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EMERGING METHODS FOR VETERINARY EPIDEMIOLOGY

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# ANIMAL MOVEMENTS

# INVESTIGATING THE ASSOCIATION BETWEEN PIG TRANSPORTS, VETERINARIAN VISITS AND FARM PRODUCTION TYPE TO INFORM RISK-BASED SURVEILLANCE STRATEGIES

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## SUMMARY

The translation of findings of livestock trade network analyses into policy is often hindered by the lack of inclusion of alternative disease pathways, and by the insufficient investigation of the relationship between network metrics and factors exploitable by policymakers. In this study, an in-depth analysis of the Swiss pig trade network was performed by adding indirect contacts that may result from veterinarian visits, and by stratifying our findings by ten production type clusters. A strong correlation was found between node centrality metrics and the production type clusters, indicating that such information may be used to define risk-based surveillance strategies. After adding veterinarian visit links, node centrality metrics increased particularly among two clusters, suggesting that inclusion of indirect disease pathways may strengthen the validity of claims or recommendations based on transport network analysis.

## INTRODUCTION

Porcine infectious diseases continue to be a threat to the international pig production industry worldwide. In the last decade, the spread of African Swine Fever (ASF) from East to West Europe has been slow but steady (Schulz et al., 2019). While surveillance efforts in Europe have been focusing on the wild boar population (Boklund et al., 2018), the recent detection of ASF cases in German pig holdings (Friedrich-Loeffler-Institut, 2021) shows that appropriate surveillance programmes should also be established for domestic pig populations.

Swiss pig holdings currently enjoy a high health status (Swiss Federal Food Safety and Veterinary Office, 2021). Porcine Reproductive and Respiratory Syndrome (PRRS), present in all neighbouring countries, is absent in Switzerland, but occasionally has been reintroduced into the country (Nathues et al., 2016). Freedom from disease is demonstrated every year through a slaughterhouse-based surveillance programme. However, the establishment of farm-based surveillance programmes may provide benefits in the future, as long as relevant factors for risk-based sampling are identified.

The growing literature on livestock trade network analysis shows that network centrality metrics, such as in- and out-degree, or temporal metrics such as ingoing contact chain (ICC)

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and outgoing contact chain (OCC), can be used to target holdings for surveillance and control measures (Lentz et al., 2016; Schirdewahn et al., 2017; Schulz et al., 2017; Sterchi et al., 2019). Recognising the complexity and demanding computational requirements of network analysis for governmental agencies, studies have evaluated the association between network centrality metrics and farm production type (Ribbens et al., 2009; Salines et al., 2017; Schulz et al., 2017). Nonetheless, classification of holdings by production type is often not available in databases used for surveillance, or only in broad categories. In Switzerland, the degree of pig holding specialisation ranges from highly specialised to completely self-sufficient with several intermediate farm types in between, but an accurate classification is currently not available in any national database.

Another factor limiting the relevance of transport network analysis is the lack of data about indirect contacts between holdings, which have been repeatedly documented to occur (Brennan et al., 2008; Ribbens et al., 2009; McReynolds et al., 2014; Relun et al., 2015), but are rarely accounted for in network models. A recent review emphasised that solely focusing on trade-related infectious disease pathways may result in the formulation of ineffective surveillance strategies (Kinsley et al., 2020). Rossi et al. (2017) showed how the addition of between-farm contacts generated by veterinarian movements in Italy resulted in increased potential for disease transmission among dairy farms.

The aim of this study was to build and analyse a contact network of Swiss pig holdings considering both pig transports and veterinarian visits, to classify pig holdings by production type using transport network data, and to quantify the association between network centrality metrics, type of contact (pig transports, veterinarians) and the holding's production type.

## MATERIALS AND METHODS

### Clustering of holdings by production type

Data on pig transports between Swiss pig holdings were extracted from the *Tierverkehrsdatenbank* (TVD), the Swiss national animal transport database. The frequency of incoming and outgoing contacts was calculated for the holdings present at least once in the TVD in 2019. The contacts were stratified into seven types of pigs transported: boars, gilts, sows or gilts in a shared piglet production ring, 10-kg piglets, 25-kg piglets, finisher pigs and old breeding pigs. The frequencies were then used as inputs for the Partitioning Around Medoids (PAM) clustering algorithm (Kaufman and Rousseeuw, 2009) to obtain holding type clusters by pig type-specific incoming and outgoing frequencies. The number of clusters is an input parameter for the PAM algorithm and was manually defined by performing a sensitivity analysis and obtaining expert opinions on the ability of the clusters (i.e., the holding types) to represent the Swiss pig production chain in the best way. The final findings were validated by assessing the plausibility of pig trading patterns between clusters in terms of pig production cycles.

### Network of animal transports (AT)

The network of pig transports in 2019 was built using TVD data as well. Nodes were defined as pig holdings and directed links were defined as pig transports between a source and a destination holding. Transports from holdings to slaughterhouses were excluded from this analysis.

### Network of animal transports and veterinarian visits (AT-VV)

A second network was built in which, in addition to the animal transports (AT above), veterinarian visits to the pig holdings were included. Veterinarians may be potential disease vectors among the consecutively visited farms, when they visited farms on the same calendar day. Data on veterinarian visits were obtained from the largest Swiss pig health services company, which manages over 80% of breeding farms and over 50% of fattening farms. All those farms are routinely visited by company veterinarians. Visit data consisted of date of visit, veterinarian ID, holding ID, holding biosecurity level and holding production type. Holdings' geographical coordinates were extracted from the TVD. The order of farm visits was not recorded in the veterinarian visit database, however by company regulations, veterinarians are expected to visit farms by decreasing order of biosecurity level (nucleus herds, multiplier herds, other disease-free holdings, holdings of unknown infectious disease status) and production type (breeding farms, fattening farms). Accordingly, biosecurity level was used as first priority rule and production type as second priority rule to reconstitute the sequence of visits. Three different methods were applied:

1) For visit tours in which all holdings had a unique combination of biosecurity level and production type, the tour sequence was defined using that information

2) For visit tours in which biosecurity level and production type information allowed to define the tour sequence only partially, the first or last holding of the part of the tour for which a sequence could be established was taken as ending or starting point, respectively. The shortest path starting from there, in terms of Haversine distance between holdings to approximate for road distance, was selected to determine the remaining sequence of the tour

3) For visit tours in which all farms had the same biosecurity level and production type, one holding was randomly selected as the starting point and the shortest path starting from that holding and including all other holdings was selected to determine the sequence of the tour

After establishing the sequence, network links were drawn consecutively between holdings, i.e., a directed link was drawn from any holding to all consecutive holdings in the sequence.

### Temporal network analysis

For both the AT and AT-VV networks, holding-specific average ICC and OCC were calculated using the *EpiContactTrace* R package (Nöremark and Widgren, 2014), by first computing ICC and OCC for any possible seven-day time window in 2019, and subsequently averaging them over the year.

Overall and cluster-specific ICC and OCC were calculated and compared between the AT and AT-VV networks. A one-sided Wilcoxon signed rank test was used to test for a statistically significant increase in the two metrics from AT to AT-VV, to assess the effect of the addition of veterinarian visit links. The p-value cut-off at the 95% confidence level was defined as 0.05.

In order to get a better overview of holding-specific overall centrality, a third, combined metric was produced by re-scaling ICC and OCC on a [0,1] continuous scale and by computing the midpoint of those two values for each holding.

## RESULTS

### Production type clusters

A total of eleven holding production type clusters were defined by means of PAM clustering. Thirty-three holdings could not be assigned to any cluster because of missing pig type data on transports to and from these holdings and were excluded from the analysis. One cluster consisted of “almost inactive” holdings, only sending fattened pigs to slaughter plants a few times a year and never receiving any incoming transports. These holdings were not deemed relevant for the rest of the analysis and were therefore excluded. The names, size and description of remaining ten clusters are summarised in Table 1. These clusters consist of nucleus herds, multiplier herds, several types of breeding and nursery holdings, and two types of fattening holdings.

Table 1. Clusters for holding production types in the Swiss pig chain

| Cluster name | N | % | Description |
|---|---|---|---|
| *Nucleus* | 33 | 0.5 | Nucleus herds |
| *Multiplier* | 45 | 0.7 | Multiplier herds |
| *Breed_repl* | 679 | 10.4 | Breeding farms with own replacement of sows |
| *Breed_norepl* | 358 | 5.5 | Breeding farms with external sow replacement |
| *Breed_10kg* | 68 | 1.0 | Breeding farms sending out 10-kg piglets |
| *Fat_10kg* | 56 | 0.9 | Farms receiving and fattening 10-kg piglets |
| *Ring_ins* | 47 | 0.8 | Insemination farms in a shared piglet production ring |
| *Ring_farr* | 281 | 4.3 | Farrowing farms in a shared piglet production ring |
| *Fat_hfreq* | 1,309 | 20.0 | Fattening farms with high transport frequency |
| *Fat_lfreq* | 3,642 | 55.9 | Fattening farms with low transport frequency |
| Total | 6,518 | 100.0 | |

Figure 1 shows the outgoing and incoming patterns for two examples of clusters with specific boxplot patterns. These give a unique holding type “fingerprint”. In the case of *Fat_hfreq*, a high frequency of incoming 25-kg piglets and outgoing finisher pigs strongly reflects that this cluster represents fattening farms with high transport frequency. In the case of *Nucleus*, the absence of any incoming transports and a high frequency of outgoing transports of pig types associated with the breeding process identify these holdings as nucleus herds.

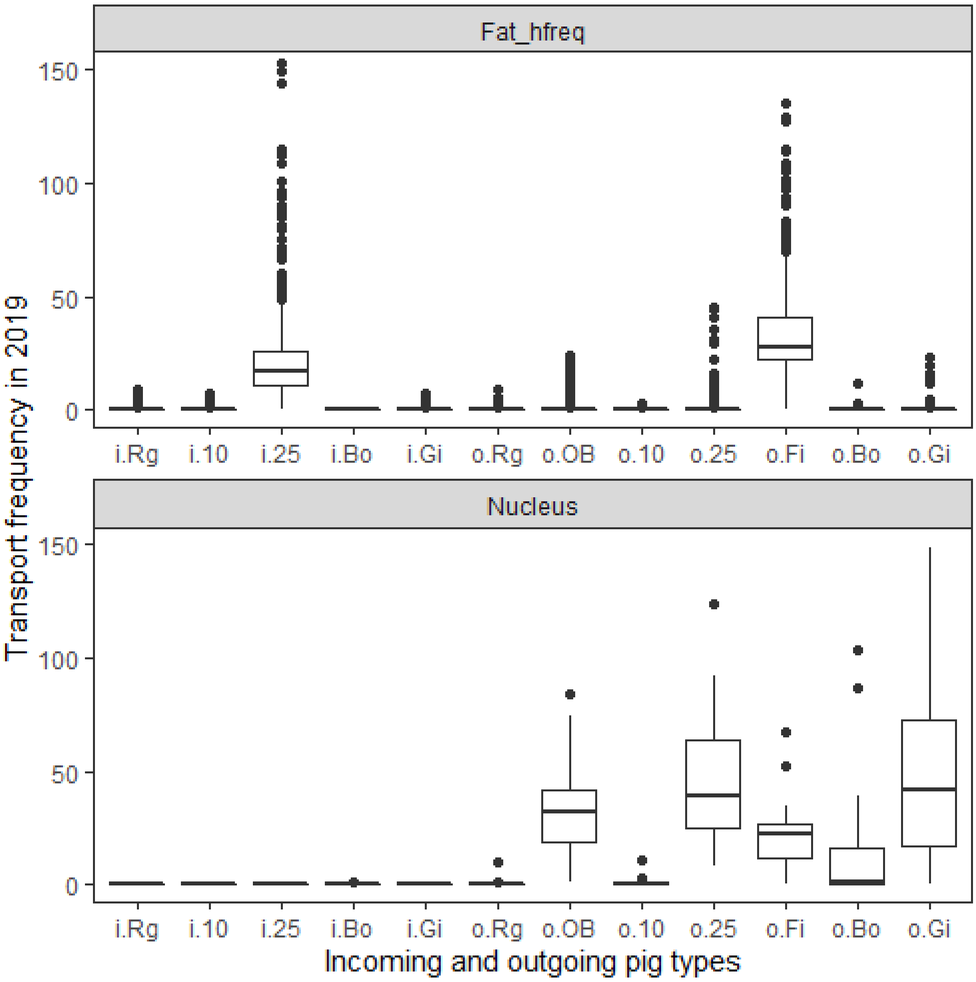

Fig. 1. Two examples of cluster “fingerprints” obtained by plotting boxplots of incoming and outgoing frequency of transports by pig type in 2019. Transport categories: “i.” = incoming, “o.” = outgoing, “Rg” = sows in a shared piglet production ring, “10” = 10-kg piglets, “25” = 25-kg piglets, “Bo” = boars, “Gi” = gilts, “OB” = old breeding pigs, “Fi” = finisher pigs

### Description of the AT and AT-VV contact networks

The AT network consisted of 48,934 links between 6,551 holdings. The number of links in the AT-VV network was 51,099, a 4.2% increase relative to the AT network.

### Temporal network analysis

Table 2 reports ICC and OCC by network and holding type cluster, together with the percentage increase in network metrics between the two networks and the corresponding p-value to test for a significant increase. *Ring_ins* was the only cluster with a large average weekly ICC, while large OCCs were found among the *Nucleus*, *Multiplier* and *Ring_ins* clusters. Overall and for all clusters, a significant increase in both ICC and OCC was observed after the addition of veterinarian visit links. The largest increase in ICC was detected in the *Nucleus* cluster (87.9%) and the largest increase in OCC was found among *Fat_hfreq* holdings (31.1%).

Table 2. Average weekly ICC and OCC by network and holding type cluster, increase in metric and p-value of one-sided Wilcoxon signed rank test

| Cluster | ICC | | | | OCC | | | |
|---|---|---|---|---|---|---|---|---|
| | AT | AT-VV | % increase | p-value | AT | AT-VV | % increase | p-value |
| Overall | 0.180 | 0.188 | 4.4 | <0.001 | 0.181 | 0.189 | 4.5 | <0.001 |
| *Nucleus* | 0.001 | 0.006 | 87.9 | 0.049 | 3.099 | 3.204 | 3.3 | <0.001 |
| *Multiplier* | 0.331 | 0.346 | 4.3 | <0.001 | 1.806 | 1.875 | 3.7 | <0.001 |
| *Breed_repl* | 0.045 | 0.053 | 14.6 | <0.001 | 0.575 | 0.594 | 3.2 | <0.001 |
| *Breed_norepl* | 0.153 | 0.161 | 5.0 | <0.001 | 0.514 | 0.532 | 3.3 | <0.001 |
| *Breed_10kg* | 0.210 | 0.217 | 3.0 | <0.001 | 0.661 | 0.682 | 3.1 | <0.001 |
| *Fat_10kg* | 0.547 | 0.564 | 3.0 | <0.001 | 0.298 | 0.303 | 1.6 | 0.003 |
| *Ring_ins* | 1.798 | 1.824 | 1.4 | <0.001 | 1.219 | 1.244 | 2.0 | <0.001 |
| *Ring_farr* | 0.310 | 0.326 | 4.7 | <0.001 | 0.727 | 0.752 | 3.3 | <0.001 |
| *Fat_hfreq* | 0.482 | 0.499 | 3.3 | <0.001 | 0.014 | 0.020 | 31.1 | <0.001 |
| *Fat_lfreq* | 0.062 | 0.066 | 6.8 | <0.001 | 0.021 | 0.024 | 12.7 | <0.001 |

By rescaling and averaging ICC and OCC, a combined metric was calculated to allow ranking single holdings and holding type clusters by network centralities in the AT-VV network. Figure 2 shows that three clusters with a total of 125 holdings, namely *Ring_ins, Nucleus* and *Multiplier*, were those with the highest average centralities.

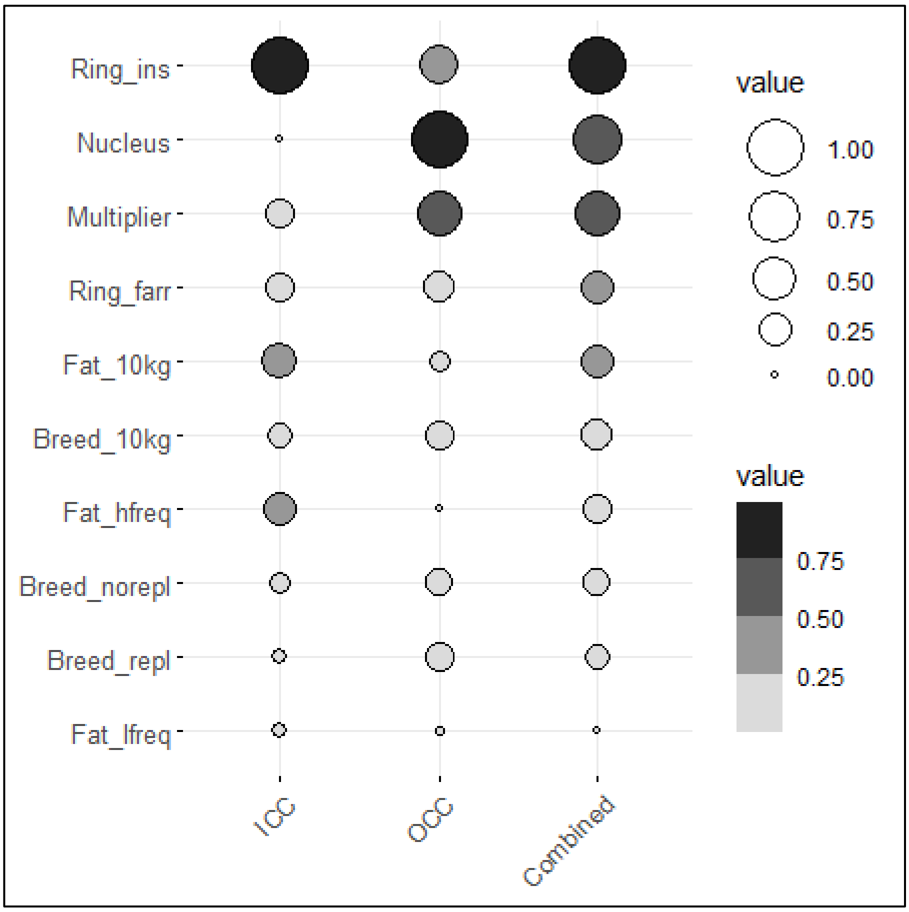

Fig. 2. Re-scaled ICC, OCC and combined metrics on a [0,1] scale, stratified by holding type cluster and ordered by decreasing value of combined metric. Circle size is a continuous representation of the combined metric, while grey-black shading is a discrete representation of the combined metric for values 0.00-0.24, 0.25-0.49, 0.50-0.74, 0.75-1.00

After ranking holdings by value of combined centrality metric, the first 125 holdings were extracted and their distribution among the ten clusters was assessed (Table 3). It was found that 83.0% of *Ring_ins* holdings, 66.7% of *Nucleus* holdings and 37.8% of *Multiplier* holdings were among the top 125 in the overall centrality ranking. Overall, 78 farms (62.4%) out of the 125 in those three clusters were also among the 125 top holdings. The fourth largest number of top-125 holdings was found in the *Fat_hfreq* cluster, with 16 holdings representing 1.2% of holdings in that cluster. The only cluster with no holdings among the top 125 was *Fat_lfreq*, and this despite the fact that it held the largest number of holdings.

The same analysis for the AT network showed that 121 holdings among the top 125 in the AT-VV network ranking were also among the top 125 in the AT network ranking.

Table 3. Distribution among clusters of top 125 holdings by combined metric in the AT-VV network

| Cluster | N | N among top 125 | % |
|---|---:|---:|---:|
| *Ring_ins* | 47 | 39 | 83.0 |
| *Nucleus* | 33 | 22 | 66.7 |
| *Multiplier* | 45 | 17 | 37.8 |
| *Fat_hfreq* | 1,309 | 16 | 1.2 |
| *Ring_farr* | 281 | 11 | 3.9 |
| *Breed_norepl* | 358 | 10 | 2.8 |
| *Breed_repl* | 679 | 4 | 0.6 |
| *Fat_10kg* | 56 | 3 | 5.4 |
| *Breed_10kg* | 68 | 3 | 4.4 |
| *Fat_lfreq* | 3,642 | 0 | 0.0 |

## DISCUSSION

In this study, it was shown that an accurate and pertinent classification of Swiss pig holdings by production type is achievable using information available in governmental databases. This classification proved to be an effective method to identify good candidate holdings for surveillance and control of porcine infectious diseases. The inclusion of veterinarian movements in the network analysis provided important insights into the impact that indirect contacts may have on disease spread. For instance, it was found that indirect contacts may expose to infectious diseases holdings that can potentially transmit them further to a large number of holdings in a short time frame.

Since the used clustering technique requires a manual definition of the number of clusters to be created, collaboration with the pig industry and veterinary public health specialists was important for the calibration and validation of the analysis. Expert elicitation is a powerful tool for knowledge gathering and policymaking in epidemiology (Bearth et al., 2014; Kuster et al., 2015), and has been recently used in a similar context of herd type classification in Ireland by Brock et al. (2021). The study underlines the importance of involving experts in the process of defining classification rules when system complexity, and thus number of clusters, is high. Additionally, the authors argue that the use of visual tools is essential to aid experts in the interpretation of the performed analysis. The cluster “fingerprints”, by means of simple box plots, proved to be an effective tool to meet this need.

Importantly, high-resolution clustering of farms by production type allowed us to discriminate between relevant and irrelevant holdings in terms of surveillance. For instance, both the *Ring_ins* and *Ring_farr* clusters consist of farms that are associated in a shared piglet production ring, a unique structure of the Swiss pig production system in which insemination of sows takes place in a central holding (*Ring_ins*) and sows are subsequently moved to other holdings for giving birth and weaning piglets (*Ring_farr*). While farms from both clusters are found among the first 125 holdings in our node centrality ranking, it is sufficient for surveillance to consider the *Ring_ins* holdings which are much fewer in numbers, as all *Ring_farr* farms are connected to them. Another example is the separation of fattening holdings into *Fat_lfreq* and *Fat_hfreq*: the former cluster has substantially low average ICC and OCC, and given that it is the largest cluster, its removal from surveillance plans allows to reduce drastically the number of holdings that may be targeted for farm-based disease surveillance programmes.

In the analysis, the three clusters with the highest combined centrality were breeding farms in shared piglet production rings, nucleus herds and multiplier herds. Breeding farms and multiplier herds were also identified as important holdings for surveillance purposes in a study of the Danish pig trade network (Schulz et al., 2017). The authors pointed out how these holdings are in contact not only with many farms, but also with several types of farms, suggesting that the disruptions their infection would cause to the national production chain would be very large. Nucleus herds do have the highest biosecurity standards and regular controls from veterinarians, however their weekly OCC is so large that even a single event of disease introduction may have dramatic consequences. Their relevance for disease surveillance is further supported by the analysis of the French pig trade network, in which nucleus herds, in addition to a large OCC, exhibited high outgoing closeness, which means that they could reach other holdings in only a few steps and thus spread pathogens faster and more broadly than other holdings (Salines et al., 2017).

Identifying holding-specific characteristics strongly associated with node centrality may allow governmental agencies to define risk-based surveillance strategies without the need to undertake complex and time-consuming contact network analysis as performed here. In the examination of the most central holdings, a strong correlation was found between node centrality and holding type in the Swiss pig production system, with almost two thirds of the holdings in the clusters with the highest network metrics also being the most central nodes overall. Although this result may be of significance for policymaking, further holding characteristics also should be explored to refine the picture.

A peculiarity of the Swiss pig trade network is its fragmented nature, which was highlighted in the previous network analysis (Sterchi et al., 2019) and was again found here through a very low average weekly ICC and OCC of 0.19. While this characteristic may inherently limit the spread of infectious diseases in case of introduction into the country, the actual connectedness of livestock holdings in terms of disease spread potential can only be evaluated after including other disease transmission pathways. Several of such pathways are known to exist, such as equipment sharing between farmers (Brennan et al., 2008; Relun et al., 2015) or visits of professionals (Ribbens et al., 2009; McReynolds et al., 2014). As a first step in that direction, here the consequences of considering veterinarian visits as a potential disease spread mechanism were assessed, and incorporating such links into the pig trade network resulted in a significant increase in ICC and OCC, both overall and among all production types. In their study conducted among dairy farms in an Italian province, Rossi et al. (2017) highlighted that adding links generated by veterinarian visits also resulted in a significantly increased disease spread potential among holdings. Moreover, the sets of most important holdings in terms of disease spread potential were very different between the cattle transport and veterinarian visit networks. This is in contrast with the findings of our study, where the sets of top 125 nodes in terms of overall centrality were nearly identical in the AT and AT-VV networks. However, only a partial comparison is possible because of the difference in available data (including only routine visits in our analysis). Nonetheless, in our case the role of veterinarian visits as a modifying factor of network centrality was identified because the largest ICC increase was observed in nucleus herds, which usually have almost zero incoming contacts via pig trade, and that the largest OCC increase occurred among fattening holdings (*Fat_hfreq* cluster), which, in terms of disease transmission potential, are frequently considered as "dead ends".

One important limitation of this study is the lack of complete data about veterinarian movements, because the available data only covers visits to 80% of Swiss breeding farms and 50% of fattening farms. This leaves the potential for the impact of veterinarian visits on average OCC of fattening farms to be even higher. Another limitation of the veterinarian visits data is the lack of visit sequences. Despite the use of biosecurity regulations and geographic features to establish the most probable visit sequences, a certain degree of uncertainty is still present regarding the order, which may have an impact on the cluster-specific assessment of network metrics. We therefore plan to perform a sensitivity analysis of variation in network metrics when randomly selecting different holdings as starting points if these cannot be defined using biosecurity level and production type rules. Finally, the study did not include other indirect trade-related disease transmission pathways, such as pig-to-pig contact in lorries, occurring when multiple farms are visited in the same day by transport lorries. Recognising the importance of such contacts is the next planned extension to our effort to enrich network analysis of disease transmission in pig production chains.

In conclusion, in this study the Swiss pig trade network was enhanced by the addition of holding production type as a farm attribute, and of veterinarian visits as a further potential disease pathway between holdings. A stratified network analysis by production type allowed for the identification of a small number of holdings that may serve as sentinels in the frame of infectious disease surveillance programmes. Veterinarian visits increased average node centrality metrics across all production types, and more significantly among holdings that are traditionally considered less relevant for disease transmission, such as fattening farms. These results highlight the importance of including further indirect disease pathways in network studies aimed at supporting the formulation of pertinent surveillance strategies.

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# PROVIDING EVIDENCE ON REQUIRED OBSERVATION PERIODS OF FREE ROAMING DOMESTIC DOGS FOR HOME RANGE ESTIMATIONS

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## SUMMARY

Several studies aiming at better understanding behaviour of dogs use the concept of home range (HR). Published studies on GPS tracking report differences in numbers of days recorded. To our knowledge, there is no accepted minimal number of days required to capture a representative HR size. We collared free-roaming domestic dogs (FRDD) in Chad, Guatemala, Indonesia and Uganda to identify a minimal number of recording days required to capture a representative HR. Seventy-five percent of dogs required up to seven days for estimating a core HR (50% isopleth) and up to 26 days for extended (95% isopleth), after which the percentage of change in the HR size for any additional observation day was at maximum, 10%. The required number of recording days depends on the isopleth level, the characteristics of the dog and the level of percentage of change accepted.

## INTRODUCTION

Domestic dogs, *Canis familiaris*, are possible vectors of zoonotic diseases and live in close contact with humans, which makes them highly relevant for animal and public health challenges (Welburn et al., 2015; Baneth et al., 2016; Otranto et al., 2017; Colella et al., 2020). Free roaming domestic dogs (FRDD) play a special role, because they are at higher risk for disease transmission to conspecifics and other species, due to their free access to the streets and their abundance in countries where canine zoonoses of high relevance (e.g. rabies or echinococcosis) are prevalent. Additional and better information on the behaviour, contacts and movements of dogs can help design disease prevention and control programs (Vaniscotte et al., 2011; Van Kesteren et al., 2013) and inform parameterisation of disease transmission simulation models (Dürr and Ward, 2015). Tracking of animal movements has been widely used in several research fields, from animal welfare, to conservation, biology and public health (Van Kesteren et al., 2013; Kays et al., 2015; Kabalika et al., 2020). Gathering knowledge on animal behaviour, its relation to their habitat and to other animals, may provide valuable insights into their ecology. While monitoring movements of animals can be done through different approaches (Hudson et al., 2017; Kabalika et al., 2020), the usage of Global Position Systems (GPS) and its possibilities have recently become more prominent (Kays et al., 2015; Bailey et al., 2018; Raynor et al., 2020).

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Over the last few years, several studies aimed at better understanding the behaviour of FRDD, using the concepts of home range (HR) and utilisation distribution (UD) (Dürr et al., 2014; Hudson et al., 2017; Raynor et al., 2020). The HR can be defined as the area which is generally used by the individual animal "in its normal activities of food gathering, mating, and caring for young" (Burt, 1943), while the UD provides information on how the area within the HR is used by the animal. Published studies on GPS tracking of dogs report starkly different observation periods, ranging from a few days to a few months (Dürr et al., 2017; Hudson et al., 2017; Raynor et al., 2020). By investigating HR across different observation periods, conclusions can be drawn on how the HR changes over time, and how the observational period influences HR estimations. To our knowledge, there is no accepted minimal number of days for an observation period to capture a representative HR of FRDD. This minimal observation period is expected to depend on several factors, such as the dog's roaming behaviour type (Hudson et al., 2017), definition of HR size, accepted variability for a HR size to be considered as representative and, potentially, the country or area where the dog lives.

The objective of this study was to evaluate the size of the HR of FRDD from four different countries and to try to identify a minimal number of observation days required to capture a representative HR. We hypothesise that for short observation periods, changes in HR size brought by each additional day of observation can be large. However, with increasing observation period, these changes in HR size are expected to decrease, until the HR size reaches a stable plateau. This plateau could be defined as the representative HR size. The findings of this study can help to inform future GPS collars studies on FRDD and potentially other species.

## MATERIALS AND METHODS

### Study design and data collection

The study was conducted in two rural areas in Chad (NDakonon and Sinetaye), two rural areas (La Romana and Sabaneta) and one city (Poptun) in Guatemala, two rural (Pogon and Hepang) and one semi-urban (Habi) areas in Indonesia, and one city (Soroti) and one rural area (Kamuda) in Uganda. Data collection took place in the frame of another research project between January 2018 and March 2019 (Warembourg et al., 2021a, 2021b). The study population was defined as owned FRDD, with dogs less than four months of age, pregnant bitches and obviously sick dogs being excluded. Dogs were selected within a pre-determined study site of 1km$^2$ (Warembourg et al., 2021a). Twenty dogs in Chad, Guatemala and Indonesia and 41 dogs in Uganda were collared with GPS CatLog™ units (www.mr-lee.com). These robust units have previously demonstrated their usefulness in studying FRDD (Dürr and Ward, 2014). A time interval of 15 minutes between two consecutive GPS fixes was selected to ensure long enough battery power. The accuracy of the GPS units had been previously assessed in different studies (Dürr and Ward, 2014; Hudson et al., 2017). Depending on the site, the units were retrieved from the dogs between 25 to 85 days post-collaring. An ethical approval was authorised in each country separately and written or oral informed consent was obtained from all owners before the start of the study. In Guatemala only, a monetary incentive of 50 quetzals (6.4 USD) was provided to the participating owners.

### Data cleaning and analysis

After retrieval of the GPS tracker, data were downloaded from the unit and subsequently imported into R (https://cran.r-project.org), which was used for data management, cleaning and analysis. Data from the first and last day of collaring were excluded from the datasets to account for biased behaviour during the days of manipulation, and datasets with less than 10 days of records were excluded from the analysis. Data from Chad, Guatemala, Indonesia and Uganda were projected from World Geodetic System (WGS) 1984 to local projected reference system UTM 34N, Guatemala Norte, UTM zone 51S and UTM zone 36N, respectively. Data cleaning was undertaken to remove obvious GPS errors and clear outliers by excluding GPS fixes with a horizontal dilution of precision (HDOP) higher than five (Lewis et al., 2007), consecutive GPS fixes generating speeds higher than 5.7 km/h (corresponding to the 0.1% highest speed values between any two consecutive GPS fixes), GPS fixes effecting the 2.5% acutest angles formed by each three consecutive points (Shimada et al., 2012) and manually removing clear outliers after GPS fixes plotted on maps.

The UD was calculated using the biased random bridge (BRB) method (Benhamou, S., 2011; Gregory, 2017) (R packages *adehabitatHR* and *adehabitatLT*), with parameter values of *Tmax* set as 75 minutes, *Lmin* set at 45 m and *hmin* as 18 m, according to previous research (Dürr and Ward, 2014). The UD of each dog was calculated for each day of the observation period in a cumulative way, i.e. using the GPS fixes of that day and of all previous days. For example, the UD of the 7th day includes all GPS fixes from day 1 to day 7. For each calculated UD, the area within the 50% and 95% isopleths – considered the core and extended HR size, respectively – were retrieved for the further HR size analysis.

To investigate the minimal observation period needed to estimate a representative HR size, the percentage of change in HR size estimates using the data until day x versus the data until day x-1, was calculated. Applying this approach, we quantified how much the HR size changes with every additional day of observation. For each dog, we identified the minimal number of observation days after which the values of the percentage of change are constantly equal or inferior to 10% or 20%, for both the 50% and 95% isopleth levels. To determine the minimal number of observation days needed to reach a percentage in change constantly below 10% or 20%, the range, median, and 75th percentile of days needed amongst all dogs and for each of the four countries separately was calculated.

## RESULTS

### Dataset

From the 101 GPS collars deployed, 50 (49.5%, Chad = 11, Guatemala = 15, Indonesia = 9, Uganda = 15) were retrieved and 44 (43.6%, Chad = 9, Guatemala = 12, Indonesia = 9, Uganda = 14) had a minimal recorded period of 10 days after the removal of the deployment and retrieval days. After data cleaning process, the number of complete days used for the UD calculation ranged from 17 to 58 days (median 33 days). After data cleaning, the number of GPS fixes per dog ranged between 1,516 and 3,415 (median 2,490) in Chad, between 1,986 and 2,010 (median 1,992) in Guatemala, between 1,149 and 2,361 (median 2,178) in Indonesia and between 2,573 and 7,647 (median 4,244) in Uganda.

### Home range size estimates

The estimated HR sizes varied between days under observation and isopleth levels (Figure 1). For the entire observation period, which varied from dog to dog, the core HR size (50%

isopleth) ranged between 0.16 and 0.89 hectares (ha) (median 0.41 ha) in Chad, 0.23 and 0.79 ha (median 0.42 ha) in Guatemala, 0.27 and 3.00 ha (median 0.43) in Indonesia and 0.27 and 1.48 ha (median 0.42 ha) in Uganda. The extended HR size (95% isopleth) ranged between 1.88 and 1,092.00 hectares (ha) (median 28.34 ha) in Chad, 1.92 and 94.16 ha (median 22.46 ha) in Guatemala, 2.42 and 86.32 ha (median 5.43 ha) in Indonesia and 2.06 and 41.65 ha (median 9.43 ha) in Uganda.


Home range, isopleth 50%

Home range, isopleth 95%

Fig. 1: Home range size of 12 dogs in Guatemala over increasing observation periods for core (50% isopleth, upper graph) and extended (95% isopleth, lower graph) HR. In black, are the lines for two dogs D015 and D017

Minimal number of days for percentages of change

The minimal number of days after which the percentages of change in HR size for each additional day of observation is below 10% and 20% was smaller for the core HR size (50%

isopleth level) than for the extended HR size (95% isopleth level), and varied between countries. Considering the total number of dogs from all countries, the observational period required varied between 2 and 32 days (median 4; 75th percentile 7) and between 2 and 17 days (median 2; 75th percentile 3) to reach a percentage of change in the core HR size of less than 10% and 20%, respectively (Figure 2). For the extended HR size (95% isopleth level), these numbers of observation days ranged between 3 and 38 (median 17; 75th percentile 26.25) and between 2 and 35 (median 8; 75th percentile 13) (Figure 2).

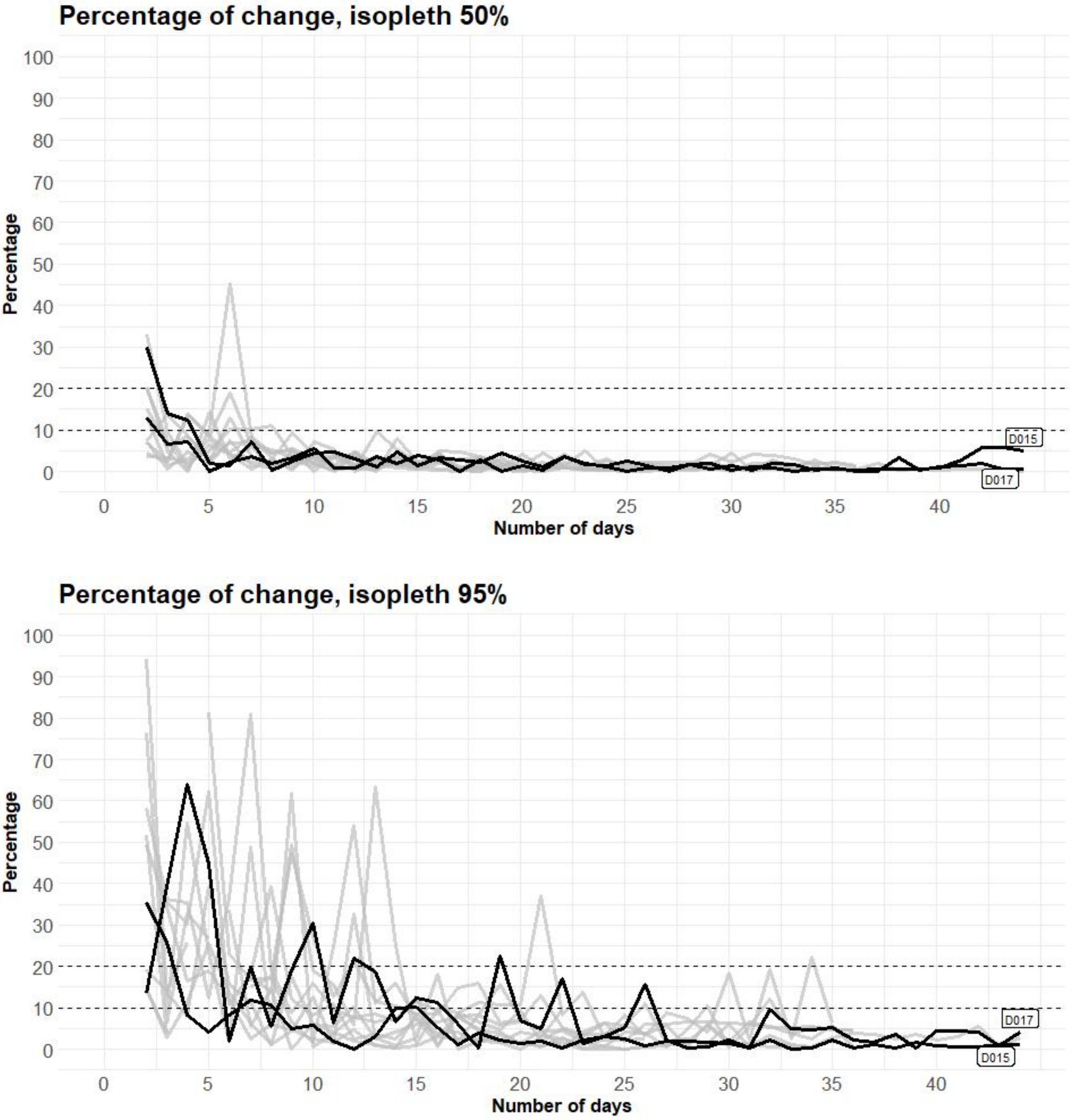

Fig. 2: Percentage of change in the home range size over time. Percentage of change in the home range size over time per dayfor the dogs collared in Guatemala, for core (50% isopleth) and extended (95% isopleth) HR. In black are the lines for dogs D015 and D017

Differences between countries were mainly visible in the upper statistical metrics (maximal and 75th percentiles) of the required observation periods. For the core HR size, the minimal number of days needed to reach a change in HR size below 10% ranged between 2 and 32 days (median 4; 75th percentile 17) in Chad, 2 and 9 days (median 5; 75th percentile 7) in Guatemala, 2 and 11 days (median 3; 75th percentile 9) in Indonesia, and 2 and 13 days (median 4; 75th percentile 5) in Uganda; while for reaching a change in HR size below 20% it was required to observe the dogs between 2 and 17 days (median 3; 75th percentile 4) in Chad, 2 and 7 days (median 2; 75th percentile 2) in Guatemala, 2 and 4 days (median 2; 75th percentile 2) in Indonesia, and 2 and 6 days (median 2; 75th percentile 2.75) in Uganda. These durations were found to be longer for the extended HR size: between 4 and 32 days (median 23; 75th percentile 27) in Chad, 7 and 35 days (median 18.5; 75th percentile 27) in Guatemala, 3 and 18 days (median 9; 75th percentile 14) in Indonesia, and 3 and 38 days (median 16.5; 75th percentile 26.75) in Uganda, for reaching less than 10% change; and between 3 and 19 days (median 15; 75th percentile 17) in Chad, 4 and 35 days (median 9.5; 75th percentile 13) in Guatemala, 2 and 13 days (median 4; 75th percentile 7) in Indonesia and 2 and 29 days (median 6.5; 75th percentile 9.75) in Uganda for reaching less than 20% change.

## DISCUSSION

In this study, the minimal number of observation days needed to capture a representative HR size of FRDD was explored in 44 dogs from four countries. The number of days required was found to be influenced by the accepted percentage of change in the HR size when data from an additional observation day were added (10% and 20% of change explored here) and the isopleth level used to define HR size (50% and 95% isopleth level explored here, representing the size of a core and extended HR, respectively). It seems that especially for the core HR, even for the more precise change of 10%, a stable value of HR can be achieved relatively quickly, with 50% of dogs reaching it within 4 days, and 75% within 7 days. As expected, it takes longer for the extended HR, with a median of 17 days for an accepted change of 10% in the HR size. Researchers may therefore be willing to accept a change in HR size of 20% to reduce the required observation period to a median of 8 days. It is thus needed to balance the resources (time and fund) available for a study with the accurateness of HR estimates accepted.

The results also highlight that the number of days needed to estimate a representative HR size highly varies between dogs, likely associated with the dog roaming behaviour type. The behaviour of FRDD is complex and influenced not only by the intrinsic characteristics of the animal, but also by the owner's behaviour and topographic conditions in the area where the animal lives (Warembourg et al., 2021b, 2021a). Studies showed that there is variation in FRDD HR size, not only between animals in the same area (Hudson et al., 2017), but also between different areas (Warembourg et al., 2021b, 2021a). A study performed in FRDD in Australia identified three main groups to which the dogs could be allocated according to their roaming behaviour: "stay at home dogs", with a stable or decreasing HR size, "roamers" with stable core HR but increasing extended HR size, and "explorers" with increasing core and extended HR sizes over the days of observation (Hudson et al., 2017). In the current study such heterogeneity between the dogs was observed, with some dogs requiring a much longer observation period to capture a representative HR size. Therefore, researchers should not only consider the accuracy and the isopleth level of interest, but also the characteristics of the study population. For heterogeneous populations it might be worth to consider longer recording periods, such as between two and three weeks, to study a representative extended HR from largely roaming dogs as well.

A limitation of this study was that a longer recording period would have enabled a better investigation of the HR sizes of the dogs with extended roaming behaviour, for which it was not possible to reach a percentage in change of less than 10% or even 20%. However, besides the battery capacity limitation, the longer the GPS unit was deployed onto the animal, the higher was the risk of loss or damage to the units. This risk was observed in our study, with only 43% of the GPS units being available for use after the end of the study period.

In conclusion, the study generated evidence that a observation period of three up to 26 days is enough to estimate representative HR sizes for 75% of the dogs, depending on the HR definition and accuracy level accepted by the researchers. However, dogs with extremely large roaming behaviour would need longer observational periods. Nevertheless, this study provides a first systematic analysis of the required observation period for FRDD, which could be used for guiding future studies of this highly relevant species in terms of animal and public health.

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# QUALITATIVE METHODS IN ANIMAL HEALTH

# THE INFLUENCE OF PEER NETWORKS ON ANTIBIOTIC PRESCRIBING PRACTICES: EVIDENCE FROM A QUALITATIVE STUDY WITH FRENCH POULTRY VETERINARIANS

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## SUMMARY

Antimicrobial resistance (AMR) is a major global public health problem, which is related to the misuse of antibiotics. The determinants affecting veterinary prescribing practices have been little explored in depth. This research addressed the social factors influencing antibiotic prescribing by poultry veterinarians in France by conducting sixteen in-depth interviews. It was evidenced that there are different influences between veterinary peers to prescribe antibiotics. It was found that junior veterinarians are accompanied by seniors in the “field training”, and that seniors can influence the antibiotic prescribing through imitation or repetition practices by juniors. Collaboration between veterinary colleagues in the discussion of cases was relevant to gain confidence and deliver coherent and unified messages to farmers. Finally, it became clear that corporate groups generate influence through formal approaches and protocols, such as the "case portfolio" that includes case treatment. Therefore, antibiotic prescribing can be influenced through veterinary leadership and networks.

## INTRODUCTION

Antimicrobial resistance (AMR) is currently a major problem in global public health, with the rapid spread of multidrug-resistant bacteria being particularly alarming and worrying (Dadgostar, 2019; Avershine et al., 2021). Misuses of antibiotics have been identified as an important driver of AMR emergence and spread. In this regard, efforts to reduce imprudent practices in relation with antimicrobial use (AMU) should target a variety of public health and veterinary actors, from physicians to veterinarians to the end-users and farmers themselves (Dadgostar, 2019).

Previous research in animal production systems has indicated that, in addition to technical factors, AMU on farms is also influenced by psychosocial determinants. However, most of the studies conducted so far have focused on farmers. For example, Wemette et al. (2020) showed that dairy farmers are often sceptical about the need for, and benefits of, reduced AMU, although they often rely on veterinarians as a source of information. Other work by Doidge et al. (2020) on perception and management of risks associated with antibiotic overuse by sheep

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and beef farmers revealed that farmers take preventative measures but deflect responsibility for reducing AMR to veterinarians and poorly managed farms. Albernaz-Gonçalves et al. (2021) explored practices of pig farmers towards AMU and AMR, and evidenced the dependence of farmers on antibiotics and a lack of preventive measures, with the misuse of and unrestricted access to antibiotics. They also showed that the use of antibiotics is legitimised, with a lack of preparedness and resistance to change in practices. In addition, previous research has stressed the need to explore farmers' professional networks and the role of interpersonal relationships in AMU reduction. Adam et al. (2020) evidenced that, in free-range broiler production in France, relationships of trust between farmers, technicians and veterinarians were crucial for transition towards reduced AMU. Such relationships were established progressively through shared responsibility, humility and reflexivity in disease management. Mutual help and support result in the sharing of decision-making processes between all the actors involved (i.e. farmers, technicians and veterinarians).

In relation to the interactions between different agents, previous studies carried out in hospital settings revealed that actors' practices in relation to antibiotic prescription may be influenced by informally established social norms or cultural rules. For example, according to Charani et al. (2018), in hospital settings, doctors are often influenced by those norms or rules coming from the work environments they are in. Within this dynamic, social rules specifically linked to antibiotic prescribing have been defined as the "etiquette of prescription". These norms or rules are socially and collectively established and accepted. It is important to note that this "etiquette of prescription" often brings together several elements, as the different degrees of hierarchy that exist between the various actors present in a setting, such as between experienced and inexperienced doctors.

While interactions between different agents operating on farms around antibiotic prescribing have been described in different settings, the determinants affecting veterinary prescribing practices remain poorly studied so far, specifically on the interactions between veterinarians themselves and their dynamics as a social collective. In this sense, the aim of this study was to investigate the formal and informal norms and rules established around the use of antibiotics in veterinary practices through the social factors that influence antibiotic prescribing by French poultry veterinarians.

## MATERIALS AND METHODS

A total of 16 in-depth interviews were conducted with poultry veterinarians belonging to several different veterinary corporate groups from the regions of Auvergne-Rhône-Alpes, Bretagne, Nouvelle Aquitaine and Pays de Loire in France. Poultry veterinarians from different age groups were contacted through snowball sampling (Naderifar et al., 2017); eight junior veterinarians (less than three years of experience), five intermediate veterinarians (between three and ten years of experience) and three senior veterinarians (more than ten years of experience). Sample size was set to meet sampling recommendations for qualitative studies regarding data saturation (Guest et al., 2006).

Prior to the interviews, an informed consent form was sent to all participants to guarantee the confidentiality of the data and information. Then, the interviews were conducted remotely and recorded via video call in February 2021. Interviews had a duration of between 41 and 119 minutes.

A semi-structured thematic guide was used for these interviews. The guide was based on the "Biographical Narrative Interpretive Method" described by Wengraf (2004), which focuses on the narrative expression of life stories and personal experiences, allowing people to express conscious and unconscious concerns. In turn, this method takes as a reference that psychosocial processes and dynamics can be explained by seemingly trivial events and anecdotes (Veith, 2004). Considering this method, this guide includes three elements: (a) "Single Question For Inducing Narrative", which is deliberately broad to allow the interviewee to elaborate, and thus make it possible to construct lifelines to identify critical events; (b) "Particular Incident Narratives", which are particular incidents, identifying changes in responses in relation to the first; and (c) "Potential Follow Up Questions", which allows relevant points to be discussed that have not previously been addressed. The interview guide was developed along the following axes: evolution of antibiotic prescribing in poultry, decision making and choice of antibiotic therapy in poultry, and informal professional learning and standards. The participants' responses were finally transcribed by an external transcription company.

The transcriptions of the interviews were analysed and coded using the NVivo software (Version Release 1.5 (935), QSR International), through thematic analysis (Braun and Clarke, 2006). The codes were grouped according to their coherence in certain themes, which were used to organise the findings. These codes were generated in three different phases: general coding, simplified coding and refined coding. This coding made it possible to identify recurrent themes, find similarities and differences, and highlight relationships in the different discourses.

## RESULTS

### Interactions between junior and senior veterinarians: The role of mentoring practices

All the junior veterinarians interviewed described the role of senior veterinarians, who take on a mentoring position. A characteristic of veterinary practices in the poultry sector is that young people consider their academic training as insufficient to start practising as professionals. In this respect, senior veterinarians, who have acquired and consolidated field experience, play an important role in acquiring and improving this knowledge. In addition to technical knowledge, the interviews showed that communication skills are useful in this role of the senior veterinarians:

> Veterinarian 5 "... I met my first boss ... who I consider my mentor as an avian veterinarian. He is really the one who taught me everything, who was very open ... He became my mentor because he was someone who was hyper open to discussion ... You could talk about everything with him, there were no stupid questions ..."

> Veterinarian 13 "... It's like that, veterinary studies are not necessarily very production-oriented, it's perhaps an understatement to say so, you leave the university with an academic knowledge of what is happening in the veterinary world in poultry production, but little idea of how to treat a batch ..."

This mentoring process is based on what interviewees called a "field training". This training plays a fundamental role. The first stage of field training consists in an immersion in complementary diagnostic techniques and veterinary consultations through farm visits, in which the senior veterinarian accompanies the junior veterinarian:

> Veterinarian 2 "... When you go out to the field, it is a bit different, in everything that is farm visits, but ... you have different phases of learning when you start ... When you start, you do a lot of necropsy, because you have to become very autonomous in necropsies ... And then, little by little, you move on to everything that is field advice, so you have to know a little more the productions, the ins and outs of each production .... And after that, all the farm visits ..."

Gradually, the junior veterinarian is taking autonomy and support from senior veterinarians becomes more sporadic:

> Veterinarian 4 "... So [after the farm visit] I do the debriefing with my colleagues when possible, when there is another one present on site, more and more they try to leave me in autonomy ... to validate the treatments I prescribe ..."

> Veterinarian 7 "... And then, little by little ... I started by doing health checks and things like that to help me and also to get to know the farmers quite simply ..."

> Veterinarian 8 "... Now I am more or less autonomous because I am the only veterinarian chair for the site, even if I still rely heavily on my colleagues [from the other practice] ... It's true that it's also thanks to them that I'm now autonomous in my diagnoses, autonomous in my way of doing things, but always in dialogue ..."

All interviewees explained that close relationships persist over time between junior veterinarians and mentors. Even when the “field training” step is over, junior veterinarians keep asking advice from their referee:

> Veterinarian 1 "... Sometimes, after a year and a half, I still call my bosses to talk about certain things. But even my bosses call each other sometimes. You can't know everything and you will never know everything in this business. It can happen to me ..."

> Veterinarian 14 "... It happens to me quite often because sometimes I want his opinion on what I have done, even if it's a case I've managed on my own, I'll still talk to him to find out if he would have done it differently or even for an opinion. If I have any doubts, I don't hesitate ..."

The mentoring practices described above result in the imitation or repetition by the junior veterinarian of the actions carried out by the senior veterinarian. A strong agreement between junior veterinarians and mentors was described by the interviewees. This situation may lead to homogeneity in prescribing practices:

> Veterinarian 2 "... There are a lot of things that I do here that I do because I know the bosses did it before. So, to the extent that we learn a lot from what they do, it's hard afterwards ..."

> Veterinarian 3 "... Because I have this habit of often asking, when I was starting out, the other people in my company how to manage cases, in the end I didn't make up my own mind, and I tend to do what they do, I often find myself agreeing with what they do ... When there is something I disagree with, I ask the question clearly, I say: "Yes, you have done that, why? Because I would have done this instead", but always, in every case, when I asked the question, there was an argument that seemed to me quite satisfactory and justified ..."

### Interactions between veterinary colleagues: Collective case discussion

All the interviewees stressed the importance of interactions and discussions with colleagues. Those interactions between colleagues are organized at different scales: within veterinary practices, and between the different veterinary practices which belong to the same corporate groups.

Those interactions are identified by interviewees as crucial, since it helps them not to feel isolated in situations where they may need help. They explain that being able to rely on their colleagues helps them to gain more confidence in their decisions:

> Veterinarian 1 "... Then, you are alone, physically, but you can always call someone really if you have a problem, if you need advice..."
>
> Veterinarian 3 "... I do a lot of internal exchanges, that is to say that I don't usually exchange on the subject with veterinarians outside the structure, but really internal with other veterinarians in the structure who have a little more experience ..."

Advice is often sought from colleagues in situations of doubt, or when facing complex clinical cases. Veterinary practice groups are organized with referents who play the role as “experts” for different aspects (e.g. specific production system), and can be contacted at any time by their colleagues:

> Veterinarian 2 "... I have a colleague who works a lot in the laying hen sector and it is true that when I have laying hens that are there, a sector that I do a little less, inevitably, if I have a doubt, I call her ... I called her because we were in a production where we could not use antibiotics as a treatment, as it was a production where the eggs were certified without antibiotic treatment ..."
>
> Veterinarian 4 "... There is a strong synergy, everyone has their field of skills. So at the beginning it is difficult because you have to identify each person's field of expertise, but you know that when you have a specific question you can ask such and such a person to exchange and discuss a case ..."

Regarding antibiotic treatments, advice provided by colleagues is based both on the practitioners’ experiences related to the indications and expected effectiveness:

> Veterinarian 3 "... When I discussed it with my colleague, afterwards, because I did not understand why ... he explained to me: "Amoxicillin, from experience, does not work in this type of production" ..."
>
> Veterinarian 4 "... In terms of the indications, the other veterinarians rather indicated to me the standard indications for which they are used ..."

Case discussion between colleagues from a veterinary practice or a corporate group aims at creating a shared view between prescribers. Interviewees stressed that consistency in discourse towards farmers is crucial for their professional credibility. Thus, in case of contrasted visions, case discussions allow the generation of a coherent and integrated discourse towards farmers:

> Veterinarian 3 "... And then, even when there are things that, in itself, I would not agree with, I also understand his choice, for example if ... I would have thought differently, I also understand my colleague's thinking and why he takes that decision ..."

> Veterinarian 4 "... I think it happens among veterinarians to have different perceptions of the same thing or apply different treatments. At the end of the day, the purpose of these exchanges is to discuss the differences in practices and see why they exist and how they can be integrated into an overall discourse that is consistent with farmers ..."

Veterinary corporate groups play an active role for structuring interactions between member veterinarians. Specific working groups organize (often based on production systems) meetings, phone conferences, and various social interactions so as to stimulate and lead discussions:

> Veterinarian 2 "... There are already meetings at [veterinary corporate group] level that are held every quarter ... We can go two and we talk about a lot of different topics that are topical .... We have phone meetings every month and then we also meet according to the groups we belong to. We also have a small quarterly meeting for the duck sector. So we see other people, but it is in smaller groups. Events like seminars, which are a bit more festive, are held once a year. But in the end, when we mix, if we are in several groups, it is quite easy to have contacts every two or three weeks ... At the big meetings, which gather about twenty or thirty people, we often have several working groups on different topics coming to show the progress of their work ..."

> Veterinarian 9 "... I think we do in-house training, what we call avian technical meetings, and we bring in outside speakers ..."

> Veterinarian 13 "... For some years now, we have had a monthly telephone meeting in our system, during which we discuss the clinical cases we have had to deal with clinical cases, evolution of pathologies, new things. This allows us all to be a little bit aware of what is going on and to know what has worked as a therapy in this or that case or what has failed, because it is important to communicate about failures too ..."

On the other hand, there may be competition between veterinarians, especially between veterinarians from different veterinary corporate groups or farmers' cooperatives. In the case of such competitions, relationships remain cordial:

> Veterinarian 6 "... Today we have [this corporate veterinary group] and [the other corporate veterinary group], which are two groups of private veterinary practices competing with each other in the field, and we are also in competition with the veterinarians of the cooperatives, who intervene on behalf of the members of their cooperatives ... The cooperative member farmers have access to the service of the cooperative veterinarian, but they can choose to have a second veterinarian, either because of a question of fees, a question of availability, but in this case, we are still in contact with the cooperative veterinarian ... [Between corporate veterinary groups] we can have farmers who are between the two, or even farmers who are closer to us, but who are their clients, and vice versa ... Compared to cooperative veterinarians, we are in competition in the sense that we are paid for the sale of medicines or food supplements and therefore, depending on who sells the product, the veterinarian gets paid or not ... The relations are cordial, let's say with my direct competitors ... we do not have direct relations on a daily basis ... The clients are well established, so there is no particular animosity ..."

Despite this competition between these veterinarians, sometimes veterinarians from one corporate group may contact veterinarians from another group. Such contact depends mainly on the geographical areas in which the farms are located, which determines the degree of involvement of the veterinarians on those farms. However, both veterinarians consider the interests of the farmers and their animals as a key element of working together:

> Veterinarian 6 "... There is a professional relationship and we meet in certain areas. We meet because there is a desire to have veterinary contact, so we have to be on the same wavelength ... It is always in the interest of the farmer. So, if one veterinarian has been before, either one or the other, and the other one comes back afterwards, inevitably there is a discussion because you have to do it in the interest of the farmer and in the interest of the health of the animals on the farm. But sometimes it can be "first come, first served" who makes the diagnosis and prescribes the treatment. There is always a relationship, at least over the phone, with the veterinarian because we work in the interest of the farmer ..."

<u>Interactions within veterinary corporate groups: Veterinary prescribing guidelines</u>

Interviewees detailed how recommendations on AMU reduction have been translated by veterinary practices and corporate groups into formal internal approaches:

> Veterinarian 4 "... Within the [corporate veterinary groups] we have a formalized approach to reducing antibiotics ... It is a somewhat global approach to formalise the use of vaccination, alternative solutions and biosecurity as an approach to reduce the use of antibiotics without depriving the use of antibiotics, but having good usage practices ..."

In addition to informal “field training” implemented at the arrival of junior veterinarians, the mentoring described above is formalized in some groups in the form of clearly identified training activities. Besides technical aspects, training also tackles soft skills, knowledge of the working environment, and finally sharing the “group culture”:

> Veterinarian 2 "... I came to a structure that had a training system for these young newcomers that was really well developed ... We have a certain number of training days per year, which is defined by the group, in particular for each young person, which is at least ten days of training per year ... We had a kind of small catalogue with different training courses that were offered ... There is a whole training objective, of youth welfare, of youth inclusion, which plays a huge role in this ... It really made sure that the young people could also meet from the different structures and exchange, share moments ... There are some kinds of meetings and seminars that were also held just for educational purposes and to promote understanding between young people. In particular, we spend a weekend together in a place paid for by the partners.... But we also do not do the same as in other companies ..."

> Veterinarian 12 "...  As a result, that's what we've always tried to do, to have at least a three-month training course on different sites, to also see the laboratory part ... that's important too ..."

> Veterinarian 13 "...  Afterwards, we train a lot internally, as I was saying, the advantage of being spread over five sites with about fifteen veterinarians, there is plenty of time to interact with each other, to see the different situations ..."

Furthermore, veterinary practices and corporate groups edit formal internal protocols such as the "case portfolio". An example of this type of internal protocols is a “treatment guide” which includes treatment recommendations for bacterial infections based on a probability approach for antimicrobial susceptibility testing. This portfolio is maintained by different veterinarians over time, and is updated on a regular basis:

> Veterinarian 6 "... We do statistics at [corporate veterinary group] level ... We produce annual statistics on the susceptibility, by species, of each bacterium, to antibiotics. This gives us an idea and we can do this at a national level as well as at regional or departmental level ... We create a small booklet, called [name], which includes all these statistics by species and by bacteria ... It is published every year and sent to all the group practices and to all the veterinarians. It is published for all species of poultry, pigs, dairy cows, sheep and goats ... We also have fact sheets, technical sheets and information sheets on the different products in the range and their use ..."

Lastly, corporate groups use internal databases, specific shared software, and even internal social networks, to facilitate access to material for comparing cases and using experience from colleagues to make decisions:

> Veterinarian 4 "... We have our software to record necropsy cases and this allows us to see what has been done in equivalent cases ... You have the assessment of the injury and the complementary examinations and the treatment that has been done ..."

## DISCUSSION

Antibiotic prescribing by veterinarians is influenced by the mentoring of senior veterinarians over juniors, discussions among veterinary colleagues and guidelines held by veterinary corporate groups.

The influence of senior veterinarians on juniors can be partly explained by the fact that seniors want to maintain a good relationship with their farmers in the long term (Coyne et al., 2016). In this sense, in the relationship between veterinarians and their farmers, trust is important for seniors, who tend to communicate their actions better than juniors (Higgins et al., 2017). To prevent trust between senior veterinarians and farmers from being affected, seniors may marginalise juniors in relation to the prescription generated, although juniors may express their intention to be more involved in the prescription process (Higgins et al., 2017). In fact, junior veterinarians sometimes do not express this intention and act behind the seniors' backs, without communicating what they have done, a situation that can hinder further interactions between the two, as in the discussion of cases. Similarly, in human medicine, hierarchical differences are respected by actors, both between junior and senior doctors and between colleagues, in order to avoid direct criticism, which can be uncomfortable and affect subsequent working relationships (Livorsi et al., 2015). In this regard, learning environments can be an appropriate time to communicate the intention of junior veterinarians and prevent these relationships from being negatively affected. However, in public health it is described that senior doctors may not be supportive or available to juniors. Indeed, senior doctors may adopt a critical stance that could affect the trust of juniors (Mattick et al., 2014); whereas between senior and junior veterinarians such support and availability exists, although the trust of juniors could also be affected by limited involvement in decisions with seniors.

It was evident that “field training” is a crucial element in the mentoring relationships established between junior and senior veterinarians. It is noteworthy that similar training has already been described in human medicine. From observations made at hospitals, different training steps have been described in the literature (Mattick et al., 2014): induction, supervision and feedback, and teaching in the field. Induction includes protection in adaptation, briefing and preventive meetings, and written procedures (e.g., guidelines for ward-specific prescribing decisions), in a similar way to the training of veterinarians. However, guidelines may not always be known to doctors. Supervision and feedback from senior doctors to juniors may be deficient, but they are helpful between junior and senior veterinarians. And teaching in the field is appreciated for extensive discussions on particular cases with solid arguments, which is also the case among veterinarians. In the same regard, it is interesting to note that junior doctors in training may be strongly influenced by seniors, feeling confident and comfortable with this and imitating or repeating their prescribing practices. However, these practices may not conform to standard guidelines. In spite of this, junior doctors may expose themselves to criticism when such imitation or repetition does not occur (Livorsi et al., 2015).

Specifically, on the discussion of cases among veterinarians, there are other animal production systems where the possibility of generating such discussion sessions seems to be complex to carry out, especially with more experienced veterinarians (Higgins et al., 2017). For example, previous work showed that veterinarians in swine production tend to work independently, and they only call on others when their expertise is not sufficient, whereas in mixed veterinarians such interaction with other veterinarians may be more routine (Coyne et al., 2016). The findings of this research reveal that French poultry veterinarians had a greater willingness to have routine interactions with their colleagues. The organization of their daily work even make it possible to have specific days dedicated exclusively for exchange of experiences with colleagues that, in turn, result in efficient field learning for junior veterinarians. A previous study has evidenced that workshops and case discussions are also a requirement in other types of animal production, and that such workshops involved not only veterinarians but all stakeholders (Rell et al., 2020).

Previous research has revealed that case discussions may focus primarily on the cases themselves rather than the antibiotics, especially when veterinarian specialists are available (Hardefeldt et al., 2018). In contrast, the present results showed that discussions between veterinarians involve both clinical aspects (e.g. diagnostic and farm management) and treatments (including antibiotics), and that these two intertwined aspects cannot be dissociated. Furthermore, when different perspectives exist between veterinarians, the discussion process aims to generate consensus prior to any interaction with the farmer. In case consensus is not reached, the contradiction may be appreciated by farmers and may affect their trust in the veterinarians (Golding et al., 2019).

In general, the veterinary interactions, both between senior and junior veterinarians as well as between veterinary colleagues, may cause veterinarians to put aside formally acquired professional knowledge, with it being more attractive to carry out what other veterinarians do (Hardefeldt et al., 2018). These interactions can maintain practices that are and have been routinely performed in the field, as senior veterinarians can draw on their experiences, while juniors can draw on the experiences of seniors (Hardefeldt et al., 2018). This maintenance of practice can also be observed in corporate veterinary groups, where it appears that the existence of formal internal approaches is mainly based on the previous experiences of their members (Coyne et al., 2016).

This study shows that antibiotic prescribing by veterinarians is conditioned by interactions between veterinarians, which may make it difficult to develop strategies to reduce and refine antibiotic prescribing. In this sense, interventions addressing antibiotic prescribing should not only focus on interactions between veterinarians and other stakeholders, but also use the clinical and non-clinical leadership and social networks of veterinarians to influence veterinary prescribing practices.

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# A QUALITATIVE APPROACH TO INVESTIGATE HOW PLACE AND RISK INFLUENCES SHEEP FARMERS' APPROACHES TO THE MANAGEMENT OF SHEEP SCAB

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## SUMMARY

The aim of this study was to understand how place influences sheep farmers' approaches to the identification and management of the risk of sheep scab in their flocks. Qualitative data was collected from 43 semi-structured interviews with sheep farmers from Great Britain and was analysed by using the constant comparative approach. The narratives shared by the farmers identified three categories which the farmers could be placed in: 'uncontrollable locations', 'liminal locations' and 'protective locations'. The identification of the three categories depicted three strategies that the farmers used to control sheep scab based on their risk, which were driven by their attachment to place. These results suggest that place-based effects have significant impacts on sheep farmers' beliefs and behaviours and thus should be considered by policymakers when developing future strategies for sheep scab control.

## INTRODUCTION

In Great Britain (GB) the effective management of sheep scab has proved to be a long and intractable challenge (Chivers et al., 2018). Sheep scab was made notifiable in GB in 1869 when all outbreaks were documented (Kirkwood, 1986; ADAS, 2008). The national control programme was responsible for the eradication of sheep scab in 1952. However, in 1973 sheep scab was reintroduced when it was discovered in Lancashire from the importation of infested sheep (Loxam, 1974). Sheep scab was deregulated by the government in June 1992 when they ruled that eradication was unsustainable and costly (ADAS, 2008). Sheep scab is now endemic in GB, with national prevalence estimated around 9.0%, with significantly higher regional incidences in Wales, Scotland, and the North of England (Bisdorff et al., 2006).

There have been several industry-led initiatives to control and manage sheep scab since it was deregulated (ADAS, 2008; Geddes, 2021), but there has been no evidence to suggest that they have been successful in reducing the prevalence of the disease. Studies have suggested that new initiatives should instead focus on specific places, such as 'hotspot' areas (defined as areas that have a higher-than-average prevalence of sheep scab) (Rose et al., 2009; Phythian et al., 2013; Chivers et al., 2018). It has been suggested the consideration of these places may allow for a more targeted approach by initiatives, where control measures could be implemented by all sheep farmers within them.

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Geographers have long considered the importance of place in human medicine as a contributing factor of health (Macintyre et al., 2002). Places have both a typology of risk and protection, provided by the physical and social properties (Fitzpatrick and LaGory, 2000). Place attachments and place meanings can determine how individuals assign risk to localities; these in turn can influence the way they adapt to place (Quinn et al., 2018).

The aim of this study was to gain an understanding of how place influences sheep farmers' approaches to the identification and management of the risk of sheep scab through semi-structured interviews.

## MATERIALS AND METHODS

### Study sample and data collection

A purposive sampling strategy was employed to allow the authors to recruit respondents from both 'hotspot' and 'non-hotspot' areas. The farmers were recruited from six areas of GB and identified by key contacts: veterinarians (who will be referred to as vets from hereon), merchants, livestock markets and contract sheep dippers within these areas. The six areas were selected on their prevalence of sheep scab based on previous outbreak maps and farm connectivity maps, and were identified as either a hotspot or non-hotspot area (Rose, 2011; Nixon et al., 2020).

Farmers were selected from four hotspot areas: Peeblesshire in Scotland, North Wales, Herefordshire, and Devon and two non-hotspot areas: Leicestershire and Northumberland. Inclusion criteria were commercial sheep farmers with a minimum of 120 sheep within the specified areas described above. All recruited farmers were contacted by telephone by the first author to arrange a suitable date to interview.

A semi-structured interview guide was developed and informed by literature on the current behaviours of managing sheep scab (Cross et al., 2010; Phythian et al., 2013) psychosocial constructs and concepts such as risk and reflections (Michie et al., 2011). Two pilot interviews were conducted with a convenience sample of sheep farmers known to some of the authors and further changes were made to improve the wording of the interview guides.

Individual interviews with farmers were conducted by the first author over the telephone between 20th July 2020 and 4th September 2020. The farmers were informed that the interview data would be used for research purposes and that they would remain anonymous in any outputs. The interviews were then transcribed verbatim by an independent transcription company.

The study was approved by the University of Nottingham School of Veterinary Medicine and Science Ethics Committee (No. 2625 181105).

### Qualitative data analysis

The constant comparative method was used to look for similarities and differences in the data based on the theoretical underpinnings of risk and place (Maykut and Morehouse, 2002). Analysis was supported using NVivo (QSR International Pty Ltd. Version 12, 2018). The initial codes were developed inductively, where codes were derived from interpreting the data. Patterns in the data were identified to construct three categories: 'uncontrollable locations', ‘liminal locations’ and ‘protective locations’. These categories depicted three strategies that farmers used to control sheep scab which were driven by their attachment to place.

## RESULTS

Forty-five interviews were conducted with sheep farmers, with two excluded from data analysis as they did not meet the inclusion criteria. Therefore, the remaining 43 interviews lasted between 15 and 50 minutes and were audio recorded with the farmers’ written/verbal consent.

### Uncontrollable locations

Farmers in this category were described as being located in “hotspot” areas which experienced a high frequency of sheep scab outbreaks in the last ten years. They recognised that they were farming in an area with an increased risk of sheep scab infestations and described sheep scab as highly prevalent and almost omnipresent in nature:

‘Well, there is always scab in the area, there is always scab around this area.’ Respondent I29 (Hotspot farmer with 10 outbreaks in their flock).

Respondents identified a range of risks posed by the physical environment. They suggested that livestock markets were high-risk spaces in which sheep scab was highly prevalent, and held their neighbours accountable for sheep scab transmission into their flocks:

‘Well, I dunno to be honest. It’s the next-door farmer or the markets’ fault to bringing the scab into my farm, ‘cause there’s hardly any sheep on my boundary so most of the farms with cattle - so it’s the marketplace, it needs sorted out to be really.’ Respondent I39 (Hotspot farmer with 4 outbreaks in their flock).

The appearance of sheep scab in their flocks was described as unpredictable, even mysterious in nature, and they felt they were unable to determine where the infestation had come from:

‘It just pops its head up.’ Respondent I1 (Hotspot farmer with 15 outbreaks in their flock).

‘We have had an incident last year or the year before, we’re not really sure where it came from.’ Respondent I12 (Hotspot farmer with 4 outbreaks in their flock).

The respondents’ fatalistic views about the uncontrollable nature of sheep scab and their ability to control risky neighbours influenced the way in which they sought to manage the disease on their farms. They doubted the need to seek veterinary advice to facilitate the identification and management of the disease on their farms:

‘I don’t actually go to the vet or anything, which perhaps I should do, but I don’t think we’ve ever had a case of lice before, and it might sound a bit Heath Robinson, but I probably inject… I think I know what scab looks like […] enough. And usually I’m right, unfortunately. <Laughs> We’ve experienced it before’. Respondent I17 (Hotspot farmer with 6 outbreaks in their flock).

They also reported using multiple treatments each year to prevent or control sheep scab. Some farmers also described using the organophosphates within showers and sprayers, which are unapproved application methods for this product:

‘We dip in the autumn, sort of now, the end of August/beginning of September to prevent sheep scab but we usually have to dip or inject in the spring. […] We spray dip them and we plunge dip all the ones on the Common and all the ones on the main holding and then spray dip the ones on another holding, but I mean that’s all Gold Fleece [diazinon].’ Respondent I29 (Hotspot farmer with 10 outbreaks in their flock).

This category represented a group of farmers who were high-risk for sheep scab infestations which was linked to their geographical location. They also increased their own risk of sheep scab by the lack of veterinary assistance and the ineffective treatment strategies used.

Liminal locations

Farmers in this category were also characterised as being located in ‘hotspot’ areas, but as having experienced no outbreaks or a low frequency in the last ten years:

‘But at the moment I would say that most of my regular neighbours around here are, as far as I know, touch wood, don’t have it, but I know of areas close to where I farm where there are scabby sheep and have been scabby sheep.’ Respondent I8 (Hotspot farmer with 0 outbreaks in their flock).

In similarity with farmers that characterised the ‘uncontrollable locations’ category, the farmers identified the same risks posed by their physical and social resources, including livestock markets:

‘Our biggest problem in [area] is the market I’m afraid. [The] market is rife with it’. Respondent I7 (Hotspot farmer with 0 outbreaks in their flock).

The farmers within this category characterised risk in terms of sheep scab potentially placing their livelihood and reputation at risk:

‘Well it’s fairly embarrassing really. If you sold a load of sheep for good money and then suddenly they ring you and say they’ve got scab in the flock, you’d feel fairly embarrassed and you know darned well that they won’t come and buy your stock next year.’ Respondent I7 (Hotspot farmer with 0 outbreaks in their flock).

The farmers also expressed that their motivations for preventing sheep scab came from a sense of responsibility for their neighbouring farms:

‘It’s the inconvenience and the effect on other people, immediately you have to tell all your neighbours because if you don’t and they get it and don’t know they can spread it to their neighbours. I mean I really believe it’s important to tell everybody.’ Respondent I8 (Hotspot farmer with 0 outbreaks in their flock).

Many of the farmers in the category reported implementing biosecurity measures to prevent sheep scab. They also avoided the identified risky practices such as buying from markets:

‘Being very careful about where we buy our replacements, only buying from well-known sources and trying to steer clear from livestock markets.’ Respondent I9 (Hotspot farmer with 1 outbreak in their flock).

Some farmers within this category also made use of their physical resources such as woodlands, roads, and arable fields within or next to the farm to protect against sheep scab, instead of implementing biosecurity measures:

‘We’re fortunate that the fields we have are mainly… aren’t touching anybody else’s sheep, that’s just how it is, we’ve got orchards and corn land so we’re fortunate that they don’t really touch anybody else’s.’ Respondent I21 (Hotspot farmer with 1 outbreak in their flock).

These farmers also made use of their vet to diagnose and advise on sheep scab and identified the importance of veterinary assistance:

‘To identify it we get the vet to confirm it and the last couple of times we’ve had it, but traditionally we’ve just looked at sheep, seen the symptoms and self-identified as being scab. The reason we started getting the vet involved now is there’s more cases of lice and scab, so we’re making sure we’re treating freshly.’ Respondent I6 (Hotspot farmer with 2 outbreaks in their flock).

This category therefore represents a group of farmers who were at high-risk for sheep scab because of their place but were protected against this risk by adopting certain measures. Like the farmers in ‘uncontrollable locations’, they identified their neighbours and markets as risky sources of sheep scab. However, these farmers characterised their risk by protecting their reputation and felt a greater responsibility for preventing sheep scab spreading to their neighbours’ farms. This influenced them to adopt biosecurity measures and engaged in more protective measures to prevent sheep scab infestations in their flocks or others.

### Protective locations

This category is characterised by farmers who farm in non-hotspot areas with a very low frequency of outbreaks in the previous ten years. They were aware that their farm was low risk geographically for sheep scab and thus provided them with some form of protection:

‘As far as I know there’s no incidences of sheep scab that I’ve heard of in this area, at all.’ Respondent I31 (Non-hotspot farmer with 0 outbreaks in their flock).

The farmers in this category identified the same risk factors for sheep scab as the farmers in the previous two categories:

‘It’s always been caught from a neighbour, you know, over the fence contact. We’ve never brought it into an area ourselves.’ Respondent I10 (Non-hotspot farmer with 2 outbreaks in their flock).

The farmers within this category characterised their risk of sheep scab in a similar way to those farmers within ‘liminal locations’. They felt sheep scab was damaging to their reputation and that they had a responsibility to protect their neighbours:

‘So as far as we are concerned prevention, prevention and that’s the end of it. I don’t want to be hated by my neighbours either, to phone up and tell them, “Oh, by the way we’ve got sheep scab”.’ Respondent I13 (Non-hotspot farmer with 0 outbreaks in their flock).

The respondents suggested that their geographical location provided a protection against sheep scab infestations which allowed them to avoid implementing some biosecurity measures, such as good neighbours:

‘Well it depends what you’re next to. If you’re next to somebody that’s a stocks-person and looks at sheep and is careful and treats for scab, then no. But if you’re next to somebody that’s rough and ready and doesn’t treat them or only treats some of them or misses sheep when they gather, then yes, I suppose it’s worth it.’ Respondent I5 (Non-hotspot farmer with 0 outbreaks in their flock).

They also described how their physical resources of the farm also provided protection against sheep scab:

‘We try to keep, as much as we can we try to keep sheep away from neighbours’ sheep. It can’t always be done but use roads for instance as B boundaries [roads], keeps you away from neighbours’ flocks.’ Respondent I5 (Non-hotspot farmer with 0 outbreaks in their flocks).

The farmers also voiced the importance of using veterinary assistance to confirm sheep scab if they suspected an outbreak in their flock. They exemplified the importance of using diagnostics, rather than making decisions based on visible signs:

‘Part of the trouble with scab is that it takes a long time to appear, from a sheep being infected it takes quite a few weeks for it to appear and you’re not keeping your eye on the ball because you're assuming that they haven’t got it and then suddenly it appears and you’ve got it and you think ah!’ Respondent I10 (Non-hotspot farmer with 2 outbreaks in their flocks).

This category therefore represents a group of farmers who are low risk for sheep scab because of their geographical place. These farmers identified the same physical and social risks as the previous two categories. In similarity to the farmers within the ‘liminal locations’, they also characterised their risk in terms of their reputation and the responsibility they held for protecting others. However, because of their low place-based risk of sheep scab these farmers did not always engage in biosecurity measures.

## DISCUSSION

To the authors’ knowledge, this is the first study to provide insights into the way sheep farmers within GB identify and manage sheep scab in their flocks through the theoretical lens of risk and place. The views and descriptions generated three categories which the farmers could be placed into based on their relations with place and their perceptions of the risk of sheep scab: ‘uncontrollable locations’, ‘liminal locations’ and ‘protective locations’. The three categories identified similar risks factors for sheep scab infestations, but they characterised and managed this risk on their farms differently.

The farmers within the ‘uncontrollable locations’ category characterised the risk of sheep scab as a disease which was mysterious in nature. The farmers within the other two categories instead characterised their risk in terms of protecting their reputation. These differences in the characterisation between the three categories restates how place impacts the beliefs these farmers hold. Therefore, these findings emphasise the need for policymakers to engage with different farmers understandings of sheep scab when proposing new control strategies.

Some of the farmers within the ‘liminal locations’ category were more likely to adopt biosecurity measures and avoided engaging in risky practices. The farmers within this category had recognised that they were able to manage their high geographical risk by not sourcing sheep from markets and utilising advice from their vets. This contrasted with the farmers within the ‘uncontrollable locations’ category who appeared to be less likely to adopt biosecurity measures. These farmers engaged in risky practices such as sourcing sheep from markets and using multiple treatments per year for the prevention and control of sheep scab. Consequently, the risky practices had become embedded in farmers’ social activities and considered as a normal part of their sheep scab control (Zinn, 2017). Many of the farmers within the ‘protective locations’ category relied on geographical features, such as roads and woodlands, as protective measures instead of employing conventional biosecurity measures. Their low geographical risk allowed this to be largely effective, so they still only experienced a low frequency of outbreaks. Markets and neighbours were identified as risks for sheep scab infestations by farmers from all three categories. Some of the farmers from the ‘uncontrollable locations’ and ‘liminal locations’ focused on the external risks to their flocks, rather than recognising the risks they had created through their own behaviours. This suggests that these farmers were engaging in a concept called ‘othering’. Othering defines and secures self-identity by distancing oneself from others or outgroups which are considered as posing a risk to their self-identity (Grove and Zwi, 2006; Ruston, 2009). By deflecting responsibility from themselves it allowed the farmers to identify markets and neighbours as the archetypical ‘other’ posing risks to the health and welfare of their flocks.

In conclusion, this study identified that place and risk have a significant impact on sheep farmers’ beliefs and behaviours, which permitted the farmers to be placed into three categories: ‘uncontrollable locations’, ‘liminal locations’ and ‘protective locations’. These beliefs and behaviours reported are of importance to policymakers to consider when developing policy to ensure that it reflects the place-based effects found. It also suggests that if sheep scab control strategies fail to acknowledge the place-based effects found, sheep scab may continue to be a long and intractable challenge in GB for years to come.

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# ANTIMICROBIAL USAGE IN FARM ANIMAL PRACTICES IN THE UK: A MIXED-METHODS APPROACH

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## SUMMARY

Monitoring antimicrobial usage in farm animals is crucial for tackling antimicrobial resistance. A cohort study using the electronic clinical records during 2019 from 23 farm practices across the United Kingdom estimated the usage of antimicrobials and highest priority critically important antimicrobials (HP-CIAs). Risk factors for using HP-CIAs were evaluated using hierarchical modelling. Using a qualitative approach, veterinarians from one of the participating practices were interviewed to explore the influences on antimicrobials use. These interviews were analysed thematically. During 2019, 98,824 antimicrobial events overall were recorded from the treatment records of the participating practices. Country, route of administration, season and the practice type were significantly associated with the usage of HP-CIAs. Four main themes were identified from the thematic analysis of the veterinarians' interviews: pressure from the industry, drug-related factors, good knowledge and clinical factors. Integrating the quantitative and qualitative findings can inform policymaking on antimicrobials stewardship in farm practice.

## INTRODUCTION

Antimicrobial resistance (AMR) is a growing public health problem (O'Neill, 2014). Inappropriate use of antimicrobials could potentially increase the risk of resistant infections emerging. Previous studies have reported an association between antimicrobials usage and AMR in *E.coli* animal isolates (Asai et al., 2006; Chantziaras et al., 2013; Holmer et al., 2019). Further, the risk of resistant infection transmission between animals and humans has been reported (Barza, 2002; Hammerum and Heuer, 2009). Therefore, monitoring the usage of antimicrobials in animals is essential to assess the risk of AMR from animals on public health.

Based on their importance to human medicine, antimicrobials are categorised by the World Health Organisation (WHO) into critically important antimicrobials (CIAs) and non-CIA (WHO, 2019). CIAs are further prioritised into highest priority (HP-CIAs) and high priority based on the frequency of use in humans and the extent of evidence of AMR infection transmission from non-human sources for which the antimicrobial is needed (WHO, 2019). However, the WHO recommend that each country or region develop its own list of CIAs based on risk assessment of antimicrobial usage at a national level (WHO, 2019). In response, the

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European Medicines Agency (EMA) further classified the WHO list of HP-CIAs into two categories based on the extent of risk for public health: Category one, where the risk is considered low or limited, which includes macrolides and; Category two, where the risk is considered high, which includes 3rd and 4th generation cephalosporin, fluoroquinolones and colistin (EMA, 2014, 2016).

Few studies in the United Kingdom (UK) have used the prescribing records from veterinary practices to estimate antimicrobials usage at the farm level (Davies et al., 2017; Hyde et al., 2017). These earlier studies used a small sample of farm practices four years ago. Estimating more recent prescribing patterns of antimicrobials across a larger sample of farm animal practices would allow a better understanding of current antimicrobials usage in food-producing animals.

Understanding risk factors for HP-CIAs usage can help in developing targeted policies for high users. Several farm characteristics have been reported as being associated with high usage of HP-CIAs. A Canadian study reported an association between high milk production, high antimicrobials use overall and high usage of dry cow therapy with ceftiofur and cephalosporin (Saini et al., 2012). That study also reported an association between farm size and high ceftiofur usage rate in Canadian dairy farms (Saini et al., 2012). A previous study reported higher usage of the HP-CIAs in dairy farms than in beef farms in Italy (Ferroni et al., 2020). Another study reported a significant difference of HP-CIAs usage between lowland and mountain dairy farms in Italy (Zuliani et al., 2020).

Understanding the rationales and perceptions behind prescribing patterns of antimicrobials by veterinarians is crucial for improved antimicrobial stewardship. Veterinarians' lack of knowledge (Mateus et al., 2014; Higgins et al., 2017) and advice from senior veterinarians were reported to influence antimicrobials prescribing (Coyne et al., 2018). Time pressures and the prescription of antimicrobials by colleagues were also reported to affect veterinarians' prescribing behaviour (Doidge et al., 2019).

Farmer-related factors have been reported to influence the veterinarians' prescribing decision, including the worry over farmers' adherence to the antimicrobials administration guidelines, farmers’ pressure to prescribe antimicrobials and the farmers' unwillingness to change their usual practices (Speksnijder et al., 2015a; Speksnijder et al., 2015b; Coyne et al., 2016; McDougall et al., 2017; Coyne et al., 2018). The economic considerations of farmers were also found to be an influence (Speksnijder et al., 2015a; Postma et al., 2016; McDougall et al., 2017; Doidge et al., 2019). Additionally, the implemented control measures and clinical signs presented on the farm were determined to be affecting veterinarians' prescribing decisions (Coyne et al., 2016; Postma et al., 2016; Coyne et al., 2018; Doidge et al., 2019). Drug-related factors such as the withdrawal period, drug formulation (Coyne et al., 2016) and route of administration (Speksnijder et al., 2015a) were also reported to be affecting the veterinarian's choice of antimicrobial.

This study aimed to evaluate the overall usage of antimicrobials and HP-CIAs in farm animals using the prescribing and dispensing records from a large number of veterinary practices in the UK. Furthermore, this study explored the drivers behind antimicrobials usage to support policy making on the responsible use of antimicrobials in farm animals.

## MATERIALS AND METHODS

### Quantitative study

Descriptive analysis: A retrospective cohort study design was used to conduct a quantitative descriptive analysis of antimicrobials usage events and to explore risk factors for using HP-CIAs by using the electronic patient records (EPR) from 23 farm and mixed veterinary practices participating in VetCompass in 2019 from two corporate groups. The VetCompass programme collates anonymised clinical records from veterinary practices in the UK for research purposes (O'Neill et al., 2014).

The current study focused on antibacterial antimicrobials defined as medicines that can destroy or inhibit the growth of bacterial microorganisms (Giguère, 2013) that were authorised for systemic usage in farm animals in the UK. Intramammary products were considered systemic treatments. Antiviral, antiprotozoal and antifungal antimicrobials were not included in the analysis. The EMA classification was used to categorise the antimicrobials agents as HP-CIA (EMA, 2014, 2016).

A master antimicrobials table with a comprehensive list of all active substance and commercial names for systemic antimicrobial agents authorised for farm animals in the UK was created by searching the Veterinary Medicine Directorate (VMD) and the National Office for Animal Health (NOAH) databases. Search terms were created for each active substance and antimicrobial commercial name to allow identification of all antimicrobials licensed for farm animals in the EPR (e.g. amox* for amoxicillin). Information on antimicrobial class, drug formulation and route of administration was added for each antimicrobial agent in the master antimicrobial table. A list of all unique treatment item names and IDs from all treatment records during the entire year of 2019 was extracted from the VetCompass database for the participating farm practices. The search terms for active substances and commercial antimicrobial agent names derived from the master antimicrobials table were then used to extract the unique list of farm animal antimicrobial item names by screening the list of all unique treatment item names. An antimicrobial mapping table was created by joining the master antimicrobials table with the VetCompass list of unique farm animal antimicrobial item names and IDs. The fields available in the final antimicrobial mapping table included the unique farm animal antimicrobial item names (as recorded by the practices), item ID, active substance, antimicrobial class, drug formulation and route of administration.

All treatment records in 2019 were extracted from the VetCompass database for the participating farm practices. Additional information extracted in the query for each treatment record included animal, client and practice IDs, species, breed, treatment ID and date, number of units sold, and partial client postcode. This treatment data table was joined with the antimicrobials mapping table via a MS Access query to identify all farm animal authorised antimicrobial treatment records during 2019 for the participating practices. Data validation and checks were conducted using MS Excel and Stata.

Risk factor analysis: A *season* variable categorised the season of treatment based on the date of treatment into winter (December to February), spring (March to May), summer (June to August) and autumn (September to November). A binary *practice type* variable was created based on information from the practices' websites (exclusively farm or mixed practice). Information on practice accreditation and the presence of farm-specific specialist veterinarians was extracted from the Royal College of Veterinary Surgeons (RCVS) website. A *Practice accreditation* variable categorised practice accreditation as general farm practice, other nonfarm accreditation, none and not applicable. A farm specialist variable was categorised as 'yes' if at least one veterinarian in the practice held a RCVS recognised farm-specific certificate or diploma and 'no' if none of the veterinarians held one. *A country* variable was created based on the practice postcode and was categorised into England, Wales, Scotland, and Northern Ireland. A *Route of administration* variable was defined for each antimicrobial event and categorised into injectable, intramammary and oral. Intrauterine antimicrobial treatment events were excluded from the risk factors analysis because this category did not have any HP-CIA events. A practice group variable was defined as the group one and group two.

Univariable logistic regression modelling was used to evaluate univariable associations between risk factors and the use of HP-CIAs as a binary (yes/no) outcome. Risk factors with liberal associations in univariable modelling ($P < 0.3$) were taken forward for hierarchical mixed effects logistic regression modelling using a manual backwards-stepwise elimination method with practice ID and farm ID added as random effects and the practice group ID as a fixed effect. Statistical significance was set at the 5% level.

Qualitative study

Data collection: The fieldwork site was a veterinary practice owned by practice group two that participated in the quantitative VetCompass study. This practice was identified as being a high-performing practice at the lowest quartile of the HP-CIAs usage based on the findings of the descriptive quantitative analysis of the data from twenty-three practices participating in the VetCompass and was purposively sampled. Nine veterinary surgeons from this practice at different career stages were interviewed during the period between July and September 2021.

Semi-structured in-depth interviews with open-ended questions were conducted to encourage participants to express their views in detail and in their own terms (Gill et al., 2008). Interview guides were developed based on the literature on factors affecting veterinarians' decision-making when prescribing antimicrobials and current influences and guidelines on using antimicrobials in farm animals. The interview structure was iteratively refined to also include into later interviews any additional issues that were raised by earlier participants (Beardsworth and Keil, 1992).

A digital device was used to record the interviews that were later transcribed into Microsoft Word format. Interviews were conducted via video calls due to the current Covid situation by the same researcher to reduce interviewer bias. Demographic data of the interviewees were collected at the time of the interview.

Thematic analysis: Reflexive thematic analysis was used for this study (Braun and Clarke, 2006) using qualitative data analysis software NVivo 12 (QSR International Pty Ltd, USA). Analysis of transcribed data was done in parallel to interview data collection (Kolb, 2012). Data saturation was considered achieved when no additional information or themes emerged from data collection and analysis; therefore, no further interviews were conducted (Glaser and Strauss, 2009). Initial codes were generated from the data to identify data features of interest. Different data codes were sorted, and relevant codes were collated into themes. The relationship between different codes, and minor and major themes were identified, and then the final themes were named and defined (Braun and Clarke, 2006; Chapman et al., 2015).

Ethical approvals for the qualitative study (URN SR2019-0474) and for the VetCompass programme (URN SR2018-1652) were granted from the Social Science Research Ethical Review Board (SSRERB) at RVC. Written informed consent was obtained from each participant prior to the interview, and participants were asked to give permission for the interview to be recorded.

## RESULTS

### Quantitative study

Quantitative results: Four (4/23) of the participating practices belonged to the first corporate group (group one), and nineteen (19/23) of the practices belonged to the second group (group two). Most of the participating practices were located in England (12/23), 5/23 were located in Northern Ireland, 4/23 were located in Scotland and 2/23 were located in Wales.

During 2019, 98,824 antimicrobial events were recorded from the treatment records of the 23 participating practices. The median count of antimicrobial events at the practice level was 3,226 (range 263-22,159). There were 17,111/98,824 (17.3%) HP-CIAs events overall, with a median of 15.4% per practice (range 4.8-22.1%). Penicillins were the most frequently used antimicrobial 29,539/98,824 (29.9%) followed by tetracyclines 19,015/98,824 (19.2%) and the combination preparations of aminoglycosides and penicillins 16,078/98,824 (16.3%). Injectables represented the majority of the prescribed antimicrobial preparations 79,144/98,824 (80.1%), followed by intramammary 11,642/98,824 (11.8%) and oral preparations 6,383/98,824 (6.5%).

Univariable logistic regression modelling identified five out of seven tested variables that were liberally associated with the usage of HP-CIAs and were further evaluated using the mixed-effects logistic regression modelling: country, route of administration, season, practice type and practice group. The final model retained four variables: country, route of administration, season and practice type. HP-CIA use was clustered more at the farm level (ICC = 0.56) compared to practice level (ICC= 0.32) (Table 1). Scotland (odds ratio, OR: 0.76, 95% CI 0.69-0.84) and Wales (OR:0.69, 95% CI 0.63-0.75) had lower odds of HP-CIAs usage compared to England. Intramammary (OR: 0.18, 95% CI 0.16-0.19) and oral (OR: 0.31, 95% CI 0.28-0.34) routes of administration had lower odds of HP-CIAs usage compared to injectable. Autumn (OR: 1.25, 95% CI 1.20-1.31), summer (OR: 1.22, 95% CI 1.17-1.28), and winter (OR: 1.22, 95% CI 1.16-1.27) showed increased odds of HP-CIAs usage compared to spring. Exclusively farm practices had decreased odds of HP-CIAs usage compared to mixed-species practices (OR: 0.81, 95% CI 0.76-0.87) (Table 1).

Table 1. Final model: hierarchical mixed-effects logistic regression results for risk factors for the usage of HP-CIAs with practice ID and farm ID added as random effects in 23 farm practices participating in the VetCompass in 2019

| Variable | Category | Odds Ratio | 95% CI | Category P-value |
|---|---|---|---|---|
| **Practice type** | Mixed | Base | | |
| | Farm | 0.81 | 0.76-0.87 | < 0.001 |
| **Country** | England | Base | | |
| | Northern Ireland | 0.98 | 0.92-1.05 | 0.603 |
| | Scotland | 0.76 | 0.69-0.84 | <0.001 |
| | Wales | 0.69 | 0.63-0.75 | <0.001 |
| **Season** | Spring | | | |
| | Autumn | 1.25 | 1.2-1.31 | <0.001 |
| | Summer | 1.22 | 1.17-1.28 | <0.001 |
| | Winter | 1.22 | 1.16-1.27 | <0.001 |
| **Route of administration** | Injectable | Base | | |
| | Intramammary | 0.18 | 0.16-0.19 | <0.001 |
| | oral | 0.31 | 0.28-0.34 | <0.001 |

| Level | ICC* | Standard error | 95% CI |
|---|---|---|---|
| **Practice** | 0.32 | 0.104 | 0.16-0.55 |
| **Farm** | 0.56 | 0.072 | 0.42-0.69 |

* Intraclass correlation coefficient

Qualitative results

Four main themes were identified as follows:

The influence from industry: Participants reflected on the substantial influence of the policies implemented by farm assurance schemes, particularly for Red Tractor which is the largest farm assurance scheme in the UK that involves farmers and industry leaders (RUMA, 2017).

> "In terms of policies, we would also follow the Red Tractor guidelines and ensure that we're not dispensing any critically important antibiotics unless there's culture and sensitivity that shows that it's the only thing that would be suitable." (Vet 6)

Participants believed that there was market pressure from supermarkets and milk buyers on farmers to reduce their antimicrobials usage. In addition, they considered veterinarians have a proactive role in monitoring farmers' usage through annual antibiotic reviews to meet the requirements in their supermarket contracts. Participants reflected on the market pressure on dairy farms in particular; sheep and beef farms were mentioned less in this discussion. Participants reported some of the supermarkets' contracts have stricter policies in using antibiotics. The following quote highlighted that some of these strict policies are considered not practical to follow by farmers:

> “[Supermarket name] is probably the hardest one; they've got these targets which are just absolutely crazy. One of our best dairy farms is only in the silver category, and to get to the gold category, he needs to be using less than 2 mg/PCU [milligram per population correction unit] for all of his animals the whole year, and it was just like I don't see how that's possible …There are animals that need it [antibiotics] otherwise they are going to come in with really bad mastitis." (Vet 4)

Knowledge of antimicrobials: Participating veterinarians showed good understanding of AMR, responsible use of antimicrobials, the term ‘critically important antimicrobials’ and the term ‘responsible use of antimicrobials’. In addition, they tended to be up to date with the national guidelines. Participants believed that they are using antimicrobials responsibly according to their knowledge and by following these guidelines. Participants attributed the problem of AMR in humans to the irresponsible use of antimicrobials in humans and some animal species, particularly poultry and pigs. Participants believed that antimicrobial usage in other sectors is more responsible and that the overall use of antimicrobials in livestock in the UK is lower than in other countries:

> “Antibiotics [HP-CIAs] that are deemed critically important for human health due to their ability to either work where there's already resistance in the human population, or they are used for fighting infections that there are very limited antibiotics that are still working for them, so they need to preserve them.” (Vet 4)

Drug-related factors: Several drug-related factors were reported to influence participants' choice of the antimicrobials they prescribe. However, they suggested that these factors do not affect their choice of the antimicrobial class or the active substance. Ease of administration was influencing participants to choose long-duration antimicrobials for beef animals that are more vigorous and not easy to handle to ensure the safety of the farmers. Participants mentioned that if they the choice between long duration and short duration preparations of the same antimicrobial class, they would choose the long duration preparation for beef animals.

> “Unlike a dairy [animal], some of the beef animals are extremely wild. And it's kind of you may just have one chance to do this. And actually, human safety plays a big factor.” (Vet 7)

The length of the withdrawal period was reported by participants as an influencing factor when they are making their antimicrobials prescribing decision. Participants reported that they consider the withdrawal time implications when prescribing antimicrobials, especially for dairy animals, to avoid milk losses by choosing antimicrobials with shorter withdrawal time.

The pharmacokinetics of the antibiotics were mentioned by participants to have an influence when choosing a first-line antimicrobial treatment for the diagnosed clinical condition to ensure that the antibiotic will reach the target organ. Drug availability was also mentioned as having an influence in choosing antimicrobials. Participants reported a recent shortage of some antibiotic products, specifically intramammary antibiotic products for treating mastitis, and that this was affecting their choice of antimicrobial treatment.

Clinical factors: The clinical condition and clinical signs presented in the farm visit influenced veterinarians’ prescribing decision. Participants reported that they prescribe antimicrobials after surgical operations, for example caesarean sections, because they perceived that contamination of a surgical wound is very likely in the farm environment. Pyrexia was reported to be a common reason for using antimicrobials, especially in undiagnosed clinical cases. Participants agreed that calf pneumonia is a viral disease in most cases that do not need antimicrobials; however, they use antimicrobials in case of an outbreak or moderate to severe clinical signs at individual animal level:

> "Like a herd level or a group level calf pneumonia, so you would maybe, you would prescribe the products and leave the farmer with the knowledge of which animals to give the antibiotics to." (Vet 3)

Participants mentioned that they follow empirical diagnosis based on their previous experience and knowledge to identify bacterial diseases that need antimicrobial treatment:

> "So, for like individual animals, I would base it on my own clinical experience and kind of knowledge of the likely causes of those conditions so, like, metritis, for example, with discharge that's going to be caused by an *E.coli* or a *Trueperella pyogenes* generally." (Vet 3)

## DISCUSSION

This research followed a mixed-methods approach to provide a more in-depth understanding of antimicrobial usage in farm animal practice. This study represents the most recent study to date to investigate the electronic treatment records from a larger number of farm veterinary practices across the UK compared to previous research, to describe the patterns of overall antimicrobial and of highest priority critically antimicrobials (HP-CIAs) use. Furthermore, the drivers and barriers for usage of antimicrobials in farm animal practices were qualitatively explored.

The current study reported a relatively high proportion of HP-CIA among antimicrobials usage (17.3%), with a median of 15.4% of antimicrobials usage at practice level and wide variation between practices (range 4.8-22.1%). These reported results are mostly related to cattle and sheep as none of the participating practices were specialised poultry or pig practices. This study reported that penicillins and tetracyclines were the most commonly prescribed antimicrobials then also which were the most common HPCIAs. The usage of these antimicrobial classes was likely to reflect the commonly presented clinical conditions seen on farms and their respective empirical antimicrobial therapy. A previous systematic review on antimicrobials usage in British sheep and cattle reported similar results (Hennessey et al., 2020).

There were reduced odds for HP-CIAs usage for intramammary preparations compared to injectables, implying that the use of the HP-CIAs was less likely to be used for controlling mastitis in dairy animals. This association reflected the antimicrobial classes of the commonly available intramammary products. HP-CIAs usage was most strongly observed at the farm level, with less seen at the practice level, suggesting that there may be more factors driving HP-CIAs usage on farm relative to at the practice level.

The results from the qualitative study supported the quantitative findings and highlighted that industry pressures on farms appear to be a strong influence on-farm HP-CIAs usage. However, other factors were seen to contribute to antimicrobials usage, including the farm production type, which is another farm-related factor that influences the use of HP-CIAs and the use of antimicrobials overall. The qualitative study indicated that veterinarians perceived that some supermarkets have stricter guidelines and that some of these may be considered impractical to follow. These strict guidelines could pressurise producers against using antibiotics even when warranted, and this might put animal welfare at risk. A previous study explored the influence of the supermarkets on dairy farmers (Begemann et al., 2020). This study also reported that supermarkets' contracts influenced farmers and veterinarians' antibiotics usage.

The current qualitative study identified some drug-related factors that influenced participating veterinarians' choice of antibiotics. Veterinarians' choice between long-acting and short-acting antimicrobials was influenced by practicality to farmers and ease of administration in dairy animals compared to beef animals to ensure farmer compliance. Participants were influenced to choose long acting antimicrobials in beef animals and short acting antimicrobials in dairy animals. Previous studies identified that concern over the farmers' adherence to antimicrobial administration guidelines influenced the veterinarians' choice of antimicrobials (Speksnijder et al., 2015b; Coyne et al., 2016).

Clinical signs and the nature of conditions presented on the farm were identified as influencing participating veterinarians' prescribing decision. Previous research reported similar results and highlighted clinical conditions presented were influencing veterinarians'' prescribing decision (Coyne et al., 2018; Doidge et al., 2019). This suggests that participants were more likely to follow empirical diagnosis and antimicrobial prescription based on their clinical experience rather than based on a laboratory diagnosis.

It was not possible to estimate species-specific usage for the current study as usage was based on farm-level activity rather than at the animal or species level. However, these reported findings are most likely to be related to sheep and cattle usage as all practices were farm or mixed practices and none of the participating practices was a specialised pig or poultry practice. In the UK, pig and poultry farms are mostly registered to a specialised practice.

In summary, this study highlights high levels of farm-level clustering of antimicrobials usage relative to the practice level. The themes emerging from the qualitative interviews support a range of farm-level drivers for antimicrobials usage, including industry pressure to reduce antimicrobials usage and the influence of farm production type, as well as veterinarians' understanding of antimicrobials and the clinical conditions presented. Integrating these quantitative and qualitative findings can help to design targeted interventions to promote further improvements in responsible antimicrobial use in farm practice and inform policymaking on antimicrobial stewardship in farm practice.

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# DAIRY COW HEALTH

# BAYESIAN DIAGNOSTIC TEST EVALUATION AND TRUE PREVALENCE ESTIMATION OF MYCOPLASMA BOVIS IN DAIRY CATTLE

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## SUMMARY

The true prevalence of dairy cattle herds with *M. bovis* infections in the Netherlands is unknown. Previous attempts to estimate prevalences were hampered by the absence of a diagnostic serological test that was validated under field conditions. This study estimated sensitivity and specificity of two commercial serum ELISAs and the true *M. bovis* herd prevalence using different latent class models. A total of 7,305 serum samples from 415 randomly chosen dairy herds were collected in fall/winter 2019 and investigated for presence of antibodies against *M. bovis* using the BIO-K-302 ELISA from Bio-X. Serum samples from 100 of these herds were also tested with a second ELISA, from IDvet. A Bayesian latent class model using the paired test results estimated a BioX sensitivity of 15.8% (95% Bayesian probability interval (BPI): 12.9–18.9%) and a specificity of 99% (95% BPI: 98.1–99.7%). IDvet sensitivity and specificity were estimated at 94.4% (95% BPI: 91.0–97.0%) and 99% (95% BPI: 98.1–99.8%), respectively. The large difference in test sensitivity between the two tests is expected to be caused by an effect of time since infection. A hierarchical Bayesian logistic model, applied on test results of all 415 herds, estimated an apparent herd-level prevalence of 46.5% (95% BPI: 41.7–51.6%) and a true prevalence of 74.7% (95% CI: 63.4–85.3%).

## INTRODUCTION

*Mycoplasma bovis* (*M. bovis*) has become increasingly important as a pathogen on beef and dairy cattle farms, causing welfare and production losses (Maunsell et al., 2011; Dudek et al., 2020). Since the first reported case of mastitis in 1961 (Hale et al., 1962), *M. bovis* has been detected worldwide, in all major cattle rearing countries (Dudek et al., 2020). *M. bovis* is a primary cause of mastitis, arthritis, keratoconjunctivitis and otitis media as well as being part of the bovine respiratory disease complex (BRD) (Maunsell et al., 2011). As antimicrobial treatment of *M. bovis* mastitis and arthritis is mostly unsuccessful, it is often advised to cull cattle with *M. bovis* mastitis and/or arthritis. However, *M. bovis* infections may persist in a dairy herd, also through asymptomatic carriers (Punyapornwithaya et al., 2010).

*M. bovis* can be identified in individual cattle or bulk milk samples by bacterial culture or PCR (Parker et al. 2018), although the bacterium may be missed due to variations in affected tissues between cattle, intermittent shedding in milk and withholding of milk from mastitic

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cows from bulk milk. Antibodies against *M. bovis* can be detected in serum and (bulk) milk using an ELISA (Parker et al., 2018). The first commercially available ELISA is produced by Bio-X (Bio-X Diagnostics S.A., Rochefort, Belgium). A second one, from IDvet (IDvet, Grabels, France), became available in 2018 and was shown to be more sensitive than the K-302 ELISA from Bio-X (Andersson et al., 2019; Petersen et al., 2020). At Royal GD, the K-260 ELISA of Bio-X is used for routine diagnostics.

Based on different diagnostic methods, herd level and animal level prevalences have been determined in different European countries. In Belgium, true dairy herd prevalence was 7.1% and 24.8% as determined by PCR or ELISA in bulk milk, respectively (Gille et al. 2018). As there was no overlap between PCR and ELISA positive herds, overall dairy herd prevalence was 31.8%. In Denmark, dairy herd prevalences were 1.6% (PCR) and 7.2% (ELISA) (Nielsen et al., 2015). In the Netherlands, an increased number of *M. bovis* infections have been observed in dairy cattle in the last decade, particularly *M. bovis*-induced cases of mastitis and arthritis. However, the true prevalence of *M. bovis* infections in the dairy cattle population is unknown. Previous attempts to estimate seroprevalences were hampered by the absence of a diagnostic test that was validated under field conditions due to lack of a gold standard. Bayesian latent class models are known for their ability to deal with this issue (Johnson et al., 2019). The objectives of this study were therefore to use Bayesian modelling 1) to estimate the sensitivity and specificity of two commercially available serum ELISAs in the absence of a gold standard and 2) to estimate the prevalence of *M. bovis* infections in Dutch dairy herds.

## MATERIALS AND METHODS

### Study design

The study was conducted in two stages. In the first stage, a random selection of dairy herds was sampled to estimate the prevalence of *M. bovis* infection. In a second stage, serum samples from a subset of the herds were used to estimate test validity of two serum ELISAs.

In the first stage, a sample size of 284 herds was chosen to estimate herd prevalence of *M. bovis,* based on an expected seroprevalence of 25% (Gille et al., 2018), 95% confidence and 5% error. A total of 1,183 dairy farms were invited to participate, based on an expected response rate of 40% and, subsequently, 40% compliance with the required sampling strategy. The sample of 1,183 farms were randomly chosen out of a population of 10,329 dairy herds with at least six calves 1-6 months old in July-August 2019. Farms were invited by e-mail in August 2019 to participate in the survey in fall/winter 2019-2020. Participating farmers were requested to arrange with their private practitioner to collect blood samples from 12 randomly selected cattle of at least 2 years old and six calves of 1-6 months old at sampling. This sampling scheme was sufficient to detect *M. bovis* infection in herds with an acute *M. bovis* outbreak (Royal GD 2018, *unpublished data*). Blood samples were to be collected between October 1 and December 31, 2019. Samples were investigated for presence of *M. bovis*-specific antibodies using the BIO-K-260 ELISA from Bio-X ('ELISA A') which was routinely used at Royal GD. Test outcomes were first expressed as a sample to positive percentage (S/P%). For this, a net optical density (OD) is calculated by subtracting the OD value in the control well (with negative control antigen) from the OD of the well with *M. bovis* antigen. Additionally, according to the manufacturer, test outcomes were categorized into one of six classes:

S/P% ≤ 37%: 0
37 < S/P% ≤ 60%: +
60 < S/P% ≤ 83%: ++
83 < S/P% ≤ 106%: +++
106 < S/P% ≤ 129%: ++++
S/P% > 129%: +++++

In the second stage of the study, 100 herds were selected based on a third test result, the IDvet ELISA on bulk milk samples, and ranged from negative to high positive in bulk milk. All serum samples from the 100 herds were investigated for presence of *M. bovis*-specific antibodies using the ID Screen® ELISA from IDvet ('ELISA B'). Test outcomes were first expressed as an S/P% as $((\mathrm{OD}_{\text{sample}} - \mathrm{OD}_{\text{negative control}})/(\mathrm{OD}_{\text{positive control}} - \mathrm{OD}_{\text{negative control}})) \times 100\%$. According to the manufacturer, test outcomes were categorized as follows:

S/P% < 60%: 0
60 ≤ S/P% <80%: +
80 ≤ S/P% <110%: ++
110 ≤ S/P% <140%: +++
S/P% ≥ 140%: ++++

## Analysis

Two Bayesian mixture models were designed. In the first model, combined test results of the two ELISAs on serum samples from the subset of 100 farms were compared to estimate sensitivity and specificity of the ELISAs ('Model 1'). In a second model, the herd-level prevalence was estimated using test results of all herds ('Model 2'). In both models, test outcomes were first dichotomized as 'positive' (categories > 0) or 'negative' (category 0) prior to analyses.

## Model 1: test characteristics

A 2x2 table with the frequencies of the observed combinations of test results ($A^+B^+$, $A^+B^-$, $A^-B^+$, $A^-B^-$) made the data frame for the model. A Hui-Walter latent class model was used for two tests in one population assuming conditional dependence of the test results (Hui and Walter, 1980). Conditional dependence of ELISA A and ELISA B test outcomes was expected because both tests detect serum antibodies (Georgiadis et al., 2003). Consequently, the combination of joint test results are stratified by the (latent) disease status *D* of the subjects, leading to the cross-classification summarized in Table 1 (Dendukuri, 1998). Note that from the frequencies in Table 1, only the 2x2 subtotals $N_{AB}$ are observed.

Table 1. Conceptual cross-classification of observed (N) and latent (Y) data from two diagnostic tests in animals that are diseased (D+) or non-diseased (D-)

| | $D^+$ | | $D^-$ | |
|---|---|---|---|---|
| | ELISA A = + | ELISA A = - | ELISA A = + | ELISA A = - |
| ELISA B = + | $Y_{++}$ | $Y_{-+}$ | $N_{++} - Y_{++}$ | $N_{-+} - Y_{-+}$ |
| ELISA B = - | $Y_{+-}$ | $Y_{--}$ | $N_{+-} - Y_{+-}$ | $N_{--} - Y_{--}$ |

The conditional dependence between the two tests was estimated using the covariance between the two tests among the diseased (*p*) and non-diseased (*n*) subjects, in accordance with the model described by Dendukuri and Joseph (2001):

$$covp = cov(\mathrm{A}, \mathrm{B} \mid \mathrm{D}^+) = \mathrm{p}(\mathrm{A}^+\mathrm{B}^+ \mid \mathrm{D}^+) - (\mathrm{SeA}*\mathrm{SeB}) \tag{1}$$

$$covn = cov(\mathrm{A}, \mathrm{B} \mid \mathrm{D}^-) = \mathrm{p}(\mathrm{A}^-\mathrm{B}^- \mid \mathrm{D}^-) - (\mathrm{SpA}*\mathrm{SpB}) \tag{2}$$

According to the model, the eight multinomial cell probabilities of the cross-tabulated data were estimated as follows:

$$\mathrm{p}(\mathrm{A}^-\mathrm{B}^- \mid \mathrm{D}^+) = \pi \times ((1-\mathrm{SeA}) \times (1-\mathrm{SeB}) + covp) \tag{3}$$

$$\mathrm{p}(\mathrm{A}^-\mathrm{B}^- \mid \mathrm{D}^-) = (1-\pi) \times (\mathrm{SpA} \times \mathrm{SpB} + covn) \tag{4}$$

$$\mathrm{p}(\mathrm{A}^+\mathrm{B}^- \mid \mathrm{D}^+) = \pi \times (\mathrm{SeA} \times (1-\mathrm{SeB}) - covp) \tag{5}$$

$$\mathrm{p}(\mathrm{A}^+\mathrm{B}^- \mid \mathrm{D}^-) = (1-\pi)\,((1-\mathrm{SpA}) \times \mathrm{SpB} - covn) \tag{6}$$

$$\mathrm{p}(\mathrm{A}^-\mathrm{B}^+ \mid \mathrm{D}^+) = \pi \times ((1-\mathrm{SeA}) \times \mathrm{SeB} - covp) \tag{7}$$

$$\mathrm{p}(\mathrm{A}^-\mathrm{B}^+ \mid \mathrm{D}^-) = (1-\pi) \times (\mathrm{SpA} \times (1-\mathrm{SpB}) - covn) \tag{8}$$

$$\mathrm{p}(\mathrm{A}^+\mathrm{B}^+ \mid \mathrm{D}^+) = \pi \times (\mathrm{SeA} \times \mathrm{SeB} + covp) \tag{9}$$

$$\mathrm{p}(\mathrm{A}^+\mathrm{B}^+ \mid \mathrm{D}^-) = (1-\pi) \times ((1-\mathrm{SpA}) \times (1-\mathrm{SpB}) + covn) \tag{10}$$

Where π being the animal-level prevalence of *M. bovis*, SeA, SeB and SpA, SpB are the sensitivities and specificities of ELISA A and ELISA B respectively, *covp* the covariance between the tests in diseased subjects and *covn* the covariance between the tests in non-diseased subjects (Dendukuri and Joseph, 2001).

Informative prior distributions for sensitivity and specificity of ELISA A and ELISA B were obtained from literature (Table 2). The covariance parameters were constrained as such that the combined sensitivities and specificities of the two tests cannot exceed the individual values of the test characteristics. A non-informative prior was used for animal-level seroprevalence. The ‘PriorGen’ package in R 3.6.1 (R Core Team, 2018) was used to obtain shape parameters for the informative prior (beta) distributions. From the cell probabilities in (Eq. 3) to (Eq. 10) the multinomial likelihood was constructed, combined with priors, and Bayes’ theorem is applied to obtain posterior distributions of the parameters.

The model was applied to three scenarios. First, the model ran on all sera from the 100 herds. Then, the model was run on test results from calves and cows separately. Posterior inferences were obtained with the package ‘rjags’ in R 3.6.1 (Plummer et al., 2019) using Markov chains, with 10,000 iterations after a burn-in period of 5,000 iterations. Convergence of the Markov chains was assessed by visual assessment of Markov chains and trace plots and by running multiple (n = 2) chains from distinct starting values (e.g., 0.05 and 0.95 for variables bounded between 0 and 1). The Brooks-Gelman-Rubin diagnostic was used to assure that the two chains had converged (Brooks and Gelman, 1998), inspecting the potential scale reduction factor being very close to 1.

Table 2. Prior information for parameters of Model 1 to estimate test sensitivity and specificity of ELISA A and ELISA B, with median prior probabilities and 95% Bayesian probability interval (BPI), reference and distribution

| Parameter | Prior median (95% BPI) | Reference | Distribution |
|---|---|---|---|
| SeA | 28% (1–92%) | Schibrowski et al., 2018 | beta(1.01, 2.59) |
| SeB | 94% (90–97%) | Andersson et al., 2019 | beta(161.11, 10.28) |
| SpA | 100% (93–100%) | Schibrowski et al., 2018 | beta(529.9, 5.35) |
| SpB | 99% (98–99%) | Andersson et al., 2019 | beta(529.9, 5.35) |
| *covp* | - | Dendukuri and Joseph, 2001 | uniform($lb_p$, $ub_p$)[a] |
| *covn* | - | Dendukuri and Joseph, 2001 | uniform($lb_n$, $ub_n$)[a] |
| Prevalence π | - | - | beta(1, 1) |

[a] $lb_p$ is the lower bound of *covp*: (SeA-1)×(1-SeB); $lb_p$ is the upper bound of *covp*: min(SeA, SeB)-SeA×SeB; $lb_n$ is the lower bound of *covn*: (SpA-1)×(1-SpB); $lb_n$ is the upper bound of *covn*: min(SpA, SpB)-SpA×SpB

Model 2: herd prevalence

A hierarchical Bayesian logistic model was developed to estimate herd prevalence of *M. bovis* amongst dairy herds in the Netherlands. The number of serum samples per herd, the number of positive test results per herd, and whether samples were tested with ELISA A or ELISA B, made the data frame for the model. Note that all herds were tested with ELISA A and a subset of 100 herds also with ELISA B. For the latter group, only the results of B were retained for the analysis. The dataset also contained the four regions in which the herds were located (north, east, south and west), whether farms had an open or closed farming system, and their herd size based on the number of lactating cows present in the herd. The model was inspired by a study by van Schaik et al (2003), in which data from two prevalence studies using an ELISA in several regions in two countries were combined in a Bayesian framework. First, the animal-level true prevalence π was estimated as follows:

$NT^{+}_{k} \mid p(T^{+})_{k}, n_{k} \sim \text{binomial}(p(T^{+})_{k}, n_{k})$ = number of animals in herd *k* that tested positive, with $n_k$ being the number of cows tested per herd (11)

$p(T^{+})_k = Se_i \times \pi_k + (1-Sp_i)(1-\pi_k)$ = probability of a positive test result for an animal in herd *k,* based on π and the sensitivity and specificity of ELISA *i* (12)

$\text{logit}(\pi_k) = \beta \times ELISA_i + t_j$ = $p(D^{+})$ for an animal in herd *k* (13)

$t_j \sim \text{normal}(\mu,\sigma)$ = random effect of herd within region *j*, with mean μ and standard deviation σ (14)

$\sigma = 1/\sqrt{(tau)}$ = standard deviation of $t_j$ (15)

The random herd effect *t* quantifies the between-herd variation within geographic regions. Diffuse prior distributions such as β ~ dnorm(0.0,1.0E-6), μ ~ dnorm(0.0,1.0E-6) and tau ~ dgamma(0.001,0.001) where used for the model parameters. Shape parameters for the informative prior (beta) distributions of $Se_i$ and $Sp_i$ where as described in Table 2. The herd-level apparent (AP) and true prevalence (TP) was obtained as follows. For AP, first the apparent herd-level sensitivity $AHSe_k$ was estimated as the probability of detecting at least one seropositive animal in a herd, based on $p(T^{+})_k$ and the sample size of the herd (Eq. 16). When this probability ($AHSe_k$) exceeded 50%, the herd was considered 'test positive'. AP was estimated as the proportion of test positive herds where $AHSe_k > 50\%$ (Eq. 17).

$$AHSe_k = 1 - (p(T^{-})_k)^{n_k} \qquad \text{where } p(T^{-})_k = 1 - p(T^{+})_k \tag{16}$$

$$AP = p(AHSe_k) > 0.50 \tag{17}$$

For TP, first the true herd-level sensitivity $THSe_k$, i.e. the confidence of detecting a diseased herd, was estimated as the probability of detecting at least one truly diseased animal in a herd (Eq. 18). When this probability ($THSe_k$) exceeded 50%, the herd was considered truly infected, otherwise the herd was considered not infected. TP was estimated as the proportion of herds where $THSe_k > 50\%$ (Eq. 19).

$$THSe_k = 1 - (p(D^{-})_k)^{n_k} \qquad \text{where } p(D^{-})_k = 1 - p(D^{+})_k \tag{18}$$

$$TP = p(THSe_k) > 0.50 \tag{19}$$

Differences in TP between the herd-level factors herd size, open/closed farming system and regions were tested. Numbers were assumed to be significantly different of each other when the 95% BPI of their difference did not include zero. The model was coded using OpenBUGS and was compiled with three sets of initial values. A burn-in period of 5,000 iterations was applied; conclusions were based on the next 20,000 iterations. The Brooks-Gelman-Rubin diagnostic was used to assure that the chains had converged. Autocorrelation plots were examined to ensure there was no strong autocorrelation between the Monte Carlo samples.

## RESULTS

### Serology

A total of 7,828 cattle from 451 dairy farms were sampled between 3 October and 31 December 2019. Five hundred and twenty-three samples were excluded from analysis due to violation of the sampling criteria, resulting in the analysis of 7,305 samples from 415 herds. From these herds, 100 herds had only three test results from calves and six test results from cows, yet these were kept in the data set. An overview of the test results for ELISA A is provided in Table 3. The majority of the cattle tested seronegative (95.4%). From the 100 herds, 1,799 samples were also tested for *M. bovis* specific antibodies using ELISA B. The joint test results are shown in Table 4.

Table 3. *M. bovis*-specific ELISA (A) test results of 2,346 calves and 4,959 cows from 415 dairy herds in the Netherlands in 2019

| Test result | Calves | Cows | Total |
|---|---:|---:|---:|
| 0 | 2,272 (97%) | 4,699 (95%) | 95.4% |
| + | 34 (1%) | 197 (4%) | 3.2% |
| ++ | 26 (1%) | 45 (1%) | 1.0% |
| +++ | 10 (0.4%) | 15 (0.3%) | 0.3% |
| ++++ | 1 (<0.1%) | 2 (<0.1%) | <0.1% |
| +++++ | 3 (0.1%) | 1 (<0.1%) | <0.1% |
| Total | 2,346 | 4,959 | 7,305 |

Table 4. Cross-classification of *M. bovis* test results from two correlated ELISA tests in serum samples from calves and cows (N=1,799)

| | ELISA B | | | | | |
|---|---:|---:|---:|---:|---:|---:|
| ELISA A | 0 | + | ++ | +++ | ++++ | Total |
| 0 | 1,025 | 91 | 130 | 110 | 310 | 1,666 (92.4%) |
| + | 19 | 5 | 16 | 12 | 35 | 87 (5.0%) |
| ++ | 11 | 3 | 2 | 4 | 11 | 31 (1.7%) |
| +++ | 1 | 1 | 0 | 0 | 9 | 11 (0.7%) |
| ++++ | 0 | 0 | 0 | 0 | 2 | 2 (0.1%) |
| +++++ | 0 | 1 | 0 | 0 | 1 | 2 (0.1%) |
| Total | 1.056<br>(58.8%) | 101<br>(5.8%) | 148<br>(8.2%) | 126<br>(7.0%) | 368<br>(18.3%) | 1,799 |

Test characteristics (Model 1)

Posterior median Se and Sp estimates for ELISA A, were 15.9% (BPI: 12.9–18.9%) and 99.0% (BPI: 98.1–99.7%), respectively (Table 5). For ELISA B, Se and Sp estimates were 94.4% (BPI: 91.0–97.0%) and 99.1% (BPI: 98.1–99.8%), respectively. The median animal-level true seroprevalence of *M. bovis* was 43.2% (BPI: 40.4–46.1%) in the subset of 100 herds, which is not a random sample of dairy herds and thus is of no practical value. Covariance between the tests was estimated at -0.023 (BPI: -0.004 – -0.006) in diseased subjects and 0.003 (BPI: -0.000–0.009) in non-diseased subjects.

SeA decreased slightly to 13.1% (BPI: 10.5–15.9%) in the model on sera from cows only (Table 5). For ELISA B, Se and Sp remained the same in the subset of cow test results. SeA increased to 39.6% (BPI: 27.3–52.8%) in the model on sera from calves, whereas SpA, SeB and SpB did not change. The potential scale reduction factor was between 0.99 to 1.00 for all parameters (results not shown), indicating proper convergence.

Table 5. Posterior medians and 95% posterior probability intervals of the prevalence π and test characteristics using Model 1 on all joint test results, and on data from cows and calves separately

| | Total | | Cows | | Calves | |
|---|---|---|---|---|---|---|
| Parameter | Median | 95% BPI | Median | 95% BPI | Median | 95% BPI |
| π | 0.432 | 0.404 ; 0.461 | 0.593 | 0.558 ; 0.632 | 0.110 | 0.082 ; 0.140 |
| SeA | 0.159 | 0.129 ; 0.189 | 0.131 | 0.105 ; 0.159 | 0.396 | 0.273 ; 0.528 |
| SeB | 0.944 | 0.910 ; 0.970 | 0.947 | 0.911 ; 0.974 | 0.937 | 0.897 ; 0.970 |
| SpA | 0.990 | 0.981 ; 0.997 | 0.990 | 0.981 ; 0.997 | 0.986 | 0.977 ; 0.994 |
| SpB | 0.991 | 0.981 ; 0.998 | 0.991 | 0.981 ; 0.997 | 0.992 | 0.983 ; 0.998 |
| *covp* | -0.023 | -0.004 ; -0.006 | -0.017 | -0.031 ; -0.004 | -0.015 | -0.047 ; 0.021 |
| *covn* | 0.003 | -0.000 ; 0.009 | 0.003 | -0.000 ; 0.009 | 0.002 | -0.000 ; 0.008 |

Estimated prevalence (Model 2)

The regional location of herds in the study population was representative for the distribution of dairy herd in the Netherlands, i.e. most herds located in the northern and eastern region (Table 6). The mean herd size was 124 cows, with is somewhat larger than the mean of the target population, which was about 100 lactating cows. Over 55% of the herds had a closed farming system, which is similar to the national average. Herds from the northern region where largest and had most often a closed farming system.

Table 6. Descriptive statistics of the investigated herds (N=415)

| Region | Number of herds | Mean herd size[a] (SD) | Closed farming system[b] (%) | Test positive herds (≥1 $T^+$ animal, %) |
|---|---|---|---|---|
| North | 121 | 138 (79) | 61.2 | 42.3 |
| East | 138 | 115 (52) | 53.2 | 50.0 |
| West | 73 | 107 (47) | 49.3 | 42.2 |
| South | 83 | 131 (62) | 56.6 | 50.8 |
| Total | 415 | 124 (63) | 55.5 | 44.0 |

[a] Number of lactating cows in third quarter of 2019

[b] No introduction of new animals in the herd in the past year in the third quarter of 2019

Posterior median cow-level TP (π) was 22.4% (BPI: 18.6–28.3%) (Table 7). The apparent herd-level prevalence was estimated at 46.5% (BPI: 41.7–51.6%) and median herd-level TP was 74.7% (BPI: 63.4–85.3%). Herd-level TP were not statistically different between regions, farming system and herd size categories.

Table 7. Posterior medians and 95% posterior probability intervals of the *M. bovis* prevalence parameters of Model 2

| Parameter | Median | 95% BPI |
|---|---|---|
| Mean cow-level TP | 0.224 | 0.186 ; 0.283 |
| Herd-level AP | 0.465 | 0.417 ; 0.516 |
| Herd-level TP | 0.747 | 0.634 ; 0.853 |
| Herd-level TP in North | 0.711 | 0.570 ; 0.843 |
| Herd-level TP in East | 0.775 | 0.652 ; 0.884 |
| Herd-level TP in West | 0.726 | 0.575 ; 0.863 |
| Herd-level TP in South | 0.771 | 0.639 ; 0.892 |
| Herd-level TP in closed farming systems[a] | 0.730 | 0.604 ; 0.844 |
| Herd-level TP in semi-open farming systems | 0.733 | 0.533 ; 0.900 |
| Herd-level TP in open farming systems | 0.781 | 0.664 ; 0.884 |
| Herd-level TP in 25% smallest herds[b] | 0.719 | 0.561 ; 0.877 |
| Herd-level TP in 25% smaller herds | 0.719 | 0.563 ; 0.854 |
| Herd-level TP in 25% larger herds | 0.724 | 0.597 ; 0.845 |
| Herd-level TP in 25% largest herds | 0.799 | 0.691 ; 0.899 |

[a] Semi-open: 1-2 cattle introduced in the herd in the past year. Open: >2 cattle introduced in the herd in the past year. Both measured in the third quarter of 2019

[b] 25% smallest: <68 lactating cattle; 25% smaller: 68-96 cattle; 25% larger: 97-131 cattle; 25% largest: >131 cattle

## DISCUSSION

In this study, test characteristics of two antibody ELISAs were validated under field conditions. As no gold standard was available for *M. bovis* infections on a herd level, Bayesian modelling was used to estimate test sensitivities and specificities and to estimate the prevalence of *M. bovis* infected Dutch dairy herds.

Results of the latent class analysis showed that the sensitivity of commercially available ELISA kits to identify antibodies against *M. bovis* vary largely. The poor sensitivity of the Bio-X ELISA has also been shown by others (Andersson et al., 2019; Petersen et al., 2020), although they used the monowell K-302 ELISA in their studies instead of the double well K-260 ELISA from Bio-X, with both antigen-coated wells and negative control antigen-coated wells. The K-260 ELISA was used for routine diagnostics at Royal GD at the time of this study. A latent class model was used for two tests in one population assuming conditional dependence of the test results, which appeared appropriate as the model estimated some (yet limited) level of covariance between diseased subjects. The model estimated the sensitivity of the Bio-X test to be no more than 15.8%. This is somewhat lower than the median of the prior distribution used in the model (28%). As an alternative, a prior distribution of the Bio-X ELISA sensitivity available from literature was used in the model with a median of 13% (95% CI: 5–30) (Wawegama et al., 2016). This did not alter the posterior distribution of Bio-X ELISA sensitivity.

The low sensitivity of the Bio-X ELISA needs to be taken into account when used in the field. Suggested explanations for the poor sensitivity of the corresponding Bio-X K-302 ELISA are a very short antibody detection and its ability to primarily detect clinically ill animals (Petersen et al., 2020). A reduced sensitivity as a result of waning immunity is supported by our finding that Bio-X test sensitivity was twice as high in samples from calves as compared to samples from cows. That is, seropositivity in young calves is most likely the result of recent infection, although maternal antibodies cannot be excluded. The sensitivity of the IDvet ELISA was estimated to be 94.3%, with no difference between samples from calves or adult cattle suggesting high sensitivity to detect both recent and past infections. Nevertheless, this raises questions to the interpretation of IDvet ELISA results when used in practice in relation to the purpose of testing. It is hypothesized that the IDvet ELISA in serum will measure (past) exposure to *M. bovis* rather than current colonization in the infected animal only (Petersen et al., 2020), which has to be taken into account when used for diagnostic purposes without parallel pathogen detection. More importantly, the duration of serum antibody elevation after natural infection is key in this matter, which is not exactly known. Vähänikkilä et al. (2019) measured serum antibodies for at least one and a half years in cattle from farms with and without apparent presence of *M. bovis*. On the contrary, Petersen et al. (2018b) showed that serum antibody responses are highly dynamic and show a high level of variation between individual cows.

In our attempt to estimate the true prevalence of dairy herds with an infection of *M. bovis*, test results of the two aforementioned ELISA tests were used simultaneously in one model. A model at herd level in which the available joint test results were used did not converge unfortunately, as 315 out of 415 lacked IDvet ELISA results. It would be worthwhile to investigate other models or software packages than the one we used to solve this matter. Herd-level true prevalence was estimated to be high, suggesting that a large proportion of the dairy herds in the Netherlands have been exposed to *M. bovis*. No statistically significant differences were found in true prevalences between regions, open/closed farming systems and herd size categories. Looking at the apparent herd-level prevalence, large dairy herds and herds that introduced cattle from other herds had a higher probability of having seropositive cattle than smaller dairy farms or closed farms (results not shown). This is in agreement with previous studies, in which herd size has been identified as a risk factor for the detection of *M. bovis* in bulk milk (Fox et al., 2003; Pinho et al., 2013; Vähänikkilä et al., 2019), although it has been described to be not associated with the bulk milk antibody test result (Petersen et al., 2016). Purchase of a carrier animal has also been described as an important risk factor (Maunsell et al., 2011). These risk factors may have remained undetected in the current study due to the low level of variation in (true) infection status of herds.

In the light of the difference in sensitivity of the ELISA tests used in our study, and the aforementioned effect of recent versus past infections, it is expected that the apparent herd prevalence of 46.5% that was found represents the proportion of dairy herds in the Netherlands with a recent infection of *M. bovis.* In addition, the true herd prevalence of 74.7% represents farms with recent exposure to *M. bovis* as well as farms with past exposure to *M. bovis.*

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# THE HERD-LEVEL ASSOCIATION OF HORMONE USE FOR REPRODUCTIVE DISEASES AND HEAT INDUCTION WITH REPRODUCTIVE PERFORMANCE IN DUTCH DAIRY FARMS

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## SUMMARY

This research aimed to associate hormone use for reproductive diseases and heat induction with herd level reproductive performance. Hormone use, herd characteristics, and test-day recording data were obtained from 560 representative Dutch dairy herds from 2017 to 2019. Calving interval, calving-to-1st insemination interval and number of inseminations were analysed using multivariable General Estimating Equations models. The models identified that a high hormone use associated with a calving interval and a calving-to-1st insemination interval that was 9.3±2.6 and 16.4±2.1 days shorter than of non-user herds (423.9±2.7 and 113.9±2.1 days), respectively. Furthermore, herds with a high hormone use associated with on average 0.31±0.04 inseminations more to get their cows pregnant compared to non-user herds (1.83±0.04 inseminations). Similar trends, but to a lesser extent, were identified in herds with a medium and low hormone use. In conclusion, hormone use was associated with a better reproductive performance in dairy herds.

## INTRODUCTION

Reproductive hormones have been advocated for several decades to maintain a good reproductive performance and are regularly applied as part of dairy cows' reproductive management (Higgins et al., 2013; Moore and Hasler, 2017; Stevenson and Britt, 2017). Reproductive hormones are used to mitigate reproductive diseases. For instance, prostaglandins are used to treat cows with anoestrus, chronic endometritis and cystic ovarian follicles (Gundling et al., 2015; Lüttgenau et al., 2016), gonadotropin-releasing hormone for corpus luteal persistence (Lüttgenau et al., 2016), and progesterone use after insemination to reduce pregnancy loss and to improve fertility (Friedman et al., 2014; Bisinotto et al., 2015). Besides, hormones are also regularly applied to induce oestrus in order to improve reproductive performance. Routine application of hormones may conceal fertility management problems which may ignore the need to overcome the primary cause. Hormone use may thus be over-prescribed and potentially misused, and cost-effectiveness may be falsely assumed if outcomes are not monitored (Higgins et al., 2013).

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Hormones are intended to improve reproductive performance but results on the effectiveness are inconclusive. Treatment of a prolonged luteal phase with prostaglandin decreased the calving-to-conception interval and the number of services-per-conception and increased the first-service conception rate (Lüttgenau et al., 2016). On the other hand, progesterone had no effect on the probability of conception-to-first-service and the probability of pregnancy in anoestrus cows (Rabiee et al., 2004). Also, there were no differences in reproductive performance between follicular and luteal cysts in cows treated with progesterone-releasing intravaginal devices compared to non-treated animals (Rudowska et al., 2019). Furthermore, negative effects of treating endometritis with prostaglandin on reproductive performance (Haimerl et al., 2018) were found. A Dutch study, conducted more than 20 years ago, investigated the effect of gonadotropin treatment on cystic ovarian cows and showed no effect on the insemination-to-conception interval (Hooijer et al., 2001).

The effectiveness of reproductive hormones is commonly studied at the cow level in randomized clinical trials (e.g. Hooijer et al., 2001; Lüttgenau et al., 2016; Rudowska et al., 2019). To the best of our knowledge, the effect of hormone use on reproductive performance at the herd level, outside those of the clinical setting, has not yet been studied. Quantifying the effects of reproductive hormones on reproductive performance at the herd level would be important in order to evaluate the actual advantage of hormone use in dairy practice. Moreover, the impact of hormone use may be different at herd level compared to cow level because the treatment effect of problematic cows might be alleviated by the reproductive performance of non-problematic cows.

The aim of this study was to associate hormone use for reproductive diseases and heat induction with reproductive performance in a large collection of Dutch dairy farms using a longitudinal dataset of three consecutive years.

## MATERIALS AND METHODS

### Data collection

Data on the sales of hormones was obtained from five large veterinary practices located in different regions of the Netherlands. The data was anonymised and contained data on sales of hormone use and herd size from 754 farms during the years 2017-2019. Three common types of reproductive hormones, which are frequently applied in dairy farms, namely prostaglandin, gonadotropin, and progesterone, were evaluated to represent hormone use. Further details on this data collection are described elsewhere (van der Laan et al., 2021).

Additional data from the regular test day recording was provided by the Dutch Cattle Improvement Cooperative - CRV (CRV Holding BV, Arnhem, the Netherlands). It included herd level data from three years and consisted of datasets containing yearly averages on reproductive performance and monthly milk production records. Also, information about whether an automatic milking system was used was provided. Artificial insemination (AI) records were provided at cow level and contained information on the breeding strategy (AI or natural breeding) and who performed the inseminations (AI company or farmer).

### Data preparation

Data on hormone use was available for each herd in sum of ml or devices sold per quarter year by the veterinary practice. Subsequently, the number of doses per quarter was calculated as the sum of ml or devices divided by the dose of the hormone's product following the package leaflet. Then, the hormone use was summed into yearly data and standardized as the number of hormone doses per 100 adult dairy cows. This dataset consisted of 2,262 observations. A detailed description of the calculation of the hormone use can be found in van der Laan et al. (2021).

All datasets were combined. During this process, records were excluded because of missing values in one of the datasets, which was mostly a result of herds not participating in the test-day recording (88.4% participated in 2019). This process resulted in a dataset containing 1,738 observations. Subsequently, only herds with more than 30 producing cows were selected, which were assumed to represent commercial dairy farms (Kulkarni et al., 2021). In addition, variables containing unrealistic records were excluded (having an average calving interval of more than 500 days; an average calving-to-1st insemination interval of more than 200 days; and an average number of AI that was more than 5 inseminations). The final analytical dataset consisted of 1,679 observations from 560 farms.

### Statistical analysis

As dependent variables, the calving interval, the calving-to-1st insemination interval, and the number of inseminations were selected. The independent variables were hormone use, year, herd size, veterinary practice ID, 305-day milk production, automatic milking system (AMS), who performed AI (AI company, farmer), and breeding strategy (AI or AI + natural breeding). Continuous independent variables on hormone use, herd size, and 305-day milk production were checked for the linearity of their relationship with the dependent variables. Hormone use was subsequently categorized into four levels (no usage, low, medium, and high use), following the 33rd and 66th percentiles of the herds applying hormones. Similarly, the 305-day milk production was categorized into three levels of low, medium and high milk production following the 33rd and 66th percentiles.

All three reproductive performance indicators were analysed using multivariable General Estimating Equations (GEE) models. To correct for repeated herd level observations over time, the autoregressive correlation structure was determined with the best model fit among competing correlation structures based on the quasi-likelihood under the independence model criterion (Cui, 2007). The general structure of the multivariable GEE model was defined in Eq.(1) as follows:

$$Y_i = \beta_0 + \beta_1 HU + \beta_2 year + \beta_3 herdsize + \beta_4 practice + \beta_5 milk + \beta_6 AMS + \beta_7 insem + \beta_8 breeding + \varepsilon_i \qquad (1)$$

where $Y_i$ represents one of the three reproductive performance indicators in year i, $\beta_0$ the intercept, $\beta_1 - \beta_8$ the regression coefficients, *HU* the categorical variable describing the hormone use (non-user, low user (>0-21.6 doses per 100 cows), medium user (>21.6-50.6 doses per 100 cows), high user (>50.6 doses per 100 cows)), *year* the categorical variable indicating the year of data observation (2017-2019), *herdsize* the mean annual herd size, *practice* the categorical veterinary practice identification number (1 to 5), *milk* the mean herd level 305-day milk production (low (4,900-8,735 kg), medium (8,736-9,616 kg), high (9,617-12,900 kg)), *AMS* the binary variable indicating whether an AMS was used, *insem* the categorical variable indicating whether cows were inseminated by an inseminator from the AI company only, or additionally also by the farmer; *breeding* the categorical breeding strategy variable (AI vs AI and natural breeding), and $\varepsilon$ the residual error term.

Bivariable models were created by pairing the hormone use variable (as the main predictor of interest) with all other independent variables in association with each of the three reproductive performance indicators. Candidate predictor variables were chosen when $P < 0.15$. A correlation check was carried out among selected independent variables by using a correlation coefficient cut off of < 0.5. Lastly, the multivariable regression modelling process consisted of a backward selection procedure until all predictor variables were significantly associated with the outcome variable. During the backward selection procedure, the presence of confounding was determined, which was assumed when estimates changed more than 25% among nested models. Two-way interaction terms between hormone use and all other predictors in the final model were also examined. All regression analyses were performed using R-studio for Windows version 1.4.1103 using the 'geepack' package.

## RESULTS

Descriptive statistics are presented in Table 1. The total hormone use from 560 Dutch dairy farms was increasing over three years. Across the three study years, the median was 36.1 (mean = 43.1; min = 0.0; max = 248.2) doses per 100 adult dairy cow-years at risk in all herds while the median was 39.2 (mean = 46.8; min = 0.4; max = 248.2) doses per 100 adult dairy cow-years at risk among the user-herds. The average proportion of non-user herds per year was 8.0%. The calving interval increased over the three years, with an average of 411.0±0.6 days. The average calving-to-1st insemination interval was 93.3±0.5 days while the average number of inseminations was 2.18±0.01. The average herd size was 120±2.0 adult dairy cows with the 305-day milk production being on average 9,124±26.4 kg.

Table 1. Hormone use, farm characteristics and reproductive performance in 560 dairy herds in 2017, 2018 and 2019

| Variable | Descriptive measures | Year<br>2017 | 2018 | 2019 | 2017-2019 |
|---|---|---|---|---|---|
| Hormone use (doses per 100 cows) | | | | | |
| Total use in all herds | Mean±SD | 40.0±1.5 | 43.8±1.5 | 45.4±1.6 | 43.1±0.9 |
| | Median | 32.3 | 37.6 | 38.9 | 36.1 |
| | (min;max) | (0.0;243.2) | (0.0;200.4) | (0.0;248.2) | (0.0;248.2) |
| Total use in user-herds | Mean±SD | 43.5±1.5 | 47.9±1.6 | 49.2±1.6 | 46.8±0.9 |
| | Median | 35.8 | 41.0 | 42.9 | 39.2 |
| | (min;max) | (0.5;243.2) | (0.4;200.4) | (1.1;248.2) | (0.4;248.2) |
| Herd size (cows) | Mean±SD | 122±3.0 | 118±3.0 | 120±4.0 | 120±2.0 |
| 305-day milk production (kg) | Mean±SD | 8,954±44.5 | 9,178±45.0 | 9,240±46.8 | 9,124±26.4 |
| Reproductive performance | | | | | |
| Calving interval (days) | Mean±SD | 408.5±0.9 | 411.4±1.0 | 413.0±1.0 | 411.0±0.6 |
| Calving-to-1st insemination (days) | Mean±SD | 92.3±0.8 | 93.5±0.8 | 93.9±0.9 | 93.3±0.5 |
| Number of inseminations (number) | Mean±SD | 2.12±0.02 | 2.18±0.02 | 2.23±0.02 | 2.18±0.01 |

Table 2 shows the results of the final statistical models on the association of hormone use with the three reproductive performance indicators. After correcting for year, herd size, practice ID, 305-day milk production, insemination, and type of breeding, the final statistical models identified that herds with a high hormone use had a calving interval and a calving-to-1st insemination interval that was 9.3±2.6 and 16.4±2.1 days shorter than non-user herds, respectively. Furthermore, herds with a high hormone use needed on average 0.31±0.04 inseminations more to get their cows pregnant compared to non-user herds. Medium-user herds had a 6.5±2.6 days shorter calving interval and a 12.0±2.1 days shorter calving-to-1st insemination interval with 0.15±0.04 additional inseminations compared to non-user herds. Low-user herds had a 6.2±2.7 days shorter calving interval and a 7.9±2.2 days shorter calving-to-1st insemination interval than herds with no hormone use.

Table 2. Results of the three final generalized estimating equations (GEE) models on the association between hormone use with reproductive performance

| Variable | Calving interval | | | Calving-to-1st insemination | | | Number of inseminations | | |
|---|---|---|---|---|---|---|---|---|---|
| | Coefficient | SE | *P*-value | Coefficient | SE | *P*-value | Coefficient | SE | *P*-value |
| Intercept | 423.99 | 2.72 | <0.0001 | 113.99 | 2.13 | <0.0001 | 1.83 | 0.04 | <0.0001 |
| Hormone use | | | | | | | | | |
| non-user | Reference | | | Reference | | | Reference | | |
| low-user | -6.17 | 2.71 | 0.02 | -7.87 | 2.20 | 0.0003 | 0.03 | 0.04 | 0.43 |
| medium-user | -6.51 | 2.61 | 0.01 | -12.00 | 2.11 | <0.0001 | 0.15 | 0.04 | <0.0001 |
| high-user | -9.25 | 2.61 | 0.0004 | -16.35 | 2.09 | <0.0001 | 0.31 | 0.04 | <0.0001 |
| Year | | | | | | | | | |
| 2017 | Reference | | | | | | Reference | | |
| 2018 | 2.92 | 1.32 | 0.03 | NS [a] | | | 0.04 | 0.02 | 0.11 |
| 2019 | 4.82 | 1.33 | 0.0003 | | | | 0.09 | 0.03 | 0.0003 |
| Herd size | -0.03 | 0.01 | <0.0001 | -0.05 | 0.01 | <0.0001 | 0.001 | 0.0002 | <0.0001 |
| Veterinary practice | | | | | | | | | |
| practice 1 | -7.17 | 1.52 | <0.0001 | -5.32 | 1.27 | <0.0001 | -0.04 | 0.03 | 0.13 |
| practice 2 | -7.75 | 1.72 | <0.0001 | -5.25 | 1.27 | <0.0001 | -0.06 | 0.03 | 0.04 |
| practice 3 | -6.18 | 1.69 | 0.0003 | -6.48 | 1.39 | <0.0001 | 0.09 | 0.04 | 0.01 |
| practice 4 | -4.42 | 1.80 | 0.01 | -3.72 | 1.46 | 0.01 | 0.07 | 0.03 | 0.02 |
| practice 5 | Reference | | | Reference | | | Reference | | |
| 305-day milk production | | | | | | | | | |
| low | NS | | | NS | | | Reference | | |
| medium | | | | | | | 0.08 | 0.03 | 0.001 |
| high | | | | | | | 0.12 | 0.03 | <0.0001 |
| Who performed AI | | | | | | | | | |
| AI company | NS | | | Reference | | | Reference | | |
| AI company and farmer | | | | -2.65 | 1.09 | 0.01 | 0.03 | 0.03 | 0.38 |
| Farmer | | | | 0.74 | 1.04 | 0.48 | -0.14 | 0.03 | <0.0001 |
| Breeding strategy | | | | | | | | | |
| AI | Reference | | | NS | | | Reference | | |
| AI and natural breeding | -3.68 | 1.62 | 0.02 | | | | -0.21 | 0.03 | <0.0001 |

[a] Non-significant

## DISCUSSION

A higher hormone use was associated with a shorter calving interval and calving-to-1st insemination interval but it was also associated with a higher number of inseminations. Therefore, this observational study showed a positive association between the total hormone use with a better reproductive performance, aside from the insemination number. The shorter calving interval and calving-to-1st insemination interval and the higher number of inseminations are a likely result of farmers aiming to achieve their farm's reproduction performance targets. Therefore, farmers appear to accept the extra use of hormones and inseminations to minimise the possibility of a delayed pregnancy or involuntary culling caused by poor reproductive performance (van Arendonk and Liinamo, 2003). Re-inseminating cows with high milk production potential is economically beneficial and can be continued for some time before becoming cost-ineffective (van Arendonk and Liinamo, 2003) with a maximum of three inseminations (Inchaisri et al., 2011). Moreover, farmers tend to use reproductive hormones more frequently in, and inseminate more often, higher parity cows than lower parity cows which have a better first insemination conception rate (Balendran et al., 2008).

The positive effect of hormone use on reproductive performance was also revealed by a previous bio-economic simulation model (Ricci et al., 2020). An increase in the conception per insemination rate with a higher hormonal use was observed in that study evaluating intensive reproductive programs, such as when synchronization protocols are applied, leading to an economic benefit. Also, a randomized controlled trial determined that gonadotropin is the best choice for early postpartum dairy cows to achieve fewer days open and a higher conception per first insemination rate, resulting in a better milk production (El Tahawy, 2014). Those studies, however, were carried out in settings, where oestrus synchronisation programs are relatively common while our data were for Dutch circumstances where hormones are mainly used in a situation without oestrus synchronisation. In this case, hormone use is a prompt curative intervention when reproductive diseases or no oestrus signs exist. The occurrence of reproduction diseases and anoestrus are, for a large part, a result of suboptimal reproductive management. The latter should be optimized to settle the primary cause and prevent further reproduction problems. Poor reproductive performance is multifactorial and optimization of herd fertility often needs an optimization of some interfering managerial points (Opsomer et al., 2006). Good reproductive management practices include reproduction data recording, genetic selection, nutritional strategies, and biosecurity measures to prevent reproductive diseases. Moreover, in order to reduce the extensive use of hormones while maintaining or improving reproduction performance, a better oestrus detection is needed (Crowe et al., 2018). Finally, if hormones are applied, its use should be based on an accurate diagnosis (Refsdal, 2000) and followed by a veterinary advice to improve reproduction management in the farm (Opsomer et al., 2006).

In conclusion, using a large representative dataset of Dutch dairy herds, at the herd level, hormone use was shown to be associated with a better reproductive performance in terms of calving interval and calving-to-1st-insemination interval. However, the average number of inseminations per cow was slightly higher on farms with a high hormone use. The usage of hormones in dairy farms needs to be evaluated for its cost-effectiveness in light of an overall improvement in reproduction management.

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# THE ASSOCIATION BETWEEN LONGEVITY AND ANIMAL HEALTH IN DUTCH DAIRY HERDS

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## SUMMARY

This study evaluated the association between longevity and cattle health. Anonymized near census data was available for 16,200 Dutch dairy herds (~98% of the dairy herds) between 2016 and 2020. Herds were divided into six groups based on their longevity i.e. high or low, increasing or decreasing, stable median longevity and varying longevity. Cattle health parameters were analysed with multivariable population-averaged models that included longevity and other management factors as explanatory variables. Herds with a high longevity were associated with lower mortality, a higher antibiotic use in cows and a higher percentage of cows with a high somatic cell count (HSCC). The latter can be explained by a larger share of older cows. Herds with a low longevity had a higher mortality, a lower percentage of HSCC cows, lower antibiotic use in cows and higher antibiotic use in calves. In conclusion, longevity was associated with cattle health.

## INTRODUCTION

Longevity is defined as the mean age at culling, slaughter, export or at death of cows older than two years. Longevity in dairy herds in the Netherlands increased from 2018 to 2020. Increasing longevity in dairy herds may be economically beneficial, given the lower replacement costs (Heikkilä et al., 2008). Having fewer youngstock also leads to fewer surpluses on the mineral balance (Schils et al., 2007). Moreover, increasing longevity is favourable for reducing greenhouse gases, given that less replacement cattle are needed (Lehmann et al., 2014). In case of involuntary culling however, having fewer replacement heifers can be a disadvantage when no replacement heifer is available. In the Trend Analysis Surveillance Component (TASC) of the Dutch Cattle Health Surveillance System (CHSS) (Santman-Berends et al., 2016), near census data of about 98% of the dairy herds are routinely collected and analysed to monitor trends and developments in numerous cattle health indicators. These data provided the possibility to analyse the association between longevity of dairy cattle herds and cattle health. The results from TASC indicated that cattle mortality was lower in herds with a high longevity, compared to herds with a low longevity. Whether this is because of better cattle health or culling before a cow dies is unknown. Most studies have focussed on reasons for early culling. Little is known about differences in cattle health between groups of farms with a different longevity. Therefore, the aim of this study was to evaluate the association between longevity and cattle health parameters in Dutch dairy herds.

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## MATERIALS AND METHODS

### Study population and available data

For this study, anonymized near census data was available from approximately 98% of the Dutch dairy herds that agreed to use their routinely collected data for monitoring of cattle health (Santman et al., 2016). In total, data from 2016-2020 of 16,200 dairy herds were available.

Data were available from six different data sources. 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% 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). Finally, data of veterinary registrations on deliveries of antimicrobials were available per product, age category of cattle at herd level and date of delivering the antimicrobials to the herd (MediRund, Zuivel NL, the Hague, the Netherlands).

### Definitions of longevity groups

Six different groups of herds were defined based on differences in longevity. Descriptive results of these groups (including number of herds, criteria and longevity) can be found in Table 1. The groups were defined based on data and expert opinion. Each group consisted of a minimum of 750 herds, to ensure anonymity and statistical power. The first group was defined as ‘herds with a high longevity’ (n=2,016). The criterium to belong to this group of herds was that the average longevity belonged to the 75th percentile of longevity in dairy herds in at least four out of five years. The second group was defined as ‘herds with an increasing longevity since 2017’ (n=785). The reference year was 2017 because in that year new phosphate regulations were implemented in the Netherlands that had an effect on the size of the herds. This group included the 10% of herds with the highest increase in longevity, the average longevity in cows in this group increased at least one year between the reference year and 2020. The third group was defined as ‘herds with a median longevity’ (n=2,022), the cows in these herds had a longevity between the 25th and 75th percentile during the whole analysed period. The fourth group was defined as ‘herds with a decrease in longevity since 2017’ (n=966). This group included the 10% of herds with the highest decrease in longevity. The longevity of cows in these herds decreased with, on average, five months in 2020, relative to 2017. The group of ‘herds with a low longevity’ (n=1,632) belonged to the 25th percentile of herds in at least four out of five years. The remaining herds were classified in the group with a ‘varying longevity’ (n=10,170), they did not meet the criteria of any of the previously described groups.

Table 1. Description of the six defined longevity groups, including the number of herds in each group, the criteria for each group and these criteria expressed in actual longevity

| Longevity groups | N herds | Criteria | Longevity |
|---|---|---|---|
| High longevity | 2,016 | At least 4 of 5 years in the 75th-percentile | 75th -perc.: >6 years and 3 months |
| Increasing longevity | 785 | 10% herds with highest increase in longevity | Increase >1 year |
| Median longevity | 2,022 | 5 of 5 years between in the 25th and 75th-percentile | 5 years and 3 months – 6 years and 3 months |
| Decreasing longevity | 966 | 10% herds with highest decrease in longevity | Decrease >5 months |
| Low longevity | 1,632 | At least 4 of 5 years in the 25th-percentile | 25th -perc.: <5 years and 3 months |
| Varying longevity | 10,170 | Herds that didn't match the criteria in the other groups | |

Data analysis

Data validation was conducted using SAS® version 9.4 (SAS, 2021). Data from the previously mentioned six data sources were combined on unique animal and herd number. Biologically impossible outliers were removed. Thereafter, the dataset was aggregated on herd and quarter of the year level. The following cattle health indicators were evaluated for their association with longevity:

- Calf mortality (0-14 days old): the number of deaths of ear tagged calves from the moment of ear tagging until the age of 14 days, divided by the total number of ear tagged calves.
- Cow mortality (>one year old): the number of deaths of cows that are at least one year old, divided by the total number of cows that are at least one year old, corrected for the number of cattle days at risk.
- Percentage of cows with high somatic cell count (SCC): the number of cows (≥ second parity) with a SCC of at least 250,000 cells/ml, plus the number of heifers with a SCC of at least 150,000 cells/ml, divided by the number of lactating cows per herd.
- Age at first calving (in months).
- Number of inseminations per heifer, regardless of successful pregnancy.
- Number of inseminations per cow, regardless of successful pregnancy.
- Being certified free or unsuspected in the IBR control program (yes/no).
- Mean antibiotic use in adult dairy cows: the yearly average animal defined daily dose (DDD) applied in adult dairy cows (>two years), per herd per quarter of the year (DDD/Y).
- Having a high antibiotic use in dairy calves (yes/no): herds are classified according to a rolling antibiotic use of ≤ 9.25 animal defined daily dose per year (DDD/Y) and a DDD/Y of >9.25. The cut-off value of 9.25 is the DDD/Y whereby 75% of the farms had a lower value and 25% of the farms had a higher value in 2013 (75th percentile).

Stata® 17 was used for analyses and generating tables and figures (Stata, 2021). Descriptive statistics were used to plot the longevity on dairy farms over time, per quarter of the year over the period 2016-2020 and to compare the defined longevity groups. Multivariable population-averaged models (PA GEE) with the appropriate distribution (e.g. gaussian, binomial or poisson) and a link function (identity, logit or log), which corrected for repeated measures per herd, were used for analyses. The cattle health indicators were included as dependent variable and the longevity group variable was added as an explanatory variable.

Other explanatory parameters that were included in the models were herd size, growth in herd size, location represented by province, milk production level, season, milk price, price of calves, open or closed farming system, status (free vs. non-free) for endemic diseases such as salmonellosis, leptospirosis, BVDV, BHV-1 and paratuberculosis, milking parlour (regular vs. automated milking system) 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). For the categorized variables, the mean of the whole population was included as the reference category, which is thus dynamic. Only the variables that explained most variation will be presented in this paper. Results were presented as an Incidence Rate Ratio (IRR), Odds Ratio (OR) or as a value. A conservative *P*-value below 0.01 was considered significant because of the large numbers of observations in the model. Colour indicators were used in the presentation of the multivariable results to indicate a significant difference compared to the reference category (e.g. the average dairy herd in the Netherlands). Black and white colours indicated significant results and dark-grey and light-grey represented non-significant results. Moreover, a black and dark-grey colour indicated an unfavourable association; a white and light-grey colour indicated a favourable association.

## RESULTS

### Descriptive results

The mean longevity on dairy farms increased for five out of six defined groups of herds during the study period. The longevity only decreased in the group of herds defined as having a decreased longevity. The mean longevity in herds that were classified as having a high longevity was seven years, and in herds with a low longevity four years and 10 months. The mean longevity increased with on average one year and two months in the group of herds with an increasing longevity. The mean longevity decreased on average eight months in the group of herds with a decreasing longevity. Both the group of herds with a median and fluctuating longevity had a mean longevity of five years and eight months at the end of 2020 (Fig. 1).

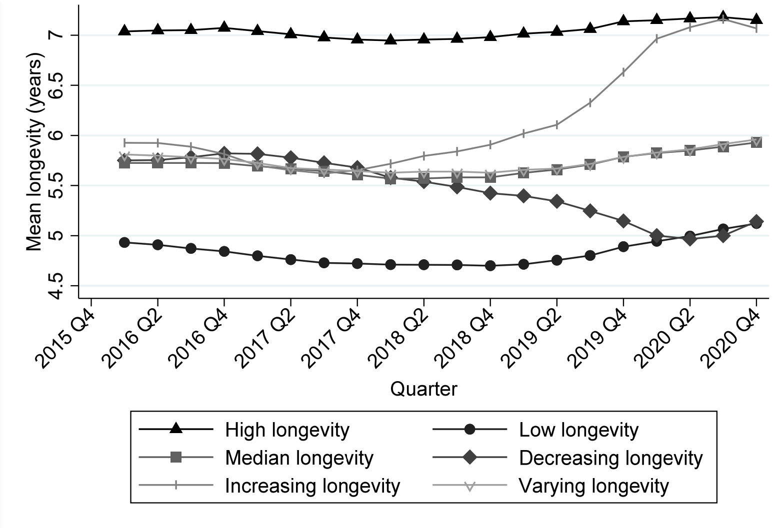

Fig. 1. Trend in the mean longevity in dairy herds, per longevity group in the study period between 2016 and 2020

Multivariable results

The group of herds with a high longevity was significantly associated with lower calf- and cow mortality compared to the average Dutch dairy herd (IRR=0.87 and 0.89, respectively). Increasing longevity was also associated with lower cow mortality (IRR=0.89). On the contrary, belonging to the group of herds with a fluctuating and low longevity was associated with a significantly higher calf- and cow mortality (IRR=1.05 and 1.01 (fluctuating longevity), IRR=1.09 and 1.15 (low longevity), respectively). A decreasing longevity was also significantly associated with higher cow mortality (IRR=1.07) (Fig. 2).

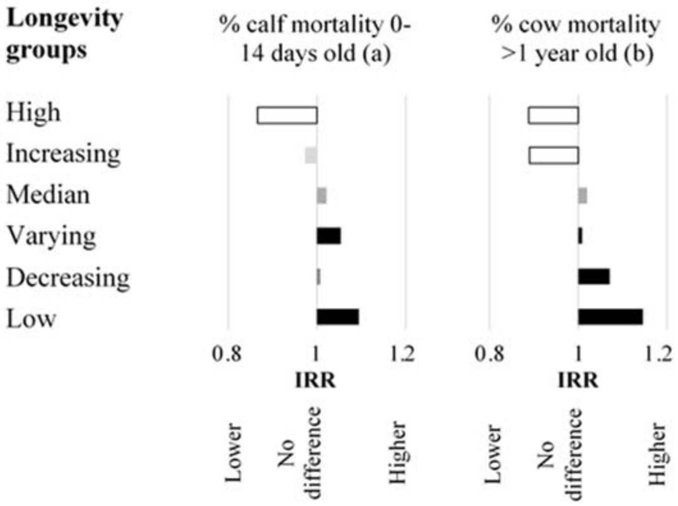

Fig. 2. Incidence rate ratios (IRR) of mortality of calves between 0-14 days old (a) and adult cows older than one year (b), in the study period between 2016 and 2020, per longevity group (corrected for other explanatory variables in multivariable model)

Herds with a high longevity had a significantly higher prevalence of cows with high SCC compared to the average Dutch dairy herd (1.1% higher). Belonging to the group of herds with a low, increasing and decreasing longevity were associated with a lower high SCC prevalence (0.6%, 0.3% and 0.3% lower, respectively) (Fig. 3).

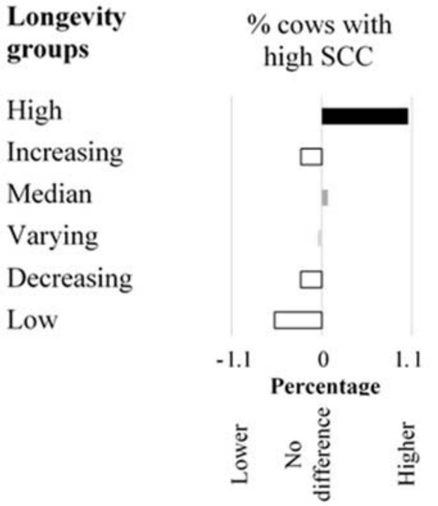

Fig. 3. The percentage of cows with a high Somatic Cell Count (SCC) in the study period between 2016 and 2020, per longevity group (corrected for other explanatory variables in multivariable model)

Production level had the strongest association with high SCC prevalence, where low producing herds were associated with a higher high SCC prevalence (6.1% higher than the average Dutch dairy herd).

A higher proportion of cows with a high SCC in herds with a high longevity may be explained by more udder infections and by a larger number of older cows with a naturally higher SCC. This issue was further investigated by comparing the percentage of cows with a high SCC (>250*103 cells/ml) per age category between longevity groups. Figure 4 shows that the percentage of cows with a high SCC increased with age. In the age group of two to three years, 6.5% of the cows had a high SCC and 22.9% in the age group between seven and eight years old. Stratified to age category, the percentage of cows with a high SCC was comparable between the different longevity groups. The variance was the highest in the age group between seven and eight years old, especially in the low longevity group, explained by a lower number of cows of that specific age group. Thus, the higher percentage of cows with a high SCC in herds with a high longevity was due to a larger number of older cows, rather than a poorer udder health for cows of a certain age.

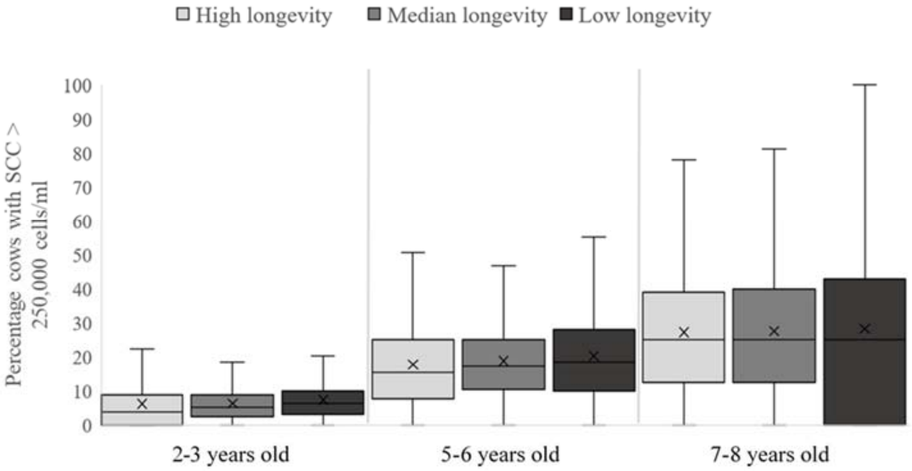

Fig. 4. Boxplots (mean (x), median, 25th and 75th percentile) of the percentage of cows with a SCC>250*$10^3$ cells/ml, in three different age groups in the study period between 2016 and 2020, for the groups of herds with a high, median and low longevity

The group of herds with a high longevity was associated with a higher age at first calving (11 days older), fewer inseminations for heifers (-2 per 100 heifers), but more inseminations for cows (two per 100 cows). On the contrary, low longevity herds were associated with a lower age at first calving (nine days) and fewer inseminations for cows (-2 per 100 cows) (Fig. 5).

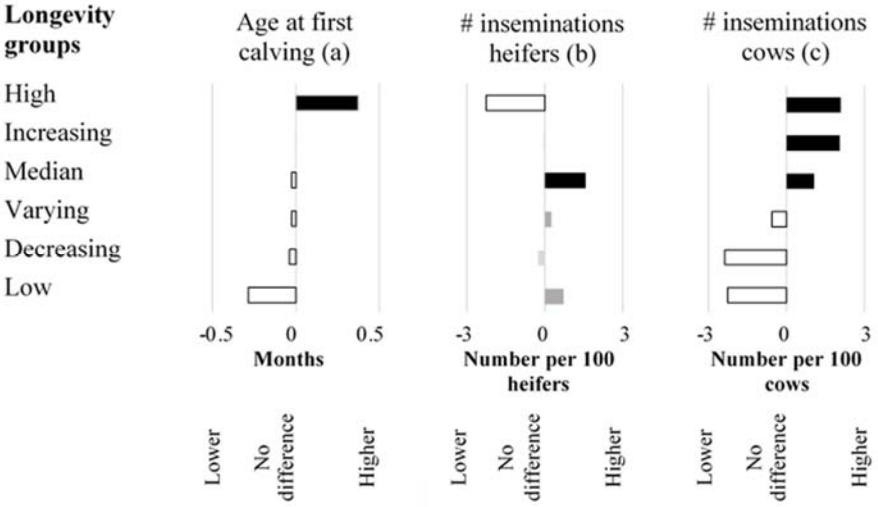

Fig. 5. Coefficient of the age at first calving (a) the number of inseminations per 100 heifers (b), the number of inseminations per 100 cows (c) in the study period between 2016 and 2020, per longevity group (corrected for other explanatory variables in multivariable model)

Longevity did not explain much variation in the age at first calving. Milk production level explained more variation, i.e. the 10% lowest producing herds had a one month and 20 days higher age at first calving compared to the average Dutch dairy herd.

Herds with a high, increasing and decreasing longevity were significantly associated with higher odds of IBR free or unsuspected certified herds (OR=1.18, 1.23 and 1.05, respectively). Herds with a median, fluctuating and low longevity were associated with a lower odds to be either IBR free or unsuspected (OR=0.90, 0.95 and 0.77, respectively) (Fig. 6).

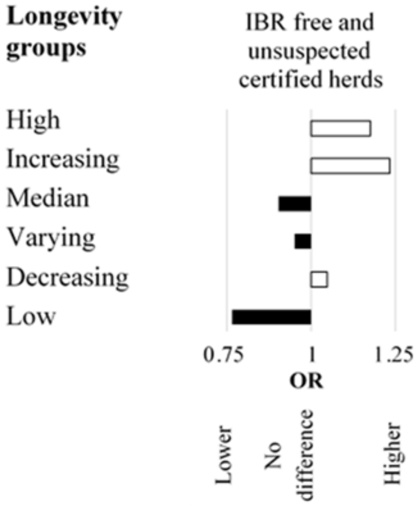

Fig. 6. The odds ratio (OR) of certified IBR free or unsuspected herds in the study period between 2016 and 2020, per longevity group (corrected for other explanatory variables in multivariable model)

Production level and type of farming system (open/closed) showed a stronger association with IBR status than the longevity status of the herd. The 10% highest producing herds were associated with a higher percentage of IBR free and unsuspected certified herds (OR=1.99). Closed farms were also associated with a favourable IBR status (OR=2.07).

The group of herds with a high, increasing and median longevity was associated with a higher antibiotic use in adult dairy cows (0.11, 0.07 and 0.03 DDDA/Y higher respectively). In contrary, herds with a high and increasing longevity also had a lower odds of a high antibiotic use (>9.25 DDD) in calves (OR=0.80 and 0.95 respectively). An opposite result was found for herds with a decreasing and low longevity, these groups were associated with a lower antibiotic use in adult cows (0.10 and 0.12 DDDA/Y lower, respectively) but a higher odds for a high antibiotic use in calves (OR=1.11 and 1.22, respectively) (Fig. 7).

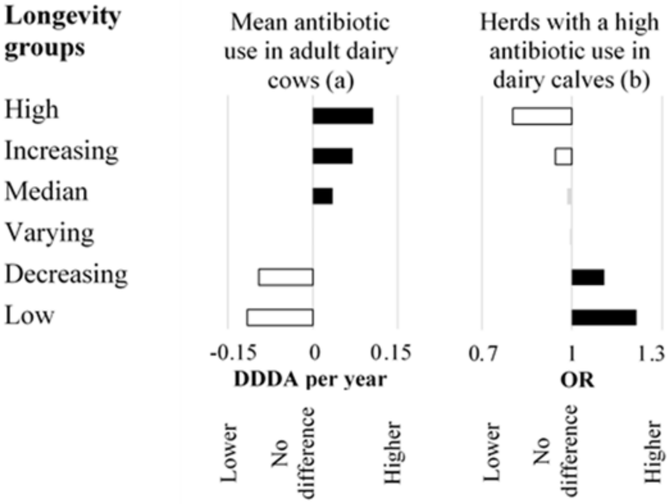

Fig. 7. The coefficient of the mean antibiotic use in adult dairy cows (a) and the odds for a high antibiotic use in dairy calves younger than 8 weeks (b) in the study period between 2016 and 2020, per longevity group (corrected for other explanatory variables in multivariable model)

## DISCUSSION

The aim of this study was to determine the association between herd longevity and cattle health. The focus was on herds with a high longevity, because public opinion favours older cows. This study shows that cattle health is associated with herd longevity. There was a tendency that herds with a high longevity had better cattle health parameters. The exception was udder health: older cows more often had a high SCC, which probably explained the slightly higher use of antibiotics in adult cows in high longevity herds.

Herds with a high longevity were associated with lower calf and cow mortality. The negative association might be related to the fact that longevity is partly determined by cows that die. However, the far majority of cows on which longevity is based, leave the herd for slaughter. The association is more likely due to better health leading to an older age at culling and lower mortality. Nevertheless, high longevity herds were associated with a higher percentage of cows with a high SCC. Yet, stratified for cattle of the same age, the probability of having a high SCC appeared comparable between the high, median and low longevity herds. This implies that more cows with a high SCC in high longevity herds is a result of a larger number of older cows that have a naturally higher SCC. Bradley and Green (2005) also observed that older cows tend to have higher SCC, irrespective of infection status. Another finding was that herds with a high longevity differed in fertility indicators. Farmers from high longevity herds tended to inseminate their heifers at a later age, with the consequence that the age at first calving was higher. A higher age at first calving may, therefore, be a consequence of management decisions, instead of a compromised fertility. The number of inseminations used for heifers remained lower. Nevertheless, more inseminations were used for cows. In a previous study, the chance of a successful first insemination decreased with parity (Inchaisri et al., 2010). This can explain why more inseminations were used for cows, because high longevity herds have more and older multiparous cows. In addition, farmers with high longevity herds may accept more inseminations to get a cow pregnant, compared to farmers with low longevity herds. The antibiotic use in adult cows was higher in high longevity herds, probably because farmers more often decide to treat instead of cull cows with an infection. Moreover, older cows are more likely to be dried off with antibiotics because they have a SCC that exceeds the threshold for which dry cow treatment is advised in the Netherlands. On the contrary, antibiotic use in calves was lower in these herds and together with the lower calf mortality, this indicates a better calf health.

Herds with a low longevity had higher calf and cow mortality compared to the average Dutch dairy herd. The percentage of cows with a high SCC was lower, which can be explained by a lower share of older cows, but may also be due to culling of high SCC cows rather than treating them. Heifers had a lower age at first calving in low longevity herds, but more inseminations were used for heifers. Possibly, heifers have not reached the optimal age for insemination yet, and therefore more inseminations were needed. Kuhn et al. (2006) found that conception rate was indeed lower for breedings at less than 15 months of age in comparison to heifers that were inseminated between 15 and 16 months of age. The cows in low longevity herds received a lower number of inseminations than the average Dutch dairy herd. Perhaps farmers are less tolerant to cows that return to service, and decide to cull the cow when fertility was deemed poor. Antibiotic use in calves was higher, and lower in adult dairy cows. Possibly, farmers treat the cows less often, and choose to remove the cow instead.

The strength of the association between longevity and animal health indicators varied and was sometimes fairly low with small differences between the longevity groups. Other herd characteristics such as milk production levels or herd size may explain more variation in the cattle health parameters. In this study, only routinely available anonymized near census data were used. Management strategies and motivations of farmers were not available. Our results indicated differences in farmers' management related to longevity and it would therefore be relevant to be able to include attitude and motivations of farmers. Such information could provide more insight in the relationship between longevity and animal health indicators. Another interesting topic for further studies is the comparison of the total lifetime production per defined longevity group. Standardized production parameters show a lower milk production per cow per day in high longevity herds (results not shown). However, it may be that lifetime production does not differ between high and low longevity herds. Further studies on this topic may help to disprove the argument that it is not economically beneficial to focus on a high longevity. Vredenberg et al. (2021) studied the effect of longevity on economic return in dairy herds and concluded that there was no difference in economic return between high and low longevity herds. However, in their study the variation in longevity was smaller (a difference of approximately one year in longevity).

To conclude, this study showed differences in cattle health between herds that differ in longevity. In general, the high longevity herds seemed to have better cattle health than low longevity herds. However, the consequence of having older cows is a higher risk for a high SCC, which can lead to more antibiotic use. Thus, aiming for a high longevity can be in conflict with a pursuit of low antibiotic use and low bulk milk SCC. The motivations and goals of farmers in different longevity groups should be investigated in a follow-up study to determine the success factors of a high longevity in combination with good cattle health.

## ACKNOWLEDGEMENTS

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# RISK MODELLING

# MODELLING PARASITE-DRIVEN IMPACTS OF AQUACULTURE ON WILD FISH: THE CASE OF THE SALMON LOUSE (*LEPEOPHTHEIRUS SALMONIS*)

A.G. MURRAY*, S.C. IVES, J. MURPHY AND M. MORIARTY

## SUMMARY

International studies show salmon lice from fish farms can substantially impact wild salmon populations. Farmed and wild salmon are respectively key components of the Scottish marine economy and environment. If larval salmon lice in the marine environment exceed threshold concentrations over large enough areas then they risk infestation of wild salmon smolts at intensities that significantly impact their welfare and populations. As a step towards providing a modelling structure for evaluation of impacts of farm origin lice on wild salmon we here combine deterministic models of larval lice production and infection with deterministic models of smolt size, growth and migration. Highest salmon lice concentrations occur in inshore waters (sea lochs and sounds), but salmon lice may also occur in more open coastal waters, and exposure to infection is dependent on smolt migratory behaviour.

## INTRODUCTION

Scottish salmon (*Salmo salar*) farming is worth ~£1BN at first sale (Munro, 2021) and is a major contributor to year-round employment in relatively remote areas of Scotland. Sustainability of the industry is influenced by the salmon louse (*Lepeophtheirus salmonis*), an ectoparasitic copepod. Impacts occur both due to high management costs of £0.34 $kg^{-1}$ to farmed production (Abolofia et al., 2017) and interactions with wild salmon populations. The presence of lice from salmon farms is associated with a risk ratio to wild salmon survival of 1.07:1.30 (Vollset et al., 2016). Risk analysis identifies salmon lice infestation of wild salmonids as a key environmental impact of salmon aquaculture, and thus limit to sustainable regional biomass (Taranger et al., 2015).

Salmon lice develop through multiple phases, each with different behaviours (Hamre et al., 2013). The lice hatch as non-feeding planktonic nauplii which mature to infectious copepodids. These copepodids must find a wild or farmed salmonid host, or die. If they infect a host the copepodids mature through attached chalimus stages, before becoming mobile pre-adults and then adults that graze on mucus and skin of their host. If numbers of these mobile lice on a fish exceed threshold intensities they can cause significant damage or cause direct mortality of their hosts. Adult female lice once mated, produce strings of eggs which hatch to produce more nauplii.

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Owing to their planktonic stages, salmon lice can be transported from their source farm over distances of tens of kilometres that are dependent on local hydrodynamics (Salama et al., 2018).

Wild salmon smolts from rivers enter the coastal environment, where they may be exposed to salmon lice originating from farms. Exposure depends on the transport processes of lice and on the movements of the fish in inshore and coastal waters. Vulnerability thresholds of fish to infection, in terms of number of lice, depend on the weight of the fish affected (Taranger et al., 2015).

Here we combine simplified deterministic models of salmon lice and salmon smolts to form a modelling structure that may be elaborated to estimate the impact of salmon lice on salmon (Fig.1). This model structure is used to produce an example map for salmon exposure to lice concentrations. Results, in terms of average infection mobile stage lice per gram of host, are illustrated in an example system.

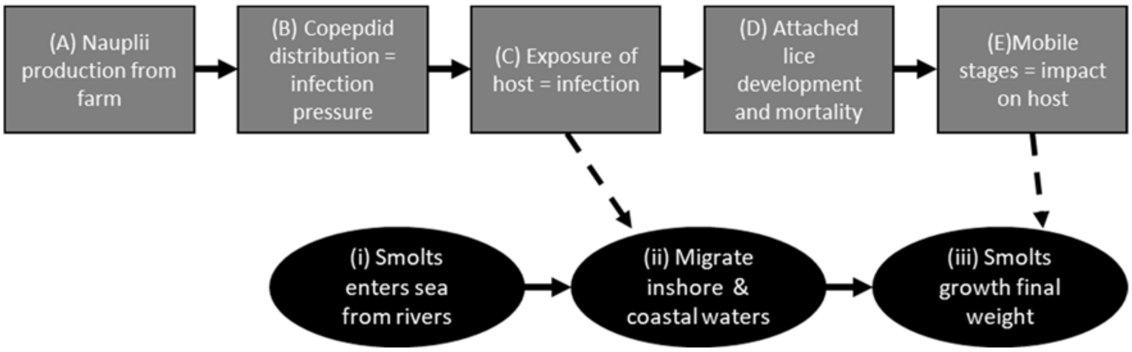

Fig. 1. Structure of interacting salmon lice (light grey, A to E) and salmon smolt (dark grey, i to iii) models

## MATERIALS AND METHODS

### The salmon lice model

The salmon lice model used is based on that from Murray and Moriarty (2021), which is here extended to include post-infection survival of lice on the host and their impact on this host. The model (Fig. 1) consists of five parts: (A) production of viable nauplii from farms, (B) transport by currents, (C) infestation of fish by copepodids, (D) maturation and mortality of attached chalimus stages on the host, and (E) mobile populations relative to thresholds for impact on fish health. Most existing modelling focuses on A and B, here we take inferences from this existing modelling and focus on development of C to E to holistically assess how concentration of copepodids in the environment relates to impact on salmon smolts.

(A) Production of viable nauplii: Production of nauplii from a salmon farm depends on the number of fish on the farm, numbers of adult female lice per fish, and viable egg production rate for ovigerous females, assumed at 30 day$^{-1}$ in seawater (Murray and Moriarty, 2021); egg viability is sensitive to environment. This stage can be actively managed by controlling the adult lice numbers on farms.

(B) Distribution of larvae: Transport depends on local hydrodynamics, complicated by phototactic swimming of the larvae, and the duration of the planktonic phases, which is temperature dependent. During this process larval lice die at a rate that was assumed to be 0.01 $h^{-1}$ (Salama et al., 2018). At 10 °C, maturation of nauplii to copepodids takes on average 4 days. This corresponds to 38% of nauplii surviving to become copepodids, which then survive on average 4.2 days (Murray and Moriarty, 2021). This equates to 48 copepodids distributed in the environment per ovigerous louse on the farm at any one time.

Copepodid distribution can be simulated in detail as a complicated and dynamic distribution of patches of copepod larvae (Salama et al., 2018). However, here, for simplicity in the application of the model, a kernel distribution is used, with copepodids, and hence infection risk, decaying with distance from the source (Salama et al., 2016). These dispersion models generate local concentrations of infectious copepodids $C$; for historical reasons this concentration is expressed in units of lice $m^{-2}$.

Here, exposure of smolts to lice is a function of average copepodid concentration $C$ and time smolts are in contact with this concentration. Resultant $C_t$ has units of lice.days.$m^{-2}$.

<u>(C) Infection by copepodids</u>: Infection rate of salmon lice copepodids depends on contact between copepodid and host (Murray and Moriarty, 2021). This is a function of motion of both louse and host. Salmon lice copepodids can achieve short-term burst speeds, $L_s$, of 1-5 cm.$s^{-1}$ over $\tau_{max}$ of 1 to 3 seconds (Heuch and Karlsen, 1997). Salmon smolts typically swim at a median speed of 1 body length.$s^{-1}$ on a Scottish west coast system (Middlemas et al., 2017). The volume of water from which lice have the potential to contact a host $U_w$ is dependent on the length $l_f$ and radius $r_f$ of the fish minus its volume $V_f$ and multiplied by its speed $B$ in body lengths.$s^{-1}$ (Eq. 1) and $X$, the distance over which lice approach smolts, is a function of lice and smolt swimming patterns (Eq. 2). These two parameters are therefore expressed as:

$$U_w = \left(\left(\pi\left(X + r_f\right)^2 \times l_f\right) - V_f\right) \times \mathrm{B} \tag{1}$$

and

$$X = min[L_s/B\,, L_s\tau_{max}] \tag{2}$$

Infectious contact.$s^{-1}$, $K$, depends on concentration of lice $m^{-2}$ divided by the depth $Z$ these lice typically mix over. Attachment of lice on contact occurs a probability, $a$.

$$K = aC/Z \times U_w \tag{3}$$

<u>(D) Attached lice development and mortality</u>: If infection is successful then lice mature through two chalimus stages to become pre-adult mobile lice. The length of time required is temperature-dependent: at 10 °C this is approximately 16 days (Stien et al., 2005). During maturation a proportion of the lice die (Stien et al., 2005). From Tucker et al. (2002), compounded mean loss for attached copepodid and chalimus stages is 34.7%. So proportion surviving $g = 0.653$ and mobile lice infection rate $M$ is given by:

$$M = gK \tag{4}$$

<u>(E) Mobile stages impact on hosts</u>: Impact of salmon lice depends on the number of mobile lice per gram of host. Threshold intensity for mortality assessed by Taranger et al. (2015) is $T1$

= 0.1 lice.g$^{-1}$ associated with 20% mortality, *T2* = 0.2 lice.g$^{-1}$ with 50%, and *T3* = 0.3 lice.g$^{-1}$ with 100% mortality.

### The salmon model

The salmon model (Fig. 1) consists of three steps: (i) smolts enter the sea at a certain initial weight, (ii) they migrate through coastal waters during which they are exposed to lice and (iii) they grow up to a final weight which determines the number of mobile lice per gram of host and hence risk of impact on smolt survival

(i) Smolts enter sea from rivers: Salmon hatch in fresh water and migrate to sea as smolts. Although smolt sizes vary between rivers and years (Malcolm et al., 2015) an initial length $l_{f0}$ = 12.5 cm is used here to illustrate the method. We also investigate effect on contact rate for smolts ranging from 10-20 cm, following Murray and Moriarty (2021) to highlight the effects of variation in initial size of Scottish salmon smolts. Given length $l_f$, fish weight $W_f$ in grams is (Eq.5):

$$W_f = (l_f - 8.38\ cm)/0.21\ cm \tag{5}$$

At 12.5 cm, a smolt ≈ 20 g by Eq. 5 (derived using data in Morris et al. (2019)). If biomass density = 1 g.cm$^{-3}$, then weight in grams and volume in cm$^3$ are equivalent. Assuming fish shape remains similar with size, average fish radius ($r_f$) is given by (Eq. 6):

$$r_f = \sqrt{V_f/(\pi l_f)} \tag{6}$$

Salmon smolts go to sea in April to May (Malcolm et al., 2015), when water temperatures typically are around 10 °C, so this temperature is used for default biological parameterisation.

(ii) Smolts migrate through coastal waters: Smolt swim speeds through inshore and coastal waters is variable; here we use the median value B = 1 body length.s$^{-1}$ for smolts in a west coast of Scotland sea loch (Middlemas et al., 2017), but also illustrate results for a range of speeds. The route they take depends on swimming behaviour and the local coastline (Kristoffersen et al., 2018; Ounsley et al., 2020), which in the Scottish case involves fjordic sea lochs, islands and sounds which will all affect route and exposure to salmon lice in different ways.

(iii) Smolt growth: Smolts final biomass is dependent on initial biomass and a growth model (Eq. 7) which determines length $l_{fD}$ after *D* days (Mork et al., 2012):

$$l_{fD} = l_{f0}\, e^{yD} \tag{7}$$

For fitted growth parameter $y$ = 0.0059 d$^{-1}$ (Mork et al., 2012) and given $D \approx 16$ day maturation time with slight difference for male and female lice (Stien et al., 2005), this formula approximates to 10% growth, so for an $L_{f0}$ = 12.5 cm smolt $l_{f16}$ = 13.7 cm which approximates to 25 g (Eq. 5).

## RESULTS

### Calculating critical lice concentrations in the environment

The model described above calculates exposure of fish to salmon lice infection. By reversing this model we can calculate concentrations of salmon lice that will result in migrating salmon being exposed to lice loads that will result in infection in excess of 0.1, 0.2 or 0.3 lice.g$^{-1}$.

An estimation of *X*: The contact distance over which lice approach hosts, *X*, depends upon the active swimming of lice copepodids. Copepodid burst swim speed can typically range from 1 to 5 cm.s$^{-1}$ (Heuch and Karlsen, 1997; Murray and Moriarty, 2021) which leads to uncertainty in estimation of contact. Therefore an estimation of *X* is needed to apply the model. We calculated a value of *X* by fitting the model to observed infection rates.

Sandvik et al. (2020) reported high infection as 10 lice.fish$^{-1}$ on 50-60 g fish held in sentinel cages, which corresponds to 17.5 cm. Given mortality of chalimus, the 10 mobiles correspond to $K = 15.3$ (Eq. 3), and for attachment a = 0.5, this *K* implies 30.6 copepodid contacts with hosts (survival on the host is not included as all lice stages are included in the 10 lice.fish$^{-1}$).

This observed infection occurred where model simulation concentrations of $C_t = 1.8$ lice.day.m$^{-2}$ (Sandvik et al., 2020). Assuming $B = 1$ s$^{-1}$ and $Z = 2$ m (Murray and Moriarty, 2021) then we fit $X = L_s = 1.84$ cm.s$^{-1}$ copepodid swimming speed, for this infection on 55 g fish at this concentration. This estimation of *X* is consistent with typical observed copepodid swimming speeds (Heuch and Karlsen, 1997), and will be refined as more data become available.

Calculating maximum lice concentrations for wild salmon population impacts: For $l_{f0}$ of 12.5 cm fish, with final weight of 25 g, the threshold of *T1* correspond to 2.5 mobile lice on the fish, while the $T2 = 0.2$ and $T3 = 0.3$ lice.g$^{-1}$ thresholds correspond to 5 and 7.5 mobile lice. These loads correspond to 3.8, 7.7 or 11.5 infection events, which implies 7.7, 15 or 23 contacts as $a = 0.5$ probability of infection (Murray and Moriarty, 2021).

Table 1. Summary of threshold numbers of mobile lice on hosts and environmental copepodid concentrations that induce population impacts on smolts (from Taranger et al., 2015)

| | 12.5 cm fish | | |
|---|---|---|---|
| Impact level | T1 20% | T2 50% | T3 100% |
| Mobile lice on fish | 0.1 lice.g$^{-1}$ | 0.2 lice.g$^{-1}$ | 0.3 lice.g$^{-1}$ |
| Copepodids in water | 0.76 lice.day.m$^{-2}$ | 1.52 lice.day.m$^{-2}$ | 2.28 lice.day.m$^{-2}$ |

Given that $L_s = 1.84$ cm.s$^{-1}$ these parameters correspond to maximum exposure levels of $C_t = 0.76$, 1.52, and 2.28 lice.day.m$^2$ respectively (Table 1), which is the product of lice concentration *C*, and number of days the salmon smolt travels through the salmon lice copepodid concentrations. However, the critical concentration depends on smolt size and speed (Fig.2a), with a minimum concentration to avoid impacts for moderate low speeds, $B = 1/\tau_{max}$, in this case $B = 1$.

An estimation of exposure time for Scottish wild salmon: Given an initial length of 12.5 cm and a cruising speed of $B = 1$, salmon swim at 10.8 km.d$^{-1}$, using the directed swimming approach of Kristoffersen et al. (2018). Most Scottish sea lochs are < 10 km, but 9 are longer than 20 km (Murray et al., 2011), for example Loch Linnhe is approximately 50 km long, so fish originating from the upper loch may be exposed to elevated lice concentrations for several days as they pass down the loch.

Threshold concentration for a specific distance to travel (Fig.2b) is not affected by $B$ when this is less than $1/\tau_{max}$, but increases above this velocity.

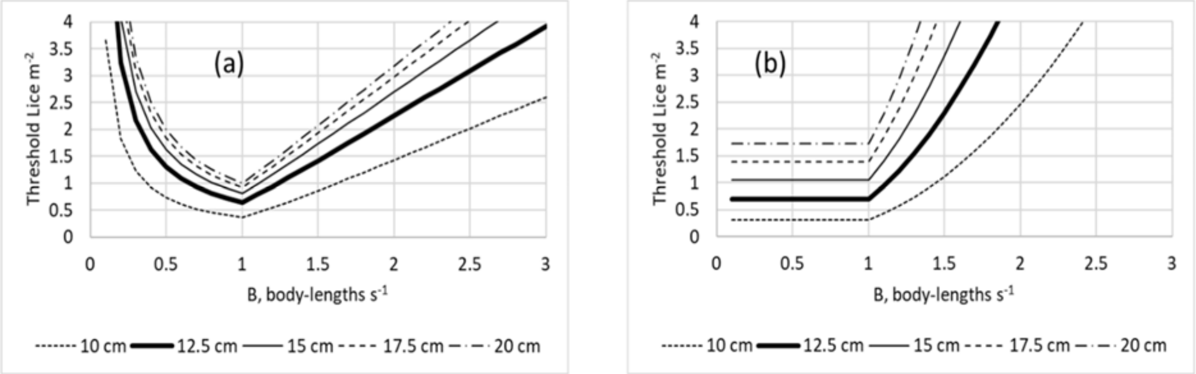

Fig. 2. Threshold salmon lice copepodid concentrations $C$ for salmon of initial length 10 to 20 cm given exposure of (a) lice m$^{-2}$ for one day's exposure or (b) for given a passage distance of 10 km

Distribution of salmon lice in Scottish inshore and coastal water

Salmon lice are produced from fish farms located along the Scottish west coast and western and northern isles (Munro, 2021). Relatively few lice are produced from wild salmonids, owing to their much smaller populations (Butler, 2002). Numbers of adult female lice per fish are reported weekly by each farm (aquaculture.scotland.gov.uk). Here 58% are estimated to be ovigerous, based on rate egg strings that are produced per adult female (Boxaspen, 2006). Numbers of fish are not reported, but are estimated from farm consented biomass using an approximation of 1.5 × consented biomass in kg /3 kg fish.

The exposure of fish to infection by lice from farms depends on the distribution of copepodid concentration $C$ and the pathway and speed of smolts. Distribution of $C$ is here set with exponential decay under kernel models (Salama et al., 2016; Murray and Moriarty, 2021), and this distribution is illustrated for a case study assessment of risk to migrating 12.5 cm smolts exhibiting directed swimming at $B = 1$ body length.s$^{-1}$ on example migration routes (Fig. 3).

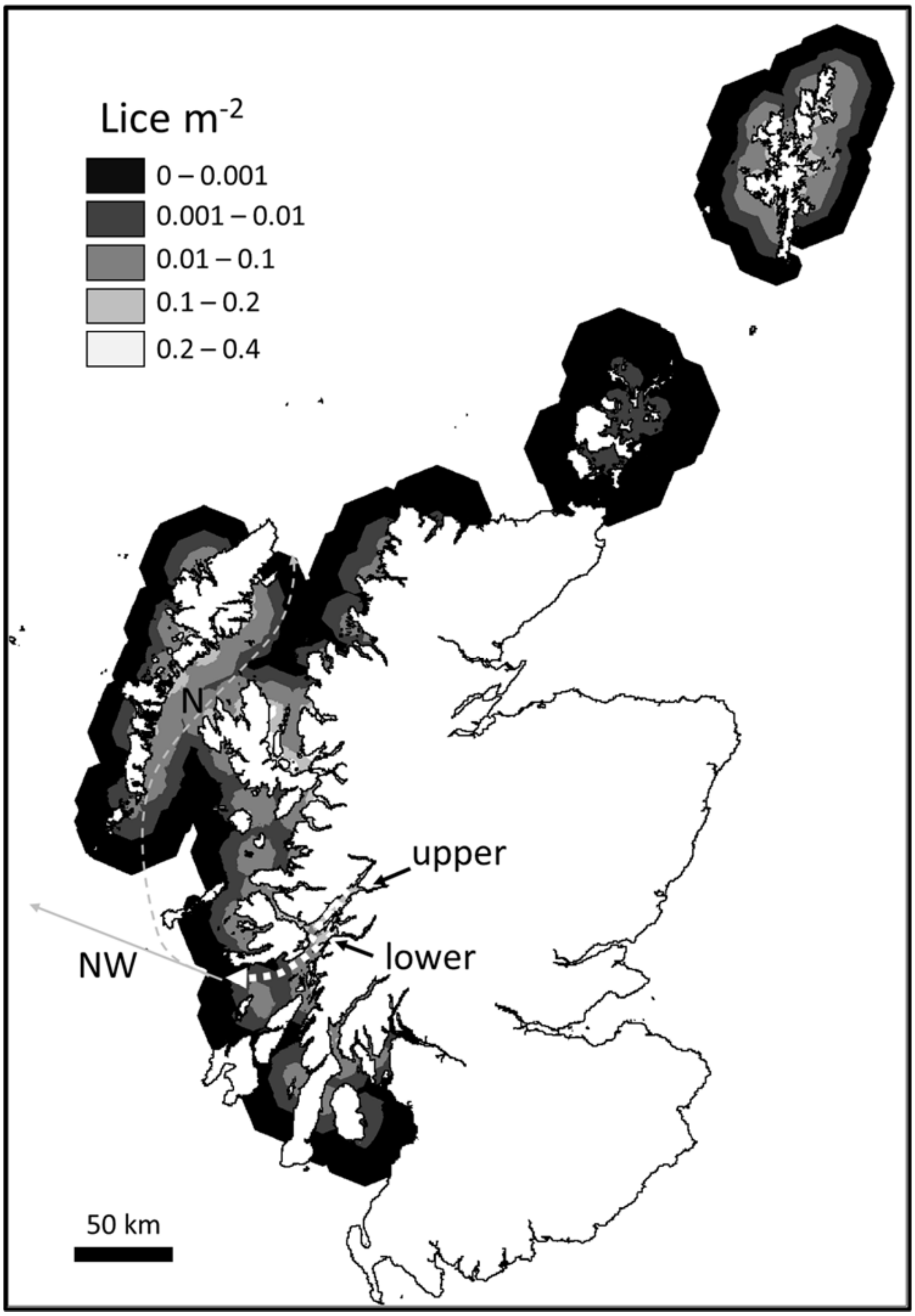

Fig. 3. Example map of salmon lice concentrations around farms based on kernel distribution modelling (weighted by site consented biomass and average adult female lice count Jan 2018-December 2020). Smolts migration routes from sources in upper and lower Loch Linnhe (white dots), and then through coastal waters to the shelf edge. North West (NW, solid grey), or North (N, dashed grey) routes are used as examples of exposure to lice infection

Highest copepodid concentrations occur in inshore waters of sea lochs or narrow sounds. Concentrations simulated for average farm lice counts in 2018-2020 are below threshold *T1* for a one-day exposure (Table 1) throughout all areas in the scenario illustrated (Fig.3). However, migrating salmon may be exposed to such concentrations for several days. Exposure over this migration time leads to infection closer to, but still below, *T1*, within Loch Linnhe under this scenario. Smolts originating further down the loch have less distance to travel and so less time of exposure, and so are on average subject to lice loads well below threshold *T1*.

Table 2. Mean mobile lice infection on 12.5 cm smolts at B = 1 for example scenarios illustrated in Fig.3

| Inshore Infection | Coastal Waters Infection | Total Infection |
|---|---|---|
| **Upper Loch**<br>**0.069 lice g$^{-1}$** | Travel North<br>0.036 lice.g$^{-1}$ | 0.105 lice g$^{-1}$ |
| | Travel North West<br>0.001 lice.g$^{-1}$ | 0.070 lice g$^{-1}$ |
| **Lower Loch**<br>**0.025 lice g-1** | Travel North<br>0.036 lice.g$^{-1}$ | 0.061 lice g$^{-1}$ |
| | Travel North West<br><0.001 lice.g$^{-1}$ | 0.025 lice g$^{-1}$ |

Lice infection of smolts in coastal waters depends on route taken (Fig.3, Table 2). Very little additional lice infection occurs for smolts travelling northwest directly to the Atlantic Ocean. However, smolts travelling north through the semi-enclosed Minch Sea can be exposed to substantial additional lice infection risk before reaching the ocean. This extra infection can be enough to reach *T1,* 0.1 lice g$^{-1}$, for the example scenario illustrated, under which smolts had already been infected at the level of 0.069 lice.g$^{-1}$ on leaving Loch Linnhe (Table 2).

## DISCUSSION

This modelling integrates research that has been carried out over decades in lice dispersal models, salmon smolt migration and biology of impacts of lice on salmon in a deterministic model structure using example system values. Further work is required to incorporate uncertainties and variation around these values within and between systems.

Concentrations of salmon lice in inshore sea lochs or sounds affect the rate at which lice infect salmon smolts passing through on their outward migration. The length of the water body traversed gives an exposure time for salmon smolts from a particular river source. Exposure to lice in coastal waters is less than in sea lochs, but lice loads could be significantly increased under some potential smolt migration routes (Table 2). Smaller smolts and/or slower smolts would have more exposure to infection than larger smolts and/or faster smolts following these routes (Fig.2). Lice infection on fish that hug the coast could be higher relative to those that take the most direct route, as we assume following Kristoffersen et al. (2018). These additional lice could have significant biological effects, especially on smolts that have already been infected at relatively high lice loads in inshore waters.

Variation in lice concentrations and fish biology will lead to variation between louse load and impact of those lice. Variation in infection can be modelled by application of detailed hydrodynamic models and models incorporating more variation in smolt size and behaviour. Nevertheless, assessment based on kernel models and smolt migration here gives a measure useful for informing management of lice. Management may be achieved by controlling input of lice from farms, farming in areas of strong lice dispersal, or in areas that smolts can avoid or transit rapidly.

Other species of sea lice from the genus *Caligus* can also infest a range of fish species, including salmon (Hemmingsen et al., 2020). While some species, such as *C. rodgecressyi* in Chile, are a major problem for salmon, in the North Atlantic region *C. elongatus* is a parasite of much less concern than *L. salmonis*. Therefore, we have restricted the modelling to *L. salmonis* the specialist salmon louse.

*L. salmonis* does parasitize other salmonid species, including sea trout (*S. trutta*) present in Scottish coastal waters. Lice susceptibility and behaviours of *S. trutta* can differ from *S. salar*, so the modelling requires adaption if it is to be applied to sea trout.

Salmon lice are one of multiple pressures impacting wild salmon populations. These pressures may include climate change impacts on both freshwater and marine environments, predators and bycatch in fisheries. Salmon populations already in decline are the most sensitive to additional mortality impact associated with salmon lice (Vollset et al., 2016).

### Data to develop the model

The modelling presented is an example based on the data available. Further data, and modelling based on those data, will allow improvement of assessment of threshold salmon lice copepodids concentrations in the coastal waters of Scotland.

Weekly adult female lice counts per fish are now published for all salmon farms in Scotland. However, more detailed data on ovigerous lice, and particularly on the numbers of salmon on farms would allow a more accurate assessment of the numbers of larval lice being released into the marine environment from farms.

Inclusion of more data on viable egg production rates and on larval survival, and their responses to salinity and temperature, would also improve modelling especially for inshore waters.

Modelling infection rate could be improved by better constraint on the model of copepodid movements and attachment to hosts. Options for this include further assessments of relationships between observed infection on sentinel cages and simulated lice concentrations in the environment. This is being carried out through the SPILLS project (https://marine.gov.scot/information/salmon-parasite-interactions-linnhe-lorn-and-shuna-spills). More detailed information on distribution of salmon lice copepodids in the environment is being produced through application of the Scottish Shelf Model (Rabe et al., 2020), with salmon lice modelling being enhanced through SPILLS and with model structure and parameterisation being improved through gap analysis, notably through a Marine Alliance for Science and Technology for Scotland supported workshop.

Further data on the effect of mobile lice loads $g^{-1}$ on smolts welfare and survival would also improve assessment of infestation intensities.

The area for which further data is most required is on the movement of smolts in inshore and coastal waters as the details of this determines their exposure time to copepodids. Size of smolts varies too, and this affects their speed and threshold number of lice on a fish before impacts. Acoustic tagging is being used to obtain observational data on smolt movements (Middlemas et al., 2017) while modelling of smolt migration is also in development (Ounsley et al., 2020).

### Future model development

The modelling presented describes the deterministic structure of interaction between salmon lice and salmon smolts. Application to specific salmon populations requires assessment of not only the typical parameters, as described, but also variation within and between systems to assess impacts at population levels. This requires assessing ranges of parameter values and tailoring them to individual populations based on data ranges within those populations.

Therefore, the aim is to develop the model into a stochastic framework with parameter ranges that influence lice infection of smolts. This will be achieved by coupling hydrodynamic models with individual-based models of virtual particles representing lice and smolts, which will generate lice-scapes and a range of smolt migration routes. These developments will allow assessment of the range of variation of salmon lice impacts across a range of smolt populations.

### Conclusion

Modelling provides a basis for developing tools for using best available data to assess the impacts of salmon lice from farms on wild salmon smolts, a key issue for sustainable production of farmed salmon. The concept used is to specify threshold lice exposure levels (Table 1), and, by assessing distribution of lice and pathways of smolts, lice infection can be evaluated relative to these thresholds for smolts originating from specific sources (Fig.3 and Table 2). The concept is illustrated here for one particular set of parameter values applied to a notional 12.5 cm salmon smolt. Smaller smolts will be more susceptible, while larger and faster ones will be less impacted by salmon lice. Salmon lice impact may be reduced through strategic location of farms, restriction on farm biomass, or control of numbers of ovigerous lice per fish - particularly when salmon smolts are going to sea, with modelling providing supporting information to assist decision making. The modelling approach can be developed further to integrate many sources of research that can be applied in practice to manage salmon lice impact in the planning and management of aquaculture, particularly new or expanded farms.

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# RISK FACTORS AND HOT-SPOT AREAS OF FOOT-AND-MOUTH DISEASE IN SMALL RUMINANTS IN NORTHERN NIGERIA

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## SUMMARY

Foot-and-mouth disease (FMD) affects cloven-hooved livestock, however the epidemiological role of small ruminants is poorly understood. A cross-sectional study conducted in three States in northern Nigeria (Bauchi, Kaduna, and Plateau) was used to estimate the true seroprevalence, investigate risk factors for small ruminant seropositivity at animal and household level and explore spatial autocorrelation of the empirical Bayes smoothed ratios at a global and local scale. The overall true seroprevalence was 10.2% (95% CI 0-19.0). State-level estimates were 17.3% (95% CI 0.0-25.8) for Kaduna, 6.9% (95% CI 0.0-15.8) for Bauchi, and 3.6% (95% CI 0.0-12.6) for Plateau. At animal level, State and species were the main risk factors identified with interaction detected between them. Households in Kaduna had higher prevalence ratios compared to Plateau (PR=1.89, 95% CI 1.40-2.55, p<0.001). A hot-spot was detected in Kaduna and a cold-spot in Plateau. These results could support future risk-based control programs.

## INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious disease that affects cloven-hooved livestock. Small ruminants (SR) can act as silent shedders of foot-and-mouth disease virus (FMDV), but their epidemiological role is generally neglected due to the scarcity of clinical symptoms (Kitching and Hughes, 2002).

In sub-Saharan Africa, FMD risk factors are well researched in cattle, while SR have received far less attention. At animal level, age, species, sex and breed have been reported as risk factors for seropositivity (Ahmed et al., 2020; Mesfine et al., 2019; Nthiwa et al., 2020; Souley Kouato et al., 2018). Factors frequently described at herd level were those increasing direct and indirect contacts between herds and with wildlife (Souley Kouato et al., 2018). The administrative division was often a significant risk factor, probably a proxy for other factors like predominant management practices, animal density, or agroecological characteristics (Guerrini et al., 2019). Spatial analyses have identified significant clusters of disease along international borders and communication networks, further highlighting the importance of human-led activities favouring animal contacts (Allepuz et al., 2015; Munsey et al., 2019). Increases in altitude and wetness were reported to be associated with a decreased frequency of

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outbreaks (Hamoonga et al., 2014), while presence of wildlife has been proposed to explain some of the observed spatial patterns (Guerrini et al., 2019).

FMD is endemic in Nigeria. Since the country hosts more than 128 million SR (FAO, 2019), the implementation of extensive national surveillance and vaccination programs in SR might turn out logistically challenging and cost-prohibitive. This makes it necessary to consider more cost-effective risk-based approaches targeting animals and areas at highest risk of infection. However, current knowledge about presence, exposure and distribution of FMDV in Nigerian SR is poor. Only four studies have focused on assessing FMD risk factors in Nigeria, two of which involved SR, with some constrains on their study design and statistical analysis, limiting the use of their results. The only available spatial analysis did not detect any significant cluster of seropositivity (Wungak et al., 2017). Within this context, the objectives of this study were (1) to determine the seroprevalence of FMD in backyard SR in three States of northern Nigeria, (2) to identify risk factors associated with FMDV antibody detection at animal and household level, and (3) to identify geographical patterns for FMDV exposure, with the final aim of generating knowledge to guide future health interventions.

## MATERIALS AND METHODS

### Study area and data collection

Nigeria is divided into 36 States, each of which is composed of Local Government Areas (LGAs) that are in turn divided into village areas. SR are mainly concentrated in the northern part of the country. The main rearing systems are represented by backyard/sedentary farmers on the one hand and transhumant/nomadic ones on the other (Adedeji et al., 2021). Between September and November 2019, Adedeji et al. (2021) completed a cross-sectional serological study associated with a questionnaire survey on the epidemiology of sheeppox and goatpox (SGP) in Bauchi, Plateau and Kaduna States, in the northern part of the country. Briefly, a multistage random sampling method was used. First, twenty villages from each of the three strata (corresponding to the three States) were randomly selected. Five households were then selected systematically in each selected village, and six SR were selected systematically within each household. A standardised questionnaire was used to collect animal characteristics (species, breed, age, sex, origin), household location, herd size, management and husbandry practices. The availability of serum samples after the conclusion of the study on SGP, allowed to test them for FMD non-structural proteins (NSP) antibodies at the Nigerian Veterinary Research Institute, Vom, Nigeria, using the PrioCHECK FMDV-NS antibody ELISA kit, Thermo Fisher Scientific.

### Data management and analysis

Seroprevalence: the apparent weighted seroprevalence was estimated to account for variability in herd size at household level using the survey commands of Stata/SE 16 (StataCorp LLC, 2019) to weight the observations based on the proportion of small ruminants sampled in their respective households. The true seroprevalence was calculated using Eq. (1) to adjust for the test sensitivity and specificity. Since the performance of the PrioCHECK FMDV-NS ELISA has not been assessed in SR in field conditions, data from available studies (Brocchi et al., 2006; Sørensen et al., 1998; Thermo Fisher Scientific, n.d.) were employed to produce a distribution of plausible values for sensitivity and specificity. The true prevalence was then estimated by running a Monte Carlo simulation with 10,000 iterations: the minimum output value was set to zero to avoid negative prevalence values. Median and range including 95% of the resulting distribution were used respectively as point estimate and 95% confidence interval (CI) of the true prevalence. Calculations were performed using the Microsoft Excel (Microsoft 365, 2020) add-in @Risk 8.0 (Palisade Corporation, 2020).

$$True\ prevalence = \frac{apparent\ weighted\ prevalence + specificity - 1}{sensitivity + specificity - 1} \qquad (1)$$

Risk factors: at animal level, the association between each variable and animal seropositivity was investigated using univariable mixed-effects logistic regression with household as a random effect to account for potential clustering at household level. At household level, the association between each variable and the number of FMD seropositive animals present in the household was investigated using Poisson regression with adjusted (robust) variances and the number of animals sampled per household as offset (Barros and Hirakata, 2003). At both animal and household level, variables characterised by a p-value <0.20 in the univariate analysis were taken forward for multivariable modelling after checking for collinearity with a Pearson correlation test: a coefficient ≥0.8 was considered indicative of collinearity.

Variables to be included in the final models at both animal and household level were selected using a forward stepwise approach: one variable at a time was added to the model and retained or discarded based on the comparison of the log likelihoods for the model with and without the variable through a likelihood ratio test with a level of significance <0.05. Then, variables retained in the final models were removed one at a time using a backward stepwise approach based on the likelihood ratio test with a level of significance <0.05 to check that the same model was obtained. Finally, the presence of effect modification between the variables included in the final models was investigated with a likelihood ratio test for interaction with a level of significance <0.05.

Intra-cluster correlation (ICC): ICC was calculated at different spatial levels by fitting mixed-effect logistic regression models with State, LGA, village, or household as random effects without including any independent variables. ICC values were compared with those expected when calculating the sample size (design effect) to estimate the impact of the study design on the final estimates of prevalence. All statistical analyses were performed in Stata/SE 16 (StataCorp LLC, 2019).

Spatial analysis: since precise household point locations were not available, the data were aggregated and analysed at LGA level using the geographic information system ArcMap 10.7.1 (ESRI, 2020) and the software package GeoDa 1.18 (University of Chicago, 2020). To improve the precision of the estimated prevalence ratios, empirical Bayes smoothed ratios were calculated to smooth ratios using a prior based on the pooled ratio across all LGAs (Pfeiffer et al., 2008a). Spatial autocorrelation of the empirical Bayes smoothed ratios was explored at a global scale by calculating the global Moran's *I* statistic with a queen's contiguity weights matrix; its significance was assessed using a Monte Carlo randomisation with 499 permutations and a p-value ≤0.05. Spatial autocorrelation at the local scale was investigated using local indicators of spatial autocorrelation (LISA), estimated through the Anselin Local Moran's I and the Getis-Ord Gi* statistics, to detect clusters of LGAs with similar ratios and identify any hot- or cold-spot at LGA level (Pfeiffer et al., 2008b).

RESULTS

Animal level

Description of study population: three quarters of the 1800 SR sampled were goats (n=1366; 75.9%) and one quarter sheep (n=434; 24.1%). The three States presented similar numbers of goats (Kaduna: n=505; 37.0%; Plateau: n=468; 34.3%; Bauchi: n=393; 28.8%), while almost half the sheep were from Bauchi (n=207; 47.7%), with the other half split between Plateau (n=132; 30.4%) and Kaduna (n=95; 21.9%). More than three quarters of the animals were female (n=1488; 82.7%), with similar proportions in goats (n=1169; 85.6%) and sheep (n=319; 73.5%). Median age was 24 months (1st quartile=12 months; 3rd quartile=42 months), but females were older than males (median age 36 months in females and 12 months in males), again with similar proportions in both goats and sheep. Most of the animals were born in the same household in which they were sampled (n=1503; 83.5%), while the remainder (n=297; 16.5%) were acquired from elsewhere.

Seroprevalence of FMD in SR: less than one fifth of the SR sampled tested positive to NSP antibodies (n=318; 17.7%) with seropositive animals throughout the study area. The overall apparent weighted seroprevalence was 17.7% (95% CI 15.4-20.3), with the highest State-level estimate obtained for Kaduna, 23.8% (95% CI 19.4-28.8), followed by Bauchi, 14.9% (95% CI 11.7-18.8), and Plateau, 12.0% (95% CI 9.1-15.8). The overall true seroprevalence was estimated to be 10.2% (95% CI 0.0-19.0), while State-level estimates were 17.3% (95% CI 0.0-25.8) for Kaduna, 6.9% (95% CI 0.0-15.8) for Bauchi, and 3.6% (95% CI 0.0-12.6) for Plateau.

Animal-level risk factors for FMD: univariable analysis showed that sex (p=0.77) and origin of the animal (p=0.96) were not significantly associated with seroprevalence, but State where the animal was sampled (p<0.0007), species (p=0.12), and age (p=0.14) had a p-value <0.20 and were taken forward to the multivariable analysis after checking for collinearity (there was no collinearity). Species and State where the animal was sampled were retained in the final model and the likelihood ratio test showed evidence of interaction between the two variables (p=0.0007). Compared to goats in Plateau, the odds of testing positive were almost double for goats in Bauchi (OR=1.83, 95% CI 1.13-2.97, p=0.01) and almost three times higher for goats in Kaduna (OR=2.97, 95% CI 1.89-4.67, p<0.001) (Table 1). Using again goats in Plateau as a reference, the odds of testing positive were almost four times higher for sheep in the same State (OR=3.78, 95% CI 2.08-6.87, p<0.001), almost a third higher for sheep in Bauchi (OR=1.61, 95% CI 0.91-2.84, p=0.10), and three times higher for sheep in Kaduna (OR=3.11, 95% CI 1.61-6.01, p=0.001) (Table 1).

Table 1. Results of the multivariable mixed-effects logistic regression model for identification of factors associated with FMD seropositivity in small ruminants, with household as a random effect and inclusive of an interaction between State and species

| State*Species | OR (95%CI) | p-value |
|---|---|---|
| Plateau*goats | Reference | |
| Bauchi*goats | 1.83 (1.13-2.97) | 0.01 |
| Kaduna*goats | 2.97 (1.89-4.67) | <0.001 |
| Plateau*sheep | 3.78 (2.08-6.87) | <0.001 |
| Bauchi*sheep | 1.61 (0.91-2.84) | 0.10 |
| Kaduna*sheep | 3.11 (1.61-6.01) | 0.001 |

### Household level

Description of study population: goats were the most numerous cloven-hoofed species (n=4125; 63.1%), followed by sheep (n=1723; 26.4%), cattle (n=552; 8.4%) and swine (n=134; 2.1%). Most households had at least one goat (n=282; 94.0%) and slightly less than half hosted at least one sheep (n=146; 48.7%), whereas only a few reared cattle (n=53, 17.7%) or swine (n=29; 9.7%). Considering all cloven-hoofed species, the median herd size was 15 animals (1st quartile=10; 3rd quartile=24). Bauchi and Plateau presented larger herds (median=17 animals in each) compared to Kaduna (median=11.5 animals) and median herd size was significantly different (Kruskal–Wallis rank test: $\chi^2$=14.617, df=2, p=0.0007).

The most common management system for sheep and goats was semi-intensive (n=285; 95.0%), with few herds managed intensively (n=10; 3.3%) or extensively (n=5; 1.7%). Most farmers attended communal grazing areas with their sheep and/or goats (n=281; 93.7%), watered them at communal sources (n=280; 93.3%), and shared male animals with other households for mating purposes (n=238; 79.3%). About two thirds of farmers carried out at least one routine treatment of SR (n=195, 65.0%), with about a quarter of them vaccinating (n=70, 23.3%), more than half deworming the animals (n=185, 61.7%), and one third using tick control (n=98; 32.7%). Nobody reportedly vaccinated SR against FMD.

More than half the households (n=182; 60.7%) presented at least one FMD seropositive sheep or goat which were mainly located in Kaduna (n=72; 39.6%), followed by Bauchi (n=61; 33.5%) and Plateau (n=49; 26.9%). The median number of seropositive animals out of the 6 animals sampled was 1 (min=0; max =6).

Household-level risk factors for FMD: univariable Poisson regression identified five variables associated with proportion of seropositive animals per household. They were State where the herd was located (p<0.0007), carrying out tick control (p=0.03), herd size (p=0.15), last time in which new SR were brought into the household (p=0.15), and having brought new SR into the household (p=0.18). Since no collinearity was detected, they were all taken forward to the multivariable analysis. The final model included State and herd size (Table 2). Households located in Kaduna and Bauchi were more likely to have a higher prevalence ratio (PR) compared to Plateau, but results were only significant for Kaduna (Kaduna: PR=1.89, 95% CI 1.40-2.55, p<0.001). Compared to herds with fewer than 10 animals, those with more than 20 individuals were more likely to have a higher PR (PR=1.30, 95% CI 0.96-1.78, p=0.09). There was no evidence of interaction between State and herd size (p=0.11).

Intra-cluster correlation: the highest ICC was recorded at household level (0.18), followed by village (0.08), LGA (0.07), and State (0.02) suggesting a low degree of clustering especially at village, LGA and State level.

Spatial analysis: although there was no significant spatial autocorrelation of empirical Bayes smoothed ratios (Moran's *I*: 0.052, p=0.26), ratios were generally lowest in the centre of the study area and highest in the west and ranged between 10.1% and 29.1%, with a median of 16.6% (Fig.1a). LGAs in Kaduna, in the western part of the study area, were characterised by high rates, with the majority (11/13; 84.6%) presenting ratios above the median and Zangon Kataf and Kauru showing the highest values (29.1% and 27.2%, respectively). However, high values were also reported in some LGAs in Plateau (Langtang North, 27.7%), and Bauchi (Bauchi, 25.1%).

Table 2. Results of the multivariable Poisson regression model with adjusted variances and number of animals sampled per household as offset showing association between household-level risk factors and FMD seropositivity in small ruminants (SR)

| Variable | Adjusted prevalence ratio (95% CI) | p-value | p-value from the likelihood ratio test |
|---|---|---|---|
| State | | | <0.0001 |
| Plateau | Reference | | |
| Bauchi | 1.14 (0.84-1.56) | 0.40 | |
| Kaduna | 1.89 (1.40-2.55) | <0.001 | |
| Herd size (includes cattle, SR, and swine) | | | 0.01 |
| ≤10 | Reference | | |
| 11-15 | 0.78 (0.56-1.09) | 0.15 | |
| 16-20 | 1.06 (0.73-1.56) | 0.75 | |
| >20 | 1.30 (0.96-1.78) | 0.09 | |

The Local Moran's I statistic identified a hot-spot involving the LGAs Kauru and Kaura (in Kaduna), meaning that those LGAs had high empirical Bayes smoothed ratios and were surrounded by other LGAs with high ratios, while the LGAs Jos East and Mangu (in Plateau) were part of a cold-spot, meaning that they had low ratios and were surrounded by other LGAs with low ratios (Fig.1b). Overall, these results were consistent with those of the Getis-Ord Gi* statistic (Fig.1c).

## DISCUSSION

This investigation provided the first estimates of state-level FMD seroprevalence in SR in Nigeria and identified FMD risk factors and spatial patterns in SR based on data collected through stratification and multi-stage sampling with a self-weighting sample to account for differences between LGAs. It provided an advance to previous studies conducted in the country, which were based on convenience sampling or limited to small study areas (Ehizibolo et al., 2019) and mainly focused on cattle (Ehizibolo et al., 2014; Fasina et al., 2013; Lazarus et al., 2012; Wungak et al., 2017, 2016).

### Seroprevalence of FMD in SR

The overall apparent weighted seroprevalence of 17.7% (95% CI 15.4-20.3) was consistent with the 16.3% value reported in sheep in Kaduna, (Ehizibolo et al., 2019). In contrast, a higher seroprevalence (64.4%) was previously reported in sheep from Kaduna, Kwara, Plateau, and Bauchi (Ehizibolo et al., 2017), with samples collected as part of an outbreak investigation, when viral circulation was likely to be very high. Furthermore, Lazarus et al. (2012) reported a 27.8% seroprevalence among small ruminants in Bauchi, corresponding to about twice the apparent weighted prevalence of 14.9% (95% CI 11.7-18.8) obtained for the same State in the present study, but their finding was based on a very small sample size (n=79) and some of the samples came from suspected outbreaks. Selection bias and different sampling strategies are the most likely explanation for this discrepancy. Serosurveys conducted among SR in Ethiopia, Kenya and Tanzania reported similar values ( Beyene et al., 2015; Abdela, 2017; Torsson et al., 2017; Chepkwony et al., 2021).

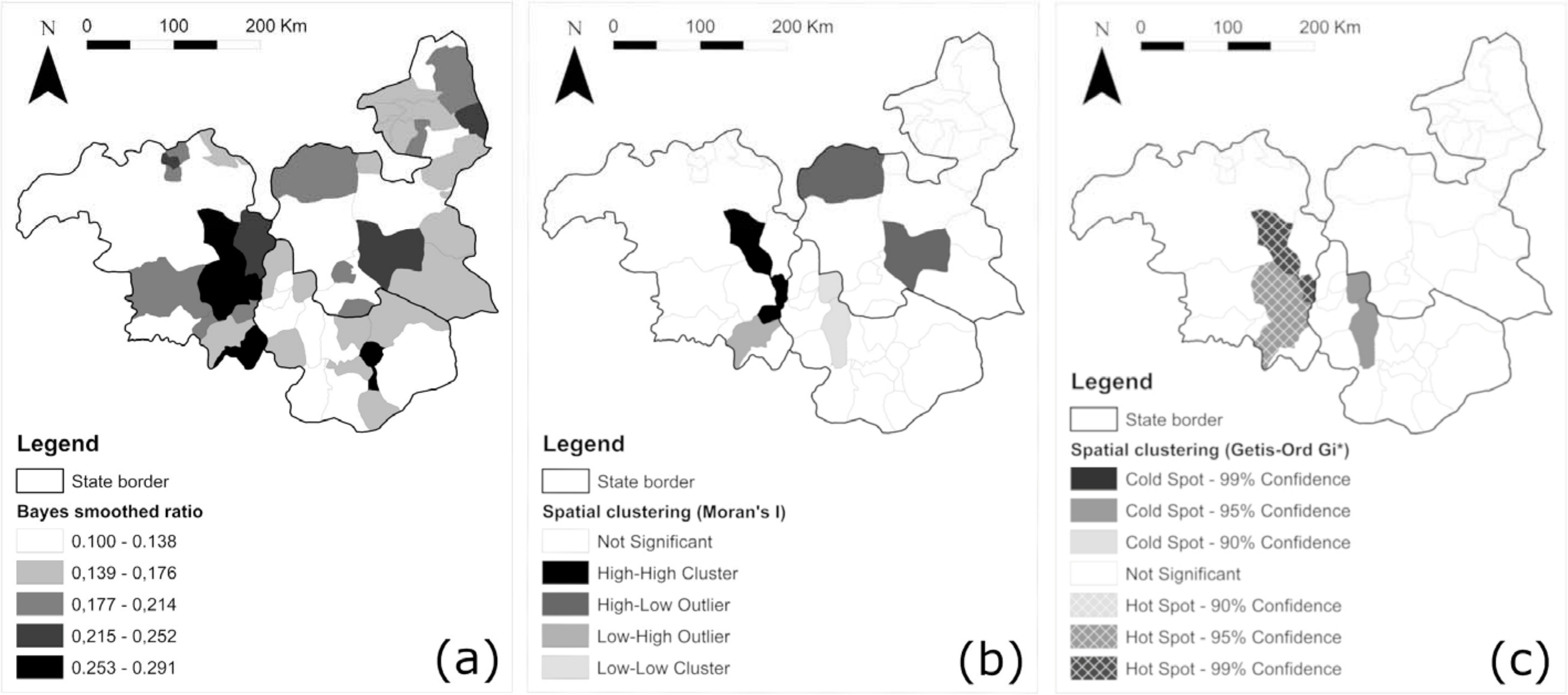

Fig. 1. Choropleth map of empirical Bayes smoothed ratios of FMD in Bauchi, Kaduna, and Plateau States (a) and spatial clustering of empirical Bayes smoothed ratios of FMD based on Anselin Local Moran's I statistic (b) and Getis-Ord Gi* statistic (c). Blank areas within State borders correspond to LGAs excluded from the study due to security problems.

FMD seropositive animals were located throughout the study area, across several households (n=182; 60.7%, had at least one seropositive SR) and almost all LGAs (n=43; 97.7%). Since in Nigeria there are no official records about village and LGA population, it was not possible to adjust for differences at these levels. Finally, FMD seroprevalence in LGAs excluded from the sampling frame because of security problems remains unknown: state-level seroprevalences may therefore be partially different from those estimated by the present study. Furthermore, this dispersion across the study area resulted in low values of ICC at all levels of clustering considered (household, village, LGA, State), and was reflected by the absence of significant spatial autocorrelation between LGAs at global level (Moran's I=0.052, p=0.26). However, a hot-spot was detected in south-eastern Kaduna, while a cold-spot was found in north-western Plateau. This suggests that, although FMD was endemic within the study area, differences existed between the three States.

### Animal- and household-level risk factors for FMD

Both final multivariable models at animal- and household-level highlighted the importance of geographical differences on exposure to FMDV, corroborating the finding about differences derived from the spatial analysis (hot-spot identified in Kaduna). Further studies are needed to investigate the role of other factors such as agroecological characteristics, distance from anthropic elements, and contacts with wildlife, to better understand the observed differences between States ( Hamoonga et al., 2014; Beyene et al., 2015; Guerrini et al., 2019; Munsey et al., 2019). The analysis at animal-level showed evidence for interaction between species and State: this was perhaps a consequence of the different proportions of sheep and goats sampled in each State, even if no clear patterns were observed in this regard. Instead, this interaction might be a consequence of differences in management practices related to cultural or religious reasons, or to the fact that different environmental conditions occurring in different States affect the epidemiology of FMD with different outcomes in sheep compared to goats. Other studies reported an equal or higher seroprevalence in sheep compared to goats, but the difference was not statistically significant. This might have been a consequence of not having enough power given the smaller sample size compared to the present study (ranging from 345 to 708 SR sampled). However, Hussain et al. (2019) analysed samples from 4015 SR without finding a significant difference between sheep and goats. Potential biological reasons for this difference need to be further investigated: as an example, the duration of the carrier status is still uncertain in sheep and unknown in goats (Stenfeldt and Arzt, 2020), while no information is available on potential differences in waning of antibody titres over time. At household level, the change in parameters of the multivariable model compared to the univariable one suggested that the association between herd size and proportion of seropositive SR per household was confounded by the State in which the household was located: in fact, the median herd size differed in different States, with Kaduna presenting a smaller median compared to the other States.

### Limitations of study

The data were collected exclusively from backyard, sedentary farmers, resulting in a high degree of homogeneity in some management practices (Adedeji et al., 2021). This limited the possibility to investigate potential differences between production systems with different management practices. Furthermore, having employed data from a questionnaire designed for collecting information about SGP might have limited the possibility of collecting specific information about FMD, but still enabled to obtain useful insights into the epidemiology of the disease.

Although the sampling strategy was originally designed to meet specific objectives related to the epidemiology of SGP, the values of ICC estimated in this study for FMD were only slightly higher than those expected by the original study design intended for SGP, while the estimated State-level weighted prevalence in Bauchi and Plateau was roughly equal to that expected, and slightly higher in Kaduna. Therefore, the sampling strategy should not have greatly affected the power and precision of the estimates.

With the aim of applying the simplest statistical techniques possible, the analyses for animal-level risk factors only accounted for one level of clustering (household) and ignored the others (State, LGA, village), while the household-level analyses did not account for any. However, the low ICC values calculated at all levels of clustering indicated that this approach was unlikely to have a substantial impact on the results.

The uncertainty expressed by the wide confidence intervals of the overall and State-level true seroprevalences was consequent to the absence of knowledge about the performance of the test in SR in field conditions. Future test validations involving different species in the field would improve the reliability of this kind of estimates.

### Perspectives

The Progressive Control Pathway for FMD (PCP-FMD) consists of a series of steps through which endemic countries can gain control over the disease (FAO/OIE, 2021): by improving knowledge on the epidemiology of FMD in SR in Nigeria, this study will likely prove useful to advance along Stage 1 of the PCP-FMD and develop risk-based control programs as required to progress to Stage 2.

Successfully exploiting samples and data from a study on SGP to gain a better insight into the epidemiology of FMD showed how integrating studies about similar transboundary animal diseases can optimise resource allocation and knowledge generation. Careful study designs might allow this strategy to be replicated, as proposed by the European Commission for the Control of Foot-and-Mouth Disease, to develop integrated programs for FMD And Similar Transboundary (FAST) animal diseases (FAO/EuFMD, 2019).

This study described the widespread distribution of FMD in sheep and goats across three States of northern Nigeria and identified hot- and cold-spots for exposure to FMDV and risk factors for seropositivity at animal and household level. These results provide a baseline for future studies and risk-based interventions aimed at reducing the impact of the disease on animals and human livelihoods.

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# PORCINE DISEASE CONTROL

# LESSONS LEARNT FROM THE FIRST MODELLING CHALLENGE IN ANIMAL HEALTH: IMPROVING PREPAREDNESS TO CONTROL AFRICAN SWINE FEVER AT THE INTERFACE BETWEEN LIVESTOCK AND WILDLIFE

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## SUMMARY

The first international modelling challenge in animal health – ASF Challenge – aimed to improve the collective capacity to predict large-scale pathogen spread between livestock and wildlife and to support public decision during health crises. We designed a realistic mechanistic model to mimic an African swine fever (ASF) outbreak spreading between wild boar and pig farms in a fictitious island and to produce synthetic epidemiological data. Five international modelling teams built their own ASF models and used this data to predict the temporal and spatial course of the epidemics and assess control measures. This challenge provided an inspiring platform for exchanging knowledge and expertise. Comparing approaches allowed to assess the predictive capacity of models and identify areas for improving modellers' responsiveness when facing a real crisis.

## INTRODUCTION

Raising livestock in a sustainable and welfare perspective requires the management of animal health, especially infectious diseases which spread at large scale between animal populations (Ezanno et al., 2020). Health crises highlight the need for robust epidemiological knowledge and predictive tools to better cope with uncertainty, especially at the livestock-wildlife interface (Gortázar et al., 2007). Developing models that forecast disease spread is pivotal to better understanding epidemics and to assess ex-ante the efficacy of control measures (Grassly and Fraser, 2008), but doing so during an epidemic is extremely difficult. Modelling challenges, which are relatively short competitions, enhance cooperation between actors and modellers' ability to advise policy makers in a timely way, improve the accuracy of model predictions and modellers' readiness when facing emerging threats, and promote international collaborations. After the first modelling challenge on seasonal influenza (Friedberg et al., 2015), annually renewed (Reich et al., 2019; Viboud and Vespignani, 2019), others were organized on Ebola (Viboud et al., 2018), Chikungunya (Del Valle et al., 2018) and Dengue (Johansson et al., 2019). However, none have concerned an animal disease so far, while animal epidemiology has specific features which may induce different conclusions on the most suitable approaches and also require specific preparedness.

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African swine fever (ASF) is an emerging disease currently spreading at the interface between wild boar and pig farms in Europe and Asia (Sánchez-Cordón et al., 2019). This viral disease is associated with a tremendous impact on swine production, livestock economy and international trade and neither a vaccine nor a treatment is available (Dixon et al., 2020). It is one of the major livestock infectious disease threats for most countries as the virus can spread internationally via geographical proximity or due to movements of persons, pigs and pork products (Vergne et al., 2017). To enhance the global preparedness to better face ASF epidemics, there is a need to consider explicitly the interface between livestock and wildlife, and to be able to assess various and combined control measures.

The objective of this paper is to present ASF Challenge, the first modelling challenge in animal health; the comparison of approaches mobilised by the five international teams involved; and to discuss lessons learnt throughout this process.

## MATERIALS AND METHODS

### Challenge organization

During the preparation step (July 2019-August 2020), a source model (called "M0") was built to generate the synthetic data mimicking a ASF-like outbreak in a realistic west-European context. The challenge itself took place between August 27, 2020 and January 13, 2021. Three situation reports and synthetic epidemiological data corresponding to days 50, 80 and 110 after the detection of a first case, were released successively to the participants. These data were produced by the original model described below and represented detection events on pig farms or wild boar, with the location and cause of detection. Finally, the predictions of the participants' models were compared and analysed.

### Model M0 and simulated epidemics

The simulated outbreak was located on a hypothetical island built by aggregating the two French regions Auvergne-Rhône-Alpes and Occitanie, using three types of land use based on public GIS data[*] (agricultural, forest and urban) to define the location of individual wild boar and domestic pig farms. Assuming that the wild boar population was reduced by half during a hunting season, we used hunting bags per department provided by the *Office Français de la Biodiversité* (OFB) to determine the initial wild boar population (500,366) and distributed the centres of their home range randomly (80% in forest areas, 18% in agricultural areas, 2% in urban areas). The coordinates of the 4,775 pig farms registered in the two French regions were also randomly distributed as follows: 33% in Auvergne-Rhône-Alpes and 67% in Occitanie, and within each region 85% in agricultural areas, 10% in forests, 5% in urban areas. Besides, we endowed each farm with variable characteristics (size; commercial or backyard status; farrow, finisher, or farrow-to-finish; access to an outdoor area) which were used to generate a biosecurity score and simulate commercial movements between farms.

The synthetic epidemiological data were simulated using a stochastic, spatially-explicit agent-based model, with three kinds of agents: pig herds (compartmental sub-model), individual wild boar, and the whole island (as a metapopulation). A recent modelling software,

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[*] https://www.diva-gis.org/

EMULSION (Picault et al., 2019), was used for making model components explicit and more revisable.

In pig farms, we neglected natural mortality, thus driving the population only by pre-calculated commercial movements, sold animals being replaced with new ones. Wild boar were subject to hunting and natural mortality only. As the epidemic was taking place during the hunting season (8 months), we indeed assumed no birth during the simulated period (Vetter et al., 2020).

Pigs and wild boar were categorised into mutually exclusive health states: susceptible (S), exposed i.e. asymptomatic but starting being infectious (E), fully infectious and symptomatic (I). All infected animals were assumed to die, resulting in an infectious carcass (C). Wild boar dying from natural causes produced either an infectious (C) or a healthy (D) carcass when E/I or S at death, respectively. In pig farms, carcasses were removed the next day, whereas wild boar carcasses could stay in the environment up to several months until fully decayed or removed by humans. The force of infection in pig farms was assumed density-dependent, with a higher transmission rate in backyard farms than in commercial farms.

We modelled several transmission pathways between epidemiological units. For pig farms, we considered: the movement of an infected pig from an infected farm, the contact with an infectious wild boar, and indirect contacts with infectious farms in the neighbourhood. For wild boar, we assumed contacts with infectious wild boar, with infectious carcasses, or with an infectious pig farm. Apart from pre-computed trade movements, all other transmission pathways were spatially explicit (based on transmission kernels) and, when farms were involved, depended on their biosecurity level and outdoor access.

Detection of ASF cases initially relied on passive surveillance: wild boar carcasses could be found and tested each day with a low probability, whereas each pig could be detected and tested each day and at death, with a probability that depended on the farm type (high in commercial farms, low in backyard farms). The detection of the primary outbreak resulted in increased detection probabilities. Current European regulatory measures were triggered immediately after the first detection, and applied to all confirmed pig farms: 1) the slaughter of all animals, 2) the installation of protection and surveillance zones subject to trade ban and increased vigilance, 3) the tracing of farms with recent trade contacts (same effect). Culled farms were repopulated after several weeks. Any infected wild boar carcass found was removed without delay and triggered an active search in the immediate neighbourhood, with a higher probability to find new carcasses. Also, a proportion of hunted wild boar were tested.

Several additional control measures were implemented successively in the simulation. First, assuming the forest near the primary case was a major threat, 300 km of fences were installed and became operational 60 days after the first detection. An increased hunting pressure started in the fenced area and in a buffer area around the fences, with systematic tests on hunted wild boar, the suspension of active search, and an increased probability of finding carcasses by passive surveillance. Then, 90 days after the first case, the detection of dead or alive infected wild boar led to the preventive culling (and testing) of all pigs from nearby farms.

### Selection of synthetic data for the challenge

An exposed wild boar was introduced near a forest at the center of the island, a few weeks before the start of the hunting season. We ran 500 stochastic repetitions of the model to identify trajectories that were realistic enough and well suited for the challenge. After discarding those without any detection or detections later than 200 days after ASF introduction, we chose at random one of the trajectories that met five selection criteria: more than 250 infected wild boar before primary case detection to ensure disease installation in wildlife; primary case found in pig farm; fewer than 500 wild boar infected outside the fences at installation; more than 250 infected wild boar 110 days after primary case; and fewer than 30 infected wild boar 230 days after primary case (Fig. 1).

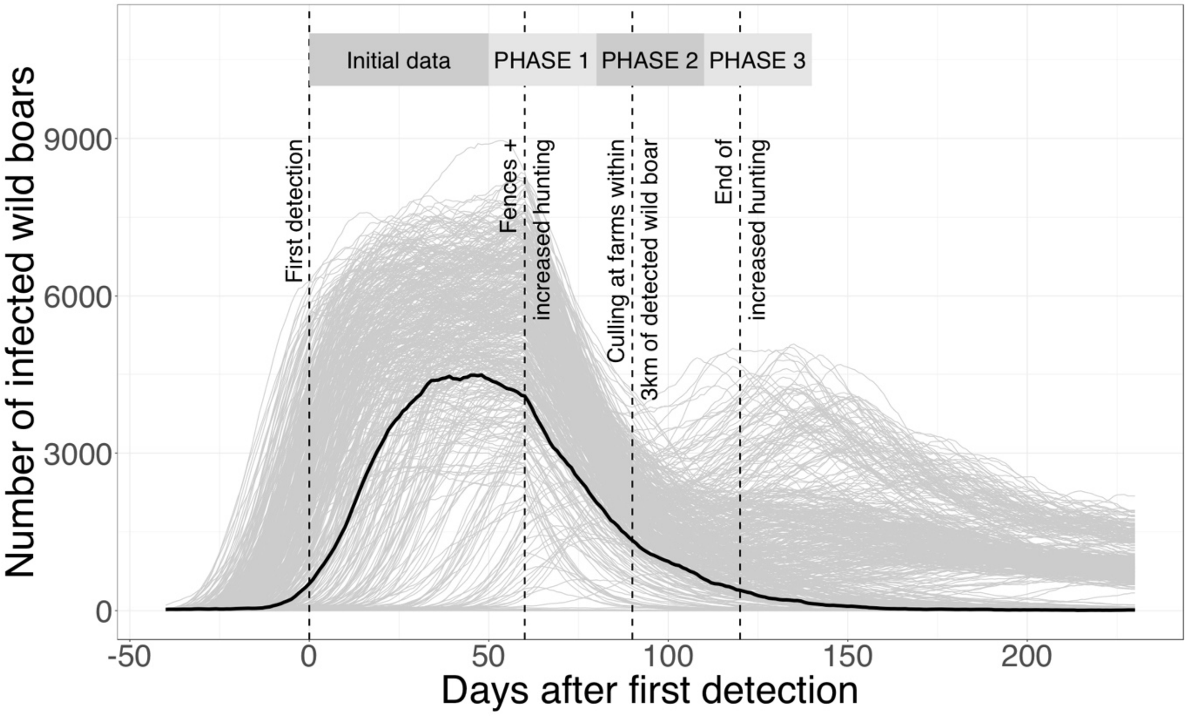

Fig. 1. Temporal dynamics of the number of live infected wild boars (exposed + infectious) for the selected trajectory (black) and the stochastic repetitions where detection occurred before 200 days (grey), with the three prediction phases that followed the initial situation report. Vertical dashed lines: changes in control interventions

Participants and their methods

Five modeller teams participated in the whole challenge (see Acknowledgements). In the narrative of the synthetic epidemics, phase 1 started 50 days after the dectection of a first case in a pig farm near a forest. Participants were provided with a first situation report and asked 1) to predict the number and location of wild boar and pig farm cases expected in the following 30 days, 2) to assess the effectiveness of surrounding the infected zone with fences, and 3) to advise on increasing hunting pressure in the fenced zone. At the end of phase 2 (110 days after first case), they were asked 1) to update their predictions on fences, now including a buffer zone and an increased hunting pressure, and 2) to advise on the effectiveness of five alternative control measures: culling all pigs from farms a) located within 3 km of a positive wild boar carcass, b) located in the protection zone, c) with a trade contact with an infected farm in the three preceding weeks; d) increasing the size of active search area around wild boar carcasses (from 1 km to 2 km); e) increasing the surveillance zone (from 10 to 15 km). At the end of phase 3, teams were asked to update their predictions and estimate the fade-out probability for the next four months. Six weeks were left to the participants for answering each phase.

The participants were free to choose their own approach, hence developed either one single model or two separate models for wild boar/pig farm interactions, with a diversity of modelling paradigms and granularity regarding both the epidemiological and spatial units. Stochastic compartmental models were used almost for all epidemiological units (except “CIRAD” team which built a probabilistic model for pig farms). Wild boar were mostly modelled at individual scale (except “WUR” team which used a spatial area) and pigs at farm scale (except for “UK” team which used individual scale). Predictions were made at several spatial scales (discretized in squares, rectangles or hexagons) ranging from 1 to 195 km$^2$.

We compared their predictions regarding the temporal dynamics of ASF spread in pig farms and wild boar with the possible outcomes of model M0 in the same conditions, i.e. keeping the challenge trajectory up to the beginning of the prediction period, then simulating M0 from that point with the appropriate settings and new random seeds. For spatial predictions, we compared 1) the predicted probability for each pig farm to be infected with the locations of positive farms in model M0 with the same conditions, 2) the probability that each spatial unit contained an infected and detected wild boar with the locations of wild boar cases in M0.

## RESULTS

### Temporal model predictions

The temporal forecasts for pig farm cases were very good in most teams (Fig. 2A), whereas wild boar dynamics appeared much more difficult to capture (Fig. 2B). The installation of fences and increased hunting pressure was highlighted by most teams as difficult to integrate in their models.

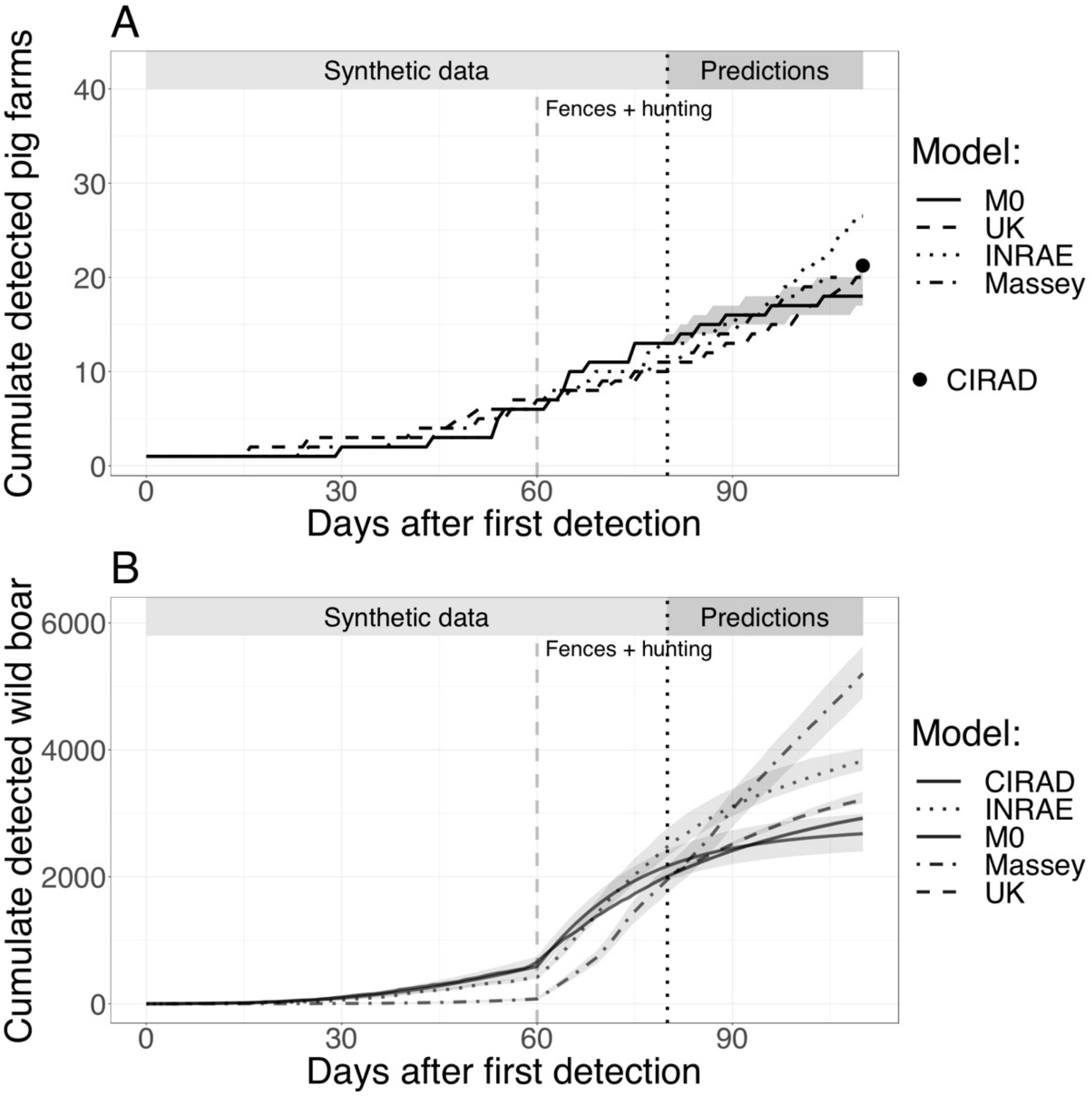

Fig. 2. Median of the predicted numbers of detected pig farms (A) and detected wild boar (B) by each team during the second period of the challenge (days 80 to 110). Acceptable range of output variations is shown (10th to 90th percentiles obtained when running model M0 during the prediction period)

Spatial model predictions

Spatial predictions for pig farms (Fig. 3) were quite good (including one team, CIRAD, which successfully predicted the occurrence of a distant case due to trade contacts). Besides, the regulatory measures based on protection and surveillance zones do not require to predict the exact location of infected farms, since an increased vigilance may be sufficient to ensure reactivity on detection and culling.

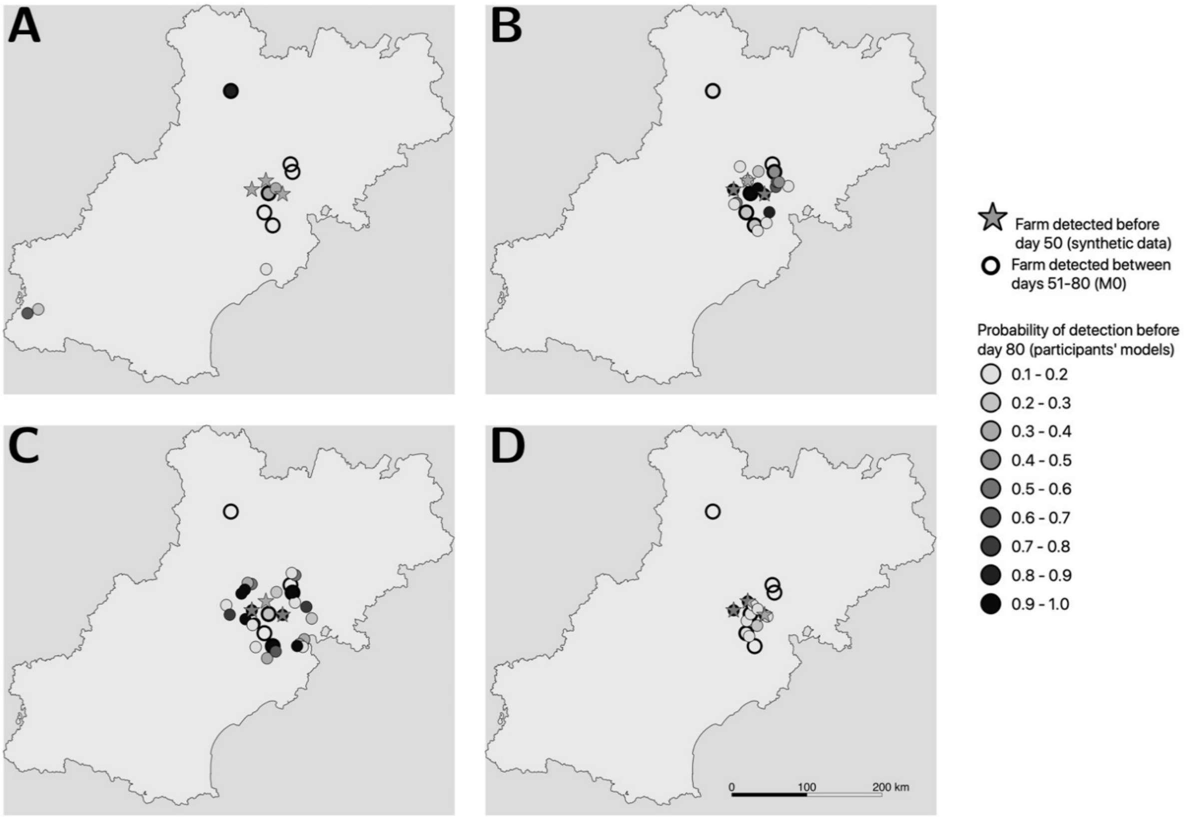

Fig. 3. Detected pig farms between days 50 and 80 as predicted by each team (black circles: farms that would have been detected in model M0 with the selected trajectory in the absence of additional control measures). A: CIRAD; B: UK; C: Massey Univ.; D: WUR

Spatial predictions regarding wild boar cases were much more heterogeneous, both in their granularity (e.g. from 1 km$^2$ to 195 km$^2$ tiles) and in the spread patterns (Fig. 4), reflecting the diversity of assumptions made by each team on wildlife and making the comparisons quite difficult.

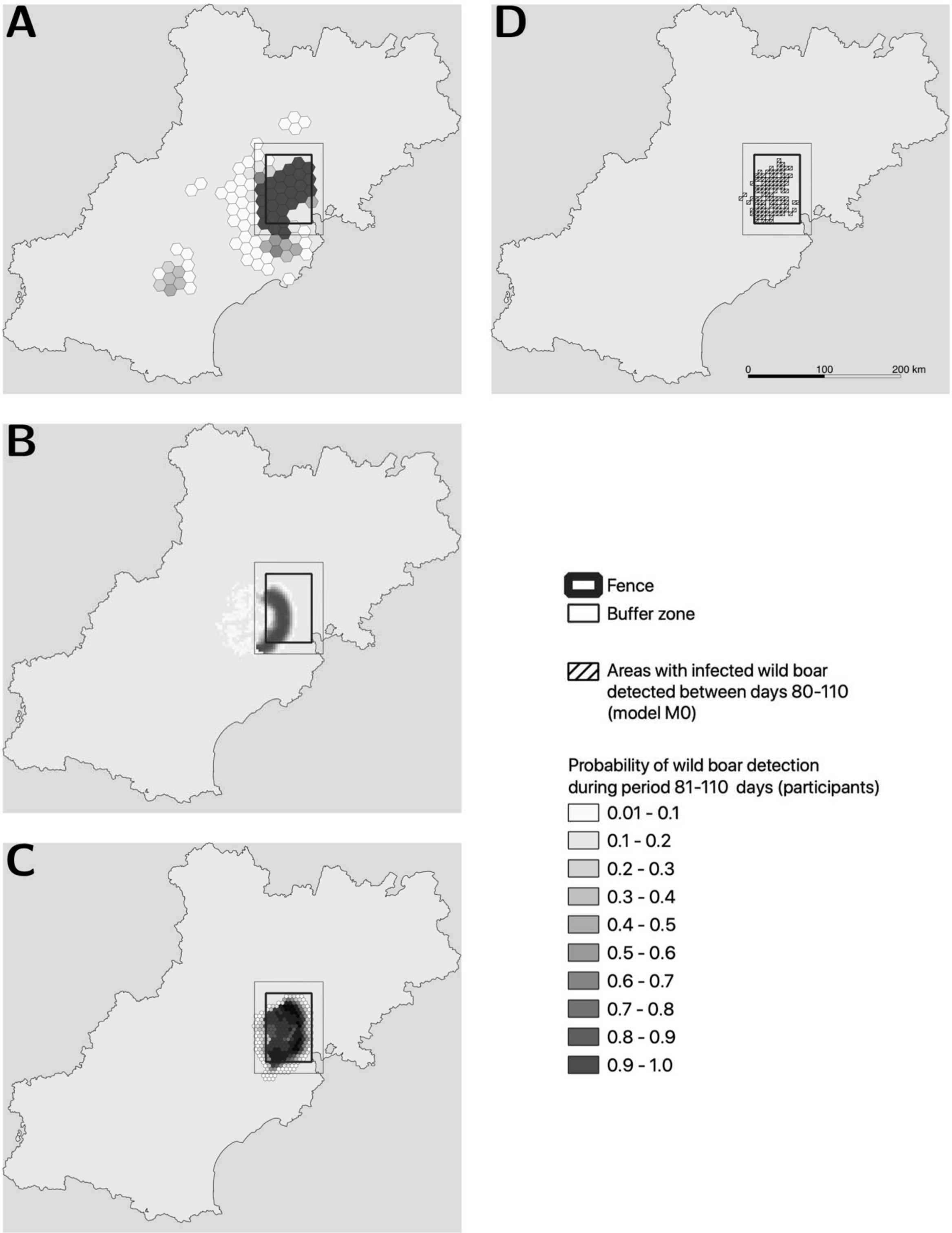

Fig. 4. Predicted probability to detect infected wild boars between days 80 and 110, without additional control measures after day 80, as calculated by two teams: INRAE (A, 195 km² tiles), Massey Univ. (B, 1 km² tiles), and CIRAD (C, 86.8 km$^2$ tiles) compared to wild boar cases found in model M0 (D, 25 km$^2$ tiles)

## DISCUSSION

Modelling challenges organized in human health over the last decade have demonstrated their considerable value for the development and assessment of modelling and forecasting methods (Ajelli et al., 2018). The ASF Challenge, that ran between August 2020 and January 2021, was the first modelling challenge in animal health. Using a new mechanistic and stochastic metapopulation and multi-host model of ASF spread, we generated synthetic data mimicking an ASF-like epidemic detected at the interface between pig farms and wild boar in a typical European context. Land use, size and location of farms, as well as wild boar hunting bags per administrative units were derived from real data in the southern quarter of France. ASF incidence data (detected cases) were provided to the modelling teams while the epidemic developed. The objectives for the teams involved in the challenge were to reproduce the synthetic epidemic assuming a set of control strategies, predict its expansion and prioritize a finite number of alternative interventions. We compared 5 independent modelling approaches and their qualitative and quantitative spatio-temporal predictions over the three one-month periods of the challenge.

To make this challenge interesting and useful, the model that generated the synthetic epidemiological data needed to be more detailed than the different models that could be developed by participants to make their predictions. Hence, data generation were computationally intensive and calibrating the model to generate interesting and realistic “ASF-like” epidemic trajectories was extremely time-consuming, especially because of the lack of data on wild boar population dynamics and mobility patterns and their expected impact on the fences’ efficacy.

The context of ASF provided an opportunity to address the specificities of infectious livestock diseases at the interface between wildlife and domestic farms. Still poorly observed (Vicente et al., 2021), this interface is key to increasing our effectiveness in controlling emerging animal diseases. All participants acknowledged the interest of modelling interactions at the interface between domestic and wild fauna explicitly to accurately predict the course and extent of the epidemics and account for the impact of control measures. The temporal and spatial predictions on domestic pigs were more accurate than for wild boar, which highlights the diversity on assumptions made on wild boar population dynamics and contact patterns due to the lack of knowledge on wildlife. Also, intensive hunting, a key control measure against ASF, was one of the most problematic feature to introduce in models (for participants and organisers), and their potential impact on wild boar ecology, possibly including increased contacts (Lange, 2015), was not modelled.

No specific modelling approach was better than all others on every prediction, and the added-value of their complementarity for policy makers will be addressed in further studies through ensemble models both for the temporal and spatial dynamics. This diversity, which was intentional in the challenge, also led to heterogeneous output formats, which made comparisons difficult and suggested stricter specifications for further challenges.

The choice of producing fictitious data as in the Ebola challenge (Viboud et al., 2018), rather than using historical records, required much work but provided the organisers with a total control and knowledge on the situation, on the noise level in the synthetic data (e.g. a proportion of small farms were not known from the beginning and discovered by participants as they became infected), and on the “representativity” of the selected trajectory compared to possible model outcomes. Such open international challenges are a privileged framework to anticipate emerging infectious diseases, including zoonotic threats at the interface between wildlife, livestock and human activity, and to improve the readiness of modelling teams to face future epidemics.

## ACKNOWLEDGEMENTS

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# CONTROLLING AFRICAN SWINE FEVER AT THE DOMESTIC-WILDLIFE INTERFACE: MULTIHOST EPIDEMIC MODELLING IN ROMANIA

B.H. HAYES*, T. VERGNE, N. ROSE AND M. ANDRAUD

## SUMMARY

To explain the infection dynamics of the current African swine fever (ASF) epidemic in Romania, a country with a preponderance of low-biosecurity backyard pig farms susceptible to spillover infection from wild boar hosts, a multihost spatio-temporal model was designed and fitted to current outbreak data. Transmission rate parameters for seven modes of disease transmission were estimated using approximate Bayesian computation, including transmission within domestic and wild hosts as well as between domestic and wildlife hosts. The model successfully captured the incidence trend among wild boar hosts, and the relative contribution of individual epidemiological units to epidemic propagation was determined. The similarity of the between-cell and herd-to-cell transmission rates indicated that infected herds were a source of infection for wildlife that was of similar magnitude as neighbouring infected wildlife populations. With a parameterized model, alternative control strategies can now be explored to assess their relative impact on epidemic dynamics.

## INTRODUCTION

African swine fever (ASF), one of the highest consequence diseases of domestic pigs and listed as a notifiable disease by the World Organization for Animal Health, is socioeconomically devastating to both individual farms and affected countries (Dixon et al., 2020; OIE, 2021). Caused by a virus of the family *Asfaviridae,* this hemorrhagic fever of *Sus scrofa* (i.e. domestic pigs and wild boar) carries a near-100% case-fatality rate (Blome et al., 2013; Dixon et al., 2020). The current panzootic strain — ASF genotype II — has managed to spread around the world, and with effective vaccine and treatment strategies still lacking, its control and eradication is exceedingly difficult (Turlewicz-Podbielska et al., 2021).

Since the introduction of the current pandemic strain into the European Union (EU) in 2014, cases have been identified in 12 EU Member States, with 10 nations still facing epidemics as of the end of 2021 (EFSA et al., 2021; Van Goethem, 2021). ASF transmission pathways vary between epidemic areas, with some nations (e.g. the Baltic states) experiencing cases predominantly or exclusively among wild boar, and others (such as Romania) seeing cases mostly among domestic pigs with likely spillover to wild boar (Chenais et al., 2019; DEFRA, 2021). As interrupting disease transmission is reliant on control strategies targeting these

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transmission pathways, understanding the dynamics unique to each epidemic provides the best chance of achieving epidemic control (Keeling and Rohani, 2011).

Mechanistic models, which allow the quantification of transmission parameters, the prediction of epidemic trajectories and the evaluation of the effectiveness of control strategies, are a proven way of combating epidemics — as seen for foot-and-mouth disease (Pomeroy et al., 2017), classical swine fever (Backer et al., 2009), and bluetongue (Courtejoie et al., 2018). However, as identified in a systematic review of mechanistic models of ASF, current models do not account for spillover events between domestic pigs and wild boar, and these events are suspected to play an important role in the propagation of some ASF epidemics (Hayes et al., 2021). It was concluded that, in addition to needing more modelling studies using empirical data derived from real epidemics, transmission between domestic pig farms and wild boar should be a component of future models (Hayes et al., 2021).

Since 2018, Romania has been facing an epidemic of unprecedented scale, affecting both wild boars and domestic pigs (DEFRA, 2021), and is considered as one of the EU countries most severely affected by ASF. Understanding the transmission dynamics is necessary to design tailored control measures, adapted to the specificities of domestic pig rearing in regards of societal and economical aspects. The ubiquity of backyard pig farming in villages — with the majority of families having one or more pigs kept in low-biosecurity backyard holdings — provides an environment highly amenable to such transmission (Andraud et al., 2021). In this study, we constructed a multihost spatiotemporal mechanistic model to estimate ASF transmission parameters in Romania and characterise the spatio-temporal infection pattern between domestic pig farms and wild boars.

## MATERIALS AND METHODS

### Data and pre-processing

Four datasets were used in the construction and calibration of the model. Case records, which include the date, coordinates, type of host (swine or wild boar) and associated farm type (backyard or industrial) were retrieved from the OIE's Animal Disease Notification System database for the period from initial case detection (10 June 2018) to the end of the first year (31 Dec 2018). Cases were restricted to the southeastern region of Romania where the initial epidemic spread was witnessed — the counties of Braila, Calarasi, Constanta, Galati, Ialomita, and Tulcea —and aggregated to the ISO-standard week.

Domestic pig herds (hereafter referred to as herds) were represented as either low-biosecurity (backyard) or high-biosecurity (industrial) operations. Backyard cases were additionally aggregated to the level of the villages, acting as single epidemiological units. Village spatial data were retrieved from the Romanian National Agency for Mapping and Real Estate (ANCPI), providing a total of 986 villages across 315 communes. Village locations were then represented by the centroid of each village. Industrial farms (n = 55) were also represented by their point coordinates, whose locations were retrieved from county-level data available through the local directorates of the National Sanitary Veterinary and Food Safety Authority (ANSVSA).

The presence of wild boar herds was modelled via habitat suitability using a hexagonal raster of landscape coverage, as inspired by Vergne et al. (2016). CORINE Land Cover imagery (© European Union, Copernicus Land Monitoring Service 2021, European Environment Agency) was rasterized into 25km² cells, sized in accordance with estimated wild boar home ranges, and the percent of forest coverage was calculated per cell (Janoska et al., 2018). Using results from wild boar distribution studies by Alexander et al. (2016) and Acevedo et al. (2019), a forest coverage threshold of 15% was selected to define suitable habitat for the cells.

Control strategies

Per Romanian regulation and in accordance with EU directives, following case detection a farm is culled, movement restrictions of people, animals and products are enacted, and a 10-km surveillance zone is established around the culled herd (ANSVSA, 2019). While an industrial farm will be culled in entirety, backyard farms within a village are considered individually and neighboring backyard herds to the infected herd are not automatically culled, allowing several backyard farms being reported successively over periods of several days or weeks. While this results in an internal diversity within the epidemiological unit of the village, this facet was addressed through considering the infectious status of a village *en-masse* from first to last case detection per outbreak.

Mathematical model

The infection model for village and industrial herds consisted of four compartments for each epidemiological unit to transit through — susceptible (S), infectious undetected (Iu), infectious detected (Id), recovered (R) — with re-susceptibility occurring after a two-week recovery period. Using expert opinion solicited from local veterinary officials, villages were defined as continuously infectious as long as successive outbreaks were declared less than two weeks apart. Thus, in the epidemic data, a village with at least two weeks without cases was then considered as recovered prior to becoming susceptible again. Wild boar cells transited through S-Iu-Id states, where a cell was considered to be continuously infectious from the first date of carcass detection in the cell until four weeks following the last date of carcass detection, as based on current understanding of wild boar carcass persistence (Probst et al., 2019).

The transmission rate *(beta)*, defined as the rate at which a susceptible agent acquires infection from an infected agent, was defined for seven modes of disease transmission: from a herd (village or industrial) to another herd, from a cell to another cell, from a cell to a herd within the cell, from a herd to the cell that contains it, from previous infection (for villages only, to account for infected meat that had been stored during initial infection and later consumed), from neighbouring infected countries, and from external sources of infection that are not represented explicitly. The transmission rates were fed into force of infection equations — the cumulation of individual infectious pressures exerted by all infectious individuals onto the susceptible individual — which were influenced from those derived by Andronico et al. (2019).

At time *t*, the force of infection experienced by a susceptible herd $j$ located in cell *p,* was given as the sum of all forces of infection exerted by infected herds and the cell $p$, modified by the relative susceptibility of the herd ($\phi_j$), as illustrated in Eq.(1).

$$\lambda_j(t) = \phi_j(\textstyle\sum_i \lambda_{i\to j}(t) + \lambda_{p\to j}(t) + \lambda_j^{prev}(t) + \lambda_j^{edge} + \lambda_j^{ext}) \qquad (1)$$

where *i* represents infected herds and $\lambda_j^{prev}$, $\lambda_j^{edge}$ and $\lambda_j^{ext}$ represent the forces of infection from previous infection, neighboring infected countries, and external sources, respectively. Between-herd transmission was assumed to be frequency-dependent, with a $\lambda_{i\to j}$ of the form:

$$\lambda_{i\to j}(t) = 1_{I_i} * \psi_i * \alpha_{SZ}(i,j,t) * \beta_1 * exp\left(-\delta * d_{ij}\right) \tag{2}$$

where $\psi_i$ represents the relative infectivity of a herd dependent on farm type (equal to 1 for villages and $\psi$ (range 0 – 1) for industrial sites), $\alpha_{SZ}(i,j,t)$ acts as a force multiplier of enhanced surveillance to account for decreases in infectiousness in surveillance zones at time $t$, $\beta_1$ is the between-herd transmission rate, $\delta$ is the exponential term representing the distance kernel for between-herd transmission, with a mean distance equal to 1/δ and $d_{ij}$ representing the Euclidian distance between herds $i$ and $j$, and $1_{I_i}$ is the indicator function which includes only infected premises for exerting infection pressure at time $t$. Transmission from infected cell $p$ to susceptible herd $j$ took the form given in Eq.(3).

$$\lambda_{p\to j}(t) = 1_{I_p} * \psi_p * \left(\frac{\beta_2}{N_p}\right) \tag{3}$$

where $\psi_p$ is the relative infectivity of the cell (1 if above the forest coverage threshold, $\psi$ otherwise), $\beta_2$ is the cell to herd transmission rate, $N_p$ is the number of herds within the infectious cell, and $1_{I_p}$ is the indicator function to consider the force of infection only if the cell is in an infectious state. Transmission from previous infection, neighbouring infected countries, and external forces of infection were calculated via a binary indicator function of meeting their respective criteria ($1_{I_j}$) and multiplied by their respective $\beta$, as in Eq.(4-6).

$$\lambda_j^{prev}(t) = 1_{I_j} * \beta_5 \tag{4}$$

$$\lambda_j^{edge} = 1_{I_j} * \beta_6 \tag{5}$$

$$\lambda_j^{ext} = 1_{I_j} * \beta_7 \tag{6}$$

The force of infection experienced by a cell $q$, was given as the sum of all forces of infection exerted by infected herds located within that cell and by infected cells, modified by the relative susceptibility of the cell ($\phi_q$), as illustrated in Eq.(7).

$$\lambda_q(t) = \phi_q(\textstyle\sum_i \lambda_{i\to q}(t) + \sum_p \lambda_{p\to q}(t) + \lambda_q^{edge} + \lambda_q^{ext}) \tag{7}$$

where $i$ and $p$ represent infected herds located within cell $q$ and infected cells, respectively. Transmission from an infected herd to a susceptible cell took the form of Eq.(8):

$$\lambda_{i\to q}(t) = 1_{I_i} * \psi_i * \alpha_{SZ}(i,q,t) * 1_c * \beta_3 \tag{8}$$

of indicator function $1_c$ to indicate if a cell contains the infected herd, and the herd to cell transmission rate $\beta_3$. Lastly, transmission from an infected cell $p$ to susceptible cell $q$ was limited to first-order adjacency and took the form in Eq.(9):

$$\lambda_{p\to q}(t) = 1_{I_p} * \psi_p * \frac{1_a * \beta_4}{N_{pq}} \tag{9}$$

with the indicator function $1_a$ to indicate adjacency, the cell-to-cell transmission rate $\beta_4$, and the number of adjacent cells $N_{pq}$.

Transitions between states S-Iu-Id-R were stochastically modelled. The probability of transition from the susceptible to infectious state for herds (Eq.(10)) and cells (Eq.(11)) was based on a tau-leap algorithm (Keeling and Rohani, 2011), with the source of infection identified through random selection among the possible sources while accounting for their relative contribution to the force of infection.

$$p_{inf}^{herd} = 1 - exp(-\lambda_j) \tag{10}$$

$$p_{inf}^{cell} = 1 - exp(-\lambda_q) \tag{11}$$

The probability of passive detection of infection (Iu-Id state transition) was considered as an exponentially-distributed function of the detection rate $\sigma$ and modified by $\zeta$, a multiplicative term to account for changes in detection and recovery within surveillance zones (Eq.(12) and Eq.(13)).

$$p_{det}^{herd} = 1 - exp\left(-\frac{\sigma_{herds}}{\zeta}\right) \tag{12}$$

$$p_{det}^{cell} = 1 - exp\left(-\frac{\sigma_{cells}}{\zeta}\right) \tag{13}$$

Assuming that all infections among domestic pigs herds were eventually detected, the probability of recovery from infection was governed by exponentially-distributed recovery rates $\gamma_{vil}, \gamma_{ind}, and\ \gamma_{cell}$ for villages, industrial sites and cells, and defined by (Eq.(14-16)).

$$p_{rec}^{vil} = 1 - exp(-\gamma_{vil} * \zeta)) \tag{14}$$

$$p_{rec}^{ind} = 1 - exp(-\gamma_{ind} * \zeta)) \tag{15}$$

$$p_{rec}^{cell} = 1 - exp(-\gamma_{cell} * \zeta)) \tag{16}$$

Of the 19 parameters in the model, 12 were able to be fixed prior to analysis. The value of the distance kernel $\delta$ was selected so as to locally-bound the force infection between herds to 20 km, the assumed maximum distance of local transmission. The average infectious periods prior to detection ($1/\sigma$) were assumed to be three weeks for herds (based on results by Guinat et al., 2018)) and eight weeks for cells (assumed). Mean infectious periods ($1/\gamma$) were estimated from the epidemic data. For villages and industrial sites, the mean infectious periods were estimated across different time periods, and were then used to parameterize the temporally-forced recovery rates for villages and industrial sites (Fig.1). Both villages and industrial sites experienced increases in their recovery rates after week eight, and villages experienced a subsequent increase in their recovery rate following week 24. Industrial sites were assumed to be 20% as infectious and susceptible as villages, and cells with forest coverage less than 15% were assumed to be 50% as infectious or susceptible. Surveillance zones were assumed to reduce transmission and increase detection and recovery by 50%.

Simulations were initialized via the infection of three cells in Tulcea county two weeks prior to the first case detection in the epidemic data; these cells being the two cells which contained the first detected infections and one other randomly infected cell in the same county.

Parameter inference was performed through approximate Bayesian computation (ABC) using standard rejection methodology (Toni et al., 2009), with uniform distributions used as prior distributions for the seven model parameters. Summary statistics for rejection reflected the spatial and temporal distribution of detected outbreaks, using quarterly incidence per epidemiological unit per county, for a total of 72 statistics. The sum of the absolute difference between the observed and the simulated sets of summary statistics was used for calculating the overall distance between a given simulation and the observed epidemic data. One hundred thousand (100,000) simulations were conducted, with the top 0.1% of the smallest distance kept for inference.

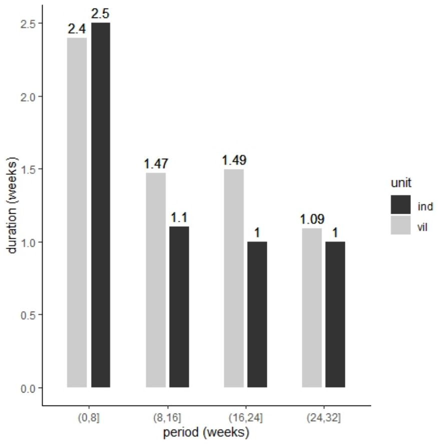

Fig. 1. Quarterly mean infectious periods for villages and industrial sites

Data compilation, model implementation, and analysis were performed in R statistical software, version 4.1.0 “Camp Pontanezen”.

RESULTS

The epidemic curve of the observed data is illustrated in Fig.2. Over the 30-week period, the initial spike followed by gradual fade-out among infected villages constrasted sharply to the paucity of cases detected among cells, with inconsistent surveillance believed to be a contributing factor.

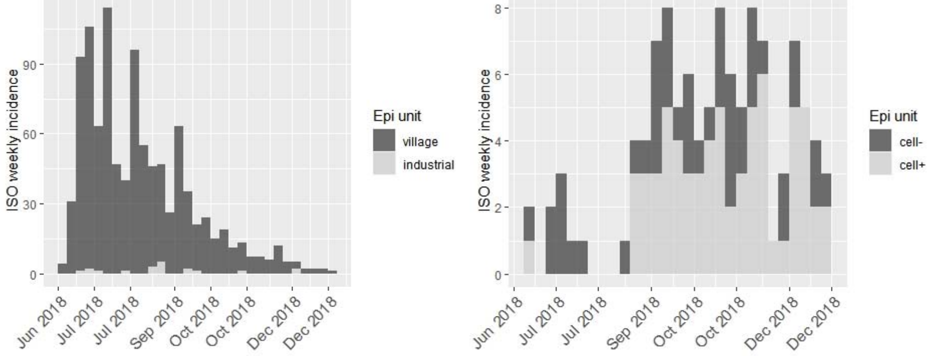

Fig. 2. ASF ISO weekly case incidence: Jun 2018 through Dec 2018. Cell - and cell + refer to cells below or above the forest coverage habitat suitability threshold

Transmission rates ($\beta$) were estimated for all seven routes of transmission, with ABC results and model parameter estimates listed in Table 1. Whereas the distribution for $\beta_2$ was not different from its prior, the posterior distributions of the six other transmission rates proved to be substantially influenced by the model fitting process. The transmission rate from herds to cells ($\beta_3$ = 0.1631 (95% CI 0.0100, 0.3828)) was similar to that of cells to cells ($\beta_4$ = 0.1315 ((0.0051, 0.3724)), though the *betas* for both the between-herd transmission rate ($\beta_1$) and the transmission rate from the effect of the international border ($\beta_5$) were 10-fold lower than others. The *beta* for external sources of infection was as expectedly the smallest.

Table 1. Fixed and estimated parameters for best fitting model

| Parameter | Parameter type | Mean | 95% Credible Interval |
|---|---|---|---|
| $\psi_i$: Relative infectivity of domestic pig herd | Fixed | 0.2[a] | - |
| $\phi_j$: Relative susceptibility of domestic pig herd | Fixed | 0.2 [a] | - |
| $\psi_p$: Relative infectivity of wild boar cell | Fixed | 0.5 [a] | - |
| $\phi_q$: Relative susceptibility of wild boar cell | Fixed | 0.5 [a] | - |
| $\beta_1$: Transmission rate between herds | Estimated | 0.0194 | (0.0009, 0.1024) |
| $\beta_2$: Transmission rate from cell to herds | Estimated | 0.2363 | (0.0074, 0.5752) |
| $\beta_3$: Transmission rate from herd to cell | Estimated | 0.1631 | (0.0100, 0.3828) |
| $\beta_4$: Transmission rate between cells | Estimated | 0.1315 | (0.0051, 0.3724) |
| $\beta_5$: Transmission rate from prior infection | Estimated | 0.0807 | (0.0058, 0.1841) |
| $\beta_6$: Transmission rate from edge effect | Estimated | 0.0179 | (0.0010, 0.0809) |
| $\beta_7$: Transmission rate from external forces | Estimated | 0.0003 | (0.0000, 0.0015) |
| $\sigma_{herds}$: Detection rate for domestic pig herds | Fixed | 1/3 $\text{week}^{-1}$ [b] | - |
| $\sigma_{cells}$: Detection rate for wild boar cells | Fixed | 1/8 $\text{week}^{-1}$ [a] | - |
| $\gamma_{vil}$: Recovery rate for villages | Fixed with seasonal forcing on weeks 8-23 and 24-30 | 1/2.4, 1/1.48, 1/1.09 $\text{week}^{-1}$ [c] | - |
| $\gamma_{ind}$: Recovery rate for industrial sites | Fixed with seasonal forcing on weeks 8-30 | 1/2.5, 1/1.07 $\text{week}^{-1}$ [c] | - |
| $\gamma_{cell}$: Recovery rate for cells | Fixed | 1/6.32 $\text{week}^{-1}$ [c] | - |
| δ: Distance kernel for between-herd transmission | Fixed | 0.2 [a] | - |
| $\alpha$: Reduction in transmission within surveillance zone | Fixed with seasonal forcing on weeks 15-19 and 20-30 | 0.5, 0.495, 0.490 [d] | - |
| ζ: Increase in detection and recovery within surveillance zone | Fixed with seasonal forcing on weeks 15-19 and 20-30 | 0.5, 0.495, 0.490 [d] | - |

[a.] Assumed value. [b.] Guinat et al., 2018 [c.] Derived from epidemic data [d.] Fit to epidemic data

The model succeeded in capturing the trend in incidence of ASF among wild boar hosts, with the observed weekly incidence falling within the 95% prediction interval predicted by our posteriors (Fig. 3).

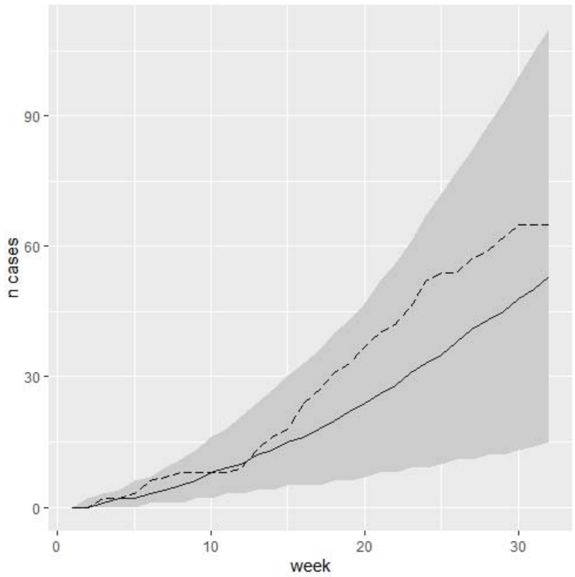

Fig. 3. Cumulative incidence of wild boar cases. Predicted simulation dynamics are shown as the median (solid line) and 5th and 95th percentile (the shaded region) of number of cases, with cumulative incidence of the observed epidemic data indicated by the dashed line

## DISCUSSION

In this paper we have attempted to construct and parameterize a mechanistic model of ASF virus transmission that simulates transmission both within and between domestic pig and wild boar hosts, using data from an on-going epidemic. We were able to obtain estimates of the seven transmission rates to explain the relative contributions of each epidemiological unit class to epidemic propagation. The proximity of the between-cell and herd-to-cell transmission rates indicated that infected herds were a source of infection for wildlife that was of similar magnitude as neighbouring infected wildlife populations. The *beta* that governed the rate of transmission from cells to herds ($\beta_2$) appeared to have a relatively wide 95% credible interval. As this is one of the key parameters we are attempting to ascertain, further refinement of our parameter estimation strategy is needed in order to clarify the relative contribution of herds and cells to ASF spread among herds.

Our model focused on the initial 30 weeks of the epidemic, a period where the initialization and subsequent augmentation of control strategies led to non-uniform application of control measures across the study period. These differences must be accounted for in the modelled transmission dynamics, and to do so our model included seasonal-forcing of parameters related to both the infectious period and surveillance intensity in an attempt to reflect the changing control pressures. A relevant development of this work would be to assess the impact of these changes by simulating the epidemic without them and measuring how more substantial the epidemic would have been.

Local traditions play heavily into the ASF scenario being modelled, as the winter holiday slaughtering of pigs likely act as a strong external influence on ASF infection and propagation at the village level. Consequently, we focused our model leading up to this time period but not proceeding beyond, as the transmission dynamics will inevitably change following a mass culling.

Our choice of transmission kernel purposely excludes long distance transmission. While this may be realistic regarding local spread between villages, this is unlikely the case among industrial sites where transport distances of pigs can be many times greater. However, we are not modelling infection transmission among the transit network, and the external force of infection exists to capture these long-distance jumps in transmission that are otherwise extremely difficult to model mechanistically.

With a mechanistic model parameterized to the epidemic under study, alternative control strategies can now be explored to ascertain the relative outcomes they may have on the epidemic dynamics in comparison to the strategy enacted. Additionally, as some estimated parameters could be further refined, additional parameter estimation strategies will need to be explored to better fit our model.

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# APPLICATION OF SYNDROMIC SURVEILLANCE TO ROUTINELY COLLECTED SWINE PRODUCTION DATA FOR FARM HEALTH MANAGEMENT AND EARLY DISEASE DETECTION

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## SUMMARY

Driven by the need for early disease-warning and the underexplored use of production data, a syndromic surveillance (SyS) system was developed using production data routinely collected in pig reproduction farms. Health-related indicators were created from the recorded data, and two different time-series types emerged: the weekly counts of events traditionally used in SyS; and continuous time-series, where every new event is a new observation, and grouping by time-unit is not applied. Control charts were used for temporal aberration detection. System performance was evaluated using simulated outbreaks of porcine respiratory and reproduction syndrome (PRRS). The system proved capable of providing early detection of unexpected trends, serving as a useful health and management decision support tool for farmers. Further research is needed to combine results of monitoring multiple parallel time-series, in particular for the case of the continuous time-series.

## INTRODUCTION

As a response to the need for timely disease detection, and enabled by growing data digitalization, syndromic surveillance (SyS) has been used more and more frequently in animal health (Dórea et al., 2011). The prime statement of any SyS system is that a given indicator of population health changes when a health hazard is introduced, and these changes can be noticed if routinely collected data are analysed continuously (Mandl et al., 2004a).

As electronic records are reaching farms, data recorded at the herd level, or even individually for each animal regarding different phases of productivity (hereby generalised as "production data"), have appeared as a growing data source for animal health surveillance. These data, if correctly managed, allow large population coverage and the shortest time lag between a health event and its potential detection (Dórea and Vial, 2016). Production records are generated continuously, not only on the occasion of a disease event. This offers advantages such as timeliness, but also results in a lack of specificity, in particular representing a challenge to the definition of the events to monitor and what deviations should be characterised as abnormal. These methodological issues have not yet been extensively addressed, as exploration

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of farm-level data for SyS has so far been limited, likely due to their distributed nature and governance.

This project aimed to investigate whether production data recorded regularly in reproduction pig farms could be used to construct health and performance indicators; and whether these indicators could be subjected to near-real time temporal monitoring (syndromic surveillance) to detect early signals of reproduction failure, which could be caused by undetected diseases or management errors.

## MATERIALS AND METHODS

### Data source

The majority of swine farms in Sweden manage their records using the production management software PigVision (Agrosoft®, http://agrosoft.eu/, 2014). To explore the structure of data recorded using this software, data from a large sow producing farm, shared voluntarily and anonymously were used. For back-end data management, PigVision creates a local server which hosts a SQL (Structured Query Language) database. Data from this SQL database were accessed within the statistical programming environment R (R Core Team, 2020) using the package {RODBC} (Ripley and Lapsley, 2020).

### Data organization into indicators

The data available in PigVision were used to construct production and reproduction performance indicators. Stakeholders helped select indicators that could be useful to monitor health, as well as production and reproduction management. Stakeholders also advised that the best unit of time for counting events and running detection would be weekly. The indicators chosen are listed in Table 1.

Some indicators could be easily grouped as “number of events per time-point (weekly)”, which is the type of time-series traditionally used in SyS (discrete time-series). Some other indicators were better expressed as continuous time-series, that is, series in which every new event represents a new observation. A specific value associated with these observations is monitored individually, rather than grouped in discrete time-steps. Examples are pregnancy length and number of live born piglets, both associated with every new farrowing event. The column “Limit(s)” in Table 1 identifies whether indicators were relevant for detection of potential increases (upper control limits are used), decreases (lower control limits) or both.

### Time-series analysis

Most of the SyS systems developed to date are based on discrete time-series (Unkel et al., 2012; Dórea and Vial, 2016). Time-series analyses of the continuous time-series was investigated to the extent possible, as described in each step. The entire workflow was programmed using the statistical environment R, and is available publicly (https://github.com/SVA-SE/PigPeaks).

Table 1. List of performance indicators constructed from production data, and chosen by stakeholders as relevant for monitoring

| Category | Indicators | Time-series | SyS[a] | Limit(s)[b] | Utility |
|---|---|---|---|---|---|
| Gilts | Age at first service | Continuous | No | --- | Management |
|  | Age at first farrowing | Continuous | No | --- | Management |
| Empty Sows | Number of sows empty longer than 4 days | Weekly | Yes | UCL [c] | Health |
| Services | Reservices per week | Weekly | Yes | UCL | Health |
|  | Time to reservice | Continuous | Yes | UCL \| LCL [d] | Health |
|  | Percentage of reservices after 4 weeks | Weekly | Yes | UCL | Health |
|  | Percentage of failures after 4 weeks | Weekly | Yes | UCL | Health |
| Pregnancy | Pregnancy length | Continuous | Yes | LCL | Health |
|  | Time to abortion | Continuous | No | --- | Health |
|  | Abortions per week | Weekly | Yes | UCL | Health |
| Farrowing | Days between farrowings | Continuous | Yes | UCL \| LCL | Health |
|  | Total piglets per farrowing | Continuous | Yes | UCL \| LCL | Health |
|  | Live piglets per farrowing | Continuous | Yes | LCL | Health |
|  | Percentage of dead piglets per farrowing | Continuous | Yes | UCL | Health |
|  | Mummified piglets per farrowing | Continuous | Yes | UCL | Health |
| Weaning | Days to weaning | Continuous | No | --- | Management |
|  | Litters weaned per week | Weekly | No | --- | Management |
|  | Piglets weaned per week | Weekly | Yes | LCL | Health |
|  | Piglets weaned per litter | Continuous | Yes | LCL | Health |
|  | Deaths in weaning piglets per week | Weekly | Yes | UCL | Health |
|  | Weaning weight per litter (average per piglet) | Continuous | Yes | LCL | Health |
| Post-Weaning | Deaths in weaners per week | Weekly | Yes | UCL | Health |
| Exit | Last event before exiting per week | Weekly | No | --- | Management |
|  | Dead sows per week | Weekly | Yes | UCL | Health |

[a] Syndromic Surveillance applied; [b] Temporal aberration detection limit(s); [c] Upper control limit; [d] Lower control limit.

### Retrospective analysis

The presence of temporal effects (Lotze et al., 2008) was evaluated for all weekly time-series using the R package {vetsyn} (Dórea et al., 2015). Models available assume that observations represent specific time units (discrete time-series). Considering the lack of systematic temporal patterns in the weekly time-series, and the need for further research to address continuous time-series, pre-processing was ultimately discarded from the analysis process, and methods will be presented for all time-series analysed without pre-processing.

### Create a baseline

Exploratory analysis of the time-series showed that event recording was considerably low in the first few years of data. Therefore, for weekly indicators the first 200 weeks were removed, with 271 weeks remaining. For the continuous indicators, a maximum number of the most recent 5500 observations per indicator were left.

To create an outbreak-free historical baseline for each indicator in which SyS was applied, the non-parametric method of moving percentiles was chosen. A 95th percentile was selected as the upper limit and a 5th percentile as the lower limit, applied in a moving window of 104 weeks (two years) for weekly time-series. For continuous time-series, the moving window was based on the mean number of observations within two production cycles.

Aberration detection

In the absence of significant temporal effects that needed to be modelled and extracted from the discrete time-series data, control charts were applied directly to the indicator time-series as the methods of aberration detection. This even for the continuous time-series, which although novel in veterinary SyS, more closely resemble the industrial processes which control charts were originally intended to monitor.

To ensure detection of sudden spikes (potentially indicative of management errors) as well as slow increases/decreases in the observed values (potential outbreak signals), both the Shewhart and Exponentially Weighted Moving Average (EWMA) control charts were applied (Yahav et al., 2011). Dórea et al. (2014) previously demonstrated that EWMA and Shewhart control charts showed complementary performance in detecting outbreaks of different shapes.

Algorithms' parameterization and implementation

Both detection algorithms chosen were applied to each indicator. For the weekly indicators, the current algorithms implementations from the {vetsyn} package were used. A baseline of 104 weeks (2 years of data) was chosen as suggested in Dórea et al. (2013a). To avoid baseline contamination, the 2 weeks of guard-band applied as default in the {vetsyn} package for weekly data was maintained. As the analyses performed were retrospective, for weekly time-series the number of observations to apply syndromic surveillance (evaluate window) were all remaining observations (the last 165 weeks of the data). Parameterization of the EWMA algorithm further requires a smoothing coefficient (lambda). Smaller values of lambda are recommended for detecting smaller shifts, and larger values for larger shifts. Based on common examples from the veterinary SyS literature (Faverjon et al., 2019), a value of 0.2 was applied.

The {vetsyn} workflow was designed specifically for discrete time-series analysed in the SyS context, as algorithms are applied recursively in each time-point to be evaluated. For evaluation of the continuous time-series, the original control charts implementations in the package {qcc} (Scrucca, 2004) were used, in which there is no separation between baseline and evaluation window. The entire historical series is used to calculate the central, upper and lower control limits. All observations in the time-series are then evaluated against these limits.

For the choice of detection limits, the approach suggested in Dórea et al. (2013b) of using a scoring system to combine multiple detection limits was followed. These limits were based on standard deviation thresholds, that is, the number of standard deviations (s.d.) above or below the mean that decide when an observation is abnormal. For both discrete and continuous time-series three different standard deviations (2.5, 3, and 3.5) were applied. The 3 standard deviation limit had been tested with good performance in using production data to detect porcine reproductive and respiratory syndrome (PRRS) outbreaks in the United States (Silva et al., 2017). To apply the scoring approach, the standard deviations of 2.5 and 3.5 were added, keeping 3 as the centre.

### Evaluation of performance in a practical scenario

No outbreaks of regulated diseases were known to be present in the historical data. In this case, it is common practice in SyS to create simulated data by designing artificial outbreaks and injecting them into the indicators (Dórea et al., 2013a).

It was of specific interest to inject outbreaks of PRRS since Sweden is free of the disease, but the virus still circulates in Europe (Carlsson et al., 2009). Based on Pejsak and Markowska-Daniel (1997) and Valdes-Donoso et al. (2018), ten indicators were deemed most suitable to inject outbreak signals:

- *Reservices per week*, *Abortions per week*, *Piglets weaned per week*, *Deaths in weaning piglets per week*, and *Dead sows per week*; all of which are discrete, *weekly* time-series.
- *Pregnancy length*, *Live piglets per farrowing*, *Percentage of dead piglets per farrowing*, *Mummified piglets per farrowing*, and *Piglets weaned per litter*; all of which are *continuous* time-series.

To simulate artificial outbreak signals within each of these indicators, the effects reported in the literature mentioned were reviewed, and used to construct a typical log-normal outbreak curve (Keeling and Rohani, 2008). Outbreaks were assumed to always start in week 1 and last 13 weeks, therefore confined to a single quarter of the year. The time-series for each indicator from the second quarter of 2015 until de first quarter of 2018 (3 years, 12 quarters in total) were used as the basal farm behaviour. For each of the 10 indicators, twelve copies of each indicator series were created, and a single outbreak signal was injected in each of those copies, always in a different quarter.

Detection performance was evaluated based on three main performance measures: false alarm rate, timeliness and sensitivity. False alarm rate was assessed based on the number of alarms triggered when the control charts were applied to the original observed data, without any injected outbreaks. The false alarm rate was calculated based on the lowest detection threshold. Timeliness of detection was assessed by plotting the simulated outbreak signal curves against the density of alarms generated per week, for each indicator and each algorithm. The system's sensitivity was evaluated based on the total number of alarms generated by each of the two control charts for each of the 12 simulated outbreaks.

## RESULTS

### False alarm rate

In the weekly indicators, the false alarm rate using EWMA was highest for the indicator *Abortions per week* (13.3%), followed by *Piglets weaned per week* (5.5%). The rates were much lower in the remaining indicators: 1.8% for *Deaths in weaning piglets per week*, and 0.6% in both *Dead sows per week and Reservices per week*. The Shewhart algorithm had lower false alarm rates overall, except for *Abortions per week*. The respective rates were 13.3%, 0%, 4.8%, 1.2% and 0%.

False alarm rates were overall higher in the continuous indicators. Using EWMA, the higher false alarm rate was *Pregnancy length* (14.8%), followed by *Mummified piglets per farrowing* (7.8%) and *Piglets weaned per litter* (7.6%). The remaining rates were 3.2% in the *Percentage* *of dead piglets per farrowing*, and 2.2% in *Live piglets per farrowing*. The respective rates for the Shewhart chart were 1%, 3.1%, 0.4%, 2.3% and 2.1%.

Timeliness

Figures 1 and 2 show the total number of alarms triggered in the weekly time-series by the EWMA and Shewhart control charts, respectively, in each simulated outbreak week, for all twelve simulated quarters simultaneously (superimposed). For instance, week 1 shows the sum of alarms triggered in week 1 summing all twelve simulated outbreak quarters. EWMA results are shown for continuous time-series in Fig. 3.

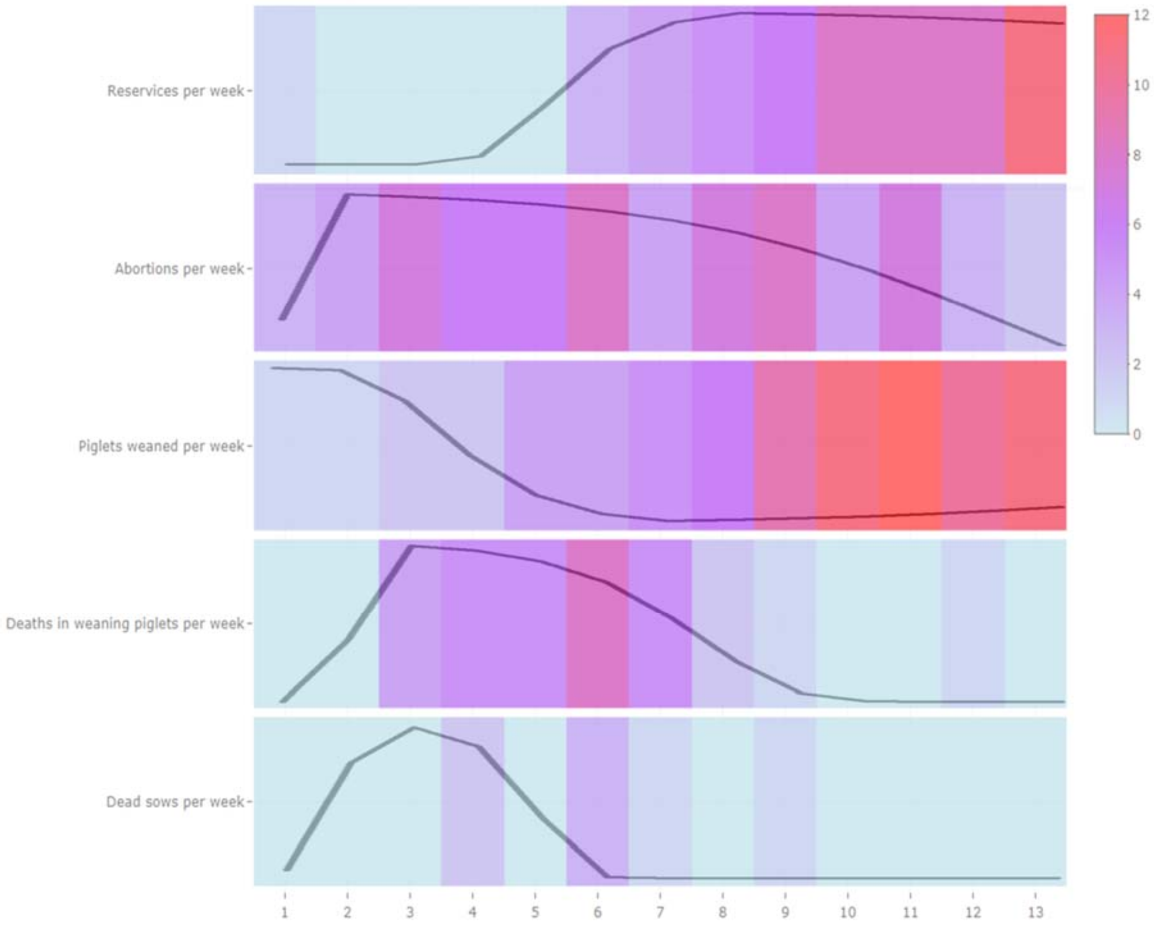

Fig. 1. Total number of alarms triggered by the EWMA control chart in each week of simulated outbreaks in weekly indicators, superimposed for all twelve quarters simulated. The corresponding log-normal curves used for aberration injection are also depicted

Regarding weekly indicators, and as illustrated in Figs. 1 and 2, in 3 out of 12 simulated outbreak quarters the system generated an alarm already in week 1 for the indicator *Abortions per week* with both control charts. The indicators *Reservices per week* and *Piglets weaned per week* had each 1/12 simulated outbreaks generating an EWMA alarm in week 1. However, in the latter indicator, the number of quarters with alarms increases in the following weeks as opposed to *Reservices per week* where the next alarm is only detected in week 6. EWMA generated an alarm in all simulated outbreaks of *Piglets weaned per week* in week 11. The indicator *Dead sows per week* with Shewhart also generated an alarm on week 1, but in a single simulation, and only 2 more weeks had alarms. The indicator *Deaths in weaning piglets per* *week* with EWMA has its first alarm in week 3 with 4/12 simulated outbreaks generating alarms in that week, and the same indicator with the Shewhart control chart had better timeliness with 3/12 simulated outbreaks generating an alarm in week 2. By week 6 all 5 weekly indicators monitored would already have generated at least one alarm in all simulated scenarios.

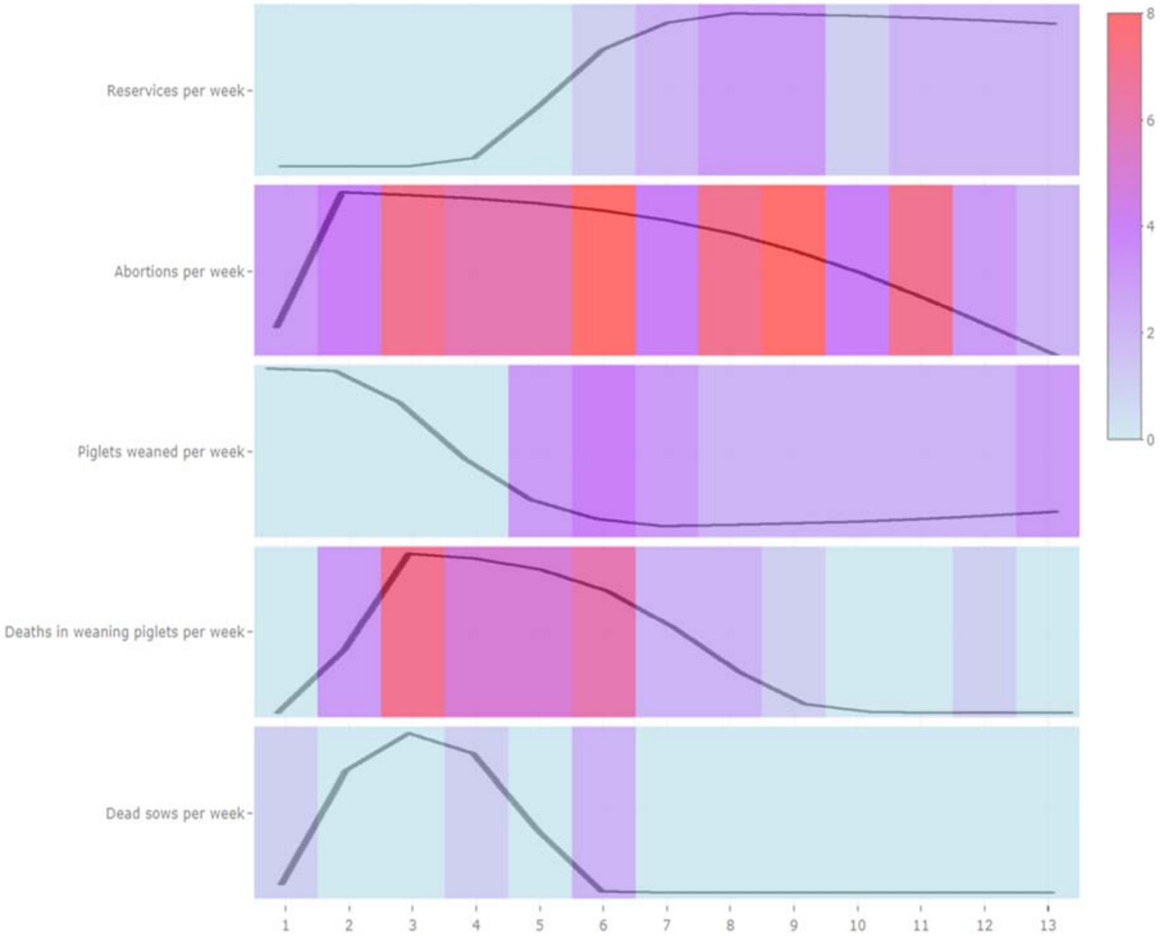

Fig. 2. Total number of alarms triggered by the Shewhart control chart in each week of simulated outbreaks in weekly indicators, superimposed for all twelve quarters simulated. The corresponding log-normal curves used for aberration injection are also depicted

For the continuous indicators, as shown in Fig. 3 the indicator which triggered the most EWMA alarms in an earlier phase was *Mummified piglets per farrowing*, with 319 out of 345 observations generating an alarm in week 1, 355/356 in week 2, and 374/374 observations in week 3 (all observations triggered alarms). The second earliest indicator with most EWMA alarms was *Piglets weaned per litter*, with 153/380 observations generating an alarm in week 1, 167/373 in week 2, and 124/379 in week 3. Then, the number of alarms increased, with the maximum number of 377 out of 377 observations generating an alarm in week 8. *Percentage of dead piglets per farrowing* and *Pregnancy length* had a similar number of EWMA alarms triggered, despite the first indicator having more alarms generated at an early phase. *Live piglets per farrowing* was the least timely indicator.

Timeliness of continuous indicators with the Shewhart algorithm was poorer in comparison to EWMA. Analyses of the results showed that the indicator which triggered the most Shewhart alarms in an earlier phase was *Pregnancy length*, with 19 out of 345 observations generating an alarm in week 1, 21/356 in week 2, and 28/374 in week 3. The indicator *Mummified piglets* *per farrowing* was the one with the highest number of alarms triggered, despite being few at an early stage. From week 4, the number of alarms start to increase considerably with 53 out of 341 observations generating an alarm in that week, 302/342 in week 5, 339/370 in week 6, and 357/374 observations in week 7 (maximum number of alarms triggered).

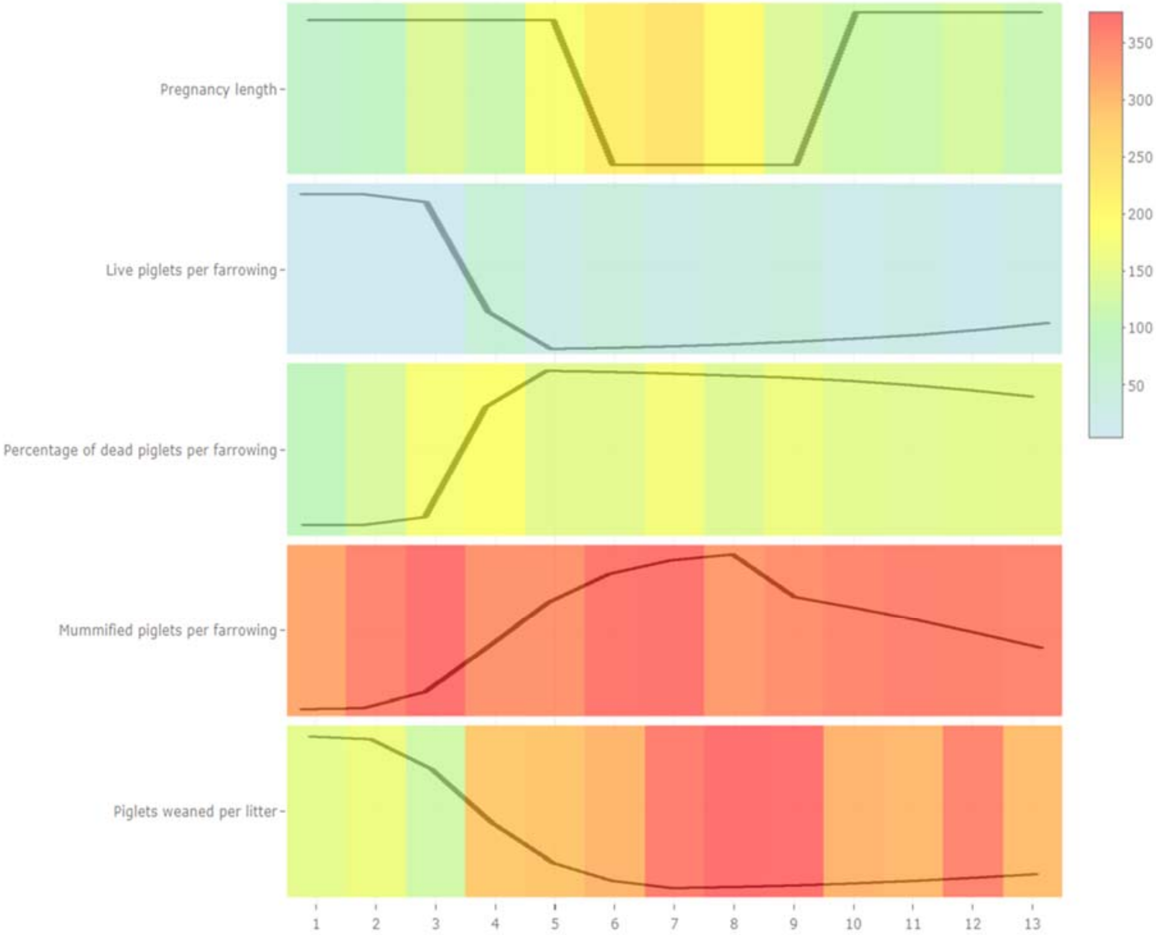

Fig. 3. Total number of alarms triggered by the EWMA control chart in each week of simulated outbreaks in continuous indicators, superimposed for all twelve quarters simulated. The corresponding log-normal curves used for aberration injection are also depicted

Sensitivity

For weekly indicators, in *Abortions per week* and *Piglets weaned per week* EWMA alarms were triggered in at least 2 weeks in all simulated outbreaks (of 13 weeks each). The indicator *Reservices per week* and *Deaths in weaning piglets per week* did not trigger any EWMA alarm in only 1 out of 12 simulated outbreaks. The least sensitive indicator was *Dead sows per week* with 7 out of 12 simulated outbreaks that did not trigger any EWMA alarm, and with a maximum number of 2 EWMA alarms per simulated outbreak (out of the 13 weeks of a simulated signal). The total number of EWMA alarms generated over the 12 quarters was highest in the indicator *Piglets weaned per week* (78, 50% of the total number of weeks within simulated outbreak quarters), followed by *Abortions per week* (69, 44%) and *Reservices per week* (54, 35%). *Deaths in weaning piglets per week* and *Dead sows per week* generated EWMA alarms in only 31 (20%) and 7 (4%) weeks, respectively. The number of weeks with Shewhart alarms was generally half to one third that of EWMA alarms, except for *Abortions* *per week* and *Deaths in weaning piglets per week*, for which EWMA and Shewhart generated about the same number of alarms.

Concerning the continuous indicators, *Mummified piglets per farrowing* was the indicator that generated the most EWMA alarms in all twelve quarters, with a total of 4565 alarms out of 4603 observations (99% of the observations triggered an alarm). It was followed by the indicator *Piglets weaned per litter* (75%), *Percentage of dead piglets per farrowing* (43%), and *Pregnancy length* (41%). *Live piglets per farrowing* generated EWMA alarms in only 7% of the total number of observations recorded within weeks of simulated outbreaks. The number of alarms generated by the Shewhart control chart was generally very low (3-13%), except for *Mummified piglets per farrowing* (54%). Before these percentages can be interpreted in terms of sensitivity, further research is needed to define how to use results of control charts detection in the case of continuous time-series, where multiple events (and therefore multiple alarms) are observed per time-point (in this case week).

## DISCUSSION

The system created proved capable of providing early detection of signals associated with PRRS infection. The results show that this PRRS outbreak, regardless of which quarter had happened, had a big chance of being noticed at an early phase, using the indicators chosen. Although not all indicators are equally effective, the combination of the ten gives a useful overview of farm animal health regarding this disease.

For the creation of the hypothetical outbreak signals, rather than simulating defined artificial shapes and applying these same shapes to all indicators, we tried to use evidence from the literature to create outbreak signals that closely represent the expected behaviour of each particular indicator under a real scenario of PRRS spread within a farm. As a consequence, when interpreting the results, we must keep in mind that the timeliness and sensitivity observed are a combination of the statistical performance of the control charts for each indicator, as well as the choices made regarding which series contained earlier or stronger outbreak signals.

It is generally recommended that algorithms should be parameterized for a maximum false alarm rate of 3% (Mandl et al., 2004b). Some of the indicator/algorithm combinations had a false alarm rate higher than 3%, which could in time cause producers to stop paying attention to system alerts. All results presented were based on the lowest detection threshold. The scoring system proposed allows users (farmer, advisor, analyst, veterinarian) to tailor the system, accepting higher alarm scores for some indicators. This has to be assessed individually for each indicator to balance sensitivity and specificity once the system is used in practice.

In terms of timeliness, both control charts exhibited good performance in both indicator types, although the EWMA often notices changes earlier. This was expected since it is known for its performance of detecting gradual changes (Yahav et al., 2011), meaning that this control chart is supposed to perform better with these slow increasing/decreasing signals. Similarly, sensitivity of the Shewhart control chart among weekly indicators was worse when compared to EWMA's.

For continuous time-series, sensitivity was presented as the total number of alarms triggered by the total number of events. However, as many events would happen in the same week, considering every statistical aberration as a true alarm is likely not a viable way to use the system in practice. Further research is needed to understand how to use aberration detection outputs for continuous time-series. Nevertheless, our results showed that including the continuous indicators in the monitoring will add value and timeliness to the system.

This study shows that production data is useful and has potential, corroborating the reported by Dórea and Vial (2016). Production data could also support the identification of non-infectious sources of variation, since indicators based on production data are not disease-specific. As such, they may be effective in detecting unspecific changes in herd health (management errors), and could provide a continuous view of the system performance (situational awareness).

The fact of using simulated data (and not real data of a PRRS outbreak) and performing the outbreak injections and analyses in one single farm are limitations of this study. Future work is required to overcome these limitations in order to improve the system.

The system investigated solves the practical issue of data access by applying SyS directly at the data source, using data already routinely collected by the leading swine herd management software in Sweden. Implementation in practice and in real-time will however require evaluation of a greater number of farms, and in particular development of the monitoring system to work prospectively, while all analyses reported here were performed retrospectively.

The work showed that the use of production data can be valuable for monitoring indicators of health and reproductive performance, serving both as a management and an early detection tool. In the future, research should focus on how to combine the evidence of multiple indicators to have one overall alarm/assessment of the risk of an ongoing outbreak.

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# AVIAN AND SWINE INFLUENZA TRANSMISSION

# DISENTANGLING THE ROLE OF POULTRY FARMS AND WILD BIRDS IN THE SPREAD OF HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS H5N8 IN EUROPE

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## SUMMARY

In the winter of 2016-17 Europe was severely hit by an unprecedented epidemic of highly pathogenic avian influenza (HPAI) H5N8 virus, causing significant impact on animal health, wildlife conservation and livestock economic sustainability. By applying phylodynamic tools to H5N8 sequence data collected from poultry farms and wild birds during the epidemic, we quantified how fast the virus spread, how many infections were not detected and how many transmission events occurred at the wildlife-domestic interface. We also investigated predictors of the virus spread between farms across borders. These results are crucial to better understand the virus transmission dynamics, with the view to inform policy decision-making and reduce the impact of future epidemics of HPAI viruses.

## INTRODUCTION

Since the beginning of the 21st century, the highly pathogenic avian influenza (HPAI) H5N8 virus (clade 2.3.4.4b) represents one of the most serious threats to animal health, wildlife conservation and livestock economic sustainability. In June 2016, the virus was detected in wild birds in regions of Central Asia (at the Ubsu-Nur and Qinghai lakes, known as migration stop-overs) and subsequently spread to other Asian countries and Europe (Napp et al., 2018). By the end of 2017, the virus had caused one of the most severe epidemics in Europe, in terms of number of poultry outbreaks, wild bird cases and affected countries (Napp et al., 2018). Most of poultry outbreaks occurred in France (37.8%), followed by Hungary (21.5%), Germany (8.5%), Poland (5.8%) and Czech Republic (3.9%) (Napp et al., 2018).

While epidemiological and phylogenetic studies have generated important clues about the H5N8 virus transmission patterns in Europe, they remained opaque to the specific role of poultry farms and wild birds in the disease spread. In particular, understanding the viral transmission dynamics among these two subpopulations is crucial to determine which of these two has the greatest potential to drive the viral transmission during epidemics, which, in turn, represents critical information to better target control strategies. When appropriate pathogen genetic and epidemiological data are collected, phylodynamic methods can fill this critical gap (Guinat et al., 2021). By fitting population dynamic models to genetic sequences collected

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during epidemics, these tools aim at quantifying disease transmission dynamics and have been particularly used to study the spread of infectious diseases in structured populations, be they stratified by time, species or geography (Nadeau et al., 2021). Importantly, birth-death model-based approaches (Kühnert et al., 2016) explicitly allow for the direct estimation of key epidemiological parameters, such as the effective reproduction number $R_e$ (which captures the number of secondary infections generated at any time during an epidemic in a partially immune population) (Anderson and May, 1979), while taking into account the sampling effort.

Using a phylodynamic framework, this study aimed at disentangling the role of poultry farms and wild birds in the spread of H5N8 in Europe during the 2016-2017 epidemic. We fitted a phylodynamic model with geographical and host structure to H5N8 genome sequences collected from both host types (190 from poultry farms and 130 from wild birds) in four severely affected European countries (Czech Republic, Germany, Hungary and Poland) to: (i) infer the number of unreported infections, (ii) discriminate the number of new infections arising from local transmission versus importation events and (iii) identify factors driving the virus spread between farms across borders.

## MATERIALS AND METHODS

### Selection and alignment of sequences

All H5N8 genome sequences collected during winter 2016-2017 from four severely affected European countries (Czech Republic, Germany, Hungary and Poland) were downloaded from the GISAID database on September 1st, 2020 (https://www.gisaid.org). Selected sequences were annotated with available sampling dates, locations and hosts, aligned using MAFFT v7 (Katoh and Standley, 2013) and manually edited using AliView v1.26 (Larsson, 2014). The final dataset consisted of 190 genome sequences from infected poultry farms and 130 from infected wild birds.

### Phylodynamic analysis

Multi-type birth-death model: The multi-type birth-death (MTBD) model was fitted to the sequence alignment (Kühnert et al., 2016). Under this model, infected hosts could transmit the virus to another host from the same discrete subpopulation, referred to here as deme, eventually become non-infectious due to recovery or death/depopulation, be sequenced and sampled upon becoming non-infectious or could transmit the virus to another host from another deme. All transmission, become non-infectious and sampling processes are assumed to be deme-specific and constant through time, except for the within-deme $R_e$ that was estimated across four-time intervals, corresponding to the four phases of the epidemic.

Under this model, sequences were organized into five demes, according to the host type and geographical location: 'poultry farms in Czech Republic', 'poultry farms in Germany', 'poultry farms in Hungary', 'poultry farms in Poland' and 'wild birds in the four countries'. All sequences from wild birds were aggregated into one deme (not depending on the geographical location as for poultry farms) since it was assumed that the majority of sampled wild bird species (mainly mallards and swans) could move freely among countries (Atkinson et al., 2006).

Predictors of H5N8 virus spread between poultry farms across borders: The MTBD model was extended with a generalized linear model (GLM) to inform the H5N8 virus spread between poultry farms across borders by 19 time-independent predictors (Lemey et al., 2014): the 2016 live poultry trade (FAOSTAT, 2021), the 2016 poultry density in the source and destination deme (FAOSTAT, 2021), the 2014 poultry farm density in the source and destination deme (EFSA et al., 2017), the 2017 farm outbreak density in the source and destination deme (FAO, 2021), the 2021 human density in the source and destination deme (Wikipedia, 2021), whether two countries shared borders and the distance between countries' centroids. To account for potential missing predictors, we also included predictors to assess the virus spread from or to one individual country. In this GLM parametrization, the between-deme $R_e$ parameters act as the outcome to a log-linear function of the predictors. To avoid collinearity among predictors, predictors were removed when the Pearson correlation exceeded > 0.7. Bayes Factors (BF) were used to determine the contribution of each predictor in the GLM. The BF cutoff for substantial contribution of a given predictor in the GLM was set at 3.2 (Kass and Raftery, 1995), meaning that its posterior inclusion probability in the model was 3.2-fold more likely than its prior inclusion probability (0.50).

Inference of MTBD model parameters, structured trees and epidemic trajectories: Phylodynamic analysis was implemented using the BDMM-Prime package (Vaughan, In prep.) for BEAST v2.6.3 (Bouckaert et al., 2014) and the BEAGLE library (Ayres et al., 2012) to improve computational performance. All analyses were run for 40-50 million steps across three independent Markov chains (MCMC) and states were sampled every 10,000 steps. The first 10% of steps from each analysis were discarded as burn-in before states from the chains were pooled using Log-Combiner v2.6.3 (Bouckaert et al., 2014). Convergence was assessed in Tracer v1.7 (Rambaut et al., 2018) by ensuring that the estimated sampling size (ESS) values associated with the estimated parameters were all > 200. For each set of posterior model parameters set and associated structured tree, an epidemic trajectory (i.e. corresponding to the sequence of transmission, become non-infectious and sampling events that occur throughout a given epidemic) was drawn from the distribution of such trajectories conditional on the tree and parameters. To test the robustness of the phylodynamic analysis with respect to changes in the $R_e$ priors, a separate set of analyses were performed using broader and tighter priors on the within- and between-deme $R_e$.

## RESULTS

### Number of unreported H5N8 infections

Figure 1 represents the temporal distribution of the inferred cumulative number of no-longer infectious outbreaks/cases per deme together with the cumulative number of officially reported outbreaks/cases for comparison. The cumulative number of officially reported poultry farm outbreaks in Germany, Hungary and Poland were within the inferred 95% Highly Posterior Density (HPD). More discrepancies were observed for poultry farms in Czech Republic and wild birds in the countries.

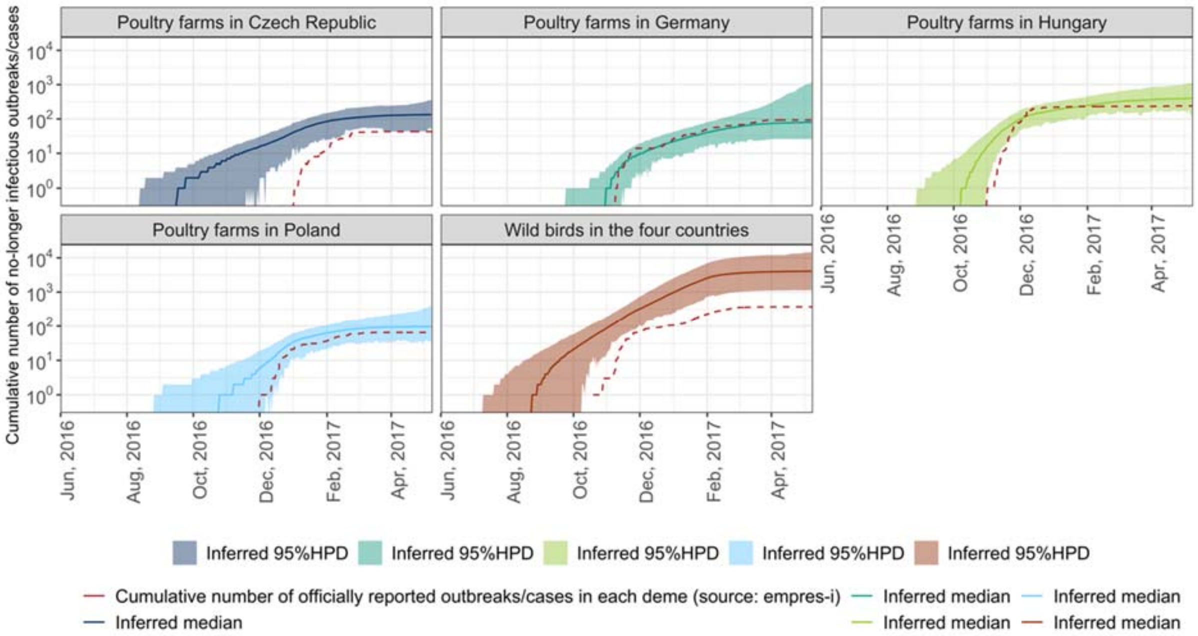

Fig. 1. Temporal distribution of the inferred cumulative number of no longer infectious outbreaks/cases per deme. The solid line represents the median inferred, the areas represent the 95% HPD. The dashed line represents the cumulative number of officially reported outbreaks/cases in log scale

Number of local H5N8 transmission versus importation events

Figure 2 illustrates the temporal distribution of the inferred median number of local transmission and importation events per deme. In Germany, Hungary and Poland, the epidemic was dominated by local virus transmission events between poultry farms with an increase around March 2017, November 2016 and December 2016, respectively. In Czech Republic, the epidemic was dominated by importations from wild birds. For all countries, an increase in the number of importation events from wild birds were observed around January – February 2017. The epidemic in wild birds was also dominated by local transmission between wild birds and the highest number of importations were coming from poultry farms in Czech Republic.

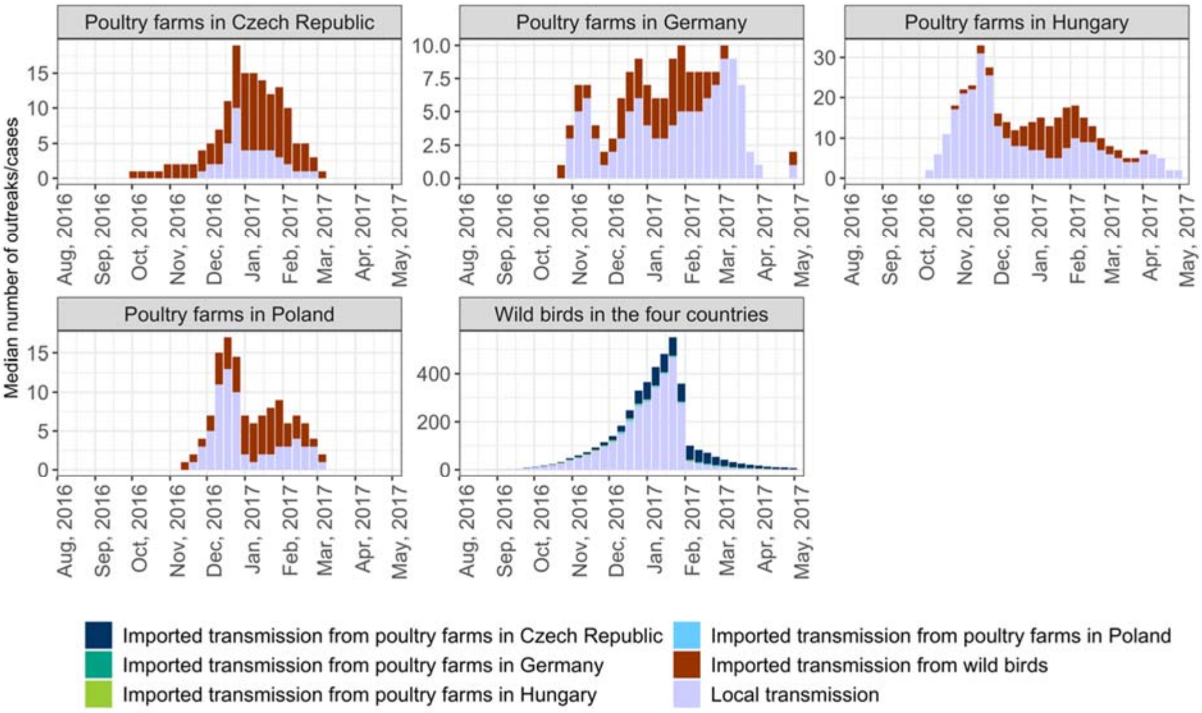

Fig. 2. Temporal distribution of the inferred median number of poultry farm outbreaks and wild bird cases arising from local transmission and importation events per deme. In this graph, for each trajectory and each deme, we computed the median number of within-deme and between-deme transmission events over time

### Predictors of H5N8 virus spread between poultry farms across borders

There were eight predictors included in the GLM: the 2016 live poultry trade, the 2016 poultry density in the source and destination deme, the 2014 poultry farm density in the source and destination deme, the 2017 farm outbreak density in the source and destination deme and the distance between countries' centroids. Figure 3A shows, for each predictor, the inclusion probability which represents the proportion of the posterior samples in which the given predictor was included in the model and the BF which quantifies which of the posterior and prior inclusion probabilities of the given predictor in the model is more likely. Figure 3B shows the log conditional effect size which represents the log contribution of the given predictor when it was included in the model. None of the predictors were statistically supported to be associated with the spread of H5N8 virus between poultry farms across borders, illustrated by the low BF metric (<3.2) (Fig. 3A) and the similar distribution between the posterior coefficient estimates (Fig. 3B) and the prior.

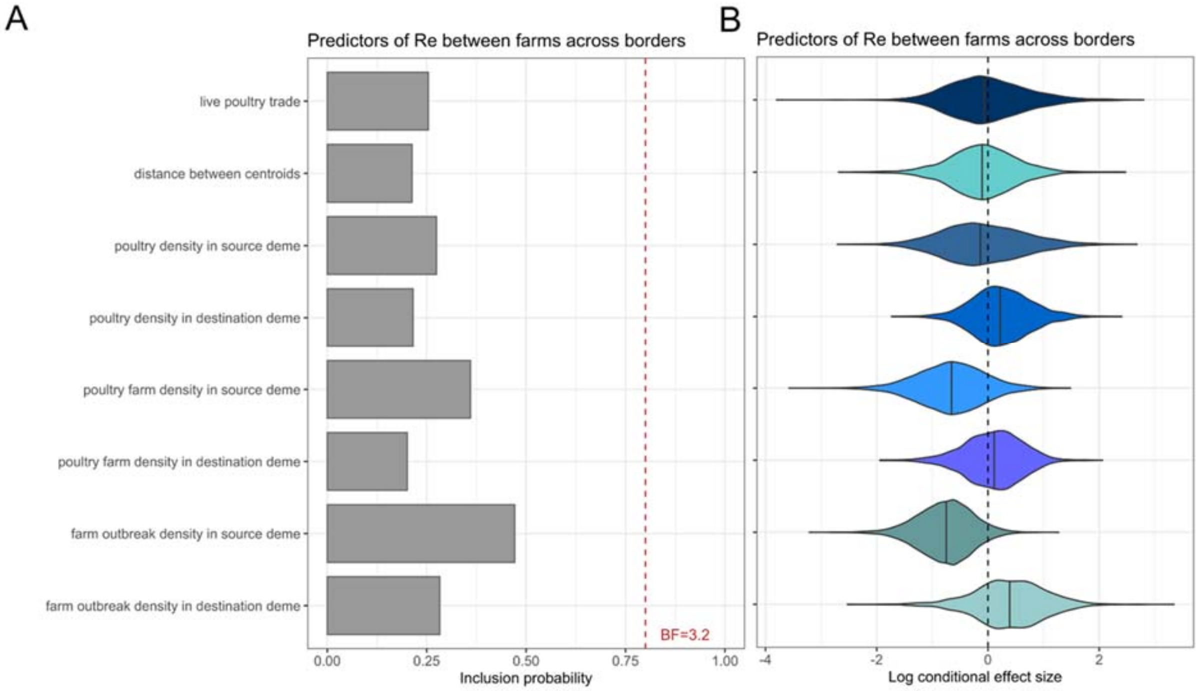

Fig. 3. A) Inclusion probability for predictors of the between-farm H5N8 virus spread across borders. This represents the proportion of the posterior samples in which each predictor was included in the model. Bayes Factors (BF) were used to determine the contribution of each predictor in the GLM. B) Log conditional effect sizes for predictors of the between-farm H5N8 virus spread across borders. This represents the (log) contribution of each predictor when the corresponding predictor was included in the model

## DISCUSSION

While a total of 442 poultry farm outbreaks and 372 wild bird cases in the four countries were officially reported (FAO, 2021), the epidemic trajectories showed that these numbers could have been under-estimated, especially in the wild bird population, likely due to challenges related to wildlife surveillance (Artois et al., 2009). High reporting rates of poultry farm outbreaks were found in Germany, Hungary and Poland, likely linked to the high mortality rates of poultry following H5N8 virus infection, along with the active surveillance implemented around reported poultry farm outbreaks (EFSA et al., 2017). However, lower and more delayed reporting rates were found for the poultry farms in Czech Republic, where most outbreaks occurred in small size farms (< 100 birds), while they mainly affected large size farms (> 10,000 birds) in Germany, Hungary and Poland (Napp et al., 2018). These results suggest that the likelihood of reporting infected farms is likely associated with the characteristics of the farm. However, whether these are the results of differences in size or other factors linked to the farm size (such as different farmers' knowledge, attitudes and practices) needs further investigation.

Following the first virus introduction, the epidemic trajectories demonstrated that in Germany, Hungary and Poland, the epidemic was dominated by local farm-to-farm transmission events. Moreover, the epidemic in these countries was also partly driven by wild bird-to-farm transmission (in particular in the middle of the epidemic) showing that the role of wild birds was likely greater than expected and was not limited to the onset of the epidemic.

Having more detailed knowledge of how poultry farms are connected with one another in those countries could help containing future outbreaks by disrupting the network of potential transmissions between poultry farms. The contribution of wild birds to poultry farms outbreaks was even more substantial in Czech Republic, in which the epidemic trajectories showed that the epidemic was dominated by wild bird-to-farm transmission events. Accordingly, they also showed that the majority of farm-to-wild bird transmission events were from Czech Republic. This provides evidence that small size farms could be more exposed to virus transmission from wild birds than large commercial farms. Again, this could be explained by differences in farm size or other factors linked to the farm size (such as different farming practices – access to outdoor area – or biosecurity levels) which requires further attention. In wild birds, the epidemic was dominated by wild bird-to-wild bird transmission events. The number of wild bird-to-wild bird transmission events however decreased drastically from February 2017, likely linked to the decrease in wild bird density with migration to warmer climates (Hill et al., 2016) and the decrease in virus survival in the environment due to temperature-dependence of H5N8 virus transmission (EFSA et al., 2017).

We also attempted to uncover factors that could potentially predict the spread of H5N8 virus between farms across countries. However, none of the investigated predictors were identified as supportive predictor of the viral spread. This is in line with outbreak investigations on affected poultry farms in Europe, which showed that the likelihood of H5N8 virus introduction from one country to another via personnel contacts, trade of live poultry, feed, or poultry products was negligible (Lycett et al., 2016), although unreported cross-border activities could not be excluded. Also, our predictors ignore other potential drivers of virus spread, such as wild bird migration, different farming systems and biosecurity levels among countries. In the future, we recommend further investigation of predictors with a higher scale of temporal and spatial resolutions, which could allow for stronger contribution levels (Yang et al., 2019).

The 2016-2017 epidemic of H5N8 virus in Europe remains, like other epidemics of AI viruses, epidemiologically complex as it involved multiple wild bird species that vary in spatial ecology and clinical disease severity. During the epidemic, the virus was detected in a large number of wild bird species, mainly those of the *Anseriformes* orders (ducks, geese, swans), including mute swans (*Cygnus olor*), tufted ducks (*Aythya fuligula*), Whooper swans (*Cygnus cygnus*), Eurasian widgeons (*Mareca penelope*) and mallards (*Anas platyrhynchos*) (EFSA et al., 2017). Among these species, some can be mostly sedentary in given areas while partially or wholly migratory in others (BTO, 2017), meaning that some species can act as sentinels in some areas or long-distance vectors of H5N8 virus in others (Keawcharoen et al., 2008). Consequently, wild bird population structure may be much more complex than what was assumed in this study. Similarly, the virus was detected in several poultry species and farm types, which may play different roles in the virus spread due to discrepancies in virus infection susceptibility and farming practices (Napp et al., 2018). Unfortunately, limited information on virus prevalence or epidemiology in various domestic and wild host species between countries makes it difficult to treat species separately, thereby necessitating the grouping used here.

Phylodynamics provides one avenue for quantifying patterns and identifying drivers of infectious disease transmission dynamics at the wildlife-domestic animal interface, which is a fundamental challenge for veterinary epidemiology. We expect our results will be valuable in better informing policy decision-making as means to reduce the impact of future epidemics of HPAI viruses.

## ACKNOWLEDGEMENTS

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# COUPLING SPATIAL AND TEMPORAL STRUCTURE IN BATCH REARING MODELLING FOR UNDERSTANDING THE SPREAD OF THE SWINE INFLUENZA A VIRUS

V. SICARD*, S. PICAULT AND M. ANDRAUD

## SUMMARY

The ability to identify through modelling the drivers of infection dynamics in livestock is essential to design adapted control measures. Yet, it requires to represent livestock management practices and their impact on population structure with a high level of detail, which often comes with the development of complex simulation codes. We developed a new approach based on Artificial Intelligence (AI) which helps to account for the coupling between social and spatial structuring at different levels in pig herds through a flexible modelling language. We demonstrate in this paper its added value to propose and assess effective and realistic control measures on swine influenza A virus, based on contrasted scenarios related to pig farm management.

## INTRODUCTION

Swine influenza A viruses (SwIAVs) are widely spreading in pig production units (Salvesen and Whitelaw, 2021). In conjunction with other pathogens, they are recognised as a main etiological agent responsible for the porcine respiratory disease complex (PRDC), with high economic impact for pig producers (Woeste and Grosse, 2007; Fablet et al., 2012). Like most RNA viruses, SwIAVs are rapidly evolving and are of primary concern regarding both animal and human health, due to the potential emergence of zoonotic viral strains (Deblanc et al., 2020).

Contrary to the expected behaviour in epidemic forms, SwIAVs has been shown to remain endemic in herds, inducing successive and regular waves in batches of growing pigs with possible co-circulation of different virus strains, favouring reassortments (Rose, 2014; Chastagner et al., 2019). This endemic situation was found to be associated with the partial protection provided by maternally derived antibodies (MDAs), slowing down the batch-level transmission process and favouring the interaction of infectious piglets with the subsequent batches (Cador et al., 2016a). Another pivotal factor is the transfer of infectious particles between the different sectors, either by the airborne route or due to management constraints, for example movements of animals between the barns (Fablet et al., 2013).

Mathematical modelling approaches have been developed to tackle SwIAV infection dynamics on farms. For this purpose, transmission parameters have been estimated from

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experimental data to ascertain the role of specific factors affecting the transmission dynamics, e.g. MDAs (Allerson et al., 2013; Cador et al., 2016b) or vaccine-induced immunity (Romagosa et al., 2011). In turn, these parameters have been used to feed dynamic models representing specific farming systems. These models were based on Maternally-derived-immunity/Susceptible/Infectious/Recovered (MSIR) principles representing the evolution of the population through the different epidemiological states, and on metapopulation approaches, with subgroups corresponding to breeding herds and growing pigs respectively. However, when farrow-to-finish pig herds were represented in Europe (Pitzer et al., 2016; Cador et al., 2017), only the breeding herd and their progeny up to weaning-age were considered in the US (White et al., 2017), thus limiting the interaction of non-contemporary piglets.

To go further in the understanding of the impact of batch management and housing on the spread of SwIAV at different scales, and identify possible realistic levers, new modelling approaches were needed. The implementation of reliable, revisable and flexible simulation code is however a complex task. To overcome this challenge, a new epidemiological modelling software was previously built implementing artificial intelligence methods, EMULSION (Picault et al., 2019). We extended EMULSION to incorporate highly structured populations such as those found in pig farm management (Sicard et al., 2021), making it possible to model a realistic farrow-to-finish pig farm.

As a proof of concept to highlight the added value of accounting for highly structured populations in epidemiological models we reimplemented a simple model from the literature (White et al., 2017), which represented a farrow-to-wean pig farm composed of two barns (gestation and farrowing). Modelling with the extended EMULSION software, demonstrated the value of providing generic methods for complex systems. We kept all original epidemiological parameter values and adapted housing and farming practices to reflect realistic farrow-to-finish farms, including post-weaning and fattening barns. The model was then extended to represent the infection dynamics in a typical farrow-to-finish pig farm, keeping the epidemiological assumptions unchanged.

This paper highlights the main outcomes of this explicit representation of spatiality and batch management on SwIAV spread and finally considers possible applications of this approach to difficult questions in livestock epidemiology.

## MATERIALS AND METHODS

### Organizational model

To model the SwIAV pathosystem in the complex batch management system, we used the epidemiological mechanistic modelling framework EMULSION (Picault et al., 2019) extended with a new organizational multi-level agent-based system (Sicard et al., 2021). This original approach makes it possible to represent both the complexity of herd management, and the multi-level aspect of the spatial and social structuring of the population with modularity and flexibility. Two organizations were introduced: the first one represented a realistic batch management based on existing timetables; the second one represented the multi-level spatial partition of the environment, composed of sectors subdivided into rooms. It was possible to amend the model without additional code and to easily explore different hypotheses regarding these structures and their epidemiological connections.

Batch management

Sows and piglets were seen as two sub-populations, structured and managed by the producer. Batch farrowing herd management was considered with all-in-all-out management procedures.

Physiological stage: the animals evolve through different physiological statuses according to their type (sow or piglet): insemination, gestating and lactating stages for sows, and suckling, post-weaning and fattening stages for piglets. Therefore, sows and piglets shared only one common environment during lactation/suckling period up to weaning at 3 or 4 weeks of age. After fattening, piglets left the system to be taken to the slaughterhouse (Sicard et al., 2021).

Batches: piglets and sows are bred in batches, to guarantee a homogeneous evolution of the physiological stages. Batches are designed to be and remain consistent, i.e. that all animals are in the same physiological stage at the same time, according to their type (sow or piglet). In the model, we considered a management in 7 batches with a between-batch interval of 21 days (Sicard et al., 2021).

Housing: the farm was divided into sectors corresponding to specific physiological stages (Fig. 1). Thus, five sectors were considered: the mating, gestating, farrowing, post-weaning and fattening sector. Each sector was divided into rooms, and each room could only host one batch at a time (all-in-all-out management), batches being assigned to a room depending on occupancy. The number of rooms in a sector was set out according to the number of batches in a sector at the same time (Table 1).

Table 1. Typical pig herd batch management timetable for 7 batches with a 21-day interval between batches (Sicard et al., 2021)

| | Mating sector | Gestating sector | Farrowing sector | Post-weaning sector | Fattening sector |
|---|---|---|---|---|---|
| Physiological stage | Insemination | Gestating | Suckling | Post-weaning | Fattening |
| Number of batches to be housed | 2 | 4 | 2 | 3 | 6 |
| Sector occupancy (days) | 35 | 77 | 28 | $4 \times 61$<br>or $3 \times 54$ | $6 \times 114$<br>or$1 \times 121$ |

SwIAV transmission

MSEIR model: we developed an individual-based, discrete-time stochastic model. Five health states were modelled: maternal immunity (M), susceptible (S), exposed (E), infectious (I), and recovered (R). Recovered/Immune sows deliver maternally derived antibodies (MDAs) to their piglets. However, the protection conferred to piglets is only partial (Allerson et al., 2013; Cador et al., 2016b; White et al., 2017) and they were assumed to be potentially infected though having lower susceptibility to infection. As described in White et al. (2017), only infected and recovered sows gave birth to piglets with maternal immunity. All individuals were assumed susceptible to infection after active immunity waning (MSEIRS).

Direct transmission: it corresponds to transmission from infected animals to susceptible roommates. A density-dependant transmission was assumed, due to population size fluctuations through demographics processes (White et al., 2017).

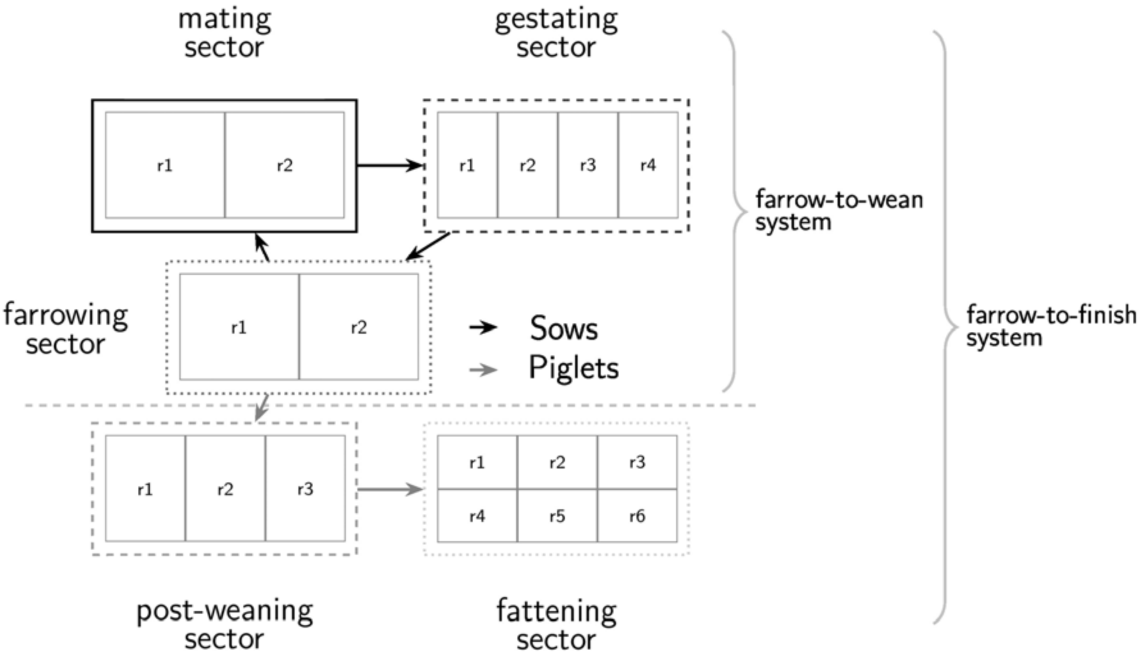

Fig. 3. Flow diagram of sows and piglets in the different sectors, with subdivision into rooms for each sector

Between-room transmission: it represents the potential indirect transmission route (airborne, fomites, human-mediated) between rooms within a sector. Values varied from $\beta_d$ (which was equivalent to having no rooms in the sector) to $\beta_d \cdot 10^{-8}$.

Between-sector transmission: corresponds to the transmission between buildings through airborne and biosecurity breaches between sectors. The parameter value was modulated depending on the scenarios.

SwIAV control

Control strategies based on increased biosecurity levels affecting the indirect transmission between rooms and sectors. Each scenario was run on two different farm systems: a farrow-to-wean system with a mating, gestating and suckling sector (piglets then leave the system after suckling) and a farrow-to-finish farm, including post-weaning and fattening sectors.

Reference scenario (Ref): it corresponds to the assumptions in White et al. (2018). Briefly, pigs are housed in sectors according to their physiological statuses and homogeneously mixed within a unique room in each sector. Direct transmission within room and indirect transmission between rooms and sectors were considered, with identical parameter (White et al., 2017) (Table 2).

Full isolation scenario (FI): it assumed only direct transmission, i.e. no transmission between sectors nor between rooms. In this scenario, we expected the SwIAV to be contained only in batch #1, where the infection was seeded.

Scenarios with partial isolation (PI0-5): to represent an increased biosecurity, limiting the indirect transmission routes, we explored a reduction in indirect transmission, due biosecurity enhancement, assumed the effect applied to indirect transmission between both sectors and rooms. The parameter values were modulated through a logarithmic scale from $\beta \cdot 10^{0}$ to $\beta \cdot 10^{-5}$ assess their impact.

## Simulations

Each animal was identified by its batch membership and its batch farrowing rank (BFR), defined as the number of mating iterations for each batch. Thus, we could discriminate piglets which were in fattening from the younger at the same time for the same batch. At the beginning, batch #1 was in BFR #1 and others in BFR #0.

The simulations were run over 645 days, starting with a burning period of two sow cycles ($2 \times 140$ days). At that time, the population reached the observed periodic pattern corresponding to the considered farming system. SwIAV was then introduced on day 280 through an infected sow in batch #1 BFR #3, i.e. at the beginning of the third cycle (insemination stage). The transmission process was monitored for 365 days (one year after introduction). Each scenario was iterated 100 times to account for variations caused by model stochasticity.

# RESULTS

## SwIAV prevalence

Figure 2 shows the prevalence per sector for a farrowing farming system after introducing an infected sow in insemination sector. In the reference scenario, the dynamics of infection was similar to the observed in White et al. (2017): a first peak during the first 25 days and a persistence thereafter. Alternative scenarios revealed the importance of the farrowing sector, where sows and piglets were mixed, and illustrate the same conclusion about the relevance of isolating the sector containing piglets.

Figure 3 shows the number of infected piglets in BFR #3 for each batch after introducing an infected sow in insemination sector for a farrowing-to-finish farming system. By varying β value through log scale, a relevant effect emerged between $\beta \cdot 10^{-4}$ and $\beta \cdot 10^{-5}$.

In scenario PI2, the impact was close to the reference scenario, due to indirect transmission parameters, except that the indirect transmission was also applied between-room. The impact of control was found substantial when transmission was reduced by $10^{-5}$ (Fig. 4 and Fig. 5). SwIAV was shown to persist in the batch #1 for each scenario, due to the short duration (56 days) (White et al., 2017). Nevertheless, the strong isolation of batches, through measures implemented at sector and room levels, is a key factor in the SwIAV spread control.

The same effects are observed on Fig. 4 which shows the herd prevalence. Scenario PI4 shows that the infection sets in gradually because of the high contagiousness of SwIAV. Each peak corresponds to piglet farrowing in each batch. The peak observed in each scenario at 120 days, corresponds to piglets farrowing in batch #1.

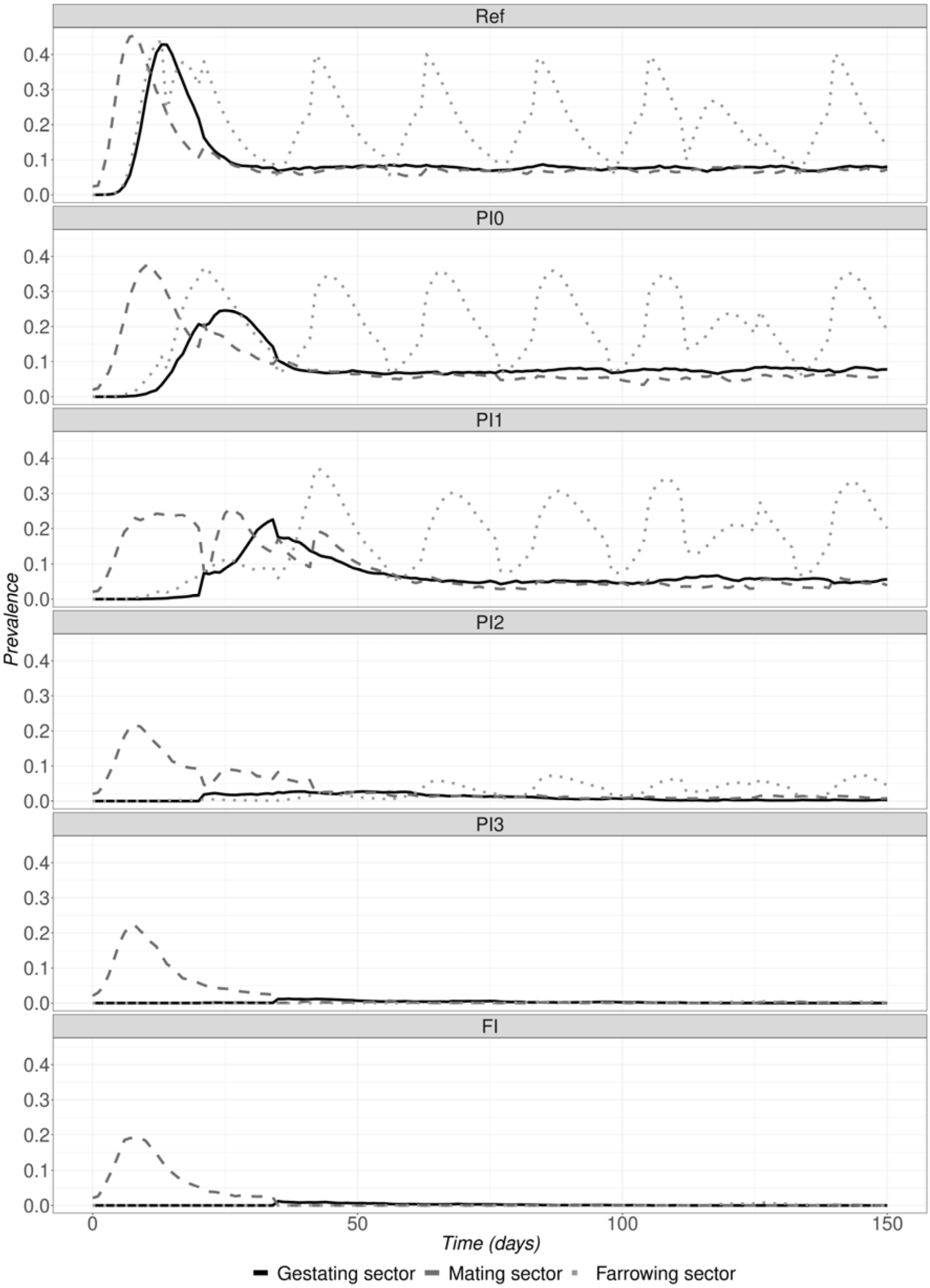

Fig. 2. Evolution of the prevalence per sector after introducing an infected Sow in batch #1 in insemination sector for farrow-to-wean farming system

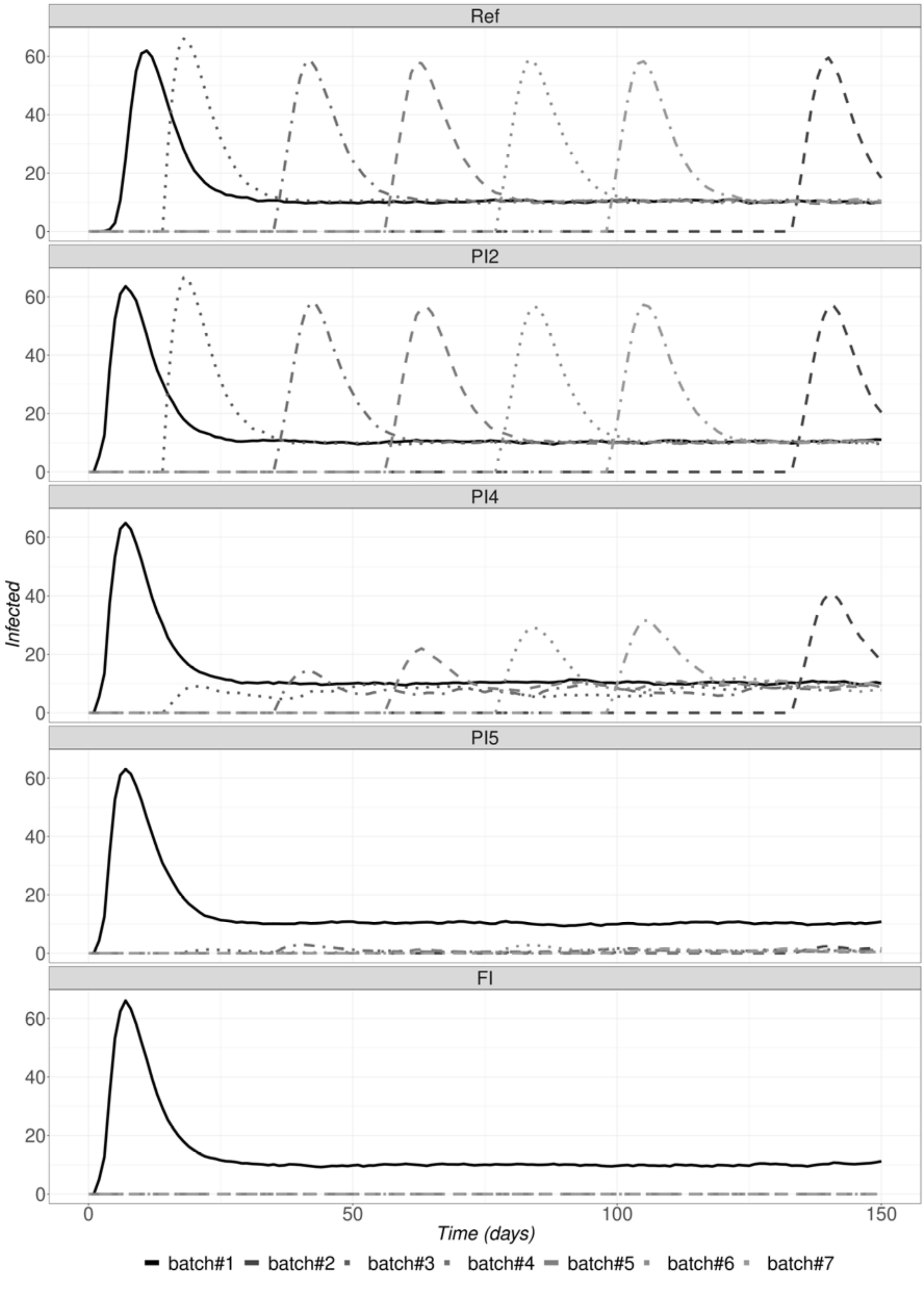

Fig. 3. Evolution of number of infected piglets by batch for BFR #3 after introducing an infected Sow in batch #1 in insemination sector for a farrow-to-finish farming system

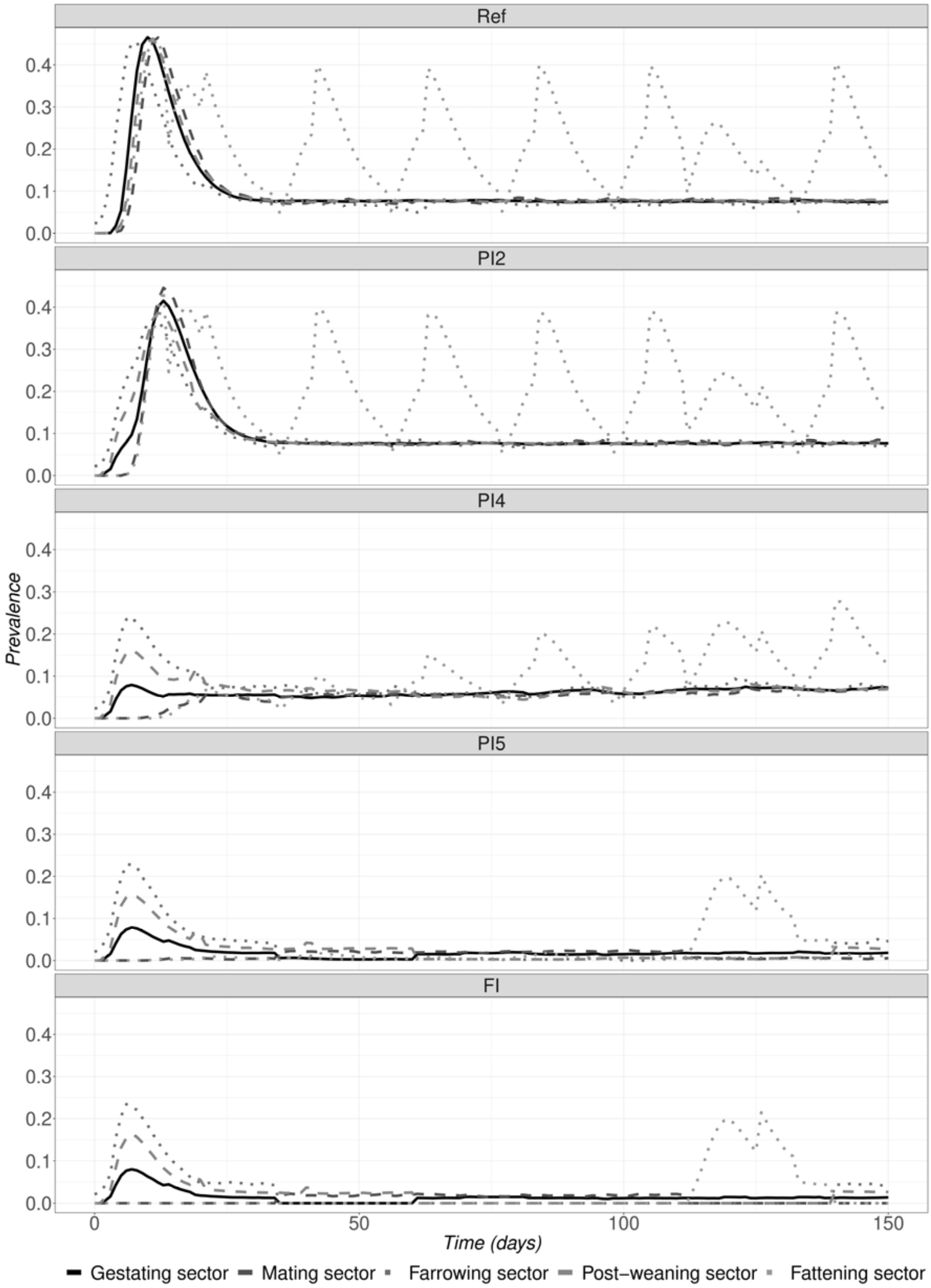

Fig. 4. Evolution of the prevalence per sector after introducing an infected Sow in batch #1 in insemination sector

## DISCUSSION

EMULSION provides a generic and flexible framework for epidemiological modelling, however it did not take into account highly structured systems (Picault et al., 2019). Therefore, the need to develop a specific solution addressing organizational aspects proved essential. The solution intended to be generic and revisable, relies on the coupling of multi-level agent-based system and organizational concepts, based on artificial intelligence (Sicard et al., 2021). It is thus possible to explicitly represent organizational aspects of the population (social and spatial), with their relationship to environmental contribution to pathogen spread. The user interface consists of a structured text file which is processed automatically by the simulation engine. This aspect makes possible for non-modellers to understand, review and potentially revise the assumptions of the model. This multi-level organizational toolset offers a simulation framework to represent a wide range of applications in the field of epidemiological modelling.

Models of SwIAV spread in pig farms had already been developed, yet coming with a high cost regarding model programming, hence difficult to maintain, adapt or revise (Allerson et al., 2013; Cador et al., 2016b; White et al., 2017). In contrast, our modelling framework represents a structured population in a batch management context while remaining flexible, revisable and reproducible.

As for illustration of the concept, we adapted the US farrow-to-wean pig herd model to account the social and spatial organization aspect. Then, we extended the model to represent farrow-to-finish pig herd, with batch-management system, at low computations cost.

Results demonstrated the major role played by indirect transmission between rooms. Control measures to reduce airborne transmission need to be highly efficient, implying high reduction factors to the transmission rate. However, such reduction values are deemed to be reachable through modification of environmental conditions (Tellier, 2009; Yang and Marr, 2011). The SwIAV modelling, with organizational aspects, provides a proof of concept of our modelling solution. Indeed, with low computational cost, our approach made it possible to account for multi-level aspects and the highly structured population.

Applied to pig herd management by batch, the impact of different control measures can be evaluated at different scales. One perspective is to assess the impact of coupled control measures applied at different levels of detail. Furthermore, our development may help to account for deviation in management procedures (e.g. cross-fostering, mingling of pigs), that could be an interesting prospect.

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# IMPACT OF DUCK FARM DENSITY ON THE RESILIENCE OF THE POULTRY SECTOR TO HIGHLY PATHOGENIC AVIAN INFLUENZA H5N8 IN FRANCE

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## SUMMARY

We analysed the interplay between duck farm density and the vulnerability of the production system to highly pathogenic avian influenza (HPAI) H5N8. To do so, we used a spatial mechanistic model, which was calibrated to reproduce the observed spatio-temporal distribution of outbreaks in France during the 2016-2017 epidemic of HPAI. Five scenarios were investigated, in which we decreased the density of duck farms in the communes with the highest duck farm density. Our results show that reducing duck farm density in the densest communes would have had a strong impact on the epidemic dynamics. However, it would not have been sufficient, even in combination with the intervention measures implemented during the 2016-2017 epidemic, to reduce the transmission rate enough to completely prevent the virus from spreading. Therefore, these measures need to be be combined with other structural preventive approaches, including reducing flock size and increasing biosecurity compliance.

## INTRODUCTION

During the winter 2016-2017, France was hit hard by a highly pathogenic avian influenza (HPAI) virus, subtype H5N8, causing almost 500 outbreaks in poultry farms. Outbreaks mostly clustered in the Southwest region of the country, where a substantial amount of ducks are raised to produce foie-gras (Guinat et al., 2018). In accordance with European regulations, the French government has implemented strict control measures to curb the epidemic, including the culling of infected flocks, preventive culling of at-risk flocks, movement restrictions in affected zones and pre-movement testing of duck flocks. Retrospectively, epidemiological studies have highlighted the role of various transmission routes and the factors that influenced the transmission dynamics. Guinat et al. (2019) used a statistical approach to show that HPAI H5N8 outbreaks were much more likely to occur in zones with a high density of duck farms. This finding was subsequently confirmed by a mechanistic modelling study that highlighted the importance of local transmission between poultry farms and the particular sensitivity and transmissibility of palmiped farms as compared to galliform farms (Andronico et al., 2019). Live-duck movements and the transit of trucks used for these movements seem to have generated only very few transmission events during the 2016-2017 epidemic (Guinat et al., 2020; Bauzile et al., 2021), despite being shown to be an effective transmission route (Bauzile et al., 2021). Based on these results, important efforts have been devoted in the subsequent

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years in France to improve external and internal biosecurity practices in poultry farms in order to prevent the risk of HPAI occurrence and its negative effects (Delpont et al., 2021).

While no significant events were reported during the following winters, France experienced another devastating epidemic of HPAI, subtype H5N8, during the winter 2020-2021. The virus, which was shown to be extremely transmissible between birds (Vergne et al., 2021), led to more than 500 poultry outbreaks, affecting the same areas than in 2016-2017. This second epidemic made a wide range of stakeholders of the poultry sector as well as decision-makers face the vulnerability of the Southwest region of France with regards to the H5N8 subtype. Indeed, this second epidemic demonstrated that the major improvements on biosecurity implemented all along the poultry chain had remained insufficient to control HPAI spread in the case of virus circulation in areas with high density of duck farms.

In order to investigate the effect of structural changes in the poultry sector on the transmission dynamics, this study aimed at evaluating how decreasing duck farm density in highly dense areas could improve the resilience of the poultry sector to highly pathogenic avian influenza viruse outbreaks.

## MATERIALS AND METHODS

To address this question, we used a farm-based mechanistic spatial model that was calibrated to the observed spatio-temporal distribution of outbreaks in France during the 2016-2017 epidemic of HPAI H5N8 (Andronico et al., 2019). We further defined six scenarios of duck farm density. The baseline scenario considered all 8379 commercial farms, including 4188 and 4191 galliform and palmiped farms, respectively, as used by Andronico et al. (2019) to estimate transmission parameters. Note that because the vast majority of palmiped farms in the region are farms raising ducks, palmiped farms will be referred to as duck farms hereafter. The five other scenarios simulated a decrease of duck farm density in the densest communes. To do so, we identified the 2, 5, 10, 15 and 20% of the communes with the highest duck farm density (Fig.1) and removed randomly duck farms in these communes until the targeted density of the corresponding threshold was reached. These percentiles represented respectively a removal of 330, 670, 1169, 1524 and 1808 duck farms in 33, 80, 174, 256 and 341 communes.

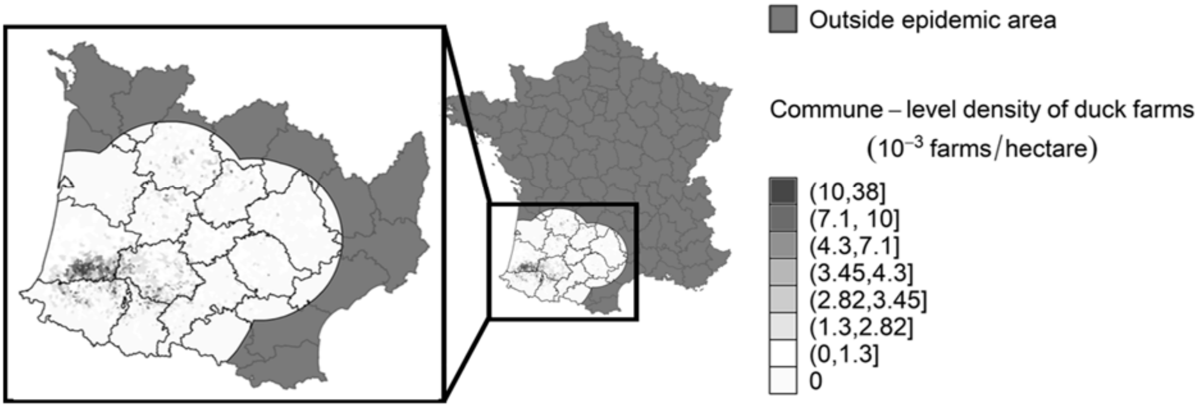

Fig. 1. Distribution of duck farm density at commune level in the southwest region

For each of the six scenarios, we first calculated the basic reproduction number ($R0$) for each farm, i.e. the expected number of farms a particular farm would be likely to infect, should all other farms be susceptible. For a given farm i, $R0_i$ was defined as in Eq.(1):

$$R0_i = \sum_j \left(1 - exp\left(-\delta * \lambda_{i \to j}\right)\right) \tag{1}$$

with $\delta$ being the duration of the infectious period for a farm (in days) and $\lambda_{i \to j}$ being the daily force of infection exerted by an infectious farm i on a susceptible farm j as defined in accordance with Andronico et al. (2019), and shown in Eq. (2):

$$\lambda_{i \to j} = \psi_i * \phi_j * \left(\frac{\beta * I\left[d_{ij} \leq d_c\right]}{N_i(d_c)}\right) \tag{2}$$

with $\psi_i$ being the relative infectivity of farm i ($\psi_i = 1$ for duck farms and $\psi_i = \psi$ for galliform farms), $\phi_j$ being the relative susceptibility of farm j ($\phi_j = 1$ for duck farms and $\phi_j = \phi$ for galliform farms), $\beta$ being the daily transmission rate, $I\left[d_{ij} \leq d_c\right]$ being a indicator function taking the value 1 if the Euclidean distance between farms i and j ($d_{ij}$) was smaller than a cut-off distance ($d_c$) and 0 otherwise and $N_i(d_c)$ being the number of farms located within a distance $d_c$ from farm i. For each scenario, the posterior distribution of $R0_i$ was determined by randomly sampling 500 values in the posterior distributions of the parameters, as established in Andronico et al. (2019) and summarised in Table 1. The spatial distribution of farm-level R0 was smoothed and displayed using R software version 4.0.2 (R-Core-Team, 2021).

Table 1. Summary of the distributions or values of the parameters used to define the model

| Parameter | Definition | Median (95%CI) | Reference |
|---|---|---|---|
| $\phi$ | Relative susceptibility of galliformes | 0.20 (0.15, 0.27) | Andronico et al. (2019) |
| $\psi$ | Relative infectivity of galliformes | 0.39 (0.09, 0.85) | Andronico et al. (2019) |
| $\beta$ | Transmission rate | 0.23 (0.16, 0.31) | Andronico et al. (2019) |
| $d_c$ | cut-off distance | 15 km | Andronico et al. (2019) |
| $\delta$ | Duration of the infectious period of a farm (between the onset of infectiousness to the culling of the flock) | 11 days | Assumed |

We then investigated the impact that the reduction in duck farm density would have had on the 2016-2017 epidemic. To do so, we ran 500 stochastic simulations from the model for each scenario with the same initial conditions than in the original model, with parameter values drawn from their posterior distributions and with the control strategies implemented during the 2016-2017 epidemic, including culling of infected flocks, implementation of surveillance and protection zones (SZ and PZ), enhancement of biosecurity measures in the SZ, and preventive culling of duck flocks in the PZ and of all poultry flocks within 1km of infected premises starting in early January. For each scenario, these simulations allowed the reconstruction of the average daily incidence as well as to compute the probability of each commune to have become infected.

RESULTS

The spatial distribution of *R*0 in the Southwest region of France for the six different scenarios is represented in Fig. 2. Our estimates suggest that reducing the density of duck farms in the densest communes has an impact on the distribution of *R*0, since, for increasing density reduction, the zone associated with higher *R*0s decreased in size. However, even when the farm density was reduced in the 20% densest communes (scenario 6 in the bottom-right corner in Fig. 2), i.e. when more than 1800 duck farms were removed from the baseline population, *R*0 still remained higher than 1.5 in a relatively wide region, suggesting that reducing duck farm density would not prevent viral spread without the implementation of surveillance and intervention strategies.

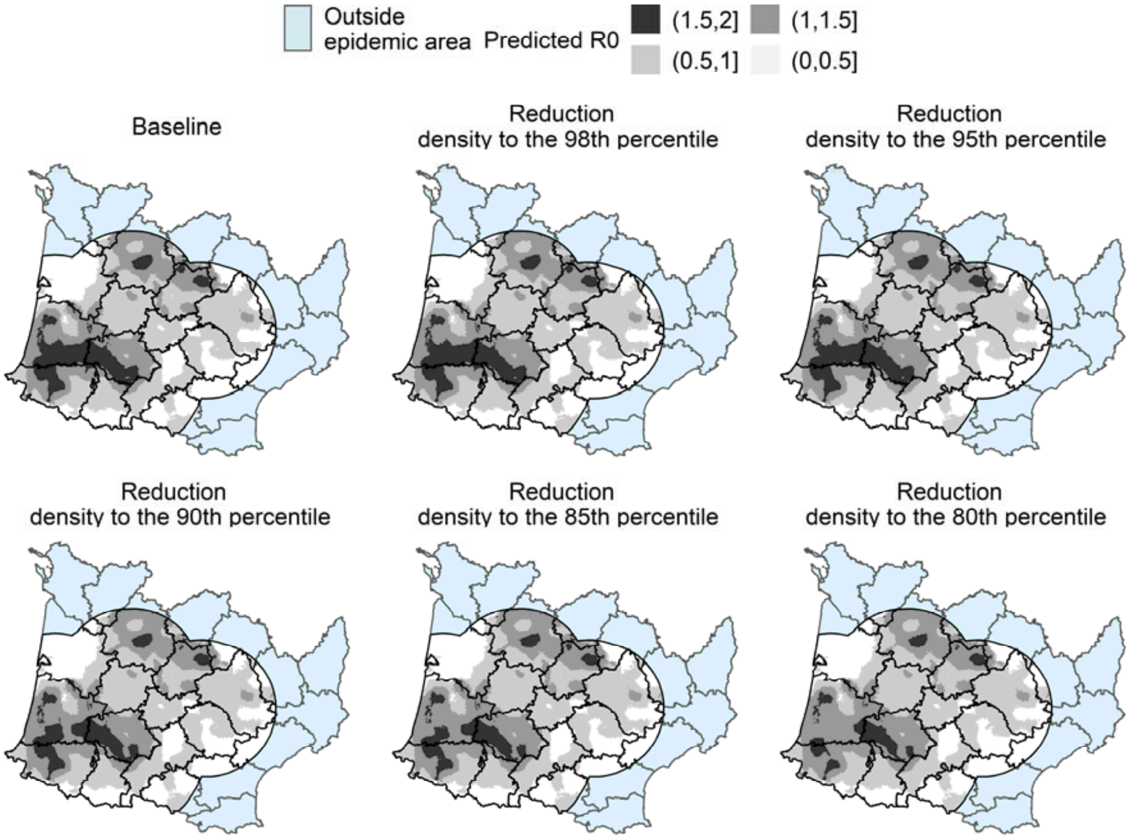

Fig. 2. Smoothed spatial distribution of the basic reproduction number (*R*0) for each of the six scenarios simulating a reduction of duck farm density in the 0% (baseline), 2%, 5%, 10%, 15% and 20% of the communes with the highest duck farm density

As illustrated in Fig. 3, when accounting for the control strategies implemented during the 2016-2017 epidemic, decreasing duck farm density in the densest communes would have had a substantial impact on the 2016-2017 epidemic dynamics, especially during the second phase of the epidemic (February 2017), where the relative reduction of the mean daily incidence was the greatest. The duck farm density reduction would have had a much more limited impact on the first phase of the epidemic (December 2016 – February 2017). However, results suggest also that none of the scenarios (even the most stringent ones) would have totally curbed the epidemic (Fig. 3). Indeed, it is expected that reducing the duck farm density in the 20% most dense communes would still have led to a total of 219 outbreaks (50% prediction interval: 169-283).

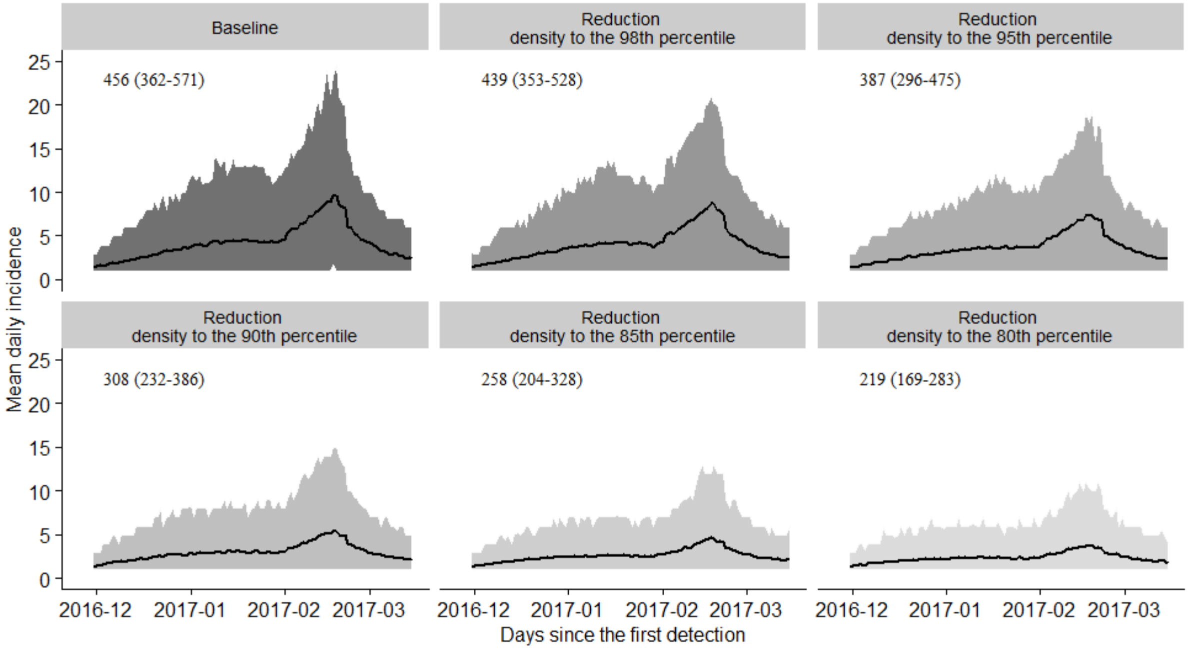

Fig. 3. Expected epidemic dynamic of highly pathogenic avian influenza in France during the 2016-2017 epidemic for each of the six scenarios simulating a reduction of duck farm density in the 0% (baseline), 2%, 5%, 10%, 15% and 20% of the communes with the highest duck farm density. Solid lines represent the mean daily incidence while the grey polygons depict their 95% prediction interval. The numbers inserted in the plot areas represent the expected epidemic size and its 50% prediction interval. This figure was based on 500 stochastic simulations from the model for each scenario with the same initial conditions, with parameter values drawn from their posterior distributions and with the control strategies implemented during the 2016-2017 epidemic

## DISCUSSION

This modelling study provides expected evidence that reducing duck farm density in the densest communes would reduce substantially the vulnerability of the poultry sector to highly pathogenic avian influenza (H5N8). It is worth noting that this result is specific to the virus that circulated during the winter 2016-2017. Indeed, it was previously shown that this HPAI H5N8 virus impacted more heavily the duck sector (Guinat et al., 2019) and was associated with farm susceptibility and infectivity that were respectively 2.6 times (95% credible interval: 1.2-10) and 5.0 times (95% CI: 3.7-6.7) greater in duck farms than in galliform farms (Andronico et al., 2019). As a consequence, it is to be expected that having fewer duck farms in the communes associated with the highest duck farm densities decreases the overall risk to the poultry sector, including the galliform farms.

Nonetheless, decreasing duck farm density, even in combination with the intervention strategies that were implemented in 2016-2017, was not sufficient to reduce the transmission rate enough to prevent the virus from spreading (Fig. 2 and 3). As an example, removing more than 1800 duck farms in the densest communes only halved the total epidemic size. However, it is likely that our approach underestimated this effect as it was assumed that the effectiveness of the intervention strategies that were put in place in reaction to the detection of outbreaks (mostly the delay between the onset of infectiousness and the culling of the flock, and the reduction of farms' susceptibility if included in a surveillance zone) were constant across scenarios. One can argue that reducing the outbreak incidence would limit the risk that the veterinary services, which are in charge of implementing these measures, are overwhelmed by the number of farms to depopulate. This, in turn, could enhance the effectiveness of their intervention and their communication to farmers. To overcome this limitation and provide more realistic results, our model could be modified to account for the decreased effectiveness of interventions as the number of reported outbreaks grows.

To further improve the resilience of the poultry sector to highly pathogenic avian influenza epidemics, it is now paramount to further investigate the effect that complementary strategies could have on the virus dynamics. One important question that remains to be addressed is related to the impact of duck flock size on virus transmission risk, since an appealing alternative to asking some farmers in the densest communes not to produce at all during the high-risk period would be to ask farmers to produce less. Also, following the HPAI (H5N8) epidemic that occurred in France in 2020-2021, the Ministry of Agriculture has requested duck flocks to be confined during the high-risk periods to avoid contacts with wild birds, except for small flocks of less than 3200 heads which could remain outside. Therefore, another important question would be to assess the impact of outdoor farming on virus transmission risk. The population data that were used to feed the model of the 2016-2017 epidemic were not sufficient to address these challenging questions. However, the relevant data are now available and will be used to reconstruct the 2020-2021 epidemic and assess the impact of these two complementary strategies.

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# SURVEILLANCE AND CONTROL

# UNDERSTANDING TIME SERIES OF RFID SENSOR DATA FOR PREDICTING MORTALITY IN LAYING HENS

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## SUMMARY

Diseases and mortality early warning systems are warranted in the egg production industry. Tracking animal movement and resource occupancy using RFID sensor technology has the potential to create such early warning systems. In this study, we analysed a dataset of individual hens' behavioural time budgets of 9375 laying hens in relation to hen mortality using a custom-built RFID system in a commercial farm. The dataset consists of mortality data of each individual hen, the average time each hen spent in the key resources of the aviary system (feeders and nest box), and on the outdoor range. Based on the data, we constructed the corresponding individual time-series data with the mean time duration and the frequency of visits to the feeders, nest boxes and outdoor range for the entire production period. The hens were then grouped according to the time of their death during the production period into four distinct clusters. Subsequently, resource usage patterns from each individual mortality cluster were revealed for the entire production period. We investigated the resulting resources usage patterns in terms of trends and similarities with ARIMA models, autocorrelation and cross-correlation functions. We also correlated the obtained mortality clusters measures with activity patterns and body weight at placement of the hens. Our study demonstrates that mortality can be modelled using the hen`s activity patterns thus leading to the potential mortality prediction in the early stages of production period.

## INTRODUCTION

Free range laying hens can be provided with the opportunity to access various structural areas including open floor space, feed areas, water lines, nest boxes, perches, aviary tiers, winter gardens and ranges (Larsen et al., 2018). Different individual location preferences can lead to the development of hen subpopulations that are characterised by various performance, health, and welfare parameters (Sibanda et al., 2020a, 2020b). Understanding the complexity of hen movement and hen interactions within their environment provides an opportunity to limit the disadvantages that are associated with housing in non-caged husbandry systems and aids in decision-making for farm staff, managers, and equipment designers. Flocks with higher prevalence of diseases have lower average movement (determined through the analysis of optical flow of surveillance footage) and that analysis of flock movements and production system usage might provide a method for predicting outbreaks (Colles et al., 2016; Courtice et al., 2018; Sibanda et al., 2020b). Common health and welfare concerns in free-range flocks

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such as Spotty Liver Disease, *Ascaridia galli*, plumage damage, beak damage and fractures to the keel bone caused by falls, collisions are related to individual hen movement and access to different resources in the barn and on the outdoor range (Courtice et al., 2018; Rufener et al., 2019). The clinical signs for several disease vary and include loss of appetite and inactivity that can potentially be detected through individual-level behavioural monitoring using sensor technologies. In previous studies we have demonstrated that usage of key resources provided to the hens is correlated to the disease and mortality (Sibanda et al., 2020b).

There are numerous ways in which farm disease surveillance and mortality can be monitored through the flock production system. The most popular method used is to manually collect a number of samples on the aviary systems, outdoor range (or a subsection of it) at a certain time points at different times of the day and age of hens, which usually starts when the farmers observe death in their chicken (Sellek et al., 1997). However, the accuracy of the values for the active disease surveillance is questionable, as it could reflect either a biased sub-sample of the flock, thereby reducing the chances of disease detection. Furthermore, this approach provides a high-level view for disease surveillance, but does not capture details for predictions at the individual level or vulnerable flock sub-populations such as reduced use of the feeders.

Advances in video cameras, sensor technology, Radio Frequency Identification (RFID) technology, and accelerometers, in combination with the reduction in cost, has provided massive prospects for in-shed disease surveillance of individual hens (Ahmed et al., 2021). Manual video annotation is time-consuming and does not identify individual hens without identifying features. Furthermore, automated computer-vision based approaches suffer from challenges such as occlusion, where hens and equipment block off individuals. However, RFID systems showed the potential to overcome this obstacle for monitoring in free-range systems, and have been widely used across agricultural production systems for identifying and tracking the movement of animals (Barnes et al., 2018; Bowen et al., 2009; Brown et al., 2014; Maselyne et al., 2014). Most recently there has been increase in use of RFID system in free-range hen production systems (Bari et al., 2020) for monitoring range and nest-box usage, along with movement within feeding system, by deploying a comprehensive system of RFID receivers and antennas throughout aviary systems and on the open range. Moreover, due to its capacity to individually identify each hen without disturbing the animals' activities (Campbell et al., 2020), these sensors have the potential to be used for early warning systems and help in decision-making to improve the welfare and health of animals. Real-time location systems can measure these parameters automatically and provide data for early detection of behaviour changes relevant to hens' health and welfare.

The use of automated systems for collecting data within such a production environment yields very large datasets with sufficient depth to apply more complex machine learning algorithms (Ruhnke et al., 2019). For example, time series analysis in combination with supervised machine learning can be used to predict future parameters such risk of mortality (Han et al., 2011). By monitoring behaviour time patterns of hens, it is possible to determine any deviations from regularity which could indicate individual health status, therefore allowing for early intervention, and improving on-farm productivity. In a previous study we have shown that the hens that used the outdoor range were three times less likely to die compared to the hens that stayed in the shed (Sibanda et al., 2020b). Therefore, we hypothesize that the use of the key resources over time on the aviary system and outdoor range might be used as an indicator of likelihood of mortality. This study builds upon this preliminary body of work by applying the time series algorithms to describe and predict mortality based upon aviary and outdoor range usage patterns as monitored with RFID monitoring system across the production cycle.

## MATERIALS AND METHODS

### Animal housing and management

All procedures carried out in this study were approved by the University of New England's Animal Ethics Committee (AEC 16-087). A total of three flocks (Flocks A – C) each housing 40,000 Lohmann Brown hens were kept on the same commercial farm. In each flock, 3125 randomly chosen hens were RFID leg-banded at 16 weeks of age for individual identification, and then placed in partitioned cross-sections of the shed, allowing for the monitoring of individual range access. Details of the experimental set up and the validation of the RFID system are provided by Sibanda et al. (2020a, 2020b). All the hens were fed the same diet according to the breed standard and they were exposed to the same management team and procedures. The shed was equipped with a three-tier aviary system, tunnel ventilated system, curtain sides and pop holes along the entire length of the shed wall, which allowed constant air flow and temperature control. Manure was removed frequently using automated manure belts, preventing any ammonia build-up.

### Mortality cluster classification

A total of 7171 hens were used in this study with 4782 having a full dataset at the end of the trial. Hen mortality was recorded daily by physical evaluation and removal of dead hens in the shed by farm staff. The mortality data was segmented into four different groups based on the time of their death. The group of hens that did not die were filtered out of the data first. The hens that had died during the production period were then grouped using the optimal univariate *k-means* clustering in R programming language and the optimal number of clusters k was estimated at three with Calinski-Harabzs method. The data from the hens that did not die was then appended to the other three clusters of hens. The clusters were identified as follows: Cluster 1 spent 2 - 115 days alive, Cluster 2 spent 117 - 227 days alive, Cluster 3 spent 228 - 335 days alive. The hens that survived the whole production period when then appended to the dataset and labelled as Cluster 4 (Fig. 1).

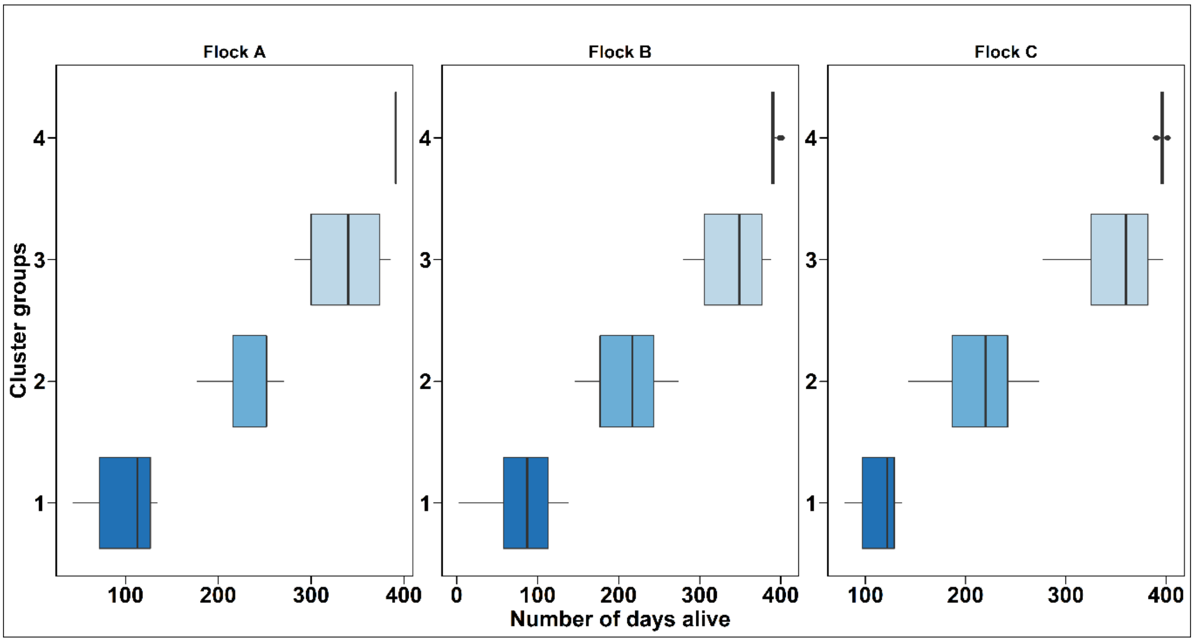

Fig. 1. Univariate cluster analysis and cluster description of hens according to their time of death. The cluster centroid for cluster 1, 2, and 3 were 68, 215, 338 days alive. The cluster 4 hens are the hens that survived the whole production period

### Timeseries data creation

In this study we analysed activity patterns of laying hens in relation to hen mortality using a custom-built RFID system. The data analysed in this study consisted of 9375 hens individually tracked for the access to key resources (feeders, nest box and outdoor range) during the entire laying period (16-74 weeks of age). We created timeseries data based on the frequency of visits to, and duration on, the feeders, nest boxes and outdoor range for each individual hen from week 16-74 as shown Fig. 2. In brief, the raw timestamp data was aggregated to sum the frequency of visits and the total time each hen spent on the key resources (feeders, nest box and outdoor range) for each day the hens were alive.

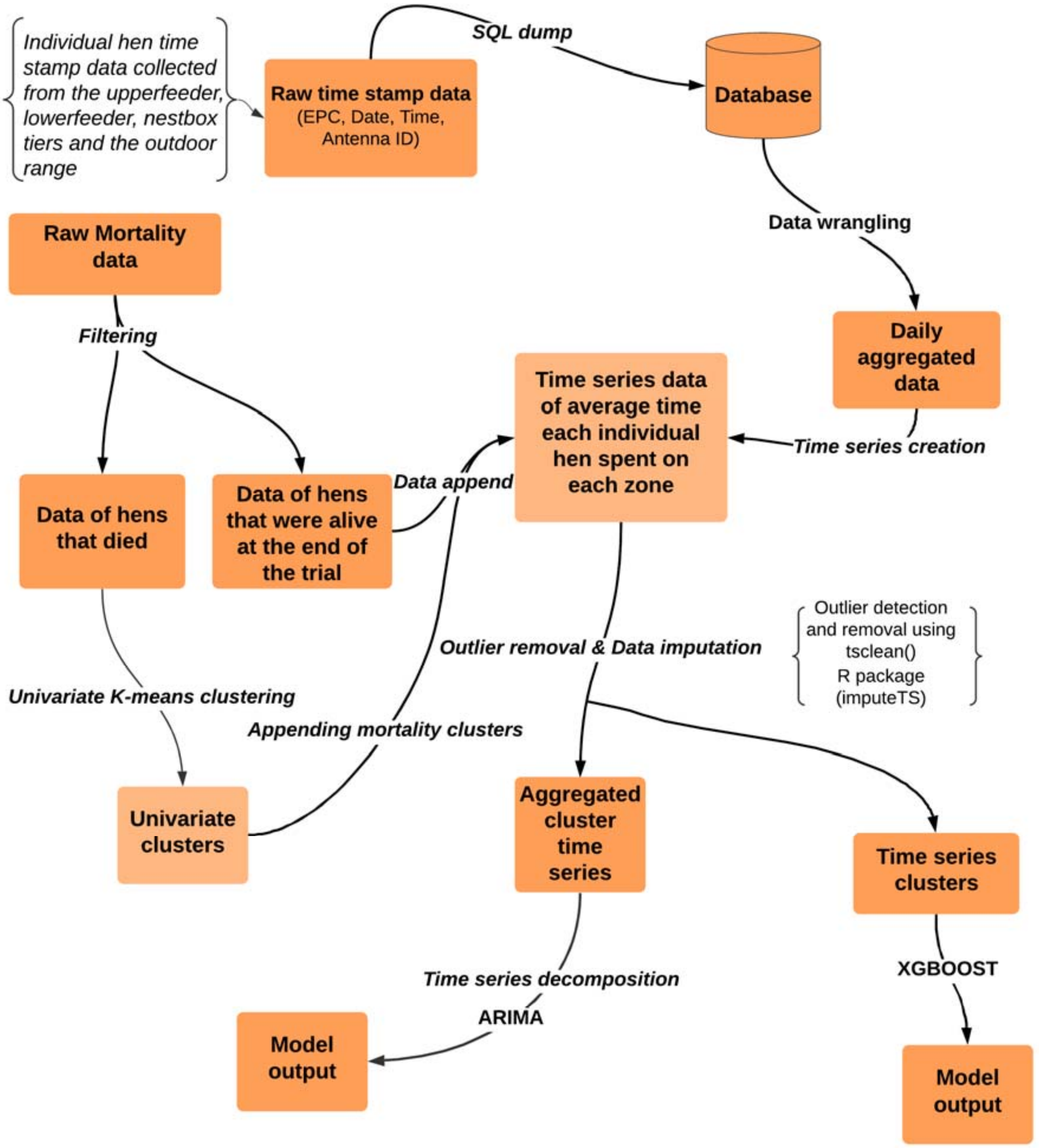

Fig. 2. The workflow for the time series analysis of the individual hen occupancy according to the mortality clusters

Activity time series comparison and decomposition of mortality clusters

In order to understand activity trend for the four separate clusters, each time series was decomposed into trend and seasonality. Secondly, based on the clusters and individual timeseries, we compared the average time duration data for each cluster to investigate daily activity patterns or use of the key resources in the hen house using the AutoRegressive Integrated Moving Average (ARIMA) models and cross-correlation function after pre-whitening with average time on each zone as the input series. Model parameters were estimated using the R programming language (R Core Team, 2021) and Rstudio integrated development environment (Rstudio Team, 2020) and the *forecast* package (Hyndman, 2022) to fit the ARIMA models.

## RESULTS

### Placement body weight

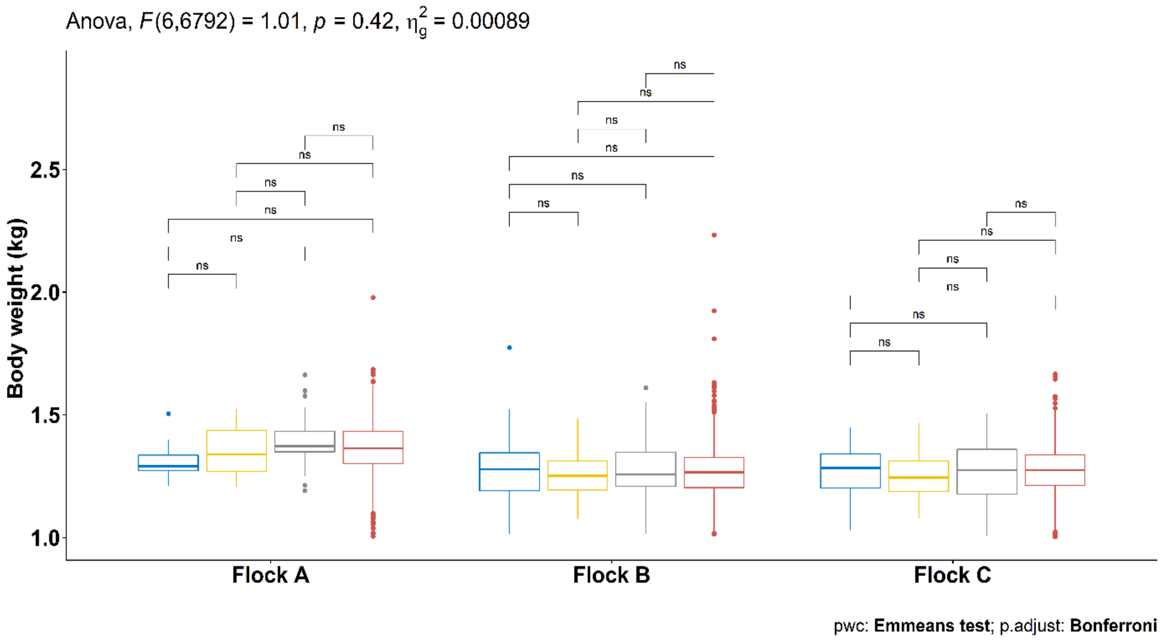

Fig. 3. The boxplot represents body weight of the 4 mortality clusters. The blue, yellow, grey and red boxplots represent hens of cluster 1 to 4 respectively. The ns in the plot represent statistical non-significance of differences between the clusters at significance level 0.05

As shown in Fig. 3, significant flock effects were evident on the average bodyweight of hens (P < 0.0001). There was no significant effect of the mortality clusters on the average body weight at placement (P > 0.05).

### Aviary system and outdoor range occupancy for a single hen

The example time series (Fig. 4) shows that hen activity patterns were cyclic with random variations. The number of visits to the nest box was cyclic and consistent throughout the production period of the hen, however the total time duration at the nest box was upward shifted towards the end of the production period. The time the hen spent at the lower feeder showed multiplicate seasonal changes. The magnitude of using the lower feeder changed with the hen age.

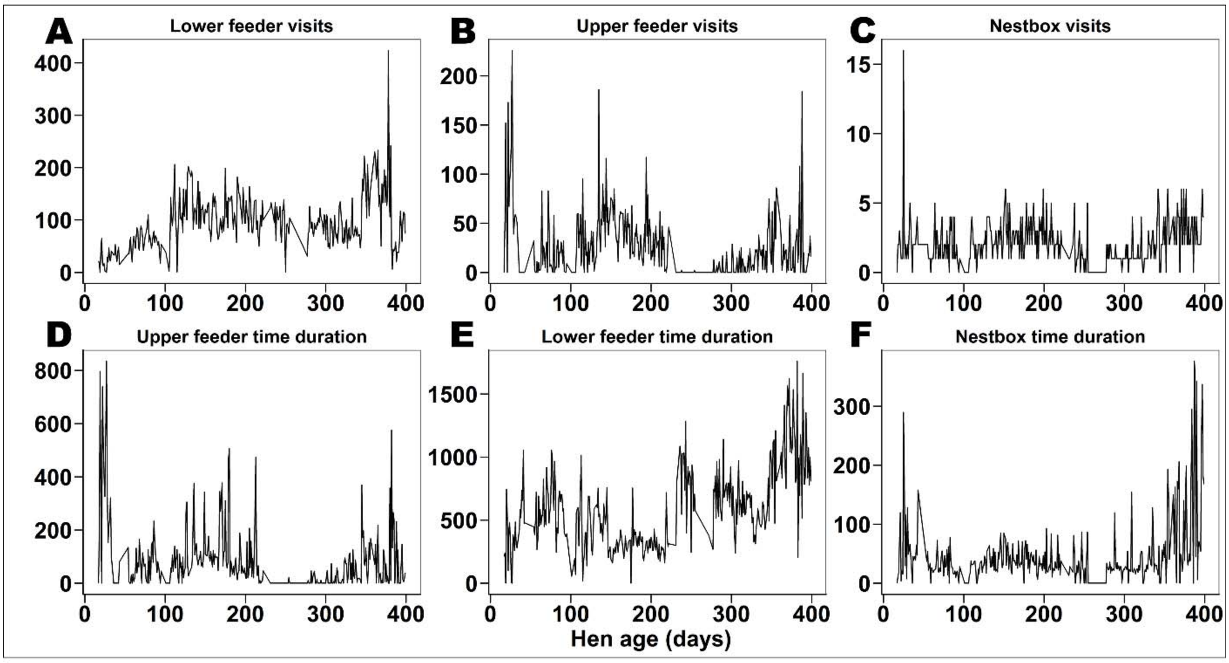

Fig. 4. An example of multivariate time series analysis of the individual hen occupancy of the aviary system during the production period demonstrating the number of visits (A-C) and the average time duration per day to the three key resources (D-F)

Time series trends for each cluster

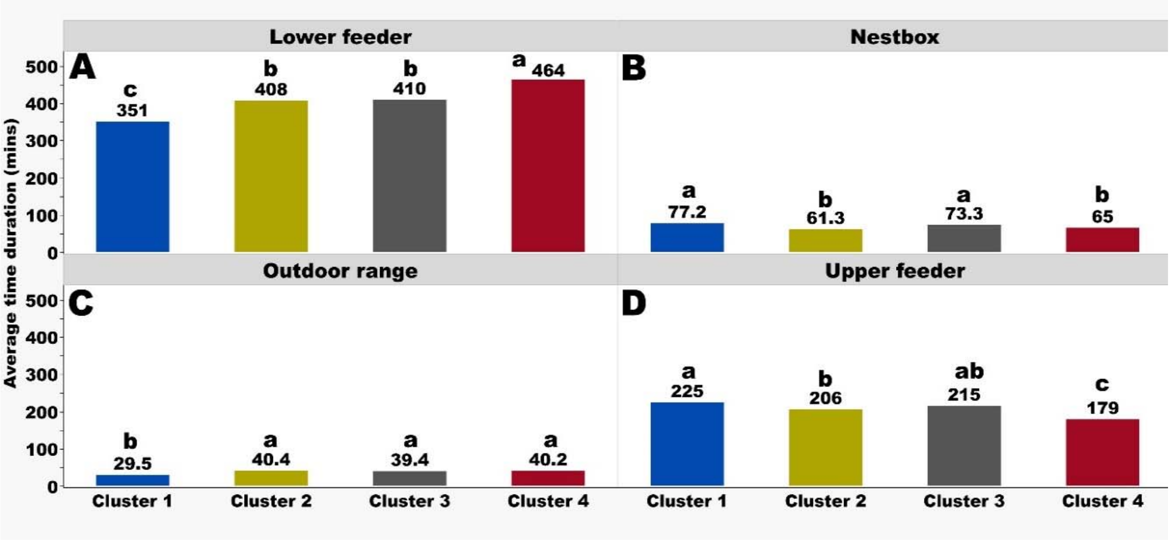

Fig. 5. Multiple comparison of the average time duration of hens on lower feeder (A), nest box (B), outdoor range (C) and upper feeder (D) for the 4 mortality clusters

Analysis of the time duration (Fig. 5) revealed a significant effect of cluster ($P < 0.0001$) with significant interaction between cluster and flock ($P < 0.0001$). In general, the hens spent the least time at the nest box and on the range. Hens of cluster 4 (hens that survived) spent significantly more time in the lower feeder tier ($P = 0.001$) and on the outdoor range ($P = 0.001$) compared to hens of cluster 1 (hens that died before peak production). On the other hand, hens of cluster 1 spent significantly more time at the nest box and upper feeder compared to the hens of cluster 4 (P = 0.001).

As shown in Fig. 6 the hens that died before peak laying period (Cluster 1) showed an upward shift in the use of the lower feeder and nest box while a downward shift in the use of upper feeder during the last few days before death. The hens that survived up to the end of the trial (Cluster 4) used the lower feeder tier and outdoor range more frequently compared to the hens that died during the trial period. There was no difference in the use of the nest box between the clusters (P < 0.005). The ARIMA models for the input time-series of lower feeder, upper feeder, and range duration for all four clusters required first-order differencing because the original time-series were not stationary (ACF range -15.6 to -5.5). The cross-correlation plots confirm that there was no statistical evidence of similarity between the timeseries of cluster 1 and 4 for the time spent by the hens on the lower feeders at lag 0 (CCF range -0.27 - 0.21). The cross-correlation plots displayed a 15-25-day delay by the hens of cluster 1 (hens that died early) in using the key resources compared to the hens of Cluster 4.

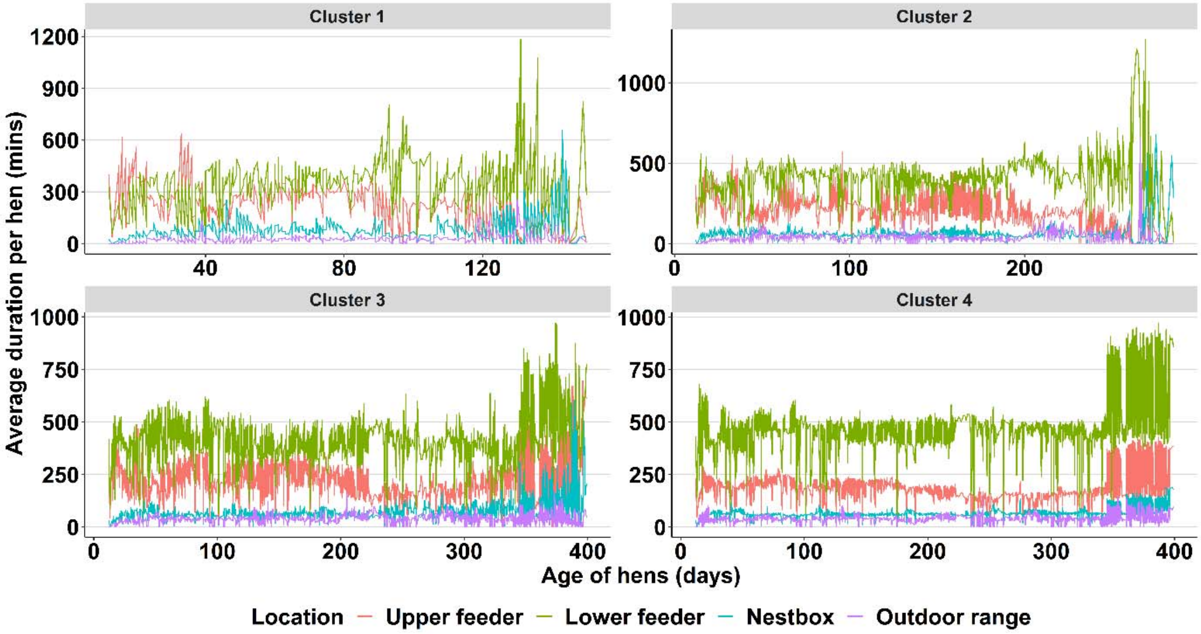

Fig. 6. The aggregated time series of daily activity patterns of hens for the four mortality clusters

## DISCUSSION

We have demonstrated the potential of applying time series algorithms to describe risk of mortality based upon aviary and outdoor range usage patterns as monitored with RFID monitoring system across the production cycle. Early warning systems are used for detecting and forecasting future hazard events and reducing risks of poor health, welfare and mortality. This work is an initial example of sensor technology being used as an early warning system for mortality. The behavioural patterns in conjunction with epidemiological surveillance have a potential to reduce the high mortality rate in free-range systems. We demonstrated the mortality clusters exhibit different behavioural pattern during the last days before death such as increased use of nest box and feeders. This indicates that change in individual behavioural pattern can be used as an indicator for poor health and welfare.

Barn and free-range systems are often equipped with multi-tier aviaries, which increase the usable indoor area for hens, subsequently allowing for a greater number of hens per unit of land area, compared to systems in which hens are just housed on the floor. Traditionally, average flock performance (body weight, laying performance, egg quality and resource use) has been measured on a flock level, as resources are allocated based on the size of the flock. However, advances in technology have provided more robust methods for analysing some of these parameters on an individual bird basis, allowing for increased accuracy and a more differentiated view of the situation in the hen house, including individual variation in selecting resources (Larsen et al., 2017).

Despite individual variability, there are consistencies with resource use within the whole flock; for example, nest boxes, outdoor ranges are less frequently visited than areas that offer water and food, based on survival instincts (Rufener et al., 2018). In this study we have shown that the use of the nest box is consistent over time due to the high motivation of the hen to lay, therefore a deviation from the consistent use of the nest box might indicate the hen may be subjected to poor health and welfare conditions (Barret et al., 2019).

In conclusion, the cross-correlation function performed well in differentiating the use of resources between clusters of mortality. There is the presence of a substantial degree of intricacy and structure in the use of resources by hens in relation to mortality which requires further investigation using machine learning methods.

## ACKNOWLEDGEMENTS

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# SIMULATING BOVINE TB BADGER CONTROL IN NORTHERN IRELAND

G.C. SMITH*, R. BUDGEY AND S. GOLD

## SUMMARY

Bovine tuberculosis is a costly disease shared by cattle and badgers, and to reduce the infection in cattle some form of badger management would be necessary. Here we compare the results of a badger field trial where test-postive badgers are culled and test-negative badgers vaccinated (a TVR approach) with the results of the simulation model originally used to predict the effect of the trial. Initial model results depended strongly on whether social perturbation occurred in the badgers following culling, and the field study demonstrated no such effects on badger behaviour. We re-run the model with the initial conditions of the TVR study and with no social perturbation, and predicted a similar outcome in terms of numbers of badgers caught, numbers infected and the substantial decline in prevalence. These results validate our model and demonstrate the utility of such predictive modelling for this disease system.

## INTRODUCTION

Bovine tuberculosis (TB) (caused by *Mycobacterium bovis*) remains a costly disease shared by cattle and badgers. In the absence of management, it appears that both species could sustain TB (Cox et al., 2005; Delahay et al., 2013; O'Hare et al., 2014) although the frequency of spread between the two species is highly variable (Crispell et al., 2019; Rossi et al., 2020; Akhmetova et al., 2021; Rossi et al., 2022). Thus, some form of badger disease management would be required to reduce TB in cattle to very low levels. Various control strategies have historically been adopted in England and the efficacy of these methods evaluated with simulation models (Smith et al., 2001), but all approaches have been non-selective culling. Such culling risks behavioural perturbation of the badger, which induces increased ranging behaviour (Woodroffe et al., 2006) and appears to increase disease prevalence in badgers and cattle (Donnelly et al., 2006).

Since 2010 an injectable vaccine *Bacillus Calmette–Guérin* (BCG) has been available for use in badgers that leads to a substantial reduction in disease in free-living badgers (Chambers et al., 2011) and a degree of herd protection for cubs (Carter et al., 2012). Along with tests that can be used pen-side to diagnose TB (the dual path platform (DPP) test) this leads to the possibility of selective culling on test-positive animals and vaccination of test-negative animals. This approach is referred to as test and vaccinate or remove (TVR).

In Northern Ireland badger control had not previously been performed, and an evaluation of this TVR approach was investigated. Initial modelling before the trial started suggested that the number of remaining infected badgers was very dependent on whether perturbation

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occurred: in the absence of perturbation a decline of about 70% in the number of infected badgers was seen, whereas with perturbation it was more modest (Smith et al., 2013) and an 83% reduction in the number of animals culled (Smith et al., 2016). With the completion of the subsequent five-year TVR study in Northern Ireland (Menzies et al., 2021) we can re-examine these predicted effects on the badger population, and use the exact initial conditions to validate the model output.

## MATERIALS AND METHODS

The TBi computer simulation model (Wilkinson et al., 2004; Smith et al., 2012; Smith et al., 2016; Smith and Budgey, 2021) was used to model the TVR study site. Input data included the initial population estimate, initial badger prevalence and the number of badgers captured each year (Menzies et al., 2021). Based on this, the model simulated the epidemiology, ecology and management of the badger population over the five-year course of the study to determine the population size and number infected. Estimates of disease prevalence at each capture point based on an analysis of trap-side TB testing (Arnold et al., 2021) were used to validate the model's output.

TBi is a stochastic, individual-based, spatially explicit model which simulates the life histories of a population of badgers at 60-day timesteps. Life histories were generated using the probabilities of reproduction, mortality, dispersal, disease progression and disease transmission collected from the population at APHA's Woodchester Park research station in Gloucestershire. Population density was taken from badger sett surveys conducted in County Down before the trial, and the demographic makeup of social groups was matched to the local population (Menzies, F., pers. comm.). The retention of some parameter values from the English model would have had minimal effect on the simulated output as the epidemiology is driven by badger density and disease prevalence, which were closely matched to the Northern Ireland study site.

The model arena comprised of a 100 x100 grid, with each cell representing 200m x 200m; the total grid representing a 400 km$^2$ landscape area. The population was 550 badgers in 85 social groups. The arena comprised a central core of approximately 100 km$^2$ where badger management was undertaken, and the boundary was defined by the extent of participating farmland. Outside the core was a surrounding buffer two social groups wide where the possible influence of control could be observed and outside this the effect of culling was expected to cease. The grid was wrapped to form a torus to eliminate edge effects. Social groups were randomly distributed across the arena and all badgers were members of a group and occupied a territory which defined neighbouring groups.

### Characterisation of badgers

Individual badgers were characterised by the variables: social group, sex, age, and health-status. The age categories were cub, yearling (one-year old), and adult. The TB-status categories were defined as: healthy, infected, single-site and multi-site excretor and probability of disease progression was according to rates in Graham et al. (2013). Badger fecundity was density-dependent based on an upper limit of litters in each social group. Births were simulated at the start of the year, and litter size was modelled probabilistically from a distribution of known litter sizes (Neal and Cheeseman, 1996), with a mean of 2.94 cubs per litter, and a sex ratio of 1:1. State-dependent mortality rates were taken from Graham et al. (2013). Badgers up to two months of age (i.e. while still underground) had a higher mortality rate than older badgers, as do males compared with females. Animals in the excretor disease classes also had higher mortality rates. Badgers were allowed to disperse, usually to smaller social groups if available (Rogers et al., 1998), based on sex-dependent probabilities (males more often than females) but independent of age and season. Badgers were also moved to neighbouring social groups in response to demographic imbalance. The probability of transmission between individual badgers was adjusted so the population disease prevalence matched the reported prevalence at the start of the study, estimated at 0.15 (Arnold et al., 2021). Disease transmission occurred between animals of the same and neighbouring social groups. As badgers interact more frequently with their own social group than with neighbouring groups, within-group transmission was given a greater probability (20-fold) than between animals in neighbouring groups. Transmission probability increased as animals moved from excretor to super excretor class.

### Simulation of management operations

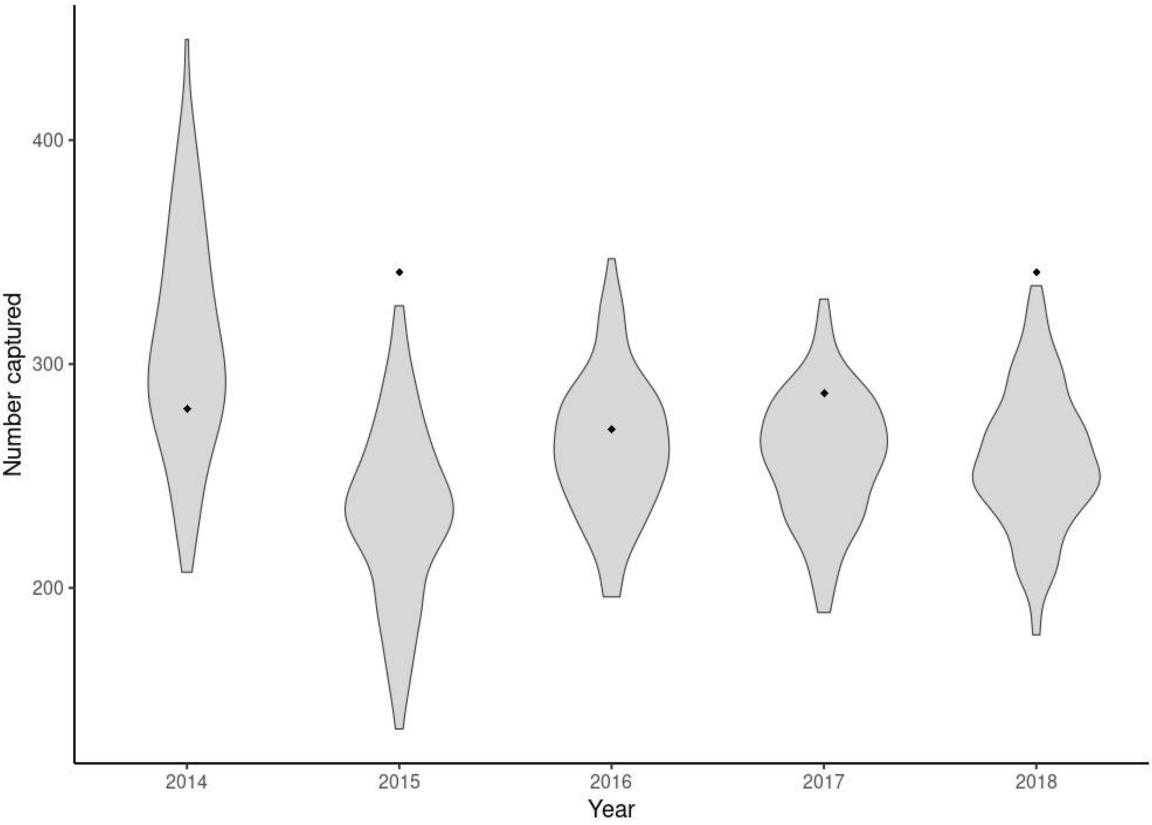
Management operations were simulated by allocating badgers a probability of capture based on the proportion of accessible land (0.94) and trapping efficacy rates (0.54) supplied by DAERA (Menzies, F., pers. comm.). Social groups were allocated to one of two trapping campaigns each year; territories not wholly within accessible land could still be subject to some level of control as badgers could be trapped away from the main sett. Badgers were individually marked during the study so recaptured animals were identifiable and this information was also available in the model. In the TVR study, animals were tested trap-side using the Dual-Path Platform VetTB test (DPP) on whole blood samples and those that tested positive removed. The model simulated badger removal based a test sensitivity (0.63) and specificity (0.94) as estimated by Arnold et al. (2021). In the study, negative testing animals were vaccinated with BCG Danish in years 2-3 and BCG Sophia in years 4-5 due to supply issues; the model gave both vaccines 0.6 probability of providing full protection from infection for susceptible badgers. Protection was for the lifetime of the badger, with further opportunity for full protection at subsequent capture for animals for which vaccination had previously been successful. A simulation of the same population with no control was also undertaken to provide a baseline.

In agreement with field results (O'Hagan et al., 2021) social perturbation resulting from badger removal operations were not simulated, beyond the filling of demographic vacancies in neighbouring social groups described above. Although TVR does result in additional vacancies, there are many fewer than with non-selective culling and this demographic rebalancing contributes little additional transmission compared to the increased ranging behaviour seen in removal operations such as the Randomised Badger Culling Trial (RBCT) in England (Jenkins et al., 2010).

## RESULTS

Model output is reported for the number of unique badger captures and the number of badgers testing positive using the simulated DPP test; these are compared to results from the study. Model output is also reported for disease prevalence in the core, ring, outer area of arena and mean of whole arena under TVR and no control; prevalence in the core is compared to the reported prevalence (Arnold et al., 2021).

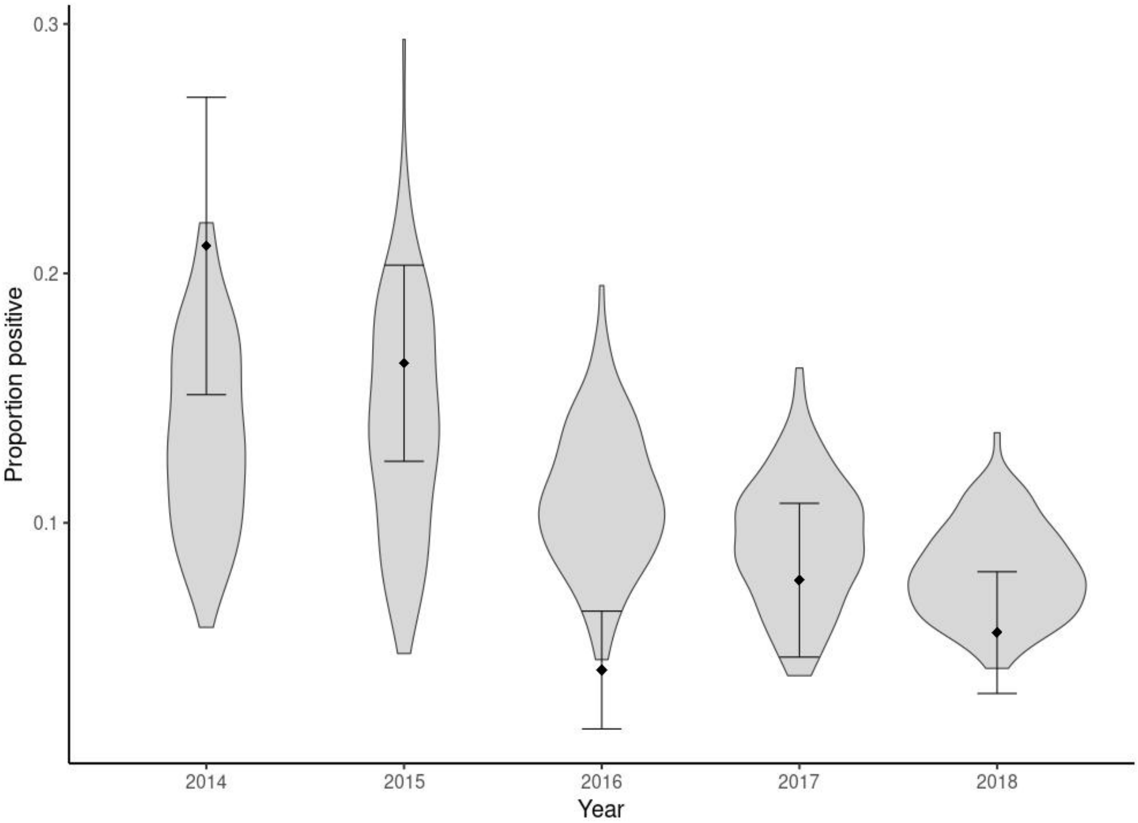
The number of unique badger captures did not vary substantially over the course of the study as relatively few were removed, and the population recovered before the following year. The model produced a similar result, but the number reported by the model was highest in the first year (2014) (Fig.1).



Points indicate results from the TVR trial and violin plots show distribution of model predictions

Fig. 1. Comparison between model results and data from Menzies et al. (2021) for number of unique badger captures in each year of the trial

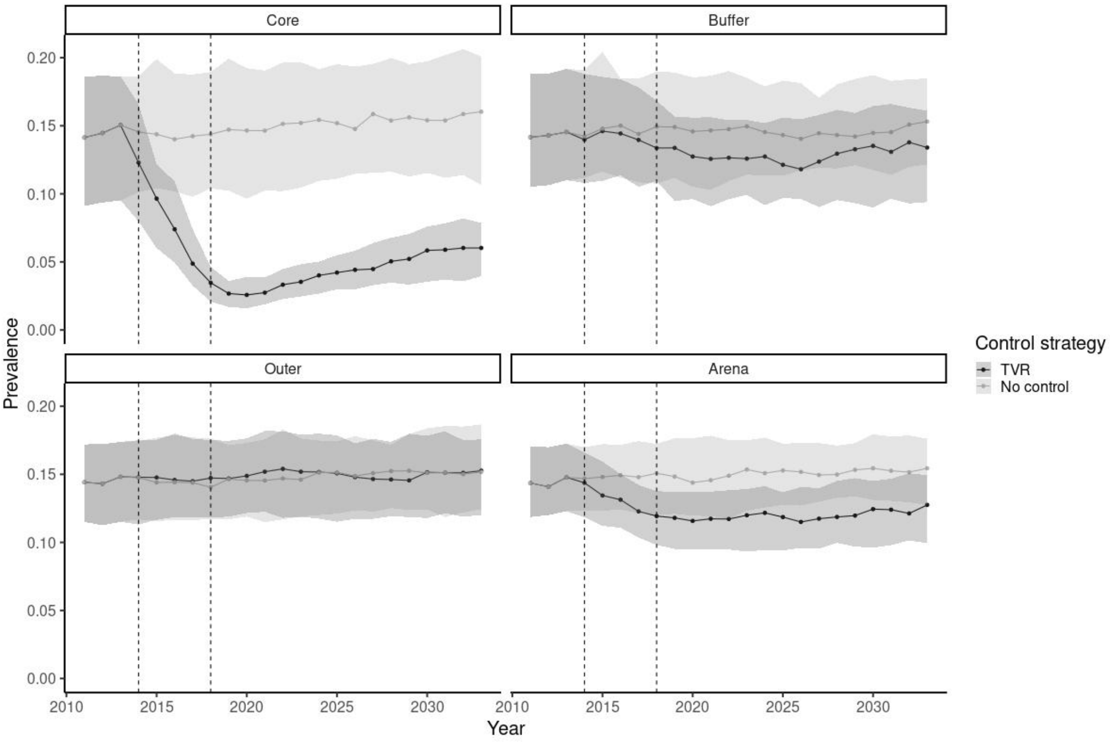
The proportion of captured badgers that tested positive was in line with the general trend in population prevalence. The number testing positive in the model was in reasonable agreement with the study in each year except 2016 but even in that year there was overlap between model results and the 95% confidence limits of the study (Fig.2).



Points indicate results from the TVR trial with error bars showing 95% binomial confidence interval, and violin plots show distribution of model predictions.

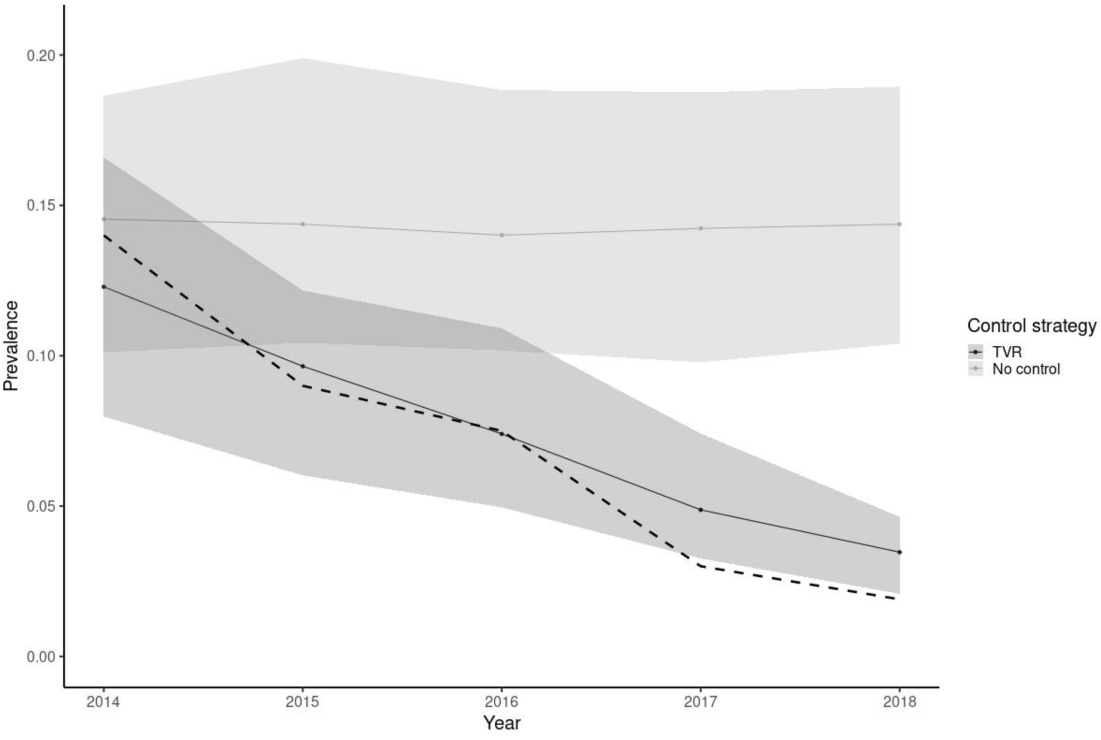
Fig. 2. Comparison between model results and data from Menzies et al. (2021) for proportion of captures testing positive in each year of the trial

The simulation was continued to year 2035 (Fig. 3). A large benefit of control was seen in the core where disease prevalence was reduced from the initial value of 0.15 to about 0.02 and slowly increased to about 0.05 some 15 years after control ended. The outcome when no control was applied was an unchanging population prevalence. A small benefit was seen in the buffer because some groups there may have partly overlapped with participating farms and therefore experienced some removal. There would also have been a small effect over the course of the study as some diseased animals in the higher prevalence buffer will have moved to the core, slightly reducing the benefit of control seen there and also some animals emigrated from the lower prevalence population in the core to the buffer, reducing the level of disease in the buffer (Fig. 4).



Vertical dotted lines indicate start and end of TVR trial. Shading indicates inter-quartile range

Fig. 3. Model results for median annual prevalence in each zone, predicted to year 2035



Dashed line represents the estimated ‘true’ prevalence from Arnold et al. (2021)

Fig. 4. Model results for median annual prevalence in core area during control period and inter-quartile range

## DISCUSSION

In recent decades there has been an increasing reliance on using computer models to predict the consequences of disease outbreaks or disease control. Such models can rarely be validated prior to any control in the field. Here we take the original model used to evaluate a TVR badger control study for bovine TB in Northern Ireland and validate it against the data from the field trial.

During the five-year field trial, a total of 824 badgers were caught, with between 271 and 341 unique captures each year (Menzies et al., 2021). This agrees well with the simulation, although the numbers caught in 2015 were higher than in 2014, whereas in the model the reverse was expected. Each year between 4% and 16% of badgers were removed: i.e., were DPP test positive (Menzies et al., 2021). This also agrees with our initial expectation of an 83% reduction in the number of badgers culled compared to a proactive cull: i.e., all badgers would have been removed.

However, the main prediction of the model was a substantial reduction in disease prevalence if social perturbation did not occur. During the trial a total of 105 individual badgers were followed using GPS collars, and there was no evidence of a change in home range size, neither annually nor monthly between the years of the study (O'Hagan et al., 2021). This strongly suggests that perturbation did not occur in this population. The field trial demonstrated a substantial decline in prevalence during the trial (Arnold et al., 2021), with the last years having a slightly lower prevalence than the simulated results, when we also assumed no social perturbation (Fig. 4).

These factors point to the success of the model in predicting the effects of the TVR approach in Northern Ireland. Since the simulated output depends most heavily on the badger social groups size and density, it therefore seems likely that the TVR approach would have similar outcomes in Ireland since badger dynamics are similar, but we cannot immediately extrapolate these results to England and Wales where social group size and density are both higher.

## ACKNOWLEDGEMENTS

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# SMART ANIMAL HEALTH – HEALTH AND WELFARE INDICATORS FOR LIVESTOCK

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## SUMMARY

The aim of this project was to develop a method that relies on animal-based indicators and data-driven metrics for assessing the health and welfare of the most common livestock species in Switzerland. Method development followed a uniform multi-stage process for all animal categories included. Literature reviews identified a variety of indicators for the different categories, some of which are well established and widely used in the field, while others lack reliability or practicability or still need further validation. Data quality and availability strongly varied between animal categories, with most data available for dairy cows and pigs. Machine-learning techniques used to predict farms for risk-based welfare inspections reached sensitivities above 80%. To estimate the animal health and welfare status of a given farm with sufficient validity, data-based indicators need to be complemented with data from on-farm assessments. Available precision livestock farming technologies can be a time-saving alternative to on-farm data collection.

## INTRODUCTION

Animal health and welfare are important topics in modern livestock and food systems. Improving and strengthening animal health, along with using novel possibilities of the advancing digitalisation in the animal health sector, are focal points of the Swiss Animal Health Strategy 2022+ (FSVO, 2021). In this context, animal health is not only understood as "being free" from diseases and injuries, but according to the Swiss Animal Welfare Act, animal health also takes into account the dignity and welfare of the animals (Anonymous, 2017).

In order to verify the effectiveness of measures to improve animal health, a method is needed to objectively measure animal health. Many different approaches to assess animal health and welfare are described in the literature. Earlier approaches mainly assessed the husbandry and environment (so-called resource-based welfare indicators). Examples are the Animal Needs Index 35 developed in Austria (Bartussek, 1999) or the Animal Welfare Index 200 developed in Germany (Sundrum et al., 1994). These methods are relatively easy to capture. However, their informative value for health and welfare is limited, as important sub-areas of health cannot be assessed with them. Other methods only focus on individual sub-areas of health, such as the

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BioCheck developed in Belgium, which assesses the biosecurity of a farm (Gelaude et al., 2014). Modern methods combine animal-based and resource- or management-based indicators of health and welfare. In the Welfare Quality® (WQ) project, welfare assessment methods and corresponding protocols were developed for cattle, pigs and chickens (Botreau et al., 2009). And in a follow-up project, similar protocols were also developed for sheep (Richmond et al., 2017) and goats (Battini et al., 2015). Advantage of these protocols are that they are very comprehensive and include both assessment of the animal as well as husbandry and environment. On the other hand, the disadvantage is that assessments with these methods are very time consuming and require farm visits with on-farm data collection. Various statements on animal health can also be made based on data that are routinely collected. An example of such a data-based method is the Animal Health Barometer (Depoorter et al., 2015). With the increasing availability of electronic records and databases, as well as data from "smart farming", the potential for such data-based methods is rapidly increasing. However, there is a lack of scientific studies that systematically assessed the validity and feasibility of these methods. Furthermore, there are also no scientific studies available yet on an established method that combines data-based indicators with on-farm indicators for assessing animal health.

The aim of this project was to develop a method to assess animal health and welfare for different livestock species in Switzerland, focusing on animal-based indicators as well as data-driven metrics that can serve as proxies for the health and welfare status of a given farm. The indicators shall allow assessing the animal health and welfare status at the level of individual farms, groups of farms and the Swiss livestock population as a whole. After successful implementation of the developed method, it should allow to: (i) monitor changes in the health status of livestock populations and individual farms over extended periods; (ii) assess the effectiveness of measures to improve animal health and welfare; (iii) identify farms with particularly good animal health in view of promoting them with financial incentives; and (iv) to implement risk-based animal welfare inspections.

This manuscript is intended to provide an overview of the work carried out as part of the Smart Animal Health (SAH) research project, outline the systematic process of the method development, highlight specific results, and discuss certain aspects of the overall project. Detailed results will be, and for some parts already have been, published in individual publications.

## MATERIALS AND METHODS

The SAH method was developed in a uniform multi-stage process for all animal species and categories included. These were dairy cows, veal calves, sheep, goats, sows, fattening pigs, broilers and laying hens. First, a context analysis was conducted, consisting of a review of scientific literature, data availability and potential Precision Livestock Farming (PLF) technologies. In order to determine the current state of research regarding existing indicators for assessing animal health and welfare, systematic literature research was carried out for all animal species and categories involved. For literature identification, search queries were made in the scientific databases PubMED, Web of Science, Scopus, CAB Direct and Science Direct and identified publications were analysed based on the PRISMA guidelines. Subsequently, a preliminary set of suitable indicators was compiled and discussed with stakeholders. Based on stakeholder feedback and relevance regarding Swiss production conditions, suitable indicators were determined and assigned to four categories, based on the Swiss Animal Welfare Act

(Anonymous, 2017): "Animal health"; "Husbandry and feeding"; "Appropriate behaviour" and "Freedom from pain, harm and anxiety". In the data context analysis, existing databases and information systems were examined for their usefulness for assessing animal health and welfare and the availability of data for the previously determined indicators. In addition, a systematic search was carried out on available PLF technologies, which could be used to simplify and improve data collection. In a next step, farm visits were carried out and the SAH method with each indicator set per animal category was applied in the field. For dairy cattle, veal calves, pigs and poultry, circa 30 farms were visited per category and data on SAH indicators were collected. At the same time, the WQ protocol was performed to assess the animal health and welfare status of a given farm, which later served as reference values. For laying hens, the MTool (Keppler et al., 2017) was used as reference method. Following the farms visits, a second round of stakeholder workshops was held, results of the field studies were discussed and the method was further refined. Finally, outcomes from the SAH and WQ methods were compared and assessed for consistency using descriptive and inferential statistical methods. Within the SAH method, a dual approach was chosen in order to take into account dependencies and restrictions in the scoring and integration of the individual indicators. On the one hand, a purely statistical description of the scores was conducted based on benchmarking. On the other hand, individual indicators were scored by means of thresholds with target and alarm values. For the benchmarking, indicator values were standardised by means of z-transformation and compared to the other farms serving as reference population. The 25% and 75% quartile served as cut-offs and scores were categorised into "lowest 25%", midfield (25%-75%) or "best 25%" (>75%). The evaluation by means of target and alarm values was based on threshold values either taken from literature (Brinkmann et al., 2020; Knierim et al., 2020; Schrader et al., 2020) or were adapted and/or defined for Swiss production conditions by expert consultation.

### Risk-index

To address the aim of implementing risk-based animal welfare inspections, a purely data-driven approach was chosen based on public databases with high data availability and coverage. Different databases and national registries containing information on farm demographics, animal identification, traceability, diagnostic results, and animal welfare inspections were pseudonymised and interconnected. Exploratory data analysis combined with machine learning algorithms were used to identify relevant proxies for estimating animal health and welfare. Input data (so-called features of the holding) were fed to machine learning algorithms that built a model of classification. Different methods, including logistic regression, random forests or artificial neural networks were applied and performances of the different algorithms were assessed through a K-fold method.

## RESULTS

The literature review identified a large number of potential indicators for the respective animal species and categories. For dairy cows, broilers, sheep and goats detailed results of the reviews have recently been published (Gebhardt-Henrich and Schlapbach, 2020; Lutz et al., 2021; Minnig et al., 2021; Zufferey et al., 2021). Some of the indicators are already well established and widely used in practice, while others lack reliability or practicability and/or require further research and validation. Moreover, it was found that some indicators have only limited suitability for Swiss production systems, for example due to smaller herd sizes or different welfare regulations. The data review revealed that data sources containing potential indicators can be allocated into three categories: (i) public data sources, where content and access are regulated under public law (ii) private data sources that are owned by farmers and/or private organizations and (iii) so-called "on-farm" data, which is considered as data that does not yet exist in a data base and must be collected on the farm (Table 1).

Table 1. Categorisation and characterisation of different data sources and its usefulness for assessing animal health and welfare

| | Public data[a] | Private data | On-farm |
|---|---|---|---|
| Data sources | animal movement database (TVD), agricultural policy information system (AGIS), laboratory information system (ALIS), information system for control data (ACONTROL) | breeding associations, slaughterhouse records, integrator's production and health data | none, must be collected via farm visits |
| Indicators | mortality, farm demographics, participation in animal welfare programmes | somatic cell counts, performance, treatments | lameness, body condition score, qualitative behaviour assessment |
| Data availability | high | limited | none |
| Coverage | high | mid-high | very low |
| Usefulness | low-mid | mid-high | high |
| Comments | different granularity and availability for species/category | different availability depending on owner and membership | on-farm data collection is very time consuming |

[a] Access and content regulated by public law

The most relevant public data sources are the animal movement database (TVD), the agricultural policy information system (AGIS), the laboratory information system (ALIS) and the information system for control data (ACONTROL) the information system on antibiotics in veterinary medicine (IS-ABV) and the meat inspection database (FLEKO). However, the latter two could not be considered due to their parallel introduction during the research project and the associated issues with data quality. Private data sources include for example data from breeding associations, private carcass evaluations, data from animal health services, electronic treatment journal, performance data, data from producer organisations and integrators (especially in the broiler and veal calf production), parts of the mandatory milk tests or other records from farmers or veterinarians. The "on-farm" data, where no data records exist and has to be collected on the farms, includes specific data on animal welfare such as qualitative behaviour assessment or freedom from pain, suffering, harm and anxiety.

In general, most relevant data and information for the assessment of animal health and welfare are found in the private data sources and the data collected "on-farm". However, public data sources are the easiest to access for governmental instances and have the highest level of coverage. In the TVD, there are major differences between animal species. For cattle, there is a high level of detail at the individual animal level. However, there is no specific category for "veal calf", a common production type in Switzerland, which means that these animals cannot be distinguished from other calves. For sheep and goats, the granularity of animal movement data is increasing due to recent regulatory changes for small ruminants, so that assessments at individual animal level will be possible. For pigs, the information is only available at farm and animal group level, and for poultry, data is limited to the number and age of the animals at stabling and the production type. The ALIS contains, among other things, test results on notifiable animal diseases. However, these have often limited informative value with regard to the animal health and animal welfare status of a farm. The AGIS contains demographic data and information on direct subsidies for public and ecological services. It mostly does not contain any direct data on animal health, but does contain auxiliary variables (proxies) which can correlate with animal health and welfare indicators. The IS-ABV was introduced in 2019, contains the antibiotic prescriptions according to the Therapeutic Products Act (Anonymous, 2000) with information on oral group therapies, individual animal treatments as well as the dispensing of veterinary medicinal products in stock and has stricter data protection regulations than other public data sources. The updated version of FLEKO was launched in 2020 and contains information on ante-mortem inspection of the live animals and condemnation of partial and whole carcasses. The exploratory data analyses have shown that in general, integrating multiple data sources increases their potential compared to using each data source as a stand-alone source. Furthermore, it is important that the entire data sets are available for these analyses and that possible parameters do not have to be selected a priori. Since the public databases were pseudonymised, they could be integrated with each other, but not linked to the private databases. The availability and quality of data from private data sources also varies considerably for the different animal categories. For dairy cattle and pigs, various relevant data from existing sources can already be used (with the agreement of the respective data owner). For poultry, potential health and production data are available, but these are mainly owned by integrators and were only accessible to a very limited extent. The least data is available for veal calves, goats and sheep. This led to the time-consuming collection of the missing data for the indicators in the farms.

The review of PLF technologies, which can be a time-saving and objective alternative to person-based collection of indicators, has revealed two main findings: Firstly, the range of PLF technologies varies greatly between different animal categories and secondly, there is a huge discrepancy between scientifically validated and commercially available PLF systems (Rowe et al., 2019; Stachowicz and Umstätter, 2020). The largest supply is for dairy cows, both in terms of availability of the number of system types and the number of suppliers within a system type. Fattening pigs and broilers follow in second place, while for sows, laying hens, veal calves, sheep and goats the choice of available PLF technologies that could be used to identify health and welfare issues is very limited (Stachowicz and Umstätter, 2020). The review has shown that there are commercially available PLF technologies for many of the SAH indicators, although information on the validity of these systems is often not available (Stachowicz and Umstätter, 2021a). To improve the accuracy of PLF technologies, a framework that proposes a categorization of the aim of detection of issues related to general welfare, disease and distress and defined disease was developed (Stachowicz and Umstätter, 2021b).

All SAH indicator sets developed for the different livestock categories consisted of data-based indicators (indicators for which data could be retrieved either from existing public or private data sources) as well as on-farm indicators for which data had to be collected "on farm". Depending on the animal category, there were considerable differences in the origin and availability of the data. In addition, the number of indicators per category varied between the different the animal categories. Table 2 shows as an example the SAH indicator set for fattening pigs with information on data origin. The validity of the SAH method was evaluated by comparing the outcomes with the WQ and MTool protocols carried out in parallel. It became apparent that certain parts of animal health could already be assessed with a relatively high degree of validity. However, data availability and the indicator set based on it are crucial. If public data, private data and on-farm records can be used, valid estimations can be made on all sub-areas of animal health and welfare. If, on the other hand, only information on data-based indicators are available, the validity decreases significantly and in particular the welfare categories "Appropriate behaviour" and "Freedom from pain, harm and anxiety" can usually only be assessed inadequately or not at all. Therefore, for sufficient validity, a combination of data-based and on-farm indicators with direct assessments of the animals is needed.

Table 2. Indicator set for fattening pigs with corresponding data origin

| Category | Indicator | Public data[a] | Private data | On-farm |
|---|---|---|---|---|
| Animal Health | lameness | | | x |
| | mortality | | x | |
| | condemnations | x | | |
| | treatments | x | x | |
| Husbandry and feeding | external and internal biosecurity | x | x | |
| | average daily gain | x | | x |
| | participation in animal welfare programmes | x | | |
| | water supply | | | x |
| Appropriate behaviour | soiling degree | | | x |
| Freedom from pain, harm and anxiety | bursitis | | | x |
| | tail lesions | x | | |

[a]Access and content regulated by public law

Risk index

Most of the machine-learning techniques applied reached similar classification performances. However, due to limited data availability for some species, a risk index could only be estimated for cattle and pig farms. As the explainability of the model was important both for the public acceptance of such a data-driven index as well as the planning of on-farm welfare inspections the random forest model proved to be the most suitable method. Median sensitivity with random forests was 81.7% for cattle farms and 81.8% for pig farms, respectively (Fig.1). The proxies with the highest correlation with the likelihood of violating animal welfare law, and thus the most important predictors, were participation in and compliance with federal ecological ("ÖLN") and animal welfare programmes ("BTS and RAUS"). Similarly, structural characteristics of the farms, such as the type of husbandry or the standard labour force, as well as reporting discipline for animal movements had high predictive weights.

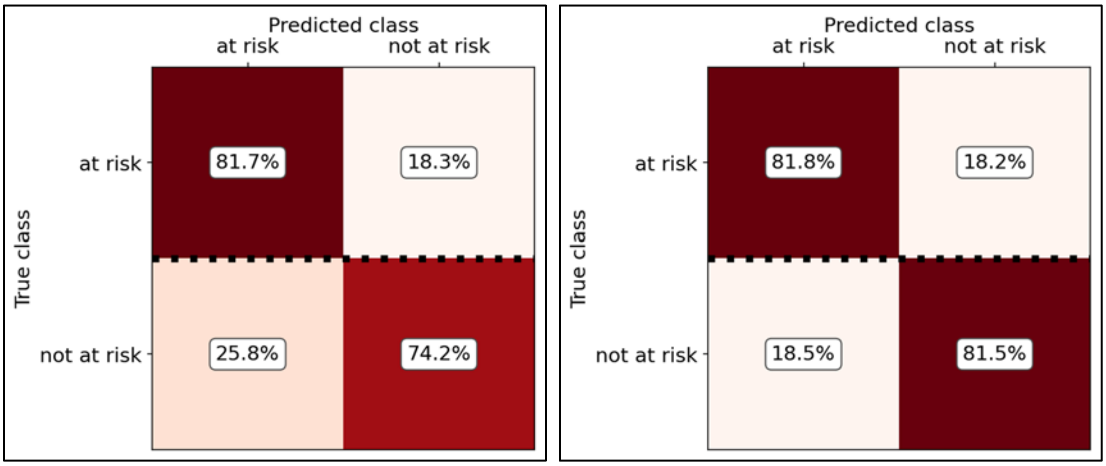

Fig. 1. Median confusion matrices of the models for identifying dairy farms (left) and pig farms (right) with an increased likelihood for animal welfare violations

## DISCUSSION

For the most important livestock categories in Switzerland a method for assessing animal health and welfare with species-specific indicator sets was developed that combines data-based indicators with on-farm indicators. The availability and quality of animal health information varies greatly between the different animal species. For dairy cows and pigs, various data-based indicators already exist and can be used to assess the different sub-areas of animal health. For poultry, good data are available in private data sources, but were not available for evaluation in this project. For veal calves, sheep and goats, only very few data related to animal health are systematically collected. For a high validity of the animal health and welfare assessments, a combination of public and private data with a direct on-farm assessment of animals is currently still necessary across all animal categories. In particular, the two categories "appropriate behaviour" and "freedom from pain, harm and anxiety" cannot be assessed reliable enough with a purely data-based indicator set. Data availability and quality are crucial for valid estimates and the predictive power of the indicators sets strongly depends on these factors. Public data sources could be improved by introducing new data variables (e.g. mortality in poultry) or categories (e.g. veal calves). Furthermore, fewer free-text fields for data entry would improve data quality and simplify analyses. The addition of new data sources (e.g. IS-ABV and FLEKO) would further improve the validity of the method. For private data sources, which have been shown to generally contain more valuable data than public sources, accessibility is a key factor. This can be achieved through data use agreements with data owners. One possible approach to increase the willingness of data owners to share their data would be to pay financial incentives when data is shared and a farm is found to have a particularly good animal health status. To generate more data for "on-farm" indicators, PLF technologies could be used for time-saving data collection on farms or key locations such as slaughterhouses. In addition, the concept of "iceberg" indicators with key indicators that effectively summarise many measures of welfare and are easy to understand (FAWC, 2009) has proven to be a promising approach for health and welfare assessments with on-farm data (Wadepohl et al., 2019). To further enhance the performance of the developed risk-index model, inclusion of additional data sources as well as finer grading of input data for the severity of historical welfare violations should be evaluated in a next step and validated in the field. The outcomes demonstrated that models based on proxy data can achieve high correlations with key factors such as animal health and welfare and can serve as a useful tool to support the planning of risk-based animal welfare inspections.

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# EMERGING METHODS FOR VETERINARY EPIDEMIOLOGY

# CONFIDENCE INTERVAL INTERPRETATION: INTRODUCING THE CONFIDENCE INTERVAL FUNCTION

L.L. MESSAM* AND H-Y. WENG

## SUMMARY

Confidence intervals are often used incorrectly to perform hypothesis tests in the biomedical sciences. In this paper, the confidence interval function is introduced. It is demonstrated that its use helps to prevent errors in inference caused by a reliance on null hypothesis significance testing, and in particular, on the dichotomisation of results into “significant”, and “non-significant” categories. By way of three examples of confidence interval function use, it is shown that dependence on p values for inference can lead to a) unfounded claims of conflicting results, b) overlooking effects of practical importance, and c) incomplete appraisal of study results. Authors of epidemiologic studies are encouraged to routinely report confidence intervals and use sketches of confidence interval functions to interpret them.

## INTRODUCTION

The effect of null hypothesis significance testing (NHST) on inferences in the biomedical sciences is currently under scrutiny. This is due to its dependence on p values as an inferential measure, most frequently manifested in the degrading of results into “significant” and “non-significant” categories based on predetermined cut-offs (usually $p < 0.05$). For decades, epidemiologists have advocated that 95% confidence intervals (CIs) be used in lieu of p values for study inferences (Lang et al., 1998; Stang and Rothman, 2011). However, 95% CI use has itself been justly criticised for a number of reasons. First, their correct interpretation (like a p value’s) depends on imagining the infinite repetition of all aspects of the study (Rothman et al., 2008; Naimi and Whitcomb, 2020). Second, “95%” or any other confidence level is as arbitrary a choice as “$p = 0.05$” for statistical significance and is similarly without a sound scientific basis. Third, researchers regularly exploit the correspondence of CIs with p values to subtly perform NHST by making inferences based on whether the CI’s limits contain the null value (one and zero, for ratio and difference measures, respectively) or not (Poole, 1987a). This latter practice replicates some of the flaws of p value-based inference including the dichotomization of results into “significant” and “non-significant” categories and the consequent disregard for the magnitudes of the estimated parameters (Goodman, 2008) . This often leads to irrelevant, and sometimes erroneous, conclusions (Rothman, 1986; Rothman et al., 2008). More importantly, using a CI to perform a significance test disregards the purpose for which it is intended (Rothman, 1986; Poole, 2001). A CI contains a set of parameter values which are all compatible with the collected data. This set includes the point estimate: the estimate of the parameter that the data provides most support for (i.e. the Maximum Likelihood

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Estimate (MLE)). For most common epidemiologic measures, the width of the interval is directly proportional to the standard error of the point estimate and provides a measure of its precision (Greenland et al., 2016; Naimi and Whitcomb, 2020). Thus, the CI provides information on both the magnitude of the estimated parameter and its precision in distinct ways (Lang et al., 1998). This characteristic alone renders inferences based on confidence intervals superior to those based on p values (Poole, 2001).

It has recently been reported that, over the last 20 years, on most occasions when CIs have been interpreted in articles published in *Preventive Veterinary Medicine*, they were used as surrogates for hypothesis tests rather than for an appraisal of the practical importance and/or precision of point estimates (Messam et al., 2021). This practice likely contributes to problems of reproducibility in veterinary epidemiology in the same way it has in other epidemiology subspecialities (Lash, 2017). One possible way to ameliorate this is for investigators to learn to visualise CIs as a function, and to use this understanding to gauge both the range of parameter values that are reasonably compatible with the data, as well as to quantify the precision with which the parameter is estimated. A CI function is a graph of all possible confidence limits (0 to 100%) derived from a single study (i.e. dataset). While advocated for use by epidemiologists since the 1980s (Miettinen, 1985; Poole, 1987a), the authors know of only one instance of their use published in the veterinary epidemiologic literature (Messam et al., 2021). The aim of this project is to introduce the CI function, show how it is used to correctly interpret confidence intervals and thus to avoid erroneous conclusions typical of p value-dependent inferences.

The manuscript is organised as follows: First, the confidence interval function and its construction are described. Next, three examples are provided which are designed to show how inferences can be enhanced by their use. Finally, the paper closes with a brief discussion on the merits and limitations of CI function versus NHST-based inference, and points the reader to easily accessible resources that help investigators to use CI functions.

## MATERIALS AND METHODS

### What are CI functions?

Description: Given a point estimate, a CI function can be constructed by plotting its confidence limits (horizontal axis) against 1 – the confidence (in decimals) level (vertical axis). A bilaterally symmetric curve is generated (for ratio measures, only if a log scale is used for the horizontal axis) with apex at the point estimate and horizontal asymptotes at 0 (i.e., 100 % confidence) (Fig.1). Any horizontal line corresponding to C% confidence on the vertical axis intersects the curve at the lower and upper limits of the C% confidence interval (Fig.1). The point of intersection of a vertical line from a parameter value on the horizontal axis with the curve equals the p value for that parameter value (Fig.1). Thus, the p value for an analysis, is the point of intersection of the line from the null value on the horizontal axis and the curve. This is more accurately called the null p value (Fig.1). Note that the p value for the null effect is also the p value for one other parameter value, the counter null (Fig.1) (Rosenthal and Rubin, 1994; Infanger and Schmidt-Trucksäss, 2019) and, each p value is shared by two parameter values, with the exception of the p value for the point estimate, which is unity and unique. Parameter values with equal p values have equal compatibility with the data and so, there is as much support in the data for the null value as there is for the counternull. The more distant parameter values are from the point estimate (MLE), the less compatible they are with the observed data and correspondingly, the smaller are their p values. Finally, the area under the

CI function (AUC) provides a quantitative measure of the precision of the point estimate (Berrar, 2017).

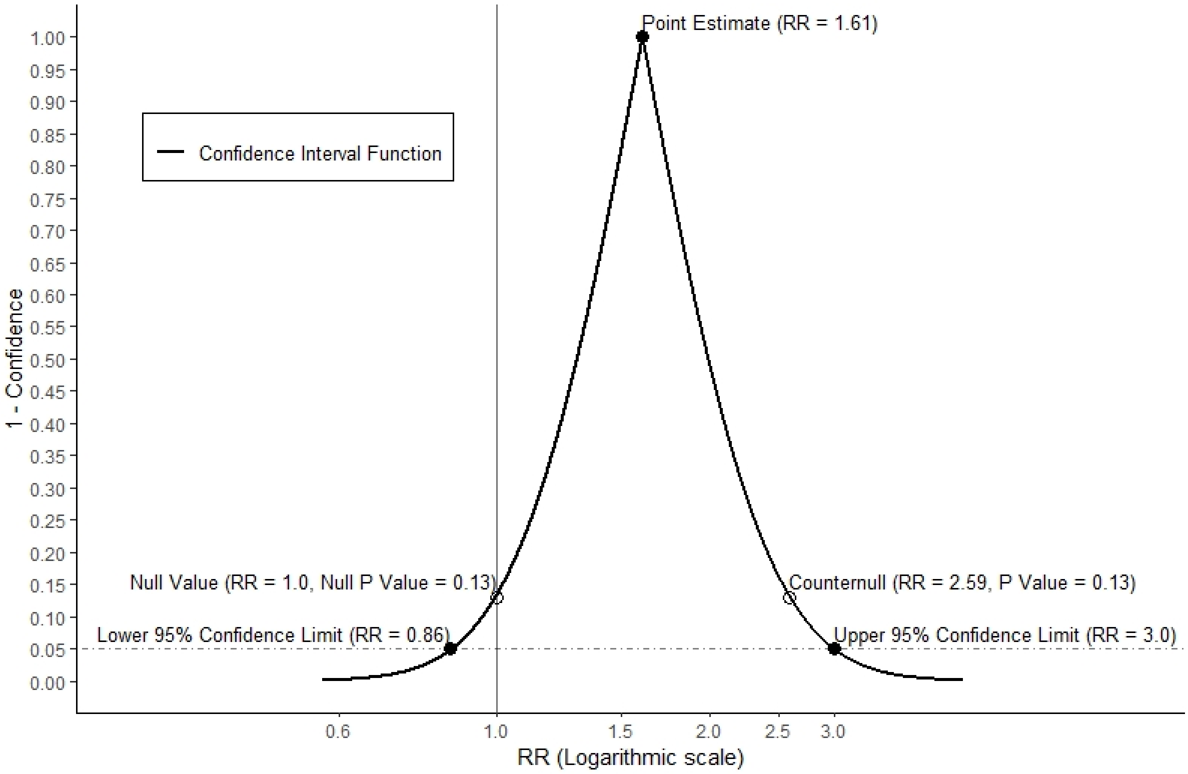

Fig. 1. Confidence interval function for a hypothetical exposure-outcome relationship

Interpretation: Without specifying any particular confidence level, the CI function provides a two-dimensional view of the location of parameter values that are most compatible with the analysed data and a visual appreciation of the precision of the point estimate (Fig.1).

Applications: The advantage of using CI functions instead of NHST is shown for inferences using three examples. In each example, the results are interpreted assuming that the correct statistical model has been used to generate point estimates and CIs, and there is no bias or confounding. The reader is referred to point 3 of the American Statistical Association's recent statement on p values (Wasserstein and Lazar, 2016), as while assumed here to facilitate the demonstration of CI function use, these assumptions are unrealistic and run contrary to advice on best practice.

## RESULTS

### Example I

Researchers in the dairy industry are often interested in the relationship between the timing of clinical mastitis relative to first service. One measure of this is the difference in time (in days) to first service observed between cows that had and didn't have clinical mastitis. In investigating this relationship, "Elmaghraby, 2004" (hereafter "Elmaghraby"), obtained a mean difference (MD) = 9.9; 95% CI: 1.3 - 18.5, while "Boujenane, 2015" (hereafter "Boujenane") obtained MD = 9.3; 95% CI: -3.8 - 22.4, as reported in a recent meta-analysis (Dolecheck et al., 2019).

NHST approach: Given that the results for the two analyses were $p < 0.05$ i.e., significant (Elmaghraby) and $p> 0.05$ i.e., not significant (Boujenane), it is concluded that the results of both studies are conflicting. Specifically, the Elmaghraby result suggests that there is a difference in time to first service between cows that had clinical mastitis and cows that did not, while the Boujenane result suggests that there is no difference.

CI function approach: If the CI function approach (Fig. 2) is applied, the following observations can be made:

1. The point estimates from the two studies are practically identical (9.9 and 9.3 days).
2. Parameter values that are most compatible with the data are essentially the same for both studies (Fig.2).
3. At every level of confidence, parameter values that fall within the limits of both CI functions are substantially more consistent with positive than negative differences in time to service (cows that experienced clinical mastitis taking longer) (Fig.2).
4. The difference between the two CI functions is essentially just one of precision, with the estimate obtained for the Elmaghraby study being more precise (narrower curve) than the one obtained for the Boujenane study (Fig.2). This is supported by their respective AUCs of 7.11 and 10.85, respectively.

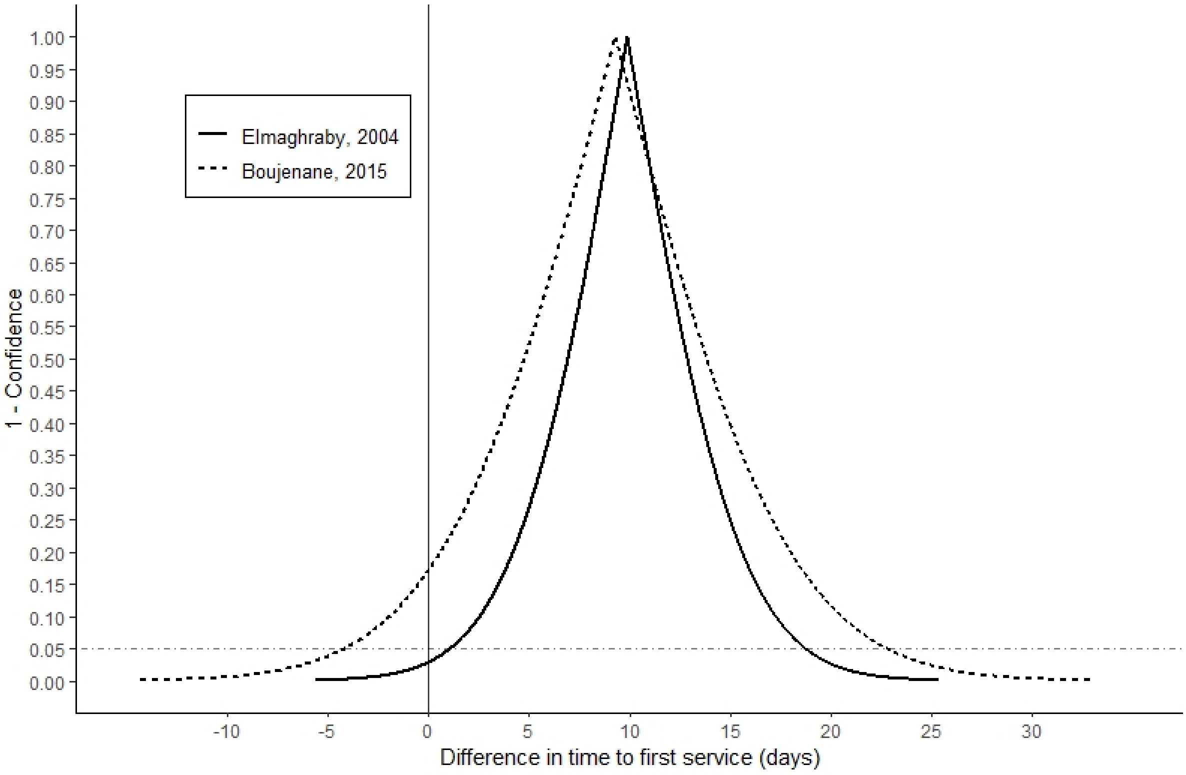

Fig. 2. Confidence interval functions comparing two studies investigating the difference in time to first service (days) between dairy cows with and without mastitis. Studies taken from Dolecheck et. al (2019)

Overall, both results suggest that clinical mastitis is associated with a delay to first service of 9-10 days. Thus, rather than being in conflict both studies support each other's findings.

Example II

Antibiotics are often used for prophylaxis against Bovine Respiratory Disease (BRD) complex. Here the results of two randomised controlled trials on the prophylactic effects of macrolides against BRD are compared. In a recently published meta-analysis (Baptiste and Kyvsgaard, 2017), it was reported that "Hibbard, 2002" (hereafter "Hibbard") obtained RR = 0.83; 95% CI: 0.71 - 0.96 ($p < 0.05$), while "Dedonder, 2016" (hereafter "Dedonder") obtained RR = 0.53; 95% CI: 0.25 - 1.12 ($p > 0.05$) for the same comparisons (i.e., the incidence of BRD among macrolide-treated cows, compared with the incidence of BRD among untreated cows) (Baptiste and Kyvsgaard, 2017). Imagine, that the question at hand is whether these trials provide support for macrolides having an effect of practical importance in reducing the incidence of BRD. Practical importance is defined as a reduction of 30% or more in incidence (i.e., RR $\leq 0.7$).

NHST approach: Given that the 95% CI obtained for the Hibbard study does not contain 1.0 ($p < 0.05$) it can be concluded that macrolides are effective for prophylaxis against BRD and that because of the converse, the Dedonder result (is non-significant and) suggests that macrolides have no protective effect against BRD.

CI function approach: Looking at the CI functions (Fig.3) the following observations are made:

1. The Hibbard result suggests that a very narrow range of macrolide effects are reasonably compatible with the data and are consistent with a 30% or smaller reduction in incidence (indicated by the curve being almost entirely to the right of the vertical line at RR = 0.7) (Fig.3). This is indicative of no effect of practical importance.
2. Based on the Dedonder result, the most likely prophylactic effect of macrolides (given the data) is a 47% reduction (RR = 0.53). Additionally, the result suggests that macrolide use is mostly consistent with effects of practical importance (≥ 30% reduction) but that one cannot rule out trivial prophylactic effects (0 to 30% reduction) or even effects that slightly increase BRD incidence. This is illustrated by a substantial majority of the CI function being to the left of the line RR = 0.7 and that there are parts of the curve that fall within the range $0.7 < RR < 1.0$ as well as extend to the right of RR = 1.0 (Fig.3).
3. It is noted that the Hibbard result is much more precise than the Dedonder result (AUC = 0.11 to 0.61, respectively).

Overall, it is concluded that while the Hibbard result does not support a practically important prophylactic effect of macrolides (only effects supporting a reduction <30% are reasonably compatible with their data), the Dedonder result provides strong (though not conclusive) support for the prophylactic effect of macrolides being of practical importance. These results illustrate almost the opposite of that concluded using the NHST approach.

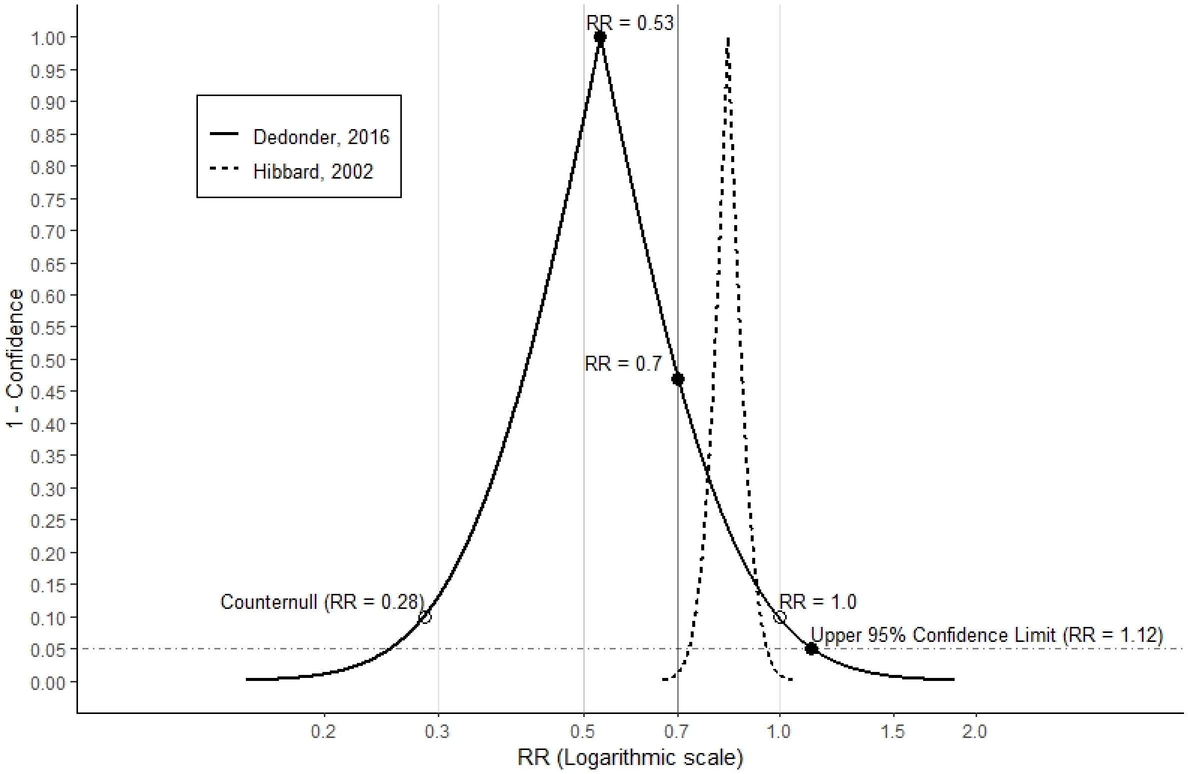

Fig. 3. Confidence interval functions comparing two studies investigating the prophylactic effect of macrolides on Bovine Respiratory Disease. Studies taken from Baptiste and Kyvsgaard (2017)

<u>Example III</u>

A recent systematic review exploring the effects of pet ownership and cardiovascular disease (CVD) included studies by "Friedmann, 2011" (hereafter "Friedmann") and "Chowdhury, 2017" (hereafter "Chowdhury") (Yeh et al., 2019). These studies examined the effect of non-canine pets on CVD mortality, comparing pet owners to non-owners: "Friedmann" (OR = 0.32; 95% CI: 0.20 - 0.51) and "Chowdhury" (OR = 0.88; 95% CI: 0.72 – 1.07) (Yeh et al., 2019).

<u>NHST approach</u>: Based on the observed p values the Chowdhury result shows no association of non-canine pet ownership with CVD mortality ($p > 0.05$), while the Friedmann result shows an association ($p < 0.05$) with CVD mortality.

<u>CI Function approach</u>:

1. The Chowdhury study suggests that only ORs very close to one are reasonably compatible with the data (Fig.4) and primarily suggests support for a weak negative association between non-canine pet ownership and CVD mortality.
2. The Friedmann study suggests that only OR < 0.4 are reasonably compatible with the data and thus provide support for a strong association (Fig.4).

It is concluded that the Chowdhury study more precisely (AUC = 0.16) estimates a weak negative association, and Friedmann's less precisely (AUC = 0.38) estimates a strong negative association. In this example, the inferences based on the NHST approach are incomplete and illustrate the limitations resulting from p values not being able to separately communicate the magnitude and precision of an association.

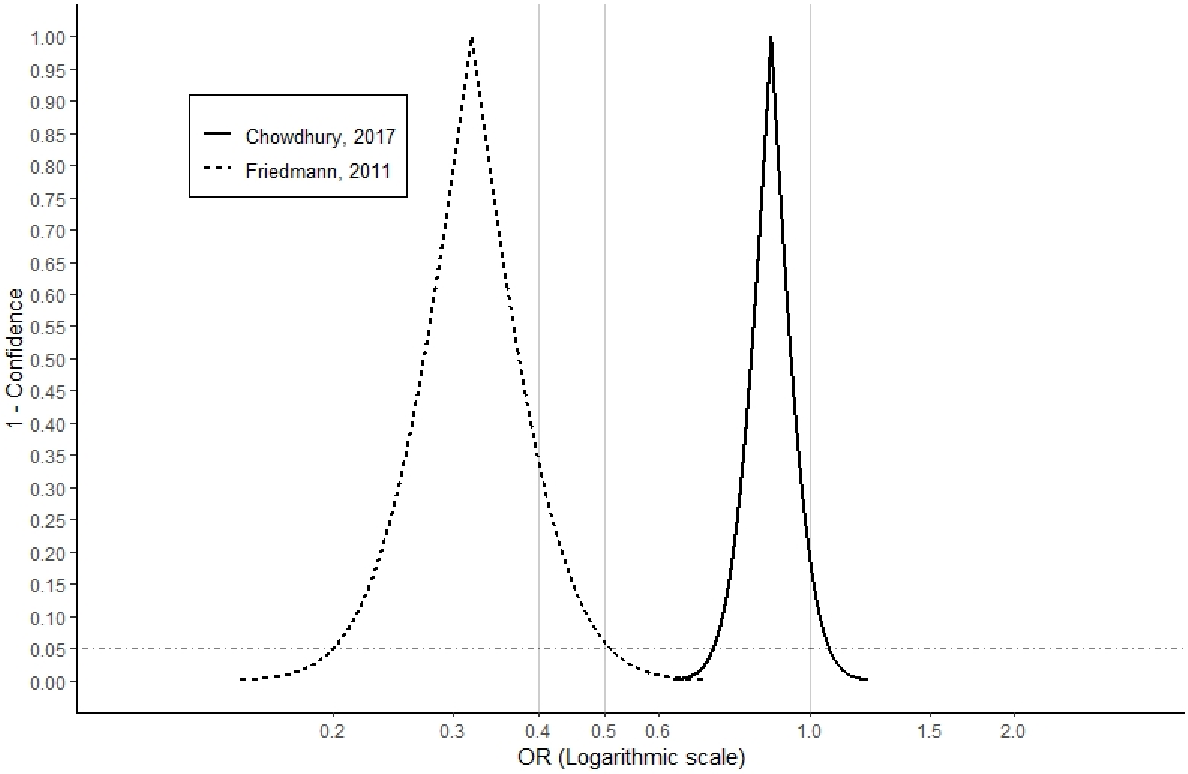

Fig. 4. Confidence Interval functions comparing two studies investigating the association between pet ownership and cardiovascular disease. Studies taken from Yeh et. Al (2019)

## DISCUSSION

CI functions are known by a variety of other names in the literature, including p value functions (Poole, 1987a; Infanger and Schmidt-Trucksäss, 2019). Like others, (Sullivan and Foster, 1990) the term "Confidence Interval function" is preferred by the authors, because it emphasizes the CI- rather than p value-dependent nature of the inferential approach. Because CI functions can visually depict both the magnitude and precision of point estimates, they can be used to clarify similarities and differences in results obtained in a variety of situations common to epidemiologic data analysis. These include comparisons of crude and adjusted effects during confounding evaluation, distinguishing between stratum specific effects during effect measure modification evaluation, comparison of different procedures for construction of confidence intervals and determining the extent to which observed results are consistent with effects of a given magnitude (Sullivan and Foster, 1990; Infanger and Schmidt-Trucksäss, 2019). Readers are referred to a very accessible tutorial on the topic by Infanger and Schmidt-Trucksäss (2019).

In this paper, it has been shown that in disciplines like epidemiology, where the focus is on measurement of parameters (Lash, 2007), NHST-dependent dichotomization of results leads to at best incomplete (Example III) and irrelevant inferences (i.e. "significant" or "not significant” statements). Often, this gives rise to misleading inferences resulting in incorrect conclusions (Examples I and II). With increasing frequency, examples of such inferences are highlighted in the human epidemiologic literature (Schmidt and Rothman, 2014; Lash, 2022) and it is likely such examples exist in the veterinary epidemiologic literature as well. CI functions provide all the information needed to correctly interpret the results of an analysis and show that when taken into consideration, more nuanced and complete conclusions can be drawn.

CI functions also help the analyst to place appropriate emphasis on the precision of estimates. Inferences are quite rightly more conclusive, the more precise the estimate (e.g. Examples II and III). This is consistent with the view that the data generating process leading to a more precise result is less affected by chance and hence, all things being equal, more likely to be substantiated by future research (Poole, 2001). This is also consistent with the practice of more precise results providing more weight in formal syntheses of evidence, like meta-analyses. For instance, in Example III, the Chowdhury (AUC = 0.16) and Friedmann (AUC = 0.38) studies contribute 18.3% and 7.2%, respectively, to the overall estimate (Yeh et al., 2019). It is also expected that the Hibbard study (AUC = 0.11) would contribute more to the final meta-analytic estimate than the Dedonder study (AUC = 0.61) (Baptiste and Kyvsgaard, 2017). Again, it is the precision of the estimates not the magnitude of their p values that determines their contribution in meta-analyses.

Throughout this paper, the phrase “reasonably compatible” is consistently used because CIs never show absolute incompatibility with data (Poole, 1987b). Thus, in using a CI function, one should not focus on the limits of any particular CI but use it to create a visual picture of the overall location (magnitude of effect/association) and shape (precision) of the graph (Poole, 1987b; Rothman et al., 2008), bearing in mind that values closer to the point estimate are more consistent with the data than values further away, and that the change in compatibility is not linear. One could say that this approach does not provide the certainty that NHST-based inferences provide. For instance, in Example II (Fig. 3), one might argue that the limit of practical importance ($\geq 30$ %) is too conservative and that it should be higher, e.g. $\geq 50\%$, leading to other conclusions based on the Dedonder study. Following conclusions based on a CI function approach, this criticism would lead to a discussion centred around the research question, i.e., the magnitude of the effect of macrolides as prophylaxis against BRD (e.g., “Which is more realistic $\geq 30\%$ or $\geq 50\%$?”). This is not possible after either a “clear-cut” “significant”, “non-significant” decision or the reporting of only a p value. A closer look at the NHST approach in Example II (Fig. 3) makes clear shortcomings in the use of the (null) p value as a basis for conclusions. The null p value (approximately 0.1) is also the p value for the hypothesis that RR = 0.28 (the counter null) (Fig. 3). This means that there is as much support in the data for a value of RR = 1.0 (no effect) as there is for RR = 0.28 (effect of practical importance). One might then ask, “On what basis do we preferentially choose RR = 1.0 and declare no effect?” This example makes evident the arbitrary basis behind the certainty in NHST-based decision making. One could further ask, “Is there greater support in the data even for a RR = 0.7 (the suggested minimum effect of practical importance) than RR = 1.0 (no effect)?” The answer is “yes”, and the CI function helps to answer this question without needing complex calculations. First, 0.7 is proportionally closer to 0.53 (the MLE) than 1.0 is and second, 1.0 is closer to the upper 95% confidence limit (1.12) than is 0.7 (Fig. 3). The CI function makes this obvious.

These examples show that dependence on p values for inference can lead to a) unfounded claims of conflicting results, b) overlooking effects of practical importance, and c) incomplete appraisal of observed associations. Like others, the authors recommend the routine reporting of point estimates and confidence intervals and the use of CI functions to interpret them (Poole, 1987a; Berrar, 2017; Infanger and Schmidt-Trucksäss, 2019). To do this, it is not necessary to present a CI function for each analysis, or for them to be reported in published manuscripts. However, authors are encouraged to routinely make rough sketches and use these to visualise their results. Rough sketches can be created by plotting a point estimate (zero confidence) along with confidence limits for 2-3 levels of confidence (e.g., 20, 50 and 95%) and interpolating the points. Manuscript quality CI functions can also be generated in R, using the package: pvaluefunctions (Infanger and Schmidt-Trucksäss, 2019).

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# THE BENEFITS OF QUANTITATIVE ESTIMATION OF POPULATION AMR (“QEPA”) IN ONE HEALTH

R.W. HUMPHRY*, C. WEBSTER, M.K. HENRY, G.J. GUNN AND G.T. INNOCENT

## SUMMARY

Antimicrobial resistance (AMR) in bacterial populations is complex: sub-populations have different minimum inhibitory concentrations. There is no single, simple AMR measure that is universally applicable. One approach is to utilise AMR measures that best predict outcomes. However, it is unclear currently which are the best measures to use. The authors consider the common approach of considering a single isolate as representative of the whole population and therefore defining it as “resistant” or “susceptible” to be a poor representation of the underlying population. Proposed here is a Quantitative Estimation of Population AMR (QEPA) that simultaneously estimates the total bacteria density and the proportion of bacteria resistant at a specified antimicrobial concentration, with associated uncertainties. A validation study demonstrates a close match between predicted and measured estimates in constructed mixtures. A cross-sectional pilot study shows heterogeneity within and between samples that would not readily be observed using a binary classifier.

## INTRODUCTION

Counting things permits us to “see” large quantities of information. Entities that we wish to describe, may have a number of characteristics, all of which we could try to measure. For each characteristic, we might devise a number of such measures. Which measure we choose to use, affects (distorts) how we perceive the characteristic that we wished to describe (Stone, 2020). This is a quantitative analogy to the theory and evidence that language itself affects the way we think (Lucy, 2001). This applies to science as well as to every-day life. One recent popular example is that in the 20th century, the focus on IQ as a metric of intelligence very likely directed (or even diverted) science and policy around “intelligence” (Gould, 1981).

Problems associated with antimicrobial resistance (AMR) are seen by medical and veterinary practitioners, and are predicted to worsen in the future (O’Neill, 2016). No matter how successful the current and future interventions are in reducing antimicrobial usage (AMU) in humans and animals, the tension between usage and resistance is likely to be with us indefinitely. It appears that in some countries, we have already started to be successful in tackling the obvious candidates for reduction of usage. For example, in the United Kingdom (UK) there has been a decrease in AMU in food-producing animals and, very importantly, a 79% decrease (2014-2020) in Highest Priority Critically Important Antimicrobials (HP-CIA)

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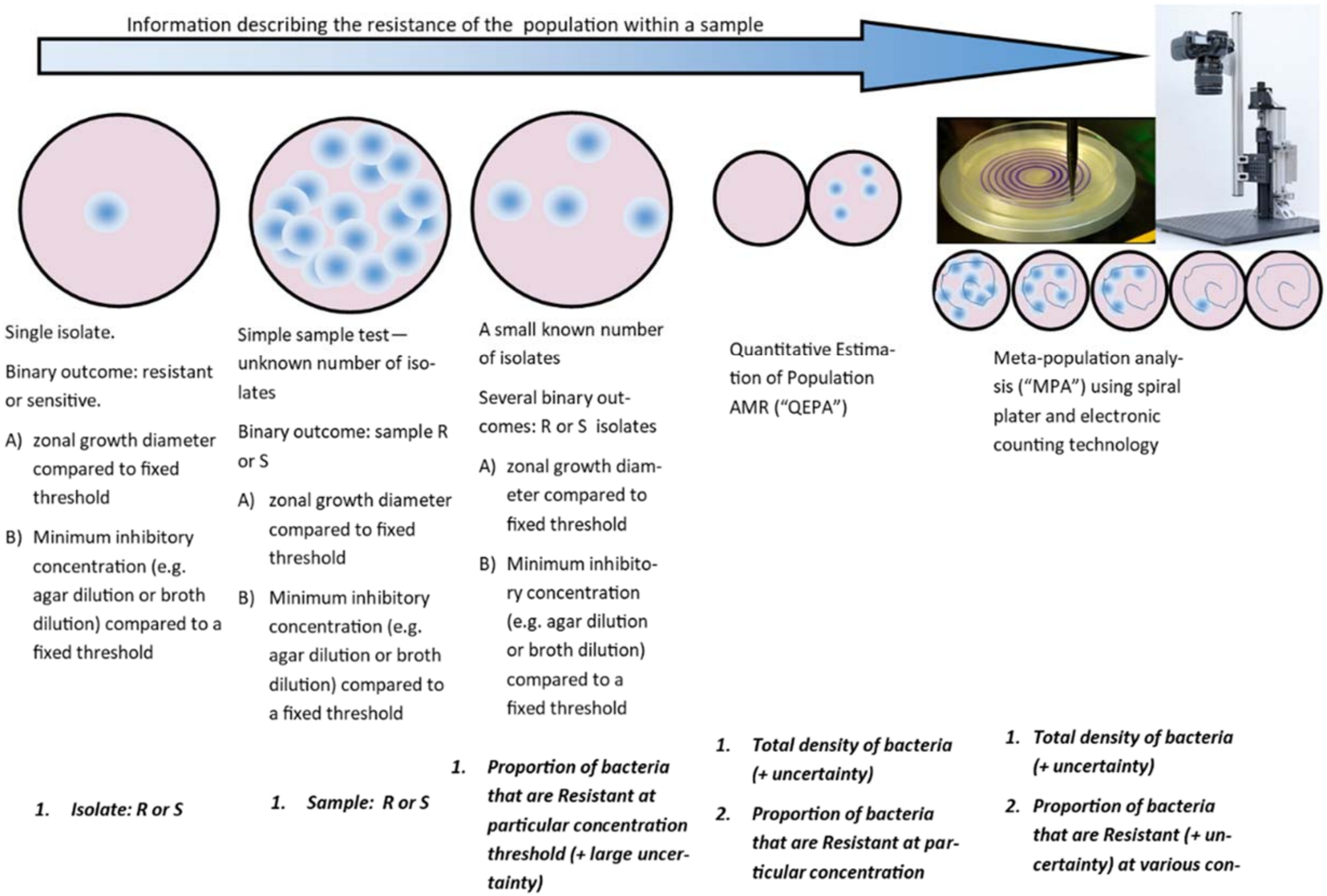
that are as used as “last-resort” antimicrobials in human medicine (UK-VARSS, 2021). As we succeed in tackling the most obvious AMU candidates, it is likely to become harder to identify the next best antimicrobials for reduction. Whether it be in animals or in humans, optimising the benefits of usage relative to the cost of selection for resistance is likely to become increasingly important.

A “One Health” approach to the identification of the optimum trade-off between usage and resistance might be to measure the empirical associations between resistance in animals and resistance in humans. This would require an optimum approach to quantifying the level of AMR and the level of AMU in both animals and humans.

In previous work, the authors presented evidence firstly that there is not a single way of measuring AMR and secondly, that the method used can determine the observed outcome (Humphry et al., 2017, 2002). For example, the estimated prevalence can be substantially affected by the choice of measure for AMR (Humphry et al., 2018).

At its most complex (providing the richest information), the phenotypic resistance of a population of bacteria from a sample can be characterised by describing the proportion of bacteria that are inhibited at different concentrations of antimicrobial (Humphry et al., 2002). At its simplest, resistance can be measured by taking a single bacterial isolate from a sample and giving a binary measure (resistant or sensitive) to each antimicrobial at a given concentration. Within the UK’s VARSS report, prevalence estimates of AMR are made by taking a single isolate from a sample from one animal per production unit tested, so as to avoid pseudo-replication by sampling animals that may be very similar in their resistance profile (UK-VARSS, 2021). This is valuable for high level, national comparisons. Between these two extremes, lie intermediate methods, which offer different degrees of information describing the resistance of the bacterial population. This spectrum is illustrated in Fig.1. Towards the information-rich end of the spectrum lies “Quantitative Estimation of Population AMR” (QEPA) which is the method presented in more detail in this paper.



Single isolate.

Binary outcome: resistant or sensitive.

A) zonal growth diameter compared to fixed threshold

B) Minimum inhibitory concentration (e.g. agar dilution or broth dilution) compared to a fixed threshold

1. *Isolate: R or S*

Simple sample test—unknown number of isolates

Binary outcome: sample R or S

A) zonal growth diameter compared to fixed threshold

B) Minimum inhibitory concentration (e.g. agar dilution or broth dilution) compared to a fixed threshold

1. *Sample: R or S*

A small known number of isolates

Several binary outcomes: R or S isolates

A) zonal growth diameter compared to fixed threshold

B) Minimum inhibitory concentration (e.g. agar dilution or broth dilution) compared to a fixed threshold

1. *Proportion of bacteria that are Resistant at particular concentration threshold (+ large uncertainty)*

Quantitative Estimation of Population AMR (“QEPA”)

1. *Total density of bacteria (+ uncertainty)*
2. *Proportion of bacteria that are Resistant at particular concentration*

Meta-population analysis (“MPA”) using spiral plater and electronic counting technology

1. *Total density of bacteria (+ uncertainty)*
2. *Proportion of bacteria that are Resistant (+ uncertainty) at various con-*

Fig. 1. An illustration of some different measurement methods for AMR ranging from the simplest (LHS) to the most complex (RHS)

## MATERIALS AND METHODS

### General QEPA method

The QEPA method involves a standard laboratory quantification of bacterial density in combination with Bayesian modelling.

Laboratory process: The sample is serially diluted (10-fold) and from each dilution, a fixed volume is spread evenly on an agar plate containing an appropriate compound for detecting the bacterial type of interest. Each plate has the number of distinct identifiable colonies counted. Where the density of bacteria is too high (a count of more than 300), the count is considered invalid. All valid counts, even if very low, are carried forward to the statistical analysis. This process is repeated for the sample on plates both with and without the antimicrobial of interest, at a concentration of antimicrobial that is normally defined by bodies such as the European Committee on Antimicrobial Susceptibility Testing (EUCAST). In the studies reported here, the antimicrobial used was tetracycline and the cut-off concentration was 8 mg/L - in the absence of a EUCAST-defined threshold for tetracycline (EUCAST, 2021) for *E. coli*, the published concentration of 8 mg/L for tigecycline was used as a proxy level for the antimicrobial of interest in these trials.

Statistical process (Bayesian modelling): The two main advantages of the statistical approach that were used in modelling within the QEPA method are: a) that all valid counts can readily be included for a particular sample, irrespective of how small the count is, and the model will weight them accordingly; and b) the flexibility that comes with this framework means that both the core of the model which estimates the bacterial density can be combined with any experimental or sampling design. The model was implemented in the JAGS language (Plummer, 2003) and run using the R package "runjags" (Denwood, 2016).

Model core: The core of the model estimates the bacterial density, uses all valid counts (in the presence of the antimicrobial or not) and is based on a Poisson distribution which in turn is based on the parameter λ ("lambda") representing the ***underlying*** density contributing to the observed counts. The ***observable*** density for any count is based on the product of the ***underlying*** density and the sample dilution for that particular count. For antimicrobial plates, the observable density also depends on the level of AMR in the sample (i.e. the proportion of bacteria that are resistant to the antimicrobial at the level present in the plate).

### Validation trial

The validation trial involved the construction of artificial samples, designed to have a range of particular proportions of *E. coli* that were resistant to tetracycline (chosen because it was administered in the dairy calves of a cross-sectional sampling – see below).

The trial involved two experimental blocks, each of which was run at a different time (in 2021) due to logistical constraints.

Within each experimental block there were two replicates. Within each replicate a series of artificial samples were created with an intended proportion of resistant bacteria ranging from 0% to 100%. To construct each replicate, a culture of resistant bacteria and a culture of sensitive bacteria were created, each culture having been clonally grown from a randomly assigned, archived isolate that had immediately and previously been confirmed as being resistant or sensitive, respectively. Prior to mixing the sensitive and resistant cultures, an approximate estimate of their respective bacterial densities was made using the McFarland turbidity method (Markey et al., 2013; Prolab, 2021). The McFarland method can only provide an approximate estimate of bacterial density.

Based on the McFarland approximate estimate of bacterial density, the two cultures (sensitive and resistant) were mixed variously to achieve approximate proportions of bacteria being resistant, from 0% to 100%. The intended proportions of bacteria resistant was modified between experimental block 1 and block 2 in response to the first set of results, and these are documented in Table A1 of the Appendix.

QEPA provided more precise estimates of the bacterial count in the two pure cultures (0% resistant and 100% resistant). These were used to make improved (i.e. more precise) estimates of the true proportion resistant in each mixture. These values are referred to as the "predicted proportion resistant".

Each mixture was split into two replicates and then each replicate tested according to the QEPA method (SOP RD ERU 058, (ERU Lab team, 2019)).

### Cross-sectional trial

Freshly defecated faeces from five housed dairy calves aged 10-12 weeks were sampled in 2018 and tested using the QEPA method. The calves had been treated with "Hexasol LA" which contains oxytetracycline as one of its active agents (Veterinary Medicines Directorate, 2020), one month prior to sampling.

## RESULTS

### Validation trial

In Fig. 2 there was close concordance between the proportion resistant as predicted by the known mix of the resistant population and the sensitive population in combination with the estimated density of each respectively, and the proportion resistant in each constructed mixture as estimated by the QEPA method. The bars present the 95% credible interval for both the predicted (horizontal) and the estimated (vertical) proportions and demonstrate that in almost all cases, the two methods estimated the proportion to be the same within the bounds of that uncertainty. The two data points that stand out as not concurring were within Validation trial 2. This may be as a result of type I errors, which cannot be excluded in one or both methods, given the number of estimates presented in this way over the four trials.

The uncertainty around the estimated low proportions of resistance is small compared to the uncertainty around the estimates of higher proportions of resistance. This is due to low proportions having one high count (non-antimicrobial plate) and one low count, the low count having a low variance (high precision) due to the equivalence between mean and variance associated with the Poisson distribution. In samples with a high proportion of AMR bacteria, both counts are high and therefore both estimates exhibit a relatively low precision. The uncertainty around the high proportions of bacteria being resistant is naturally bounded by one, leading to asymmetry in the credible intervals.

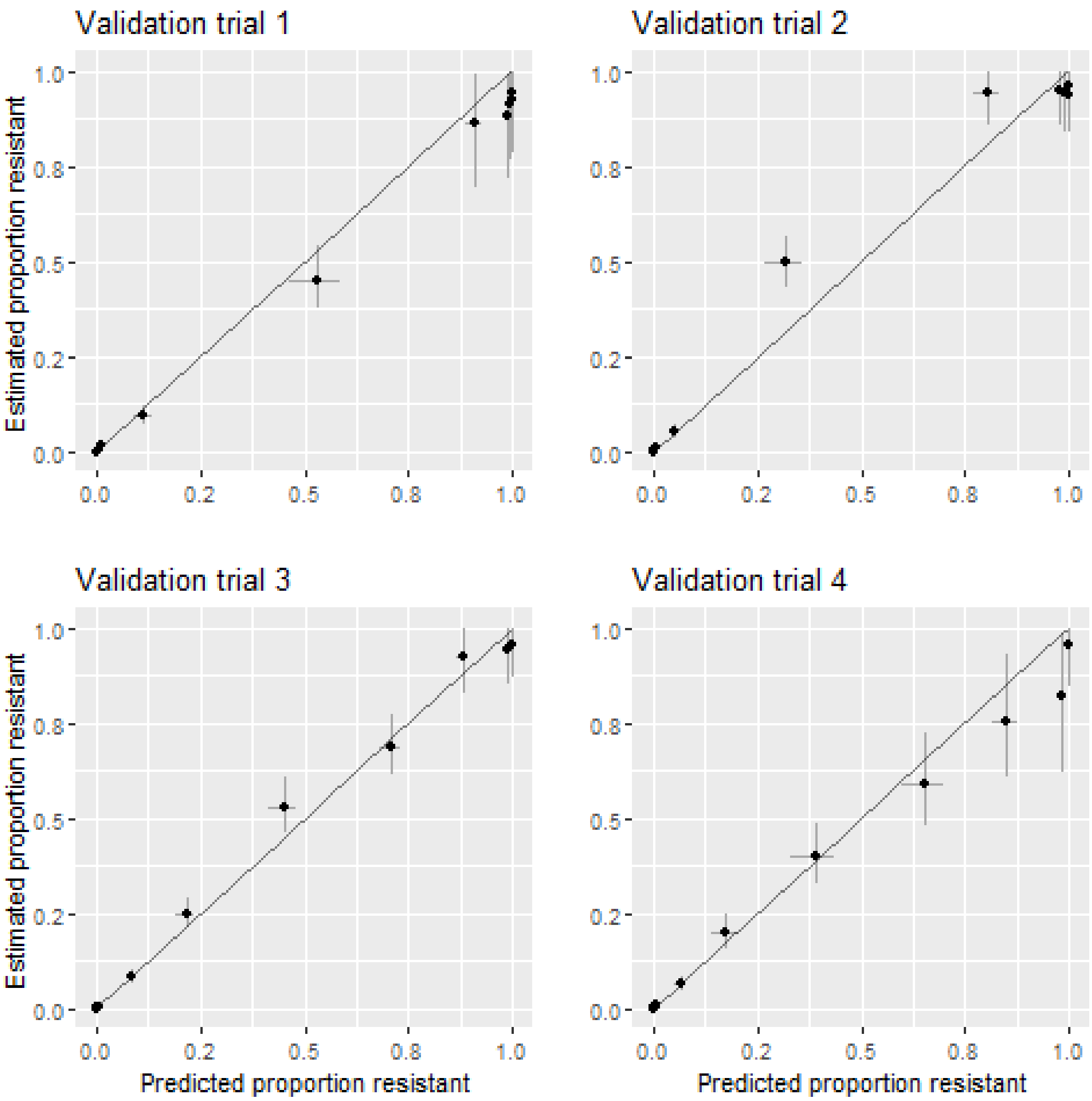


Fig. 2. The estimated proportion of resistant bacteria in the constructed samples against the predicted proportion resistant. The continuous line represents y=x, i.e. the line on which a perfect match would lie

## Cross-sectional trial

The results for the cross-sectional trial are presented in Figs.3 and 4. Figure 3 shows the estimated mean proportion of bacteria resistant (and associated 95% credible intervals) in each of the animals sampled (except Animal 2 for which the analysis did not converge). There is evidence of heterogeneity *within* samples – the estimates of proportions are not all zero or one, and evidence of heterogeneity *between* samples – the estimates are not all the same (e.g. the estimated proportion resistant from animal 5 appears to be substantially higher to animals 1, 3 and 4).

The probability of all four animals for which the model converged testing negative (sensitive), had the single isolate per sample method been used, is estimated by the product of each animal testing sensitive:

$$\Pr(X_{1:4} = 0) = \Pi_{i=1}^{4}(1 - \Pr(X_i = 1)) \qquad (1)$$

where $\Pr\,(X_i = 1)$ is the probability of an isolate, i, testing resistant as shown in the figure by the proportion of bacteria resistant. The probability of all four animals testing sensitive is, in this way, calculated at 0.61.

Figure 4 presents the estimated mean total bacterial density. It is clear that the variation between animals in their estimated bacterial density is large. It is also apparent when comparing Fig. 3 with Fig. 4 that (albeit on the basis of only the four animals reported), there is no striking relationship between total bacterial density and proportion of bacteria resistant.

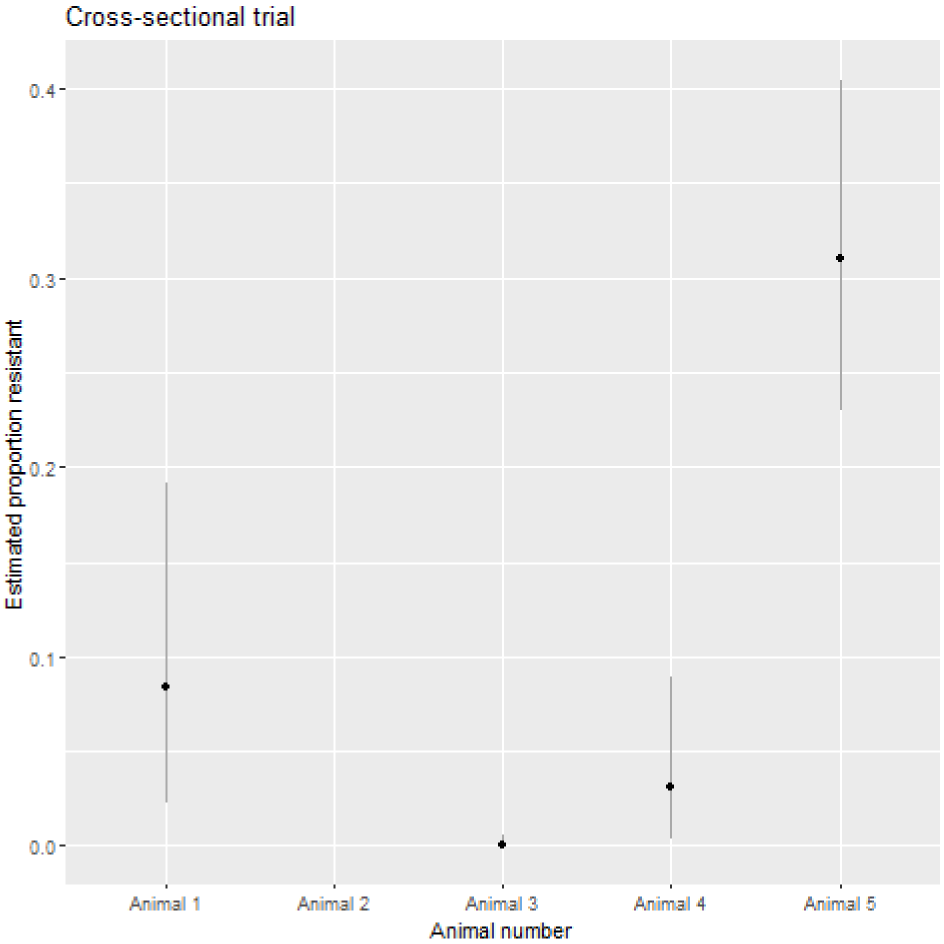


Fig. 3. The estimated mean proportion of bacteria resistant (with 95% credible intervals) in samples from each of four (out of five) housed dairy calves. The model did not converge for Animal 2 and hence no estimate is provided

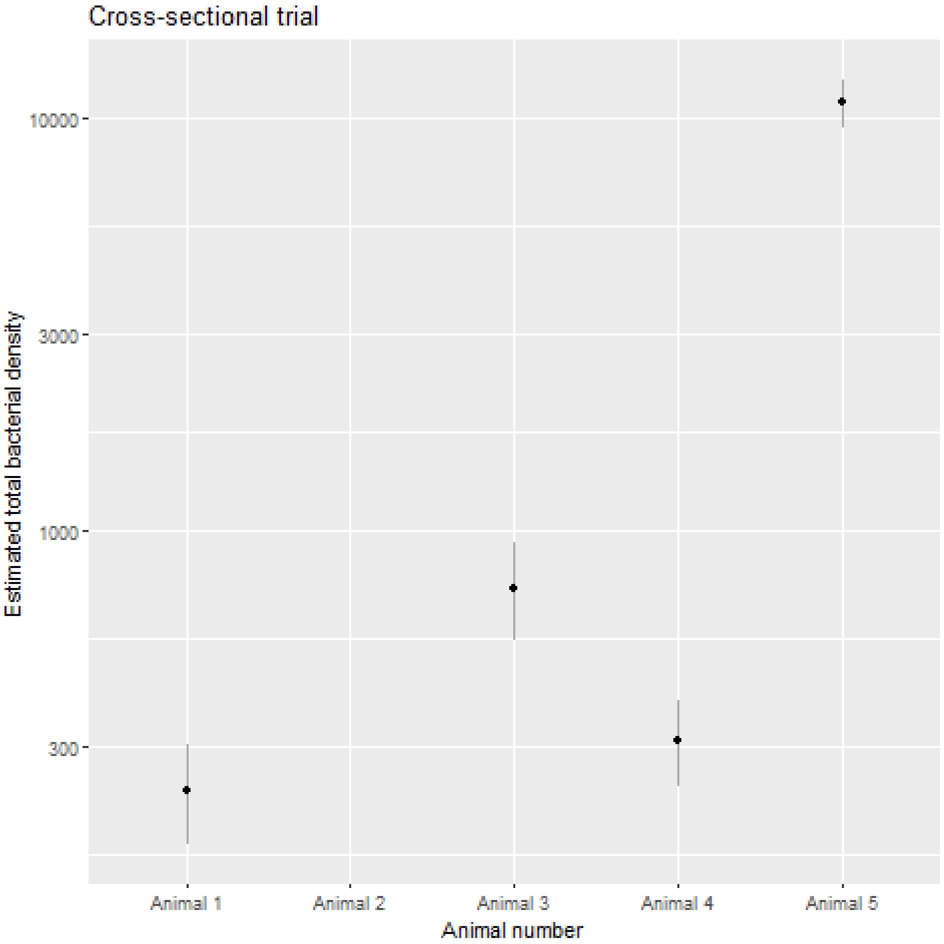


Fig. 4. The mean estimate of the total density of bacteria (with 95% credible intervals) in samples from each of four (out of five) housed dairy calves. The model did not converge for Animal 2 and hence no estimate is provided. Note that the values on the y-axis are plotted logarithmically

One month prior to sampling, calves one and two had been treated with “Hexasol LA”, calves three and four had not, and there was uncertainty about whether calf five had received this treatment. Hexasol is an intramuscular injectable product used for respiratory disease and contains the antimicrobial oxytetracycline.

## DISCUSSION

Here the results from a validation trial and a cross-sectional trial using a proposed new method called “Quantitative Estimation of Population AMR” (QEPA) are presented.

The validation trial demonstrated that the method appears to be working well, whilst the cross-sectional trial gives an indication of the level of information that the method provides (total bacterial density and proportion of bacteria resistant).

It is important to consider how much information arises from using QEPA and is presented here compared to how much information would have been collected from the single isolate per sample method which is commonly used (Humphry et al., 2018).

The cross-sectional trial showed that there was variation in the proportion of bacteria resistant both within and between samples from different calves and that this would not have been observed using the oft-used method of one isolate per sample. This trial also demonstrated that there were differences in total bacterial density between the samples from different animals. Had a single isolate per sample been tested as sensitive/resistant, the most likely result was all 4 isolates testing sensitive (Pr=0.61) and this compares starkly with the richness of information provided by the QEPA method.

There has been strong progress in reducing the measured antimicrobial usage (AMU) in livestock (ECDC et al., 2021; UK-VARSS, 2021) with some initial signs that this reduction may now be slowing down. This is to be expected as many different antimicrobials are being used in different species in different ways. Therefore, it might be expected that policies to reduce antimicrobial usage would provide greatest early traction in those situations where it is easiest to reduce usage.

Thereafter, further reductions in usage are likely to be more difficult. It might be that better evidence is sought on which parts of the AMU network give the greatest benefit (reduction in problematic AMR) to cost (loss of treatments available to farmers/vets). The putative chain between AMU in livestock and AMR in humans is complex with many steps (Department of Health, 2014). This means that it is unlikely the effect of AMU in livestock at one end can be directly measured and related to problematic AMR in humans, at the other end. An alternative is to measure relevant associations between nodes which are close to each other in the network (e.g. AMR in the environment or in food) and to measure AMR in a way that is most strongly associated with the usage and with the problem. At this stage it is not known whether the proposed method gives more useful data on AMR but it is hoped that in this paper the authors have clearly demonstrated potentially how much more information this method provides. Further studies, should therefore make use of the method to populate the causal chain between AMU and AMR in human medicine.

With the objective of using this method to target future research, the authors are currently designing a study in livestock to quantify variability in AMR within the different strata of the dairy system (samples, animals, groups of animals, farms). If it becomes clear that certain strata have very low variability in AMR, then this could act as a guide to sampling programmes (there is little value in taking multiple samples, or “pseudo-replicates”, from groups where variation is low). Where certain strata have very high variability in AMR, this directs research to seek causes of that variability within those strata, with the possibility that some of those causes can be managed to reduce the risk of AMR.

The authors’ suspicion is that there will be some areas of research regarding the targeting of AMU reduction that will benefit from using more informative measures of AMR and that will only become apparent as the QEPA method, or similar, are applied in more complex field studies.

## ACKNOWLEDGEMENTS

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## APPENDIX

Table A1. The intended percentage of bacteria resistant within the validation trial, blocks 1 and 2

| Block | Intended percentage of bacteria resistant (%) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0.1 | 0.5 | 1 | 10 | - | 50 | - | 90 | 99 | 99.5 | 99.9 | 100 |
| 2 | 0 | 0.1 | 0.5 | 1 | 10 | 25 | 50 | 75 | 90 | 99 | - | - | 100 |

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| 1986 | Edinburgh | Thrusfield |
| 1987 | Solihull | Thrusfield |
| 1988 | Edinburgh | Thrusfield |
| 1989 | Exeter | Howe |
| 1990 | Belfast | McIlroy |
| 1991 | London | Jones |
| 1992 | Edinburgh | Thrusfield |
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| 2018 | Klaus Depner<br>African swine fever: Lessons learned about the epidemiology, politics and practical implementation of control measures | Päivi Rajala-Schultz<br>Veterinary epidemiology at the intersection of livestock disease, production and animal welfare |
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|---|---|---|
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| 2008 | Paul Fine<br>Infectious disease eradication – meanings and implications | Kenton Morgan<br>For the benefit of Mr Kite |
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| 1999 | Aalt Dijkhuizen<br>The 1997/98 outbreak of classical swine fever in the Netherlands: lessons learned from an economic perspective | Mark Woolhouse<br>Understanding the epidemiology of scrapie |
| 1998 | Wayne Martin<br>Art, science and mathematics revisited: the role of epidemiology in promoting animal health | |

## SOCIETY FOR VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

## APPLICATION FOR MEMBERSHIP

Name ..........................................................................................................

Address ..........................................................................................................

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Telephone: ..........................................................................................................

Fax: ..........................................................................................................

E-mail: ..........................................................................................................

Signed .................................................................. Date .............................

Please enclose the membership fee (£60 sterling to cover two years' membership) along with this application form. Overseas members without British bank accounts are requested to pay 2 - 3 years in advance. Cheques should be in £ sterling and drawn from a British bank. British members should pay future dues by standing order (forms are available from the Secretary or Treasurer). Payment can also be made by credit card; the appropriate form is available from the Society's website, http://www.svepm.org.uk/, or from the Secretary or Treasurer.

Please send this form to the Society's Treasurer:

Dr 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***