaccinated based on the targeted vaccination coverage,

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and then the number of vaccinated herds that would have suffered an SGP outbreak. The net benefit of vaccination at herd-level obtained during the PBA was then multiplied with the numbers of vaccinated herds that would have been infected by SGP in order to obtain the total benefit of the vaccination program at regional level. Costs of vaccinating to farmers whom paid for vaccination, but would not have suffered an SGP outbreak, were subtracted from this total benefit.

The models were run for a 3 year period, and a discount rate of 4.99% was applied (Central Bank of Nigeria, 2020). Vaccination costs estimates separated into vaccination cost (subsidised), consumables, staffing and sensitisation, were obtained via collaborators based on other established vaccination programmes. For SGP with PPR, only additional consumables and staffing which would be required to administer another vaccine were calculated. Cost and benefit values from PBA models for severely affected unvaccinated herds were used to populate this regional-level PBA, as data utilised in the severely affected production models was obtained from within the region, and no data exists on the likelihood of herds to experience a slight compared to a severe outbreak.

Stochastic simulation and sensitivity analysis

@Risk software for Excel (Palisade Corporation, USA) was utilised to incorporate stochasticity to allow for variability and uncertainty in the model through Monte Carlo stimulation. Programme Evaluation and Review Technique (PERT) or Uniform distribution were fitted to variable or uncertain parameters. Each model was run for 10,000 stochastic simulation iterations. Model outputs are presented as median values and their 90% prediction intervals.

Sensitivity analyses was conducted to assess the effect of individual variables on the model outputs. Advanced sensitivity analyses were undertaken by varying specific disease and economic parameters between their 1st and 99th distribution percentiles. Manual sensitivity analysis was additionally undertaken for vaccination efficacy, as this is a critical variable for vaccination economic viability and there is limited field data. Analysis of vaccination efficacy was undertaken with subsidised vaccination costs at 50%, as experts suggested that this was a realistic amount of government funding which could be obtained.

Ethical approval

Ethical approval was granted by the Social Science and Research Ethical Review Board at the Royal Veterinary College (URN SR2020-0266).

RESULTS

All economic values are presented in pounds sterling (1 ₦ = £0.0019, as consulted on 4th December 2020).

Gross margin analyses

Results from GM analysis are shown in Table 1. GM was negative in SGP severely affected unvaccinated herds.

Table 1. Overall herd gross margin (GM) and gross margin per reproductive female (GMRF) in £ from stochastic stimulations for vaccinating for sheep and goat pox (SGP) of sedentary and transhumance small ruminants herds in northern Nigeria.

	Without SGP (baseline) Median (90% CI)	With SGP and unvaccinated (Severely affected) Median (90% CI)	With SGP and unvaccinated (Slightly affected) Median (90% CI)	With SGP and vaccinated (Severely affected) Median (90% CI)	With SGP and vaccinated (Slightly affected) Median (90% CI)
Sedentary					
GM	329 (263 - 403)	-69 (-131 - -4)	256 (197 – 388)	290 (230 – 355)	317 (253 – 390)
GMRF	22 (18 -26)	-5 (-8 – -0.31)	17 (13 – 21)	19 (16 – 23)	21 (17 – 25)
Transhumance					
GM	1032 (560- 1708)	-370 (-727 – -111)	707 (349 – 1261)	877 (459 – 1479)	992 (533 – 1649)
GMRF	22 (15 - 29)	-8 (-15 – -2)	15 (9 – 22)	19 (12 – 25)	21 (14 – 28)

SGP financial impact

SGP costs and profit reductions are presented at the herd-level in Table 2 and SGP regional-level costs, stratified by state in Table 3. At the herd-level, SGP cost were greatest in transhumance herds. Changes in GM exceeded 100% in severely affected scenarios explaining negative values obtained in GM. In all scenarios, revenue foregone represents the largest proportion of SGP losses (83-92% of all additional costs due to SGP). In sedentary herds, reduced offtake of non-reproductive animals, as more need to be kept to replace the reproductive herd, was the greatest contributor to costs (20%-26%). In transhumance herds the greatest contributors were reduced price of SGP affected animals offtaken (23%), and reduction in value of SGP affected animals remaining in the herd (22%), for slight and severe scenarios respectively. At the regional-level, SGP disease cost was greatest in Plateau State, followed by Bauchi and Kaduna States, respectively.

Economic viability of vaccination

Results from PBA for SGP vaccination at the herd-level are shown in Table 4, and cost-effectiveness analysis results at the regional-level shown in Table 5. SGP vaccination resulted in herd-level net benefits in both herd types and disease scenarios. Benefits of vaccination were greatest in transhumance herds. At the regional-level, vaccination was found to be profitable when comparing government costs to societal benefits, over three-years for both strategies explored, with greater BCR's realised when combining SPR vaccination with the PPR programme. Net present value and BCR was greatest in high-incidence compared to low-incidence LGA, with losses only realised in low-incidence LGA's when vaccination is 100% subsidised by the government.

Table 2. Net sheep and goat pox (SGP) costs in £ at herd-level (herd), per reproductive female (per repro female) and percentage of profit loss due to SGP (profit loss) over an annual production cycle, from stochastic stimulations.

	Net SGP cost for severely affected unvaccinated herds Median (90% CI)	Net SGP cost for slightly affected unvaccinated herds Median (90% CI)
Sedentary		
Herd	404 (479 – 323)	71 (109 – 43)
Per repro female	27 (31 – 22)	5 (7 – 3)
Profit loss	121 (143 – 101)	22 (32 – 13)
Transhumance		
Herd	1409 (2236 – 838)	303 (579 – 147)
Per repro female	30 (41 – 21)	7 (10 – 3)
Profit loss	138 (174 – 109)	31 (47 – 16)

Table 3. Net sheep and goat pox (SGP) costs in £ over an annual production cycle, from stochastic stimulations rounded to the nearest 100,000, annual disease incidence rate and estimated small ruminant population for Bauchi, Kaduna and Plateau States

	Bauchi State	Kaduna State	Plateau State
Net SGP costs (millions) Median (95% CI)	19.9M (26.0M – 15.1M)	3.8M (5.4M– 0.3M)	0.8M (1.5M– 0.9M)
Disease incidence (%)	10	1	5.67
Small ruminant populations (millions)	8.5M	3.09M	2.9M

Table 4. Net benefit of sheep and goat pox (SGP) vaccination at herd-level (herd), per reproductive female (per repro female) and benefit to cost ratio of SGP vaccination (BCR) from stochastic stimulations.

	Net benefit of SGP vaccination for severely affected unvaccinated herds Median (90% CI)	Net benefit of SGP vaccination for slightly affected unvaccinated herds Median (90% CI)
Sedentary		
Herd	358 (289 – 431)	59 (35 – 92)
Per repro female	24 (20 – 28)	4 (2 – 6)
BCR	7 (5 – 9)	5 (4 – 8)
Transhumance		
Herd	1251 (737 – 2017)	264 (124 – 516)
Per repro female	27 (18 – 37)	7 (4 – 15)
BCR	5 (3 – 14)	5 (3 – 13)

Table 5. Net present value (NPV) and benefit to cost ratio (BCR) in £ from stochastic simulations, farmers investment and government investment for regional sheep and goat pox (SGP) vaccination strategies and high and low-incidence states over 3-years.

	NPV Median (90% CI)	BCR Median (90% CI)	Farmers investment	Government investment
SGP vaccination only				
0% subsidies	35,746,719 (26,478,158 -47,220,901)	30 (22-39)	2,403,263	1,250,038
50% subsidies	36,367,184 (27,055,807-47,887,446)	19 (15-24)	1,201,631	2,031,471
100% subsidies	36,947,626 (27,463,966-48,877,499)	14 (11-18)	0	2,812,904
SGP vaccination with PPR program				
0% subsidies	36,850,273 (27,581,711-48,324,454)	253 (189 - 330)	2,403,263	146,485
50% subsidies	27,058,281 (27,776,659-48,544,641)	29 (22-36)	1,201,631	1,348,116
100% subsidies	37,205, 257 (27,779,447-48,475,300)	16 (12-20)	0	2,548,747.65
High-incidence LGA				
0% subsidies	6,860,817 (5,598,121-8,816,104)	889 (727-1063)	135,664	7,705
50% subsidies	6,761,773 (5,516,571-8,097,539)	90 (74-108)	67,832	75,538
100% subsidies	6,677,448 (5,454,066-7,994,640)	48 (39-57)	0	143,370
Low-incidence LGA				
0% subsidies	192,890 (156,481-232,280)	26 (21-31)	135,664	7,705
50% subsidies	58,763 (22,514-97,804)	2 (1.3-2.3)	67,832	75,538
100% subsidies	-75,287 (-111,119 – -36,709)	0.5 (0.2-0.7)	0	143,370

Sensitivity analyses

Sensitivity analysis for vaccination efficacy at herd-level shows SGP vaccination realises a positive benefit with vaccination efficacy between 10-100% and government subsidies at 50%. The exception was in slightly affected sedentary herds, where a slight loss of £0.10 per reproductive female occurs when vaccine efficacy is 10%. Results shown in Fig.1.
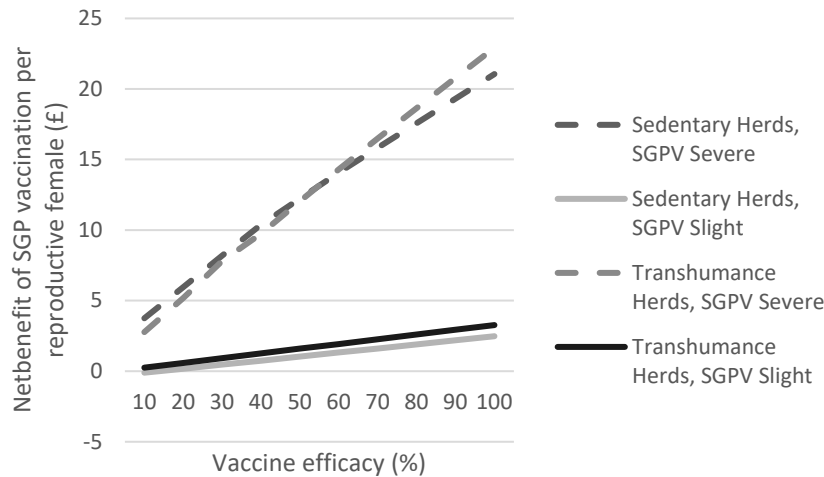
Figure 1. Results from manual sensitivity analysis, varying vaccine efficacy between 10-100% and recording effect on net benefit per reproductive female at the herd-level, with government vaccination subsidies at 50%.

Advanced sensitivity analysis showed that for sedentary herds, the most influential economic parameter was price of non-reproductive female goats, and non-reproductive goats SGP morbidity the most influential epidemiological parameter. For transhumance herds, non-reproductive female sheep, doe and ewe price were the most influential economic parameters, and non-reproductive sheep the most influential epidemiological parameter.

DISCUSSION

This study estimated costs and assessed economic viability of vaccination for SGP in Northern Nigeria, integrating production and economic models. Integrating these models is considered a reliable method of estimating disease impact and exploring disease control effects; showing how disease impacts population dynamics and subsequently economics. To the author's knowledge, this is the first-time cost and benefits of SGP vaccination, at herd and regional level, have been estimated in Northern Nigeria.

Total GM's are greatest in transhumance herds, due to greater herd size and animal value, but lower per reproductive female compared to sedentary herds. High labour costs and lower reproductive efficiency in sheep in transhumance herds contributes to this. GM values do not provide a complete measure of profitability, as fixed costs are not considered. However, fixed costs are likely to be minimal within these systems.

SGP costs estimated suggest significant losses. Slight and severe SGP scenarios were considered without providing values for the likelihood of herds being slightly or severely affected. We know that the severely affected scenario is seen in Nigeria, but the slightly affected scenario is theoretical. Further understanding of SGP epidemiology and disease dynamics within the study area will aid in quantifying this likelihood. Transhumance herds have the greatest herd-level loss, which might be due to different management and disease coping strategies. Our estimated costs exceeded median estimates previously cited within the region at £93 and £168 greater for sedentary and transhumance herds, respectively, compared to herd-level loss calculated in Bauchi (Limon et al., 2020). Differing values can be attributed to different methodologies. Developing production and GM models enabled comparisons between infected and baseline herds, so costs arising from changes in population dynamics,

such as reduced number of replacements from own stock could be estimated, which previous literature within the study area did not quantify (Bolajoko et al., 2019; Limon et al., 2020). Previous studies in the region focused on severely affected herds, so no studies are available to compare results for slightly affected herds. Top contributors to SGP cost identified (mortality, offtaking animals at reduced price, and reduction in affected animals remaining in herd), agree with previous studies (Garner et al., 2000; Senthilkumar and Thirunavukkarasu, 2010; Ben Chehida et al., 2018). Additionally, this study identified reduction in numbers of non-reproductive animals offtaken as a large contributor to costs (18-27%) which has not previously been reported. Reduced replacements from own stock demonstrates SGP's potential long-term impact on population dynamics and revenue. This study considered losses over a production cycle, costs over a longer period should be estimated in the future. Our study estimated that SGP costs within the region exceed £24 million per annual production cycle. SGP cost at the regional level has not previously been reported in Nigeria. Only direct production losses were estimated at herd and regional level; indirect SGP costs such as impact on trade were not considered, therefore this is likely a conservative estimate of SGP costs. SGP infection was modelled as occurring at the start of the year, so might have over-estimated the cost to herds not infected at the start.

Assessment of economic viability of SGP vaccination determined a net benefit. Benefits outweighed costs of vaccination, given the assumptions considered, demonstrating vaccination is financially viable on a herd-level. No previous BCR values for SGP in Nigeria or similar settings could be found within the literature. Sensitivity analysis demonstrated that benefit is realised at the herd-level even with low vaccination efficacy. Societal benefits are realised also with the regional-vaccination strategies proposed. Higher BCR's observed in high-incidence areas suggest that targeting higher-risk areas would result in a greater value for investment and would be less costly to implement. High costs of vaccination explain why when combining SGP vaccination with PPR vaccination, there is little difference in BCR and net present value, as consumables and staffing represent a small investment proportion. Exploring the economic benefits for governments, and other stakeholders within the supply chain, would potentially create more incentive to invest. Wide-spread implementation of SGP vaccination will increase livestock productivity, with potential impacts on market dynamics; vaccination will increase supply and thus potentially reduce prices of animals. Benefits of the program may this way be overestimated. Further analysis of these should be considered for a more accurate estimate of the benefits of regional vaccination programmes. Additionally, a feasibility analysis compared with analysis of other control methods, such as enhanced surveillance would be beneficial.

Lack of some data values for modelling within the literature was addressed by some unavailable parameters being obtained from field experience or collaborator discussions with farmers. This introduced potential recall or reporting bias and given the COVID-19 pandemic participants were limited in geographical representativeness. Only one transhumance farmer participated, so transhumance animal prices collected may not be representative, but a greater value for transhumance compared to sedentary animal prices corresponds with reports from the area (Limon et al., 2020). The survey from which incidence rates was obtained only included sedentary farmers, with rates obtained utilised as a proxy for transhumance SGP incidence rate. The model also presents some uncertainty, given its basis on numerous assumptions and variable parameters. Whilst assumptions will not reflect all farmers behaviour, they were agreed to reflect most common practices. Sensitivity analyses demonstrated that low and high inputs of variable parameters resulted in net SGP cost and benefit from vaccination. Dynamic, stochastic models, used in this study, are considered a good option for herd production modelling. Within the study area, there is another demographic of farmers who are also traders.

This demographic was not modelled, given the study time constraints, and their small population.

In conclusion, findings further evidence the significant economic impact of SGP to subsistence farmers within Northern Nigeria. The initial assessment of the viability of SGP vaccination conducted, determined vaccination to be economically viable at the herd and regional level for most scenarios considered, providing a net benefit for both sedentary and transhumance herds. We aim for the findings of this research to contribute policy discussions on implementing an economically viable SGP vaccination programme, and that models developed could additionally be used to explore financial impact of other small ruminant diseases within Northern Nigeria.

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APPLYING GROUNDED THEORY AND NATURAL LANGUAGE PROCESSING TO UNDERSTAND FARMERS' DECISION MAKING AROUND PROPHYLACTIC ANTIBIOTIC USE IN LAMBS

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SUMMARY

The aim of the study was to understand farmers' naturalistic decision-making around prophylactic antibiotic use in lambs. Data from 431 posts by 133 different users of an online discussion forum were analysed quantitatively using natural language processing and qualitatively using grounded theory. Results identified four categories that influenced farmers risk perceptions around prophylactic antibiotic use in lambs: anticipated regret, negative emotions and experiential avoidance; economic considerations; farmer identity; and perception of capability. Analytical thinking and authenticity scores were significantly lower in the study group compared to the control groups (P<0.01) suggesting a more personal style of thinking and guarded discourse. These results can be used to inform knowledge exchange around prophylactic antibiotic use to improve antibiotic stewardship in the sheep farming sector.

INTRODUCTION

In the UK, efforts have been made to reduce the unnecessary use of antibiotics in food producing animals (RUMA, 2020). Routine prophylactic antibiotic use to prevent neonatal disease in lambs is a key area of antibiotic use in the UK sheep sector. This is despite information campaigns illustrating that routine prophylactic antibiotic use to prevent neonatal diseases is usually unneccesary when alternative strategies are followed (AHDB, 2015; RUMA, 2019). These "good practice" strategies include hygiene practices, such as regular hand washing and cleaning out between ewes, and providing ewes with appropriate nutrition to ensure good colostrum quality. However, previous research suggest that many farms in the UK still use antibiotics prophylactically in neonatal lambs (Douglas and Sargison, 2018; Lima et al., 2019). Research is needed to understand why farmers may continue to use prophylactic antibiotic to prevent diseases in their lambs as routine.

Antibiotic use in agriculture receives a lot of attention from the public and media. As such, antibiotic use may be a sensitive topic for farmers, especially those who use prophylactic antibiotics as routine. Therefore, observational studies that ask farmers about their antibiotic use practices may be subject to social desirability bias. Observational studies are also likely to

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be prone to selection bias as farmers with undesirable antibiotic use behaviours are less likely to take part.

The use of online data from discussion forums can provide an insight into the naturalistic decision making of farmers. Online discussion forums have been used to study a variety of sensitive topics such as infertility (Hanna and Gough, 2016) and depression (Moore et al., 2016). Forums allow users to express their opinions without any perceived judgements from researchers, which removes the apparent biases of observational research. Forums have been used to understand the decision-making process of patients' use of antibiotics in human medicine (Santer et al., 2017; Ghouri et al., 2019), but there has been no such investigation in veterinary medicine.

Online texts may be analysed quantitatively to identify general trends in behaviour, motivations or emotion, or qualitatively to identify the textual meanings of posts. Combining both qualitative and quantitative data analysis – data analysis triangulation – can enhance trustworthiness of the results and provides multiple perspectives of the topic being investigated (Renz et al., 2018). The aim of this study was to use a combination of quantitative and qualitative methods to analyse internet forum posts to understand how farmers make decisions around using, or not using, prophylactic antibiotics in neonatal lambs.

MATERIALS AND METHODS

Study sample and data collection

A single large UK-based agriculture forum was selected for this research. The forum was searched for the terms "watery mouth", "joint ill" and the name of an oral product that contains spectinomycin. Threads were reviewed to determine those with relevant discussion of antibiotic use in neonatal lambs. The researcher remained a guest on the forum and did not register as a user, post on the forum or declare presence of a researcher to ensure data collection was unobtrusive. Forum threads were selected using the purposive theoretical sampling method. There were 431 posts in 10 threads from 133 different forum users. The forum posts dated from 2015 to 2019 and were accessed October 2019.

Two groups of unrelated posts were also collated to act as control groups. These groups were "weather" and "grassland management". They were chosen as weather and grassland management are topics which sheep farmers are likely to engage in, but were thought to offer a different type of discourse to neonatal lamb diseases. Posts relating to grassland management and weather were collected until the number of users was similar to that in the study (neonatal lamb disease) group.

Ethics

This analysis follows the guidelines set out by the British Psychological Society on internet mediated research (British Psychological Society, 2017). The posts within the forum were publically available and no informed consent was taken. Quotes from the forum users have been paraphrased to ensure anonymity of the users. The study was approved by the University of Nottingham School of Veterinary Medicine and Science Ethics Committee (no. 3232 200915).

Qualitative data analysis

The ten threads were copied onto a Microsoft Word document and uploaded into NVivo (NVivo qualitative data analysis Software; QSR International Pty Ltd. Version 12, 2018) to support analysis. A grounded theory approach was used for qualitative analysis and the constant comparative method was used for coding (Maykut and Morehouse, 1994). The final categories were defined and related to each other to establish an explanatory theory for farmers' decision making around prophylactic antibiotic use in lambs (Charmaz, 2006).

Quantitative data analysis

The natural language processing software Linguistic Inquiry and Word Count (LIWC) was used for the content analysis of the forum posts (Pennebaker et al., 2015a). User posts were pasted into a Microsoft Excel document, where each user was a single row. LIWC analyses the content by placing each target word into categories and measuring the relative frequencies of words in these categories. LIWC also measures the text using four summary variables: analytic thinking, clout, authenticity and emotional tone. These summary variables are measured on a 0-100 scale using percentiles derived from large comparison samples (Pennebaker et al., 2015b). A high score for clout reflects a more confident style, whereas a low score reflects a more tentative style of writing. A high score for analytical thinking reflects a more formal way of thinking and a low score reflects a personal, narrative style of thinking. A high score for authenticity indicates an honest, personal style, whereas a low score indicates a guarded, distanced style. Finally, a high score for emotional tone indicates a positive tone and a score below 50 indicates a negative tone.

The variable distributions for the study group were compared with the weather and grassland group using a Kruskal-Wallis test in Stata 16 (Stata SE/16.1, Stata Corp., College Station, TX, USA). Dunn's Test was used as a *post-hoc* test to identify differences between the three groups and P-value≤0.05 was considered significant.

RESULTS

Qualitative analysis

The qualitative analysis showed that there were two opposing risks that farmers considered: (1) the risk of only using antibiotics for treatment in lambs and (2) the risk of using prophylactic antibiotics in lambs. There were four major categories that influenced farmers risk perceptions around prophylactic antibiotic use in lambs: anticipated regret, negative emotions and experiential avoidance; economic considerations; farmer identity; and perception of capability.

Economic considerations: Some farmers were concerned about the long-term economic risks of using antibiotics in their lambs as routine. They were aware that this may cause antibiotic resistance, which would result in needing to use more expensive antibiotics.

"There has to be a balance, use cheap antibiotics now and make money until you get antibiotic resistance and the antibiotics no longer work. Then pay through the nose for more expensive new drugs or have to do without all together?"

Some farmers were concerned about the short-term economic risks of *not* routinely administering prophylactic antibiotics to their lambs. Neonatal lamb diseases were costly for farmers. Spectinomycin was seen as a cheap and effective way of preventing neonatal diseases.

"If I waited until I lost a lamb to watery mouth I would be down [£X] minimum plus the bottle of antibiotics I would waste because nothing dies uncared for on my farm, versus the [£X] it costs to treat all my lambs."

Good farming identity: Two identities of a good farmer were distinguished in this study. One identity was that of the importance of good animal welfare, which every farmer identified with. The other identity was around the importance of low antibiotic use. This identity caused conflicts between farmers as some thought that a good farmer did not need to rely on antibiotics for animal welfare, and others thought that animal welfare was the priority. These farmers argued that using antibiotics for the prevention of disease was better for the welfare of the animals.

"It all comes down to the issue of welfare. Is it right not to use spectinomycin and then the lambs get joint ill and have welfare issues, or is it better to prevent these welfare issues?"

Oral antibiotic use (spectinomycin) was seen as a normal part of lambing time and was separated from parenteral antibiotic use. The use of spectinomycin had become a cultural norm and was part of the rules of the game that define their good farming identity.

"Everything that lambs inside gets a dose of spectinomycin but other antibiotics are used rarely."

Whereas, farmers who also identified with low antibiotic use challenged other farmers' reliance on antibiotics and gave a description on how they managed to prevent neonatal lamb disease without using antibiotics.

"We used to use spectinomycin but now we now focus on bedding, making sure lambs are dry and not in draughts, and feeding ewes to ensure colostrum is good. We are getting on better so far with better husbandry."

Perception of capability: Some farmers had a positive perception of their capability to manage neonatal lamb diseases without antibiotics. They described their process of reducing their need for antibiotics at lambing time.

"We haven't used it for many years. Vaccinate ewes for clostridial diseases and feed them well for milk production before and after lambing. Make sure the lambs suck well early. Have ewe breeds that have a good maternal instinct, good lambers and produce lambs that want to suck."

"I lamb indoors and used to use antibiotics on multiples starting part way through lambing. This year I have only dosed the triplets and only because I had some left in the fridge. Next year I will not use it unless there is a problem."

There were also farmers who thought that preventing neonatal disease could not just be attributed to the skills of the farmer. Some farmers were considered luckier than others when it came to disease control, and the probability of lambs succumbing to disease was attributed to fate and chance. Each farm was perceived as unique:

"Some farmers may need antibiotics more than others, whereas others would get away with it. Sufficient colostrum has more to do with it than clean pens; you can be cleaning pens out after every ewe and still get it."

"Whatever system we use, whether we lamb outside, dip their navels, use antibiotics; I don't think we can ever be freed of this threat."

"You don't have to do anything wrong. One of my vets told me it depended what area you were in to a degree. With having sheep in different locations I could agree."

There was also external factors that influenced farmers' perception of capability in controlling neonatal diseases. Sheep farmers had low profit margins which led to lack of investment in new infrastructure. At the same time, they needed to produce lamb to meet market demand, which meant farmers had shifted their lambing period to Autumn/Winter rather than the Spring. The unfavourable weather conditions over Autumn/Winter meant that lambs were more prone to disease.

"We lamb early because our contract tell us when lambs are required for the supermarket. We are producing for public demand rather than for the sake of it."

Anticipated regret, experiential avoidance and negative emotions: Farmers experienced negative emotions when faced with cases of neonatal disease and lamb deaths. The use of routine prophylactic antibiotics was an act of experiential avoidance - an attempt to avoid uncomfortable thoughts, feelings, experiences or memories (Hayes et al., 1996).

"The allow your flock to evolve [without antibiotics] argument almost broke me – both financially and mentally – when it killed [x] percent of my lambs in 48 hours a few years back. Best practice is an aspiration but surviving is a necessity."

Some farmers felt that neonatal lamb disease was inevitable if they did not use antibiotics. They were confident that prophylactic antibiotics played an important role in their disease prevention and anticipated that they would get neonatal disease if they did not use antibiotics.

"If early lambing flocks can't use spectinomycin then it will mean increased use of other drugs to treat lambs once they become infected."

Quantitative analysis

The scores for analytical thinking and authenticity were significantly lower in the study group compared to the control groups (P<0.01) (Table 1). Emotional tone was lower than 50 in the study group, suggesting a negative emotional tone.

Table 1. Median scores for the four summary language variables in the study group and the control groups, and the results from the Kruskal Wallis test and post-hoc Dunn's test

Variable	Neonatal disease (N=111)	Grassland (N=108)	Weather (N=93)	P-value	Dunn's test
	Median (IQR)	Median (IQR)	Median (IQR)		
Analytical thinking	65.3 (48.9-79.6	70.6 (56.3-83.6)	82.4 (61.2-93.3)	<0.01	EP
Clout	49.2 (33.2-66.2)	50.0 (30.6-66.2)	46.6 (33.00-60.7)	0.81	-
Authenticity	48.27 (26.5-65.6)	65.7 (39.4-88.9)	85.2 (64.6-93.7)	<0.01	EP
Emotional tone	37.41 (25.8-66.1)	47.9 (25.8-79.3)	51.0 (25.8-80.6)	0.07	-

EP= probabilities were different within each pair of forums groups

DISCUSSION

To the authors' knowledge, this is the first paper to use natural language processing and online discussion forums to understand farmers' decision making around antimicrobial use. The use of data triangulation allowed the generation of more meaning from the data and enhanced the inferences made from the data. This analysis showed that farmers were undertaking two types of risk assessment: (1) the risk of using antibiotics only for treatment and (2) the risk of routine use of prophylactic antibiotics in lambs.

The farmers' perception of identity played an important part in whether they routinely use prophylactic antibiotics in all lambs or not. There were two good farming identities: good animal welfare and low antibiotic use. 'Good farming' draws on Bourdieu's concepts of cultural capital, habitus and field (Bourdieu, 1984). Animal welfare is a well-recognised good farming symbol (Bellet, 2018), but responsible antibiotic use is a good farming symbol which is still developing and this created a conflict.

The economic risk of prophylactic antibiotic use was considered both in the long-term and short term. Prophylactic antibiotic use was an act of temporal discounting as it reduced the immediate risk of economic losses due to lamb losses. Farmers may value the present certainty of healthy lambs more than the uncertain future positive effect of preventing antibiotic resistance. External economic constraints, such as market demand and low profit margins, also influenced farmers' decisions to use antibiotics prophylactically because of the effects on their perceptions of capability to control neonatal disease.

Many farmers believed they were capable of preventing neonatal disease without the need for antibiotics. However, farmers also believed that neonatal disease was not solely under their control and they considered the concepts of luck, fate and chance played a role. Some farmers were deemed luckier than others, and some diseases were down to chance. It may be difficult to convince farmers that alternatives to antibiotics are effective if they perceive that diseases are due to luck or chance. Additionally, each farm was seen as unique, which meant farmers of equal capability may get different results from their behaviour. This perception of uniqueness

of every farm could possibly explain why the scores for analytical thinking were lower in the study group compared with the control groups (Pennebaker et al., 2014).

Previous negative emotions around the consequences of neonatal lamb diseases influenced farmers to routinely use antibiotics in their lambs. If a negative outcome occurs because the farmer has chosen not to use antibiotics prophylactically, the farmer will feel personally responsible for the lamb deaths and this leads to regret. Farmers can anticipate the threat of regret, which leads to reduced tendency for risky behaviour. The attempt to avoid the negative experiences and feelings associated with preventable neonatal disease through the use of prophylactic antibiotic use could be an example of experiential avoidance (Hayes et al., 1996). The low emotional tone score in the neonatal lamb threads may be suggestive of their low mood due to anticipated regret.

In conclusion, this study identified that farmers must consider two opposing risks in the decision to use antibiotics in neonatal lambs: (1) the risk of only using antibiotics in neonatal lambs for the treatment of disease and (2) the risk of routinely treating lambs with prophylactic antibiotics. Farmers used antibiotics prophylactically in their lambs due to anticipated regret, concepts of luck and uniqueness, external constraints to their capabilities and temporal discounting of the economic effects. Whereas, farmers who did not use antibiotics were influenced by their positive perceptions of capability, their perceptions of the long term economic effects of antibiotic use, and incorporation of responsible antibiotic use into the good farmer identity.

ACKNOWLEDGEMENTS

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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

PAST PRESIDENTS

1982-'83	G. Davies	2001-'02	A.D. Paterson
1983-'84	P.R. Ellis	2002-'03	L.E. Green
1984-'85	G. Gettinby	2003-'04	J.L.N. Wood
1985-'86	R.W.J. Plenderleith	2004-'05	E.G.M. van Klink
1986-'87	M.V. Thrusfield	2005-'06	D.J. Mellor
1987-'88	K.S. Howe	2006-'07	E. J. Peeler
1988-'89	A.M. Russell	2007-'08	J. R Newton
1989-'90	S.G. McIlroy	2008-'09	L. Alban
1990-'91	J.E.T. Jones	2009-'10	D.U. Pfeiffer
1991-'92	J.M. Booth	2010-'11	L.A. Kelly
1992-'93	E.A. Goodall	2011-'12	C. Fourichon
1993-'94	R.G. Eddy	2012-'13	T.D.H. Parkin
1994-'95	J.T. Done	2013-'14	K.L.P. Verheyen
1995-'96	G.J. Gunn	2014-'15	K. Mintiens
1996-'97	M.S. Richards	2015-'16	H.H. Thulke
1997-'98	J.D. Collins	2016-'17	A. Lindberg
1998-'99	F.D. Menzies	2017-'18	L.R. Nielsen
1999-'00	K.L. Morgan	2018-'19	M.L. Brennan
2000-'01	S.W.J. Reid	2019-'21	K.M. McIntyre

EXECUTIVE COMMITTEE 2020-2021

K.M. McIntyre (President), M. Brennan (Senior Vice-President), G. van Schaik (Junior Vice-President), P. Robinson (Honorary Secretary), D. Brodbelt (Honorary Treasurer).

Honorary Auditors: Fraser Menzies & Keith Howe

LIFE MEMBERS

L.E. Green, 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), K.S. Howe, M.E. Hugh-Jones, W. Martin, D. Mellor, F. Menzies, K. Morgan, A.M. Russell, M.V. Thrusfield, J. Wilesmith

PLENARY TALKS

Year	Gareth Davies Lecture	Conference Opening Plenary
2021	Christine Fourichon Controlling infectious diseases: how does the interplay between field problems and epidemiological research leads 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	Dominic Mellor
	From science to policy - the case of classical swine fever (CSF) control	The trouble with epidemiology: the tyranny of numbers
2010	David Waltner-Toews	James Wood
	Beyond one world, one health and ecohealth…what's out there?	From pathogen adaption to host ecology: epidemiological and experimental contributions to the understanding of emerging infectious diseases
2009	Jørgen Westergaard	Katharina Stärk
	The interaction between veterinary science, legislation and management in animal disease control in the European Union	Food safety challenges in a global market – are we ready?
2008	Paul Fine	Kenton Morgan
	Infectious disease eradication – meanings and implications	For the benefit of Mr Kite
2007	Yrjö Gröhn	Laura Green
	Food supply veterinary medicine: Modelling of production, health and food safety	Improving Animal Health
2006	David Galligan	Nigel French
	From partial budgets to real options - concepts in animal health economics	Understanding human exposure to zoonoses from food and the environment: The application of molecular tools and modeling
2005	Bill Reilly	Simon More
	From TB to VTEC: The changing epidemiology of foodborne zoonoses	Towards eradication of bovine tuberculosis in Ireland: A critical review of progress
2004	Ulrich Kihm	Gary Smith
	BSE and the stable to table concept	Spatial models of infectious disease in the USA: a crisis of conference and confidentiality
2003	Sir David Cox	Ynte Schukken
	The current state of statistical science	Molecular and mathematical epidemiology of bovine mastitis
2002	George Gettinby	Bryan Grenfell
	Informatics and epidemiology – the first 400 years	Deterministic and stochastic influences on the dynamics and control of infectious diseases
2001	Will Houston	Mart de Jong
	Science politics and animal health policy: epidemiology in action	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 (£40 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 David Brodbelt
Royal Veterinary College
Hawkshead Lane
North Mymms
Hertfordshire
AL9 7TA
UK

TEL   +44 (0) 1707 667 155
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