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MACHINE LEARNING FOR DISEASE PREDICTION

USE OF MACHINE LEARNING TO EMULATE A SPECIALIST VETERINARY DIAGNOSIS

R.M. HYDE[*], P.M. DOWN, C. HUDSON AND M.J. GREEN

SUMMARY

Machine learning has gained substantial popularity in both human and veterinary medical fields and has great potential to aid clinical decision making, for both individual and population level diagnoses. Within dairy herd health, correct diagnosis of the predominant mastitis infection pattern at herd level is a crucial step in implementing appropriate preventive strategies. Machine learning has the potential to emulate a specialist veterinary diagnosis, and the translation of machine learning algorithms into practical decision support tools requires an accurate and robustly validated model. A range of methods can be employed to improve both the performance and interpretability of algorithms in making such a diagnosis.

INTRODUCTION

Machine learning research within medical fields have reported applications with diagnostic data such as outperforming specialist haematologists when achieving a correct diagnosis from haematological results (Gunčar et al., 2018). There has also been interest in the field of medical image recognition, particularly in the accurate classification of retinal disease (Fauw & Ledsam, 2018), skin cancer (Esteva et al., 2017), diabetes (Barakat & Bradley, 2010) and cardiac arrhythmia (Özçift, 2011).

Within veterinary medicine applications are more limited, however machine learning has been used in cattle medicine to predict the early stages of calving (Fenlon et al., 2017), high somatic cell counts (Ebrahimie et al., 2018) and a variety of fertility outcomes (Fenlon et al., 2016). There have also been applications associated with epidemiological research, for example transmission patterns of subclinical mastitis (Esener et al., 2018), exposure to liver fluke (Ducheyne et al., 2015) and the prediction of herd exposure to bovine viral diarrhoea virus (Machado et al., 2015). There is an increase in "big data" being captured in livestock farming, and the ability to turn this into "smart data" may be amplified by the application of machine learning (VanderWaal et al., 2017).

Whilst there has been substantial interest in the impact of machine learning on the clinical management of patients, there are few examples of translation into effects on clinical

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management (Clifton et al., 2015). Only through a collaborative approach between data scientists and clinicians will machine learning classification algorithms make a meaningful impact in a real world setting (Lynch & Liston, 2018).

Mastitis is a costly endemic disease of dairy cattle, costing an estimated £170 million annually in the UK (Kossaibati & Esslemont, 1997), alongside significant costs to animal welfare (Leslie & Petersson-Wolfe, 2012) as well as being an important driver of antimicrobial usage within the dairy industry (Hyde et al., 2017). Mastitis infection patterns can generally be classified, for example, as being either 'contagious' or 'environmental' and 'dry period' or 'lactational' in origin (Todhunter et al., 1995; Down et al., 2016). Since control strategies for these origins differ markedly (Green et al., 2007), it is crucial to correctly diagnose the major herd level pattern in order to provide preventive herd level veterinary advice (Down et al., 2016).

The correct diagnosis of herd mastitis patterns is critical to implementing effective control strategies on dairy farms. This diagnosis is a complex, multifaceted diagnosis based on herd data and requires specialist veterinary training and expertise. This paper explores various methods in constructing a machine learning algorithm to emulate this specialist veterinary diagnosis.

MATERIALS AND METHODS

Machine learning algorithms

An enormous number of machine learning methods are available for classification problems, with specific algorithms having superior performance for certain types of classification. A simple example of a machine learning algorithm is a decision tree, which uses "training" data (observations with a known outcome used to construct a model) to construct a "tree" which would be able to classify an outcome based on the values of input variables. Decision trees often have surprisingly good performance despite their simplicity; however, performance can be improved by "growing" many trees as part of a "forest" such as a random forest algorithm. This algorithm employs a given number of trees (*ntree*) which are each allowed a "vote" (i.e they are weighted) to predict the classification of a given observation (Breiman, 2001). Whilst the performance of random forests often outperforms that of a decision tree, they are extremely difficult to interpret due to their multi-tree nature. This example illustrates an important concept in machine learning: that of predictive power vs interpretability. Whilst the random forest might outperform a decision tree, it is difficult to determine the precise effect of each variable on the outcome of interest. With a decision tree however, it is possible to directly visualise the tree and determine the effect each variable has, with the disadvantage of a generally poorer predictive power. The biological definition of the problem being investigated is crucial to algorithm selection. In problems such as the prediction of calving onset it is perhaps not that important for a clinician or farmer to determine exactly which parameters are involved in the classification, as long as accuracy of detection is high. With the diagnosis of disease in epidemiological studies however, it might be important to identify causal factors in order to alter preventive health advice and thus an interpretable model might be more desirable. Whilst there are many algorithms available, random forest algorithms are often found to provide excellent performance in classification exercises (Wainer, 2016).

Performance metrics and assessment of performance

Cross-validation (CV) is a method of robustly assessing model performance. Ten-fold CV describes the training of an algorithm on 90% of the data and testing the performance on the remaining “unseen” 10% and repeating this process 10 times, which has been shown to be relatively robust to overfitting (Kohavi, 1995). There is however the possibility of discreet specific patterns being present within the dataset, and thus whilst 10-fold CV is relatively robust against overfitting, the use of an entirely separate external validation set to use as a final test of model performance can further ameliorate this risk.

The use of metrics such as sensitivity, specificity, positive and negative predictive values alongside a confusion matrix of results allow a deep understanding of model performance and the sole reporting of accuracy as a metric of incidence is inadequate. The “optimum” model however is ultimately dependant on the application of the model, and the “no free lunch theorem” describes the point in model tuning where improvements in one performance metric are to the detriment of others (Wolpert & Macready, 1996). Whilst sensitivity and specificity are frequently reported alongside accuracy as optimal metrics of performance, within clinical veterinary predictions the positive-predictive value (PPV) and negative predictive values (NPV) are likely to be equally valuable to clinicians faced with a clinical decision. Specific plots such as ROC curves and Lift plots allow further understanding of model performance and can be a useful method to determine which algorithm or tuning parameter to select. A Lift plot uses observations ranked by probability predictions to highlight how many observations must be tested to identify a given number of outcomes. The “lift” describes the number of samples detected by a model that are above what a completely random selection of samples might achieve (Kuhn & Johnson, 2013).

Parameter tuning

Machine learning algorithms often have several parameters that are “tuned” in order to increase model performance. In the case of random forests, there are two main parameters: the number of variables randomly sampled as candidates at each split, and the number of trees to grow. Following the notation of the R package *randomForest* (Liaw & Wiener, 2018), these parameters will be referred to *mtry* and *ntree* respectively. *mtry* is often initially estimated using the number of features, the square root of the number of features, half the number of features, and twice the number of features, selecting the optimal *mtry* based on best model performance (Liaw & Wiener, 2002; Breiman & Cutler, 2003). After this a dense grid of *mtry* values around the optimum *mtry* can be used to fine tune this parameter. *ntree* is defined as the number of trees to grow, often set at 500 as it has been shown that increasing *ntree* beyond this does not reliably improve performance unless enormous computational power is available (Oshiro et al., 2012). The effect of model tuning on model performance should be carefully selected based on the effects on model performance as assessed by key metrics of interest as previously discussed.

Feature engineering and selection

Feature engineering represents the opportunity to create novel features that might be better suited to predicting a specific outcome. For example, if a series of somatic cell counts were 6 monthly time points, a raised mean somatic cell count across all time points might be a better predictor of a subclinical mastitis issue for a machine learning algorithm to utilise rather than an individual reading from 6 months previous. There are few limitations with the features that can be engineered, and features are not necessarily required to be biologically interpretable. It is often worth considering biologically plausible features that might be engineered, in this example the ratio of high somatic cell counts to clinical cases of mastitis might be more useful in diagnosing contagious versus environmental mastitis than either feature independently. It is often advantageous to eliminate extraneous features when tuning machine learning algorithms, for several reasons. Firstly, it leads to a more parsimonious model, but perhaps more importantly it can often lead to model improvement. If a dataset had 5 truly important features and 95 “noise” features that were essentially meaningless, a random forest algorithm will still be “forced” occasionally to use these meaningless features as it can only select *mtry* features at each node split. The use of algorithms such as recursive feature elimination not only improve interpretability of the model but often result in improved model performance.

Subsampling and imbalanced datasets

Care must be taken when modelling imbalanced datasets, as there is the potential for performance metrics to be misleading. For example, a classification algorithm might achieve a 97% accuracy in a dataset with only a 3% prevalence of disease, simply by classing all outcomes as disease negative.

Subsampling is a method that can improve model performance when using imbalanced data. Potential disadvantages to subsampling include increased learning time and a risk of overfitting (Weiss et al., 2007); by evaluating model performance using cross-validated predictions this should be limited. Examples of subsampling techniques include up, down and the synthetic minority over-sampling technique (SMOTE) (Chawla et al., 2002).

Thresholds

The probability threshold is the threshold value at which a classification algorithm might select one of two binary outcomes. This is often left at a standard of 0.5, although the threshold value used can dramatically affect sensitivity and specificity of machine learning algorithms, depending on the biological question being asked.

Missing data and imputation

One particular advantage to random forest algorithms is their ability to cope with a relatively high level of missing data, even up to 80% (Breiman & Cutler, 2003). It is however often worth considering the use of imputation techniques such as random forest imputation using the *randomForest* package (Liaw, 2018), which imputes missing data points based on data from known observations.

Variable importance

Although random forest algorithms are known to be difficult to interpret, an estimate of the relative importance of features can be explored using variable importance. Variable importance is a ranked 0-100 relative score, calculated for each tree within a random forest by calculating the difference between prediction accuracies when a feature is permuted. This difference is then averaged across all trees and normalised by the standard error to provide a variable importance figure for each variable (Kuhn, 2011).

Calibration plots

The calibration of machine learning models is a crucial component in the translation of theoretical model performance into a clinical setting. Calibration plots can be used to evaluate the strength of the linear correlation between observed and predicted probabilities of a diagnosis. For example, a machine learning diagnosis would be challenging to use in the field if a 90% probability of diagnosis was only correct 30% of the time. Calibration plots are useful methods of assessing this by "binning" predicted probabilities, and plotting against observed percentages. The optimal result is a linear correlation, denoting that if the algorithm returns a 60% probability of diagnosis, then this is correct 60% of the time.

Illustrative example

An illustrative example follows using routine herd level milk recording data from 1,000 UK dairy herds. Mastitis diagnosis by a specialist recognised by the Royal College of Veterinary Surgeons is used as a gold standard for each herd. To illustrate the principles previously discussed, the random forest algorithm was used alongside two other classification algorithms to compare predictive performance. All analysis was performed using the statistical programming language R (R Core Team 2018). Random forest, support vector machines and nearest shrunken centroids were used to predict contagious versus environmental mastitis, using the *randomForest* (Liaw 2018), *kernlab* (Karatzoglou 2019) and *pamr* (Hastie 2019) packages respectively. The algorithms originally coded in these packages were called through the wrapping functions provided in the *caret* package (Kuhn 2018).

RESULTS

Performance metrics and assessment of performance

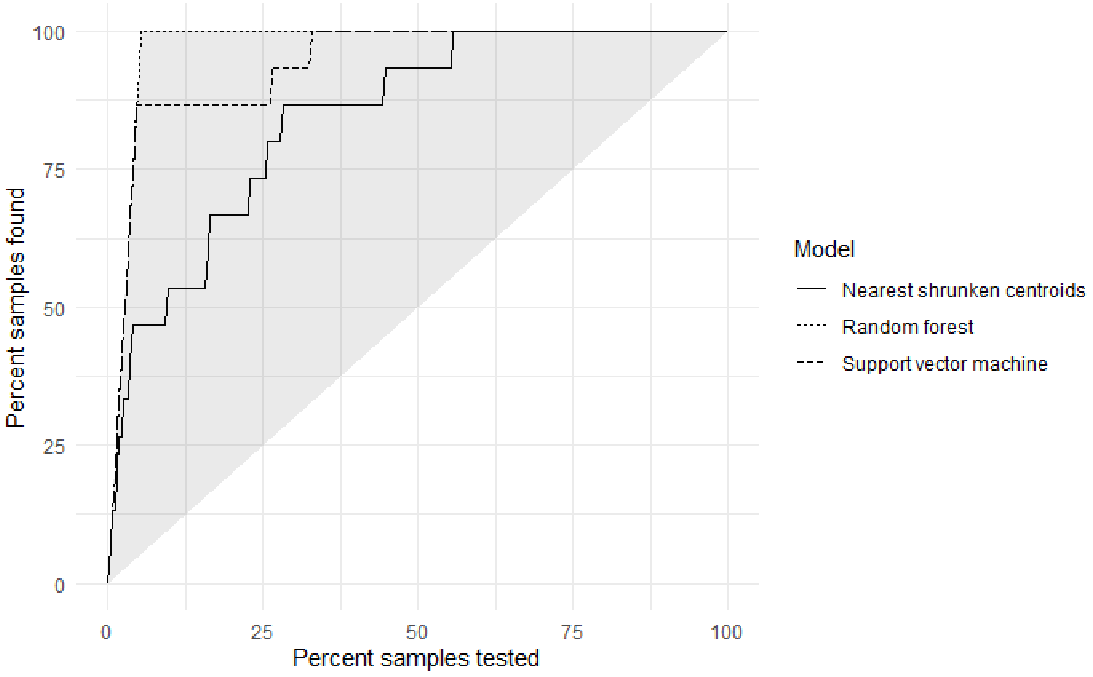

Fig. 1 Lift plot for contagious versus environmental diagnosis for random forest (dotted), support vector machines (dashed) and nearest shrunken centroids (solid)

As shown in Fig. 1, the random forest algorithm was superior to nearest shrunken centroids and support vector machines in the identification of a contagious diagnosis. There are a number of contagious observations that are detected by all algorithms, however random forest and support vector machines quickly outperform nearest shrunken centroids with the majority of contagious diagnoses, with random forest further outperforming support vector machines with the detection of the remaining contagious diagnoses.

Thresholds

As shown in Fig. 2 at the standard threshold of 0.5, whilst overall accuracy is 97.2% sensitivity is 0%. By shifting the threshold to 0.09, the overall accuracy decreases to 60%, but sensitivity increases to 100% (to the detriment of specificity dropping to 58%). This might be advantageous in a situation where the sensitivity is crucial, and specificity less important, and each model must be tuned with the biological/clinical question in mind. Figure 3 shows the effect of altering the threshold in this example of contagious vs environmental mastitis.

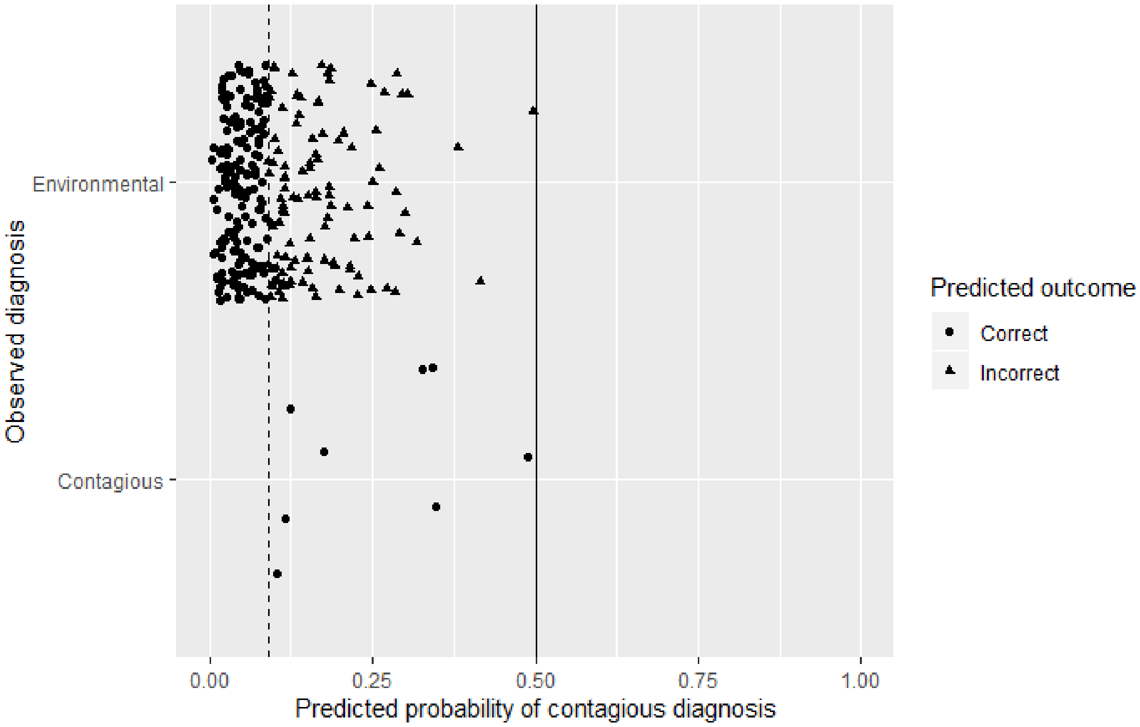

Fig. 2 Threshold plot exploring thresholds of 0.5 (solid line) or threshold of 0.09 (dashed line) in the diagnosis of contagious or environmental mastitis. Predicted outcome is correct (triangular point) or incorrect (circular point) at a threshold of 0.09

Calibration plots

As shown in Fig. 4, the algorithm returning a probability of contagious diagnosis of 0-30% is generally reasonably accurate, however when returning a probability of 35% it is only correct around 10% of the time. Similarly, when returning a probability of 40-50% it is actually often correct 100% of the time, providing potentially cautious estimates of probabilities for clinicians to interpret.

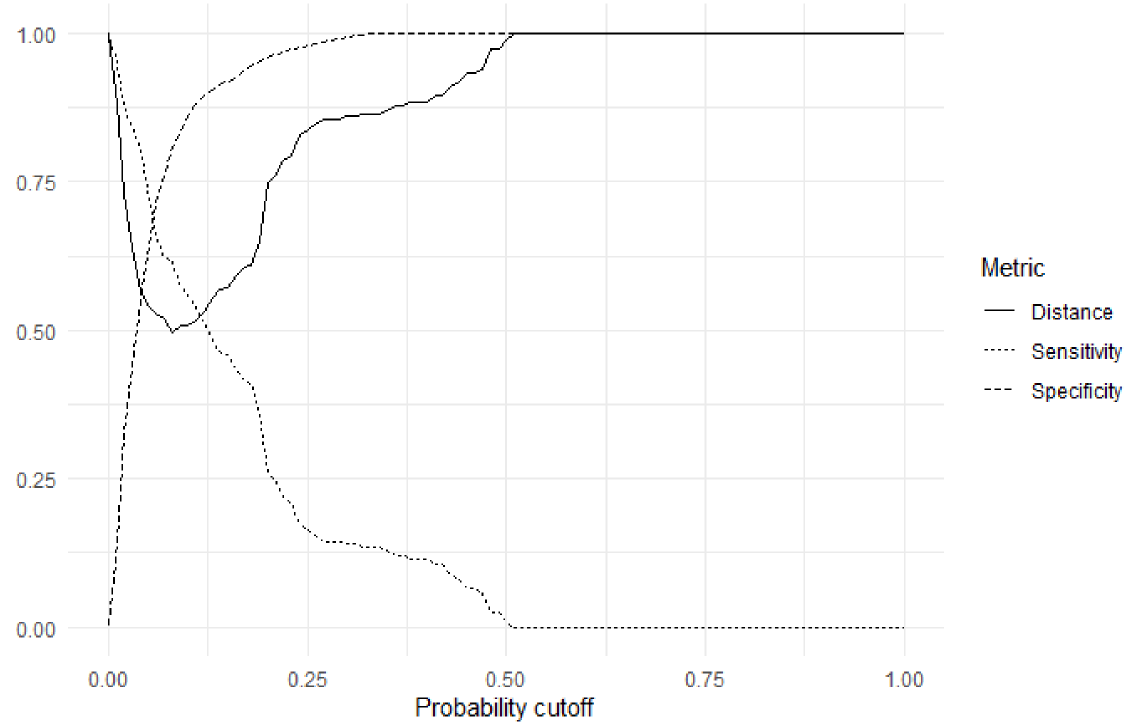

Fig. 3 Sensitivity and specificity at a range of thresholds to achieve closest distance to optimal predictive model

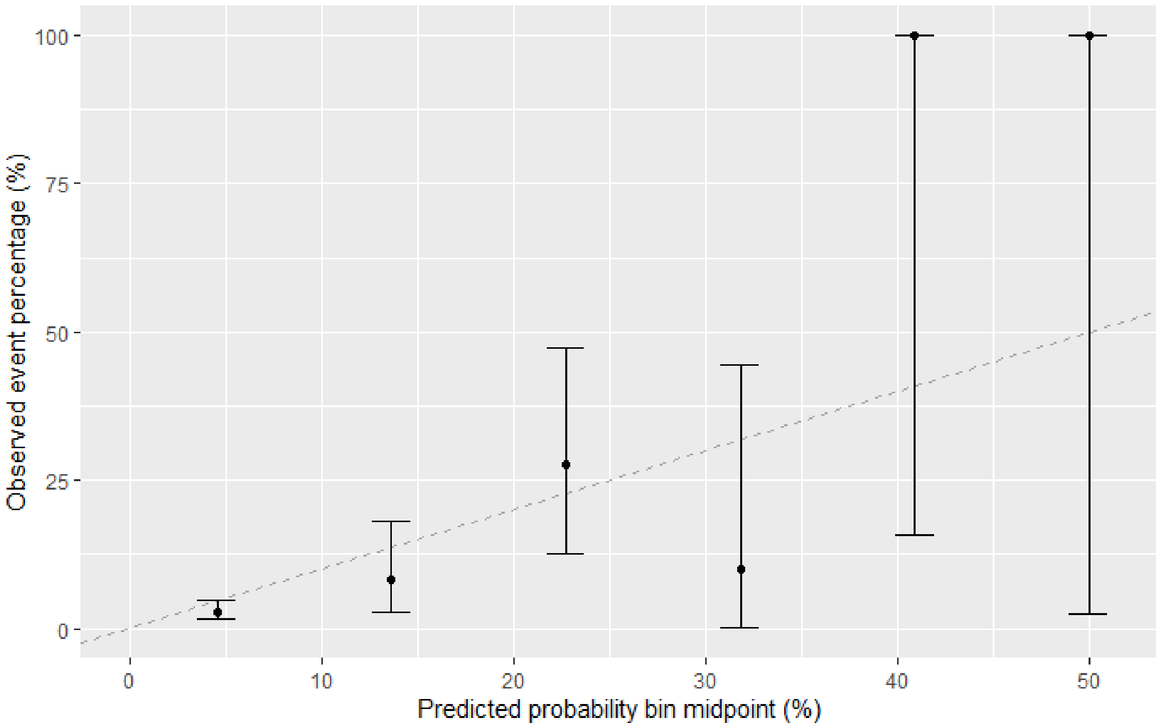

Fig. 4 Calibration plot for contagious vs environmental mastitis diagnosis comparing predicted probability of contagious diagnosis with observed percentage diagnosis of contagious diagnosis

DISCUSSION

Machine learning algorithms have great potential within the field of veterinary medicine, with potential to provide accurate decision tools for clinicians. For these tools to be translated into useful clinical tools the algorithms must be tuned to provide a performance sufficiently useful for the clinical diagnosis in question. Model performance should be robustly validated, ideally with a completely new dataset. There are many methods to improve the performance of algorithms using a variety of metrics, but whilst machine learning algorithms must demonstrate a high level of performance, an estimate of the confidence or probability of a diagnosis should also be reported or clinicians to make a reasoned clinical decision.

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USING MACHINE LEARNING TO PREDICT DISEASE IN TRANSITION PERIOD DAIRY CATTLE – THE CHALLENGES OF AN IMBALANCED DATASET AND THE IMPORTANCE OF METRICS

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AND C.D. HUDSON

SUMMARY

In our study, while attempting to fit predictive models for transition cow disease, it became evident that the metrics chosen for reporting can lead to misleading interpretation of the model's performance. When building classification algorithms, in which the goal is to correctly assign new data to predetermined classes, it is common that only accuracy, sensitivity and specificity are reported. For imbalanced datasets however, where the number of data points for each class differs substantially, the omission of metrics such as kappa or balanced accuracy can lead to inaccurate conclusions, especially since the overall accuracy is commonly very high. In this research, we illustrate the importance of reporting all relevant metrics associated with a model's performance in order to reflect the model's genuine predictive capability with new data.

INTRODUCTION

Recognising patterns in data using machine learning techniques has been a major part of data science over recent years (Biffani et al., 2017). This approach generally produces an algorithm based on identifiable 'training' data which allows predictions to be made on unseen data (Hudson et al., 2018). In recent years, the volume of data routinely collected and available for analysis has increased, with machine learning now a commonly used analytical approach. A study reported that from 2000 until 2016 the number of scientific publications each year that were relevant to machine learning grew from 10,690 to 1,211,400, increasing steadily with a peak rate between 2011 and 2013 (Biffani et al., 2017). These techniques have recently started finding a place in veterinary research, helping improve areas of farm management, such as reproductive management (Caraviello et al., 2006; Dolecheck et al., 2015; Borchers et al., 2017; Fenlon et al., 2017a,b; Zaborski et al., 2018; Higaki et al., 2019) and transition period management (Wisnieski et al., 2019).

Machine learning focuses on predictive modeling. This area differs from explanatory modeling as the latter attempts to explain the outcome based on the independent, or explanatory variables, without necessarily focusing on the predictive performance of the model (Shmueli,

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2010). Explanatory modeling generally involves using statistical methods to test causal hypotheses, where the underlying factors may be thought to cause the defined effect (Shmueli, 2010). In contrast, predictive models aim to predict specific outcomes based on available predictors with no interest in the underlying causal mechanisms. The model building process as well as the variables included in the resulting model and its predictive power are often different between the two (Shmueli, 2010; Sainani, 2014; Borchers et al., 2017) although a variety of methods (such as logistic regression) can be used for either predictive or explanatory purposes.

In order to assess a machine learning model's performance, it is essential to communicate how well the predicted outcomes match observed data points. For classification algorithms in particular, where the outcome is divided into distinct classes, the confusion matrix is a popular way of communicating model performance. It is a simple cross-tabulation of the observed data against the predicted data showing the frequency of true and false positives as well as the true and false negatives. From these values, additional "summary" metrics such as the overall accuracy, sensitivity, specificity, positive and negative predictive values and kappa can be calculated (Kuhn & Johnson, 2013). By measuring the sensitivity and specificity for different cut-off points, the receiver operating characteristic (ROC) curve can also be plotted, with the area under the curve (AUROC) being another evaluator of predictive performance (Kuhn & Johnson, 2013). An additional metric that can be used to describe an algorithm's predictive performance is balanced accuracy, which is the averaged accuracy of both classes in binary classification (Brodersen et al., 2010).

Table 1. Metrics reported in farm animal studies using machine learning methods

Metric	Studies reported
Accuracy/success rate/CCI	Caraviello et al. (2006), Mammadova et al. (2013), Dolecheck et al. (2015), Borchers et al. (2017), Fenlon et al. (2017a), Ebrahimie et al. (2018), Shahinfar et al. (2014), Sharifi et al. (2018), Zaborski et al. (2018)
Sensitivity and specificity	Caraviello et al. (2006), Kamphuis et al. (2010), Mammadova et al. (2013), Vergara et al. (2014), Dolecheck et al. (2015), Panchal et al. (2016), Borchers et al. (2017), Zaborski et al. (2018), Wisnieski et al. (2019)
AUROC	Shahinfar et al. (2014), Vergara et al. (2014), Hempstalk et al. (2015), Panchal et al. (2016), Zaborski et al. (2018), Wisnieski et al. (2019),
PPV/NPV	Wisnieski et al. (2019)
Diagnostic Odds Ratio	Panchal et al. (2016)

Several studies in farm medicine that have used machine learning methods to develop predictive algorithms for classification have reported metrics such as the overall accuracy, sensitivity, specificity and AUROC (Table 1). When predicting health outcomes in particular, the metrics reported were again accuracy (also sometimes reported as "success rate" or Correctly Classified Instances (CCI)); sensitivity and specificity; AUROC; or the diagnostic odds ratio, which is the ratio of the odds of a positive predicted value being a true positive, over a positive predicted value being a false negative (Table 1). Out of the studies evaluating transition period health management Wisnieski et al. (2019) used logistic regression models to predict metabolic stress and reported sensitivity, specificity, AUROC and well as the positive and negative predictive values (PPV/NPV). Similarly, Vergara et al. (2014) in a study exploring postpartum issues in dairy cows reported the predictive models' AUROC, sensitivity and specificity (Table 1).

Additional metrics, including the kappa and balanced accuracy were not reported in any of the studies, even though they would complement existing findings and give a more complete picture of the model's performance. It is clear that whereas imbalanced datasets with unequal number of observations per class are not uncommon, especially when dealing with disease outcomes, many studies focus on the accuracy. Some studies do include information on the AUROC that could help account for outcome balance. It has been argued, however, that the AUROC can be misleading for a number of reasons. One such reason is being correlated with prevalence, meaning that in binary classification thresholds need to be adjusted when the classes are not equal (Lobo et al., 2008). Brodersen et al. (2010) also described a common issue when dealing with binary classification in imbalanced datasets, where the algorithm tends to be biased towards the most frequent class.

Since a model's apparent predictive value can differ dramatically depending on the metrics reported, the purpose of this research was to consider the importance of metric selection in predictive modeling when dealing with an imbalanced dataset.

MATERIALS AND METHODS

Data collection

This study used a dataset collected as part of a commercial service designed to monitor the success of transition period management in UK dairy farms. Six assessors conducted monthly visits to each farm enrolled in the service, evaluating cow- as well as pen-level variables. The assessors were trained together and performed frequent informal joint visits to maximise inter observer consistency. A total of 133 herds participated in the service and 79 of these herds which had complete milk recording data were used for this analysis. Variables available in the dataset are displayed on Table 2.

Data cleaning

All data analysis was conducted using R version 3.5.1 (R Core Team, 2018). The cow and pen level data were amalgamated into a single dataset and were then combined with the additional files containing the milk recording organizations' data on calving dates and milk data.

The calving date was not available in the cow-level dataset, hence the milk recording dataset was used to provide this information. All files were merged to create the final dataset, based on the cow ID. The units of data were a transition event for a particular cow, including the pre- and post- calving data. For some cows, multiple pre- and/or post-calving recordings were captured per calving event. In this case, only the closest pre-calving score to 20 days before calving and the closest post-calving score to 25 days after calving were kept, such that there was one entry per cow per lactation. Furthermore, by applying these restriction scores far from the calving date, potential outliers were excluded from the analysis.

The body condition score (BCS), rumen fill and hock hygiene were all scored in a scale of 1 to 5. Animals with a mobility score >=2on a 0-3 scale were categorized as lame in a binary representation. Some additional variables (water trough space per cow, feed fence space per cow, stocking density, BCS change [from pre- to post-calving] and temperature-humidity index [THI]) were calculated from data provided. THI was calculated according to NRC (2001), where T is the dry light bulb temperature (°C) and H is the relative humidity of the air (%) (Eq.1).

$$\mathrm{THI} = (1.8 * \mathrm{T} + 32) - (0.55 - 0.0055 * \mathrm{H}) \qquad (1)$$

Mean daily milk yield over the preceding lactation was recoded as categorical with three levels (heifers [which had no previous yield], <30 litres, ≥ 30litres). Similarly stocking density was summarised as a categorical variable with 6 levels (cubicles <90%, cubicles 90-100%, cubicles > 100%, loose yard stocking density < 10 m^2/cow, loose yard stocking density 10-12 m^2/cow, loose yard stocking density > 12 m^2/cow).

Table 2. Variables available in original datasets

Cow level	Pen level	Milk recording data
Farm ID	Farm ID	Farm ID
Period of data collection	Period of data collection	Animal ID
Date of data collection	Type of cows	Ear Tag
Assessor	Pen Name	Date on Farm
Cow Number	Type of Pen	Date of Birth
Dry or Fresh	Pen length	Date of Exit
Heifer	Pen width	Calving Date
Found for assessment	Pen area	Lactation Number
BCS	Feed Fence Space	Lactation ID
Rumen Fill	Water Trough Space	Daily Milk Yield
Hock Hygiene	Neck Rail Height	Protein
Lameness	Number of Cows in Pen	Butterfat
Milk Fever	Cows waiting	Milk ID
LDA	Cows not waiting	Date of Milk recording
RFM	Feed available	DIM at Milk recording
Calf mortality	Water available	
Twinning	Temperature	
Metritis	Humidity	
	Bedding quality	
	Air quality	
	Feed quality	
	Water quality	
	Light quality	
	Cubicle count	
	Pen score	

The variables for bedding, feed, water, light and air quality were binary, marked as negative if the at least one recording of the pen throughout the collection period was scored as “Not Good”. Similarly, feed and water were scored as unavailable if there was at least one such recording for the pen. As only a minority of farms had designated pens for the fresh cows, the pen level data available post-partum were limited and it was decided to drop these variables due to the large number of missing data. All variables considered for the final analysis are shown on Table 3.

Model building

A binary outcome variable was created representing a cow experiencing at least one postpartum disease event (milk fever, left displaced abomasum, metritis, retained foetal membranes) per lactation.

Table 3. Predictive variables considered in final analysis

Variable	Missing data
Rumen Fill in pre-calving cows	86 (0.007%)
Hock Hygiene	1151 (0.089%)
Neck Rail Height in the pre-calving pen	5421 (43.41%)
THI in the pre-calving pen	5450 (42.42%)
Feed Fence space per cow in the pre-calving pen	5507 (42.87%)
Water Trough space per cow in the pre-calving pen	5527 (43.02 %)
Good Bedding quality in the pre-calving pen	4763 (37.07%)
Good Light Quality in the pre-calving pen	4763 (37.07%)
Good Feed Quality in the pre-calving pen	4763 (37.07%)
Good Water Quality in the pre-calving pen	4763 (37.07%)
Good Air Quality in the pre-calving pen	4763 (37.07%)
Feed available in the pre-calving pen	4763 (37.07%)
Water available in the pre-calving pen	4763 (37.07%)
BCS pre-calving	77 (0.006%)
BCS change pre- to post-calving	1201 (0.09%)
Month of pre-calving recording	0 (0.00%)
Lactation number	0 (0.00%)
Calf Mortality	0 (0.00%)
Twining	0 (0.00%)
Mean Milk Yield of previous lactation	644 (0.05%)
Mean Protein % in milk in the previous lactation	644 (0.05%)
Mean Butterfat% in milk in the previous lactation	644 (0.05%)
Stocking density in pre-calving pen	5494 (0.43&)
Lameness in pre-calving cows	0 (0.00%)

In order to determine the combination of variables that produce the most predictive models feature selection using a wrapper method was implemented. The wrapper method of choice was forward selection, adding variables to the model one at a time while assessing model performance based on the kappa value. The final models from each machine learning technique were compared. The machine learning techniques used included logistic regression, decision trees, random forest, artificial neural networks, naïve Bayes, support vector machines and k-nearest neighbours. A 10 fold validation was performed in all methods to measure predictiveness of the models using R's "caret" package (Kuhn, 2008). Models were generated and evaluated both without resampling and using upsampling, in order to explore how the balance between the two outcome classes affected the models' performance. Upsampling takes samples with replacement from the less frequent class until both classes have the same number of observations. The best tuning parameters were selected for each technique based on which combination produced the largest value of kappa. The metrics that were calculated and compared between the models were accuracy, sensitivity, specificity, PPV, NPV, Kappa (the difference between the observed agreement and expected agreement by chance), balanced accuracy (the average of the individual accuracies per class), detection rate and F1 (the harmonic mean of the sensitivity), PPV and AUROC. As the outcome was unbalanced (the vast majority of cows not experiencing a disease event), special consideration was given to kappa as a measure of likely overall predictiveness, using the thresholds stated in Viera and Garrett (2005) (Table 4).

RESULTS

The original number of observations in the cow level scoring dataset was 71,665 collected from 133 farms, including 43,185 fresh cow observations and 28,480 dry cow observations. After having a calving date match for 97.8% of all the data points, and filtering the data collection date to be within 100 days from calving, the maximum number of observations fell to 26,818 (62.1%) for fresh cows and 16,511 (57.97%) for dry cows. The final filtering in order to include one dry and one fresh scoring per cow per lactation was applied and the total number of transition events included in the dataset was 12,847 from 79 farms. The data were collected from 3rd March 2016 until the 24th October 2018. The binary post-calving disease outcome had a prevalence of 10.98% in the final dataset.

The combination of variables that produced the most predictive models were the rumen fill pre and post-partum, neck rail height in pre-calving pens, lactation number, hock hygiene, BCS change, BCS pre-calving, THI in the pre-calving pen, stocking density in the pre-calving pen, mean milk yield in the previous lactation, calf mortality and twinning.

Table 4. Interpretation of kappa values (Viera & Garrett, 2005)

Kappa	Agreement
<0	Less than chance agreement
0.01 – 0.20	Slight agreement
0.21 – 0.40	Fair agreement
0.41 – 0.60	Moderate agreement
0.61 – 0.80	Substantial agreement
0.81 – 0.99	Almost perfect agreement

Without the use of any sampling methods to make the outcome classes equal, the accuracy of all methods ranged between 87 and 88%, the kappa between 0.053 and 0.1043, the specificity from 98.1% to 99.6%, the sensitivity from 4.2% to 23.14%, AUROC from 51.7% to 56.6%, NPV from 88.8% to 90.0%, PPV from 23.3% to 61.8%, balanced accuracy from 51.7% to 56.6%, F1 from 0.08 to 0.23 and finally detection rate from 0.004 to 0.027. Without a sampling technique the decision tree algorithm could not converge and was not included in this comparison. After performing upsampling, all techniques including the decision tree were able to produce predictive algorithms with improved predictive value. While the overall accuracy remained fairly similar or in some cases dropped significantly between the two sampling methods (from 61% to 86%) the kappa values improved for almost all models. For the random forest model, which had the highest kappa value, kappa was increased from 0.10 without sampling to 0.16 with upsampling. The sensitivity of all models, even though still overall low, greatly improved appearing to be balancing out with the specificity. The PPV for these models, however, significantly dropped. All metrics for all 7 techniques are displayed in Fig. 1.

DISCUSSION

From these results it is evident that, where the outcome is unbalanced, even relatively poorly predictive models can demonstrate a high accuracy metric. The random forest, which had one of the highest accuracies, PPV/NPV balance and kappa values, was lacking in predictive power. More careful evaluation of the metrics (especially kappa and balanced accuracy, which account for unbalanced outcomes), showed that all models lacked predictive power and even though upsampling seemed to improve model performance, the resulting algorithms were unlikely to be sufficiently predictive to be useful in practice.

Accuracy was fair in all models even after using resampling methods, further indicating how misleading it may be. Sensitivity and specificity alone were also not able to accurately reflect the model's performance in all cases. For the random forest, decision tree and naïve Bayes algorithms, while specificity was high the sensitivity was extremely low, portraying the issues with the balance of the classes. For the rest of the algorithms, however, there was a better balance between the two, with the sensitivity being close to 0.6, not reflecting the model's inability to make accurate predictions. This indicates that these metrics can vary between methods and will not be sufficient to judge model performance. Since accuracy, sensitivity and specificity appeared to be amongst the most commonly reported metrics in literature, this raises some issues. These issues become apparent in our study as the kappa in all models was below the 0.4 threshold that is needed for the classifier to be considered to have at least moderate agreement (Viera & Garrett, 2005). This further validates the point that accuracy alone or even combined with sensitivity and specificity are not enough to assess predictive models, especially when analysing imbalanced datasets, similar to our case where the disease prevalence was just 10.98%.Brodersen et al. (2010) suggested that balanced accuracy should completely replace the overall accuracy, in particular to tackle the issue of imbalanced datasets. These results would support that view; balanced accuracy in all models reported here was just over 50%, which could be explained by the algorithm's tendency to assign the most frequent class to all predictions. The balanced accuracy calculates the accuracy of both classes equally and then simply averages them. When dealing with an imbalanced dataset it is common that maximum overall accuracy is achieved when misclassifying all data points from the minority class by assigning all predictions as the majority class. In this case however, the accuracy of the majority and minority groups would be 1 and 0 respectively, making the balanced accuracy 0.5. In our study it becomes clear that since the balanced accuracy is close to 0.5, while for most models the overall accuracy is very high, there is an issue with the balance between the two classes.

Viera and Garrett (2005) described how kappa is influenced by the prevalence of the disease examined, hence the frequency of its class in the dataset. In the literature we found a clear preference for reporting the overall accuracy, without complementation with additional metrics that take the proportion of the classes into account (Table 1). Whereas some studies do include the AUROC which can reflect this issue, it has been argued that it is not an appropriate metric in every situation (Lobo et al., 2008). From our results it is evident that kappa is one of the metrics most affected by dataset imbalance.

Another issue that is raised in our study is algorithm selection. In imbalanced datasets if the selection were to be based mainly on the overall accuracy, models with potentially low predictive power could be favoured since as mentioned above high overall accuracy can easily be achieved by misclassifying the minority class. One such case is the naive Bayes model in our example, which has one of the highest overall accuracies but also one of the lowest balanced accuracies and a very low kappa. In fact, it does not actually surpass in predictive power the logistic regression model, which has much lower overall accuracy. Therefore, it is evident that if not based on appropriate metrics, even model comparison can be misguided.

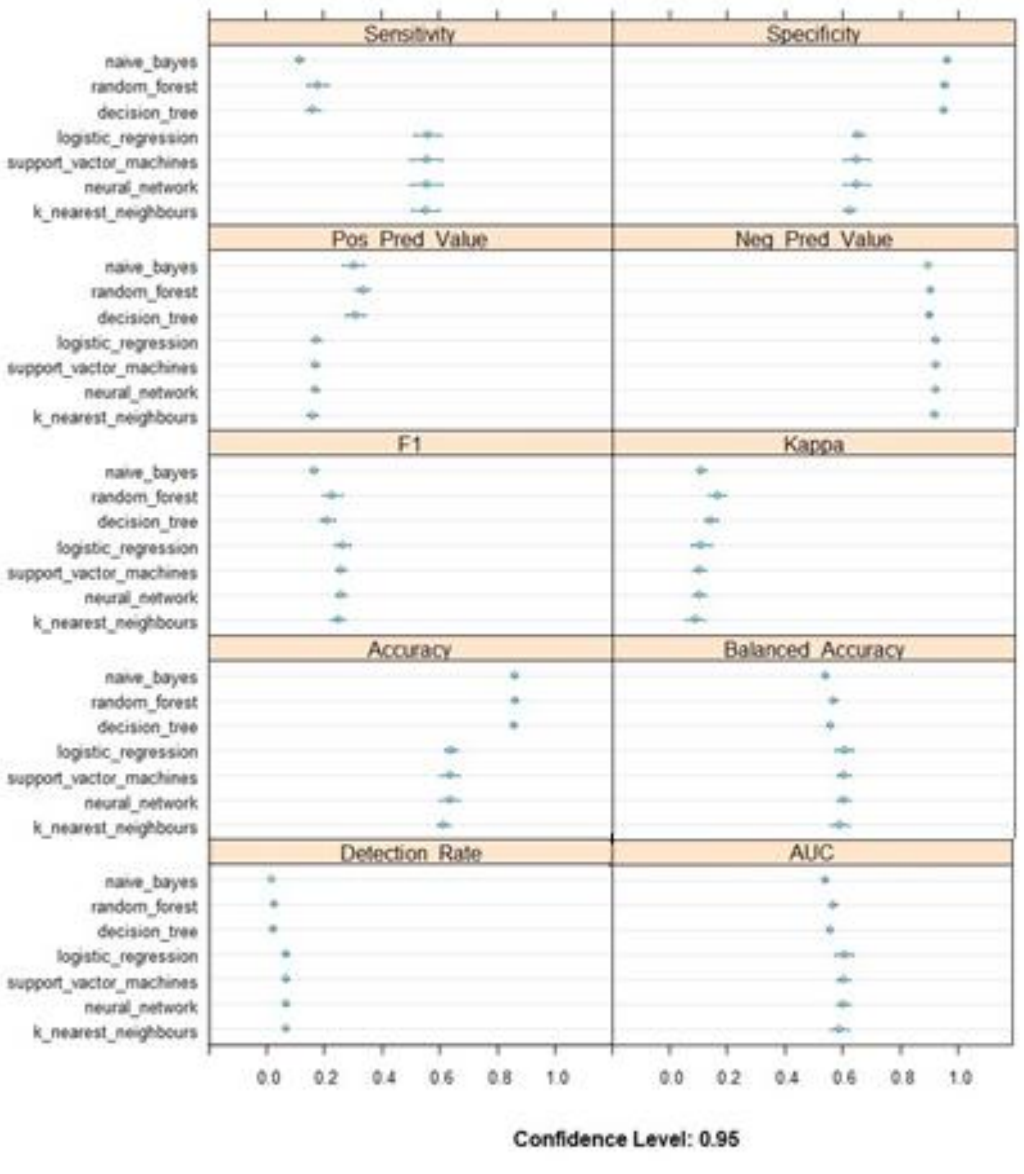

Fig. 1 Metric comparison of all models after using upsampling with 95% confidence intervals

There are a number of limitations in our study, such as the number of missing data in the predictors and potential under-reporting of certain diseases. These limitations however could potentially affect the actual predictive value of our models and their generalisability, which is not the focus of this manuscript. The methodology of the models' assessment remains the same, regardless of any such issues. Likewise, the model comparison presented here is indicative, serving as an example of algorithm selection and how it can be generally affected depended on the metrics chosen for comparison, when built using the same dataset. It does not serve as a definitive choice of algorithm for our specific research question and data as, as established, the predictive value of all models, even if assumed accurate, is very low.

It is clear from the literature that machine learning algorithms in the veterinary epidemiology field are commonly still reported using accuracy as the headline metric. This work would support the more widespread adoption of alternative metrics such as balanced accuracy or kappa, especially where the outcome being predicted is either very common or very rare.

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FOOD AND FEED SAFETY

INDUSTRY EXPANSION AND SALMONELLA SURVEILLANCE TRENDS FOR RAW MEAT PET FOOD IN GREAT BRITAIN OVER THE LAST TEN YEARS

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SUMMARY

The raw meat pet food industry has expanded in Great Britain (GB) over recent years as a result of the increasing popularity of these products with pet owners. Surveillance data suggest that there has been an associated increase in the occurrence of *Salmonella* in these products. Findings include serovars, including multi-drug resistant serovars, which are not endemic in GB food animals and of serious concern for public health and food animal production, particularly poultry.

INTRODUCTION

The feeding of raw meat pet food (RMPF) to companion animals such as dogs and cats has recently increased in popularity, but such diets are controversial. Proponents assert that they are more natural and claim a number of health benefits for pets over more conventional diets of dry or canned food (e.g. Morgan et al., 2017). However, such diets carry potential animal and human health risks due to contamination of uncooked meat with pathogens.

Studies have shown that RMPF can harbour dangerous zoonotic pathogens (Davies et al., 2019), and that feeding dogs RMPF is a risk factor for faecal shedding of these pathogens (Leonard et al., 2011). Of particular concern is contamination with *Salmonella* bacteria. In the United Kingdom (UK), human infections with *Salmonella* represent the leading cause of hospitalisation amongst foodborne zoonotic pathogens, and there were an estimated 47,000 human cases in 2017 (PHE, 2018). Dogs and cats may also become clinically ill when infected (Morley et al., 2006), but are often asymptomatic carriers of these bacteria (Finley et al., 2007). Contaminated RMPF, which does not undergo any heat treatment to deactivate pathogens, may therefore represent a potential source of human *Salmonella* infection, with the pathogen being spread through handling contaminated feed (or food bowls - Weese & Rousseau, 2006) or through infected pets shedding the pathogen into the household environment.

Increasing the volume of RMPF products on the market as a result of growing consumer demand may lead to more frequent introduction of *Salmonella* into households where these products are consumed, thus increasing the risk of human infection. Of particular concern are serovars of *S. enterica* such as *S.* Typhimurium, *S.* Enteritidis and *S.* Infantis, which are important causes of human disease. In addition, since imported animal material is routinely

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incorporated into commercially-produced RMPF, there is also a concern that non-endemic strains of *Salmonella* may be introduced into GB livestock by contact with pets that have been fed these products, or by people who have handled it.

Numerous studies of commercially produced RMPF have been conducted in North America, which have found the proportion of samples containing *Salmonella* to range between 7.1% (Strohmeyer et al., 2006) and 21% (Finley et al., 2008). However, there are few published investigations of *Salmonella* contamination in commercial RMPF produced in Europe (but see Fredriksson-Ahomaa et al., 2017 and van Bree et al., 2018) and no published studies from GB. An assessment of *Salmonella* occurrence in GB-produced RMPF is therefore needed to understand the risk posed by these products to consumers, their pets, and to livestock.

Pet food production in GB is regulated in accordance with European legislation. Commercial manufacturers of pet food must register their premises with the Food Standards Agency (FSA) under the requirements laid down in the European Feed Hygiene Regulations (EC 183/2005). In addition, the premises of any manufacturer wishing to incorporate animal by-products (ABPs) into their pet food products must be approved by the Animal and Plant Health Agency (APHA) under the European ABP Regulations (EC 1069/2009 and EU 142/2011), and additional controls apply to producers of raw pet food. These regulations define raw pet food as having not undergone any preserving processes other than chilling or freezing, and stipulate that only certain types of low-risk Category 3 ABPs are permitted for use. These include material classed as fit but not intended for human consumption, but also any material unfit for human consumption as long as slaughter took place in a slaughterhouse, it was considered fit following ante-mortem inspection, and post-mortem examination did not find signs of communicable disease.

The EU ABP Regulations require random testing of products at all approved premises for the presence of bacterial pathogens including *Salmonella*. In GB, this testing is overseen by the APHA. Sampling regimes vary across premises, being developed with the APHA and customised to individual premises according to batch size, type of animal derived product used and throughput (PFMA, 2017). In general, samples comprising ten sub-samples of 30g taken at random are collected from each product line at a frequency reflecting production volume. Sampling frequency may therefore vary (e.g. from weekly to monthly), with those plants producing multiple different products and/or greater overall volumes expected to collect samples more frequently than those producing fewer products or lower overall volumes. Samples are then sent to UKAS-accredited laboratories for *Salmonella* testing. Detection of *Salmonella* requires the Local Authority and the APHA to be notified. Isolates are then sent to the APHA for confirmation and serotyping under the requirements of the Zoonoses Order (1989), and antimicrobial resistance (AMR) testing is also carried out. The recall and disposal of any contaminated products or material is undertaken at this time, and the cause of contamination must then be investigated, alongside a clean-down of production facilities, additional sampling, and development of APHA-agreed plans to resolve the problem and prevent reoccurrence (Defra, 2018).

Here we describe trends in the reporting of *Salmonella*, including serovar diversity and antimicrobial resistance profiles, in samples submitted to the APHA as a result of this statutory testing between 2008 and 2018. This is the first time that national surveillance data have been used to investigate the occurrence of pathogens in commercially produced RMPF in GB.

MATERIALS AND METHODS

Raw pet food manufacturing plants in GB

The number of pet food manufacturing plants registered to produce raw pet food using ABPs in GB were obtained from data previously extracted from the APHA's System for Recording Animal By-Product Information (SRABPI) database. Data were available for each year from 2011 to 2018. This database is continuously updated; the data for each year were extracted in April of that year and therefore represent annual snapshots of the industry.

Salmonella serotyping and antimicrobial resistance (AMR) testing

Salmonella isolates received by the APHA were serotyped according to the White-Kauffmann-Le-Minor scheme (Jones et al., 2000). Isolates of the regulated serovars *S.* Enteritidis and *S.* Typhimurium (including its monophasic variants *S.* 4,12:i:- and *S.* 4,5,12:i:-) were further characterised by phage-typing according to the Ward and Anderson phage-typing schemes respectively (Anderson et al., 1977; Ward et al., 1987).

Salmonella isolates were tested for their in vitro sensitivity to a panel of 16 antimicrobials which are used in veterinary and/or human medicine in the UK or are useful epidemiological indicators. All tests were performed using a disc diffusion technique on Iso-Sensitest agar and antimicrobial-containing discs (Wray et al., 1991; Jones et al., 2002).

Data retrieval and preparation

The serovars, phage types and AMR profiles (resistance count and resistance pattern) of *Salmonella* isolates from samples of commercially produced raw pet food were retrieved from the APHA *Salmonella* database for the years 2008 to 2018 inclusive. Each isolate was obtained from a unique sample of raw pet food. In addition to test results, the database holds epidemiological information for each sample, including the location and date collected. Information about the animal species of meat tested and whether the meat is imported or produced in GB is also stored in the database when provided with a *Salmonella* submission. The characteristics of all *Salmonella* isolates arising from samples of compound feed intended for cats and dogs were also retrieved from this database for the years 2014 to 2018 inclusive.

RESULTS

Raw pet food manufacturing plants in GB

The number of plants approved to produce raw pet food using animal by-products in GB increased from 21 to 42 between 2011 and 2015. There was then a steep increase, with the number of plants reaching 109 in 2017 and remaining high in 2018 (Fig. 1). Turnover of producers also appears to be high; in total, 161 different plants were approved in at least one year during this time period, with 62 of these no longer approved by 2018.

In total, *Salmonella* was isolated from 75 different plants between 2008 and 2018. The number of plants reporting *Salmonella* isolations per year increased over time, rising from three plants in 2008 to 37 in 2018 (Fig. 1). Per year, most *Salmonella*-positive plants reported few isolations and the annual median number of isolations per site never exceed three. However, three different plants each reported ≥30 isolations in 2018 (data not shown).

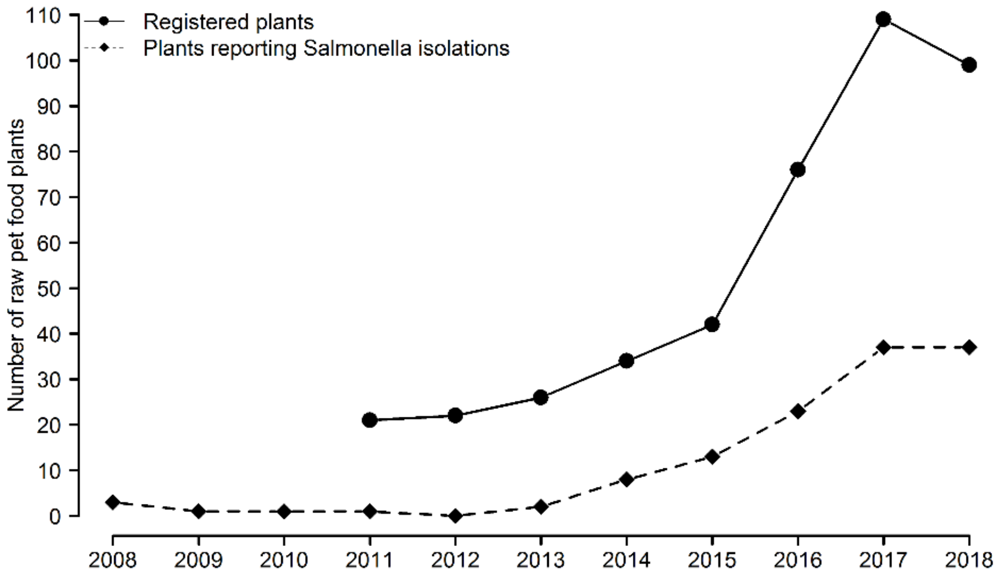

Fig. 1 The number of plants registered to produce raw pet food using ABPs in GB since 2011, and the number of plants reporting at least one Salmonella isolation since 2008

Salmonella isolations and incidents

There were 709 *Salmonella* isolations from raw pet food between 2008 and 2018. The majority of isolations occurred from 2014 onwards, with only 6% of isolations (n=44) being made prior to this. Since 2014 there were between 26 and 244 *Salmonella* isolations per year from raw pet food. In contrast, the annual number of isolations from compound feed intended for cats and dogs was considerably lower (between four and 27 per annum; Fig. 2).

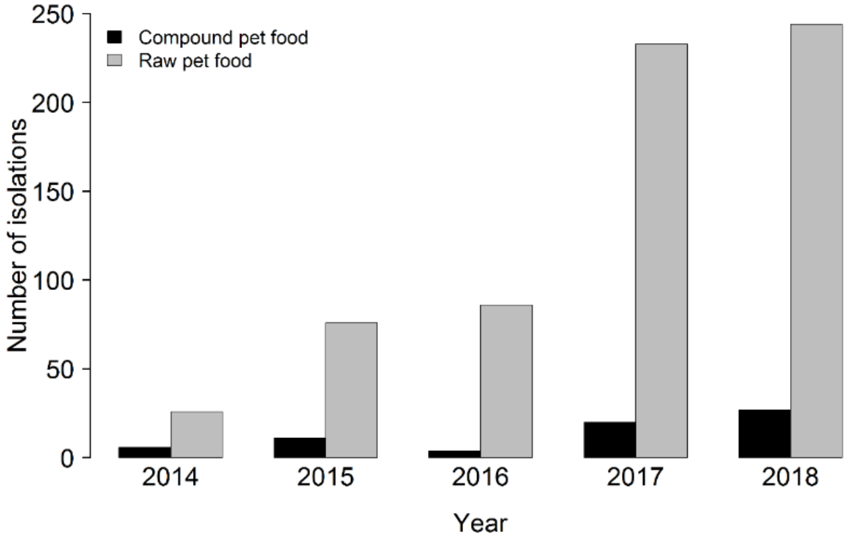

Fig. 2 The annual number of Salmonella isolations from samples of RMPF and compound pet food intended for cats and dogs

There were 37 isolations in 2008, and 33 of these isolations were collected from the same premises on the same day. From 2009 to 2013, the annual number of isolations was low (between zero and four; Fig. 3), but this increased more than six-fold between 2013 and 2014 (from four in 2013 to 26 in 2014). The annual number of isolations then increased in each subsequent year, reaching 244 by 2018 (Fig. 3). Isolations of regulated serovars were reported every year since 2013 and accounted for between 10% and 75% of all isolations annually (Table 1; Fig. 3).

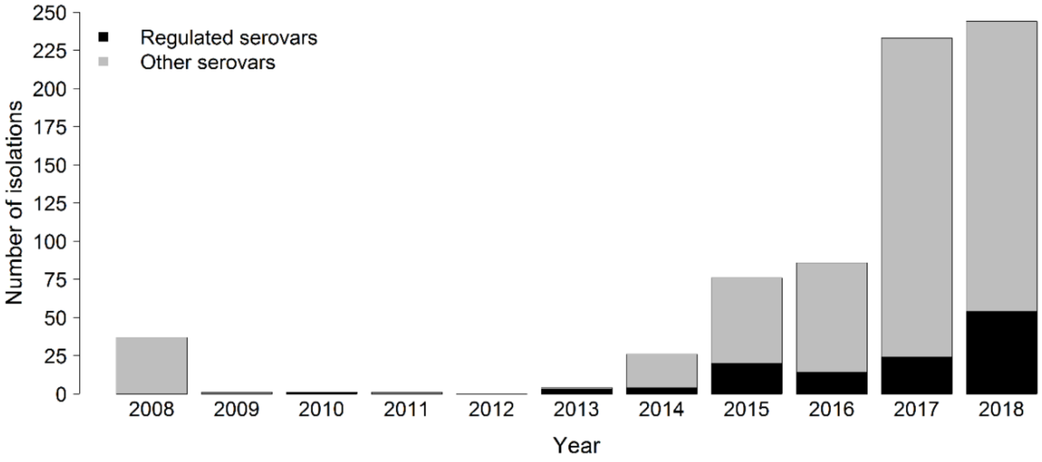

Fig. 3 The annual number of Salmonella isolations from samples of RMPF. Regulated serovars are S. Typhimurium, S. 4,12:i:-, S. 4,5,12:i:-, S. Infantis, S. Hadar and S. Enteritidis

<u>Salmonella serovars</u>

Sixty-six different serovars were identified during the study period. The number of different serovars isolated per year ranged between one and 39, and increased markedly from 2013 onwards. The most common serovar to be isolated was *S.* Typhimurium. Including its monophasic variants *Salmonella* 4,12:i:- and *Salmonella* 4,5,12:i:-, there were 103 isolations of *S*. Typhimurium during the study period, and annually it was the most frequently isolated serovar in all but one year (Table 1). In total, isolations of *S*. Typhimurium and its monophasic variants were reported from 29 different manufacturing plants (Table 2).

In addition to *S*. Typhimurium, three other regulated serovars were isolated from samples of RMPF, all since 2013 (Table 1): there were 14 isolations of *S*. Infantis (from nine manufacturing plants), two isolations of *S*. Hadar (from two different plants) and one isolation of *S*. Enteritidis (PT21). Overall there were 120 isolations of regulated serovars (Table 1), originating from 32 different manufacturing plants. Fourteen other serovars were isolated at least ten times throughout the study period (Table 1).

Table 1. Annual number of *Salmonella* isolations from samples of RMPF. All serovars listed were isolated at least 10 times, except for *S*. Hadar and *S*. Enteritidis, which are included here because they are regulated serovars

Serovar	2008	2009	2010	2011	2013[a]	2014	2015	2016	2017	2018
All serovars	**37**	**1**	**1**	**1**	**4**	**26**	**76**	**86**	**233**	**244**
S. Typhimurium[b]	-	-	1	-	1	1	9	-	4	31
S. 4,5,12:i:- [b]	-	-	-	-	1	-	2	8	-	12
S. 4,12:i:- [b]	-	-	-	-	-	3	5	5	12	8
S. Infantis[b]	-	-	-	-	1	-	2	-	8	3
S. Hadar[b]	-	-	-	-	-	-	1	1	-	-
S. Enteritidis[b]	-	-	-	-	-	-	1	-	-	-
S. Indiana	-	-	-	-	-	1	3	9	51	24
S. Montevideo	33	-	-	-	-	2	-	8	16	7
S. Derby	2	1	-	-	-	-	5	7	13	23
S. Kottbus	1	-	-	-	1	1	8	13	11	14
S. Mbandaka	-	-	-	-	-	-	8	9	9	19
S. Reading	1	-	-	-	-	-	-	-	16	7
S. Kedougou	-	-	-	-	-	4	6	2	2	6
S. Orion	-	-	-	-	-	2	5	-	7	6
S. Newport	-	-	-	-	-	-	-	-	9	10
S. Panama	-	-	-	-	-	-	1	2	9	5
S. Bovismorbificans	-	-	-	-	-	-	4	1	10	-
S. Dublin	-	-	-	-	-	-	-	2	1	9
S. Give	-	-	-	-	-	1	1	1	3	6
S. Ohio	-	-	-	-	-	1	-	-	5	4

[a] No *Salmonella* was isolated in 2012 [b] Regulated serovars

Phage types of *Salmonella* Typhimurium

Most isolates of *S*. Typhimurium and monophasic *S*. Typhimurium were phage-typed (Table 2). Ten phage types of *S*. Typhimurium, four of *S*. 4,5,12:i:- and three of *S*. 4,12:i:- were identified (Table 2). Eight isolates could not be characterised according to the available phage-typing scheme (they either did not conform to a known phage type (RDNC) or did not react to any of the phages tested (UNTY) (Table 2).

Table 2. Number of S. Typhimurium, S. 4,5,12:i:- and S. 4,12:i:- isolates from samples of raw pet food each year and the number of manufacturing plants that reported each serovar and phage type

Phage type	No. isolates	No. premises	2010	2013	2014	2015	2016	2017	2018
***S*.typhimurium** [a]									
U302	11	4	-	-	-	8	-	-	3
DT104	6	3	-	-	-	-	-	2	4
U288	3	3	-	-	-	-	-	-	3
DT1	1	1	-	-	-	-	-	1	-
DT12	4	1	-	-	-	-	-	-	4
DT29	1	1	-	-	1	-	-	-	-
DT104b	1	1	-	1	-	-	-	-	-
DT193	1	1	-	-	-	-	-	1	-
DT204e	1	1	-	-	-	-	-	-	1
DT208	1	1	-	-	-	1	-	-	-
RDNC	9	2	-	-	-	-	-	-	9
UNTY	4	4	1	-	-	-	-	-	3
NOPT [b]	4	-	-	-	-	-	-	-	4
***S*. 4,5,12:i:-**									
DT193	19	8	-	-	-	1	7	-	11
DT12	1	1	-	-	-	-	-	-	1
DT29	1	1	-	-	-	1	-	-	-
DT208	1	1	-	-	-	-	1	-	-
UNTY	1	1	-	1	-	-	-	-	-
***S*. 4,12:i:-**									
DT193	22	10	-	-	3	4	4	8	3
DT120	6	3	-	-	-	1	1	-	4
U311	2	2	-	-	-	-	-	1	1
UNTY	3	1	-	-	-	-	-	3	-
TOTAL	**103**	**29** [c]	**1**	**2**	**4**	**16**	**13**	**16**	**51**

[a] *S*.typhimurium was not isolated in 2008, 2009, 2011 or 2012

[b] Not phage typed

[c] Seven premises reported isolations of more than one variant of *S*. Typhimurium

The most common phage type overall was DT193, which accounted for the vast majority of incidents involving monophasic variants *S*. 4,5,12:i:- (11/15 incidents) and *S*. 4,12:i:- (15/21 incidents) (Table 2). In contrast, a greater diversity of bi-phasic *S*. Typhimurium phage types were identified, with only three phage types being involved in more than one incident (U302, DT104 and U288; Table 2) and only two of these being reported in more than one year (U302 and DT104; Table 2).

In total, eight different premises reported multiple incidents of *S*. Typhimurium or its monophasic variants during the study period. In all but two cases, incidents occurring at the same premises involved different phage types. In addition, on three separate occasions (and at three different plants) multiple phage types of *S*. Typhimurium were isolated from the same location on the same day (data not shown).

Antimicrobial resistance patterns of *Salmonella* isolates

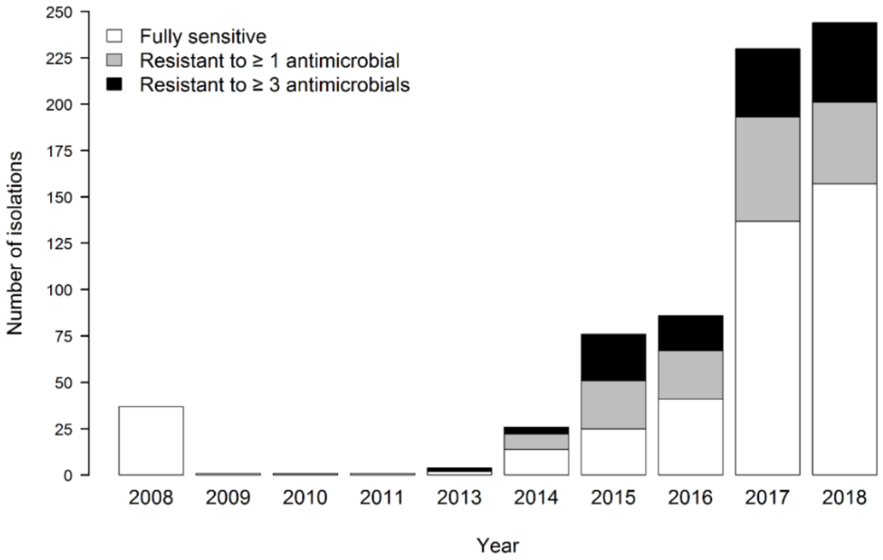

Fig. 4 Number of Salmonella isolations per year that were fully sensitive to all antimicrobials ested against, resistant ≥ 1 antimicrobial, and resistant to ≥ 3 antimicrobials

Antimicrobial resistance (AMR) patterns were determined for the majority of *Salmonella* isolates (706/709). In total, 41% of isolates collected between 2008 and 2018 (291/706) were resistant to at least one antimicrobial. Since the annual number of *Salmonella* isolations from raw pet food started to increase in 2013, the proportion of isolates displaying resistance has declined slightly, from 50% in 2013 to 36% in 2018 (Fig. 4). However, the absolute number of AMR isolates has increased exponentially over this time period, as has the number of multi-drug resistant isolates (MDR; resistant to three or more antimicrobials) (Fig. 4).

Table 3. The serovars for which multi-drug resistant (MDR) isolates were recovered. The number and proportion of MDR isolates is shown for each serovar

Serovar	MDR/Total isolates	Serovar	MDR/Total isolates
S. 4,12:i:- [a]	29/33 (0.88)	*S*. Indiana	4/87 (0.05)
S. Typhimurium[a]	26/47 (0.55)	*S*. Kentucky	3/3 (1.00)
S. 4,5,12:i:- [a]	23/23 (1.00)	*S*. Anatum	3/7 (0.43)
S. Derby	10/51 (0.20)	*S*. Panama	3/16 (0.19)
S. Kedougou	8/20 (0.40)	*S*. Paratyphi B variant Java	2/2 (1.00)
S. Infantis[a]	7/14 (0.50)	*S*. Bovismorbificans	2/15 (0.13)
S. Saintpaul	4/4 (1.00)	*S*. Stourbridge	1/1 (1.00)
S. Brandenberg	4/6 (0.67)	*S*. Kottbus	1/49 (0.02)

[a] Regulated serovar

Isolates of 16 different serovars displayed MDR (Table 3), the most common being *S*. Typhimurium and its monophasic variants. In total, 60% of MDR isolates were *S*. Typhimurium or its monophasic variants (78/130 isolates), and the majority of all *S*. Typhimurium and monophasic *S*. Typhimurium isolates (76%; 78/103 isolates) were MDR (Table 3). Multi-drug resistance was also identified in 50% of *S*. Infantis isolates, which is also a regulated serovar (Table 3).

Throughout the study period, resistance was observed to 12 of the 16 antimicrobials in isolates of *Salmonella* obtained from raw pet food (Table 4). The antimicrobial to which resistance was seen most often was ampicillin (observed in 27% of isolates), closely followed by tetracycline (observed in 25% of isolates). However, resistance to sulphonamide compounds, streptomycin and nalidixic acid was also common, being observed in 19%, 17% and 10% of all isolates respectively (Table 4).

Table 4. The number and proportion of Salmonella isolates that were resistant to each of sixteen different antimicrobials, and the resistance patterns observed in ≥5 isolates

Antimicrobial	No. resistant isolates	Resistance pattern	No. isolates
Ampicillin (AM)	188 (0.27)	Am, Na	59
Tetracycline (T)	174 (0.25)	T	29
Sulphonamide compounds (SU)	135 (0.19)	Am, S, Su, T	26
Streptomycin (S)	117 (0.17)	Am, T	24
Nalidixic acid (NA)	74 (0.10)	Am, C, S, Su, T	21
Chloramphenicol (C)	42 (0.05)	S, Su, T	17
Sulphamethoxazole / Trimethoprim (TM)	39 (0.04)	Su, T, Tm	13
Furazolidone (FR)	25 (0.02)	Am	12
Neomycin (N)	17 (0.01)	Am, S, Su	7
Apramycin (APR)	6 (0.01)	Su, T	7
Gentamicin (CN)	5 (0.01)	C, Fr	6
Ciprofloxacin (CIP)	1 (0.001)	Am, S, Su, T, Tm	6
Ceftazidime (CAZ)	0	Am, S	5
Amikacin (AK)	0	S, Su	5
Amoxicillin / Clavulanic acid (AMC)	0		
Cefotaxime (CTX)	0		

In total, 45 different resistance patterns were observed; resistance patterns that were observed in five or more isolates during the study period are listed in Table 4. The most common resistance pattern was resistance to both ampicillin and nalidixic acid (59 isolates), followed by resistance to tetracycline only (29 isolates) and multidrug resistance to AmSSuT (26 isolates); all isolates with this latter resistance pattern were *S*. Typhimurium (n=1) or its monophasic variants *S*. 4,12:i:- (n=12) and *S*. 4,5,12:i:- (n=13). The pentaresistance phenotype R-type AmCSSuT was observed in 21 isolates (Table 4), all of which were *S*. Typhimurium (n=18) or its monophasic variants *S*. 4,5,12:i:- (n=2) and *S*. 4,12:i:- (n=1). Resistance to ciprofloxacin, which is rare in *Salmonella* isolated from GB livestock, was also identified in an isolate of *S*. Kentucky from raw turkey sampled at a RMPF plant in 2017.

DISCUSSION

This is the first comprehensive analysis of *Salmonella* isolations from commercially produced RMPF in GB. The number of APHA-approved raw pet food production plants has increased substantially in recent years. At the same time, the number of *Salmonella* isolations from RMPF products collected at manufacturing plants has also increased, with frequent isolations of regulated serovars that also occur commonly in people, particularly *S*. Typhimurium and its monophasic variants. Furthermore, almost one fifth of isolates between 2008 and 2018 were resistant to at least three antimicrobials, with the majority of *S*. Typhimurium and monophasic *S*. Typhimurium isolates falling into this category.

The serovar most frequently isolated from samples of RMPF was *S*. Typhimurium, including its monophasic variants *S*. 4,12:i:- and *S*. 4,5,12:i:-. Since human infection with this serovar can lead to serious clinical disease, it is considered a major public health concern, with contaminated meat considered a primary source of transmission. In GB livestock, *S*. Typhimurium is most commonly associated with pigs and cattle, and monophasic variants with pigs (APHA, 2019). However, there has been a recent increase in the number of human cases of *S*. Typhimurium linked to contaminated meat from sheep (Carson and Davies, 2018), and the Food Standards Agency has recently reiterated their advice on proper cooking of meat and maintaining adequate hygiene during food preparation in order to reduce the risk of human infection (FSA, 2018). Lamb, along with other livestock meat such as poultry, beef and pork, is frequently incorporated into RMPF products produced in GB. In the absence of a heat-kill step or other critical control point (CCP), it is vital that consumers are made aware of the bacteriological risks associated with handling these products.

In addition to *S*. Typhimurium, a diverse range of serovars were recovered from RMPF products during the study period. Many *Salmonella* serovars have a clear association with particular livestock species (EFSA, 2019), and the serovars isolated from RMPF products are therefore likely to reflect the animal sources of ABPs used in their production. Indeed some of the most common serovars to be isolated from RMPF during the study period included those most frequently recovered from livestock species commonly used for GB meat production. For example, *S*. Indiana was the most commonly isolated serovar from RMPF after *S*. Typhimurium, and is frequently isolated from ducks, and *S*. Derby and *S*. Mbandaka, which were also common in RMPF, are frequently isolated from turkeys and chickens, respectively (APHA, 2019). As well as an increase in isolations of common serovars, isolations of rarer serovars have also increased in more recent years. This may indicate the use of ABPs from an increasingly broad range of animal species in these products, or ABPs from non-GB sources.

There was an increase in the overall diversity of *Salmonella* serovars isolated over time. In addition, isolations from RMPF products more recently have included strains of *Salmonella* that are not present in GB livestock, including multi-drug resistant *S*. Infantis and an isolate of *S*. Kentucky that was resistant to ciprofloxacin. Occurrence of such strains may indicate the use of imported ABP material into RMPF produced in GB, reflecting the existence of potentially complex ingredient supply chains which may be more challenging to control with respect to quality and microbiological safety. Furthermore, some MDR serovars which are not endemic in GB have shown the propensity for widespread dissemination and persistence in livestock in other countries. The introduction of such non-endemic strains into the GB livestock industry is therefore of major concern, particularly in the poultry industry where concerted efforts have resulted in reduced burden of *Salmonella* in recent years. RMPF products that incorporate imported ABPs provide a possible route for the introduction of such serovars into

GB livestock via contact with pets fed on these diets, via people who have handled these products, or via movement of waste material from pet food production plants to disposal sites.

Pathogen risks associated with ABPs imported into GB have been identified previously; meat imported for use in raw pet food production was contaminated with *B. suis* and subsequently implicated in transmission of *B. suis* to livestock (Frost, 2017). While the APHA ask for the origin of feed ingredients to be indicated on *Salmonella* submission forms, this information is infrequently supplied for isolates from raw pet food. Only one quarter of submissions between 2008 and 2018 indicated whether ingredients were imported or produced in GB. More complete data on the use of imported material in the production of RMPF products would therefore be valuable.

Phage typing of isolates found that while *S*. Typhimurium or its monophasic variants were often isolated multiple times from the same plant, different phage types were often involved, thus indicating multiple introductions from diverse sources. However, persistence of contamination within plants cannot be ruled out, since multiple isolations of the same phage type on different dates were identified at two plants during the study period. Detailed sampling combined with investigations at the molecular level could assist in elucidating the detailed epidemiology within plants.

Salmonella was isolated from an increasing number of manufacturing plants over time, and regulated serovars were isolated from more than 40% of plants that reported *Salmonella*. A code of practice recently produced by the Pet Food Manufacturers' Association (PFMA) gives comprehensive details of legislation and guidelines for best practice in raw pet food production (PFMA, 2017). This is encouraging and demonstrates that many in the industry are playing a role in trying to reduce the animal and human health risks associated with their products. However, it is vital that such guidelines also reach, and are implemented by, the many smaller, potentially less experienced producers that appear to make up a significant part of the industry. Still, without a true CCP there will always be a risk of these products being contaminated with *Salmonella*, other pathogens, or AMR organisms.

Following the detection of *Salmonella* in a batch of RMPF at a manufacturing premises, EU ABP regulations require the disposal of any products from this batch. However, it remains possible for *Salmonella*-contaminated products to enter the market; the imperfect nature of end-product testing means that contamination on the production line, which may be inconsistent even within a batch, may not be identified by random sampling. Investigations of *Salmonella* prevalence in RMPF products at the point of sale would help to refine estimates of exposure risk to consumers and their pets, as would data on the total annual number of *Salmonella* tests carried out at RMPF manufacturing plants. However, the trends described in this paper may well underestimate the true level of contamination. The potential risks to animal and human health due to *Salmonella* from raw meat pet food products should therefore be acknowledged, and the increasing presence of these products on the market is cause for concern.

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COMPARATIVE EXPOSURE ASSESSMENT OF ESBL-PRODUCING ESCHERICHIA COLI THROUGH SEAFOOD CONSUMPTION

C.A. MCCARTHY*, J.E. CHARDON AND C.J. DE VOS

SUMMARY

Extended-Spectrum β-Lactamase (ESBL) producing Escherichia coli (EEC) are a significant public health concern. Although previous studies have calculated the exposure through meat consumption, the risk from seafood has not been quantified. A Quantitative Microbiological Risk Assessment (QMRA) model for meat was adapted to incorporate the complexities along the seafood chain. Total exposure of the Dutch population (EEC / year) was highest for raw salmon (48.1%) and smoked eel (43.9%). The top five seafood products (raw salmon, smoked eel, caviar / cod roe, smoked salmon and anchovies) all had a higher annual exposure estimate then any meat product. The high exposure through raw salmon is due partly to the current European Union (EU) legislation which permits farmed salmon to be sold without prior freezing. With current antimicrobial usage remaining high in aquaculture industries, the results question whether the current EU legislation is adequate to mitigate against emerging microbiological hazards such as antimicrobial resistance.

INTRODUCTION

Extended-spectrum beta (β)-lactamases (ESBL) are plasmid-encoded enzymes found in Enterobacteriaceae which confer resistance to a variety of beta-lactam antibiotics (EFSA, 2011). ESBL-producing Enterobacteriaceae may cause a range of clinical infections in people from urinary tract infections (UTI) to more serious bloodstream infections (Pitout and Laupland, 2008). Current Dutch prevalence levels suggest that 3% of patients who attend their General Practitioner and 7% of hospitalised patients in intensive care units (ICU) have an infection associated with ESBL-producing *Escherichia coli* (EEC) (De Greef et al., 2019). EEC was the third most frequent cause of serious large scale antimicrobial resistant outbreaks in hospital settings in the Netherlands in 2018 (13%, 8 /59 outbreaks) (De Greef et al., 2019).

The majority of human EEC carriage is acquired from other humans, with recent studies indicating that the attribution from the open population (defined as clinically healthy individuals who had not travelled to high-risk regions nor are engaged in farming activities) may be as high as 60.1% (Mughini-Gras et al., 2019). Nonetheless, reports of EEC carriage in animals and meat products, particularly poultry products, have been reported since 2000

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(EFSA, 2011), with food consumption and preparation accounting for 18.9% of human cases in the Netherlands (Mughini-Gras et al., 2019).

Exposure assessments combine the prevalences and concentrations in raw products with human consumption, storage and cooking habits in order to compare the relative impact of different products on human exposure. Models comparing the risk of exposure to EEC through meat consumption found that beef products (78%) contributed more to the exposure risk than chicken (18%), pork (4.5%), veal (0.1%) and lamb (<0.1%) products (Evers et al., 2017). Equivalent exposure assessments have not been performed for seafood products. However, there are many perceivable reasons why exposure to EEC through consumption of seafood products could be high, including the frequent raw consumption of seafood products, heavy use of antibiotics in aquaculture and extensive international trade in fish goods. Mughini-Gras et al. (2019) found that the consumption of seafood (6.6% attribution, 95% confidence interval 0.3-21.6%) may be responsible for more cases of EEC carriage than either chicken meat (4.5% attribution, 95% confidence interval 0.2-13.1%) or bovine meat (3.6% attribution, 95% confidence interval 0.1-12.5%) (Mughini-Gras et al., 2019). Moreover, the ESBL subtypes CTX-M-15, CTX-M-14 and CTX-M-27, which were strongly associated with human-to-human transmission, were also common in seafood products (Mughini-Gras et al., 2019). The risk from seafood consumption needs to be examined in order to ensure the safe supply of seafood products.

The aim of this project was to quantify the total annual EEC exposure to the Dutch population through seafood consumption. It used a Quantitative Microbial Risk Assessment (QMRA) model to capture complexities along the seafood chain from harvest to consumption.

MATERIALS AND METHODS

Model description

The model used was based on a previous model comparing EEC exposure through consumption of meat products (Evers et al., 2017). The model was adapted to estimate the exposure of the Dutch population to EEC through consumption of seafood products (Fig. 1) and performed in @Risk 6.2 (Palisade Corporation). “Exposure” referred to the number of microorganisms on a food product at the point of consumption, and did not accommodate any dose-response parameters which are less relevant to antimicriobial resistance carriage (Evers et al., 2017). Due to the high proportion of fish products consumed raw, and the likelihood of a high pathogenic load in these products, the model concentrated on products which were not cooked prior to consumption (Table 1). Note that these products may undergo a variety of pre-retail processing (including hot smoking) and still be classed as consumed raw if they are not formally cooked prior to consumption. The total number of raw products evaluated was 14 although products may account for more than one commercial item (for example, raw salmon may be sushi or poké bowls). Additional raw seafood products (such as oysters) were excluded if they were not consumed in the most recent food consumption survey. There are some cultural differences in the terminology of some fish products. Hereafter, the study refers to hot-smoked herring as a kipper, raw herring as a soused herring and cold-smoked herring as a bloater.

In order to accommodate for the significant international trade in seafood products, the model was adapted to incorporate the effect of transport from point of catch to importation into the Netherlands. Fish were assumed to go from a starting temperature at the point of catch, T_{catch} to a transit temperature, $T_{transit}$ within a specified harvest time, $t_{harvest}$. An exponential primary growth model plus the temperature-dependent part of the gamma model as secondary growth model were taken from Evers et al. (2017). The temperature trajectory was divided into 10 parts, i, assuming a linear decrease in temperature from T_{catch} to $T_{transit}$ within $t_{harvest}$ hours. An EEC generation time at temperature increment, $t_{gen,i}$ was calculated (Eq. 1) to create an overall EEC growth multiplication factor, G, during harvest (Eq. 2).

$$t_{gen,i} = \frac{t_{gen.min}}{\left(\frac{\left(T_{catch} - (T_{catch} - T_{transit})(i/9)\right) - T_{min}}{T_{opt} - T_{min}}\right)^2} \quad (1)$$

$$G = \frac{1}{\mathrm{N}} \sum_{i=1}^{n=10} e^{\left(\frac{ln2}{tgen,i}\right) * \frac{tharvest}{n}} \quad (2)$$

EEC growth and survival parameters, namely the minimum generation time, tgen_min, minimum growth temperature Tmin and optimum growth temperature, Topt were as previously described (Evers et al. 2017).

Table 1. Description of seafood products in model

Product	Fish	Fish type[a]	Storage profile[a]
Smoked kipper	Herring	2a	Perishable
Salted herring	Herring	2a	Direct consumption
Smoked eel	Eel	1a	Direct consumption
Steamed mackerel	Mackerel	2b	Perishable
Bloater	Herring	2a	Perishable
Smoked salmon	Salmon	1a	Perishable
Pickled herring	Herring	2a	Shelf-stable
Smoked halibut	Halibut	1a	Perishable
Smoked mackerel	Mackerel	2b	Perishable
Raw farmed salmon	Salmon	1a	Direct consumption
Anchovy	Anchovy	2b	Shelf-stable
Caviar / cod roe	Sturgeon	1a	Shelf-stable
Raw tuna	Tuna	2b	Direct consumption
Salmon pate	Salmon	1a	Perishable

[a] For further details on fish types and storage profiles, see main body of the text

Pre-retail processes, namely techniques designed to retain raw characteristics, cold smoking, hot smoking and pickling were modelled by applying a log reduction to the raw products.

Storage at the consumer’s home was modelled as per Evers et al. (2017). Briefly, storage could occur at room temperature, in the refridgerator or in the freezer. Growth of microorganisms could occur if the storage temperature exceeded the EEC minimum growth temperature, Tmin. EEC is limited to a maximum population density. Inactivation due to exceeding the maximum population density per product was described as an exponential decrease with storage time for room and refridgerator and time-independent for the fraction stored in the freezer.

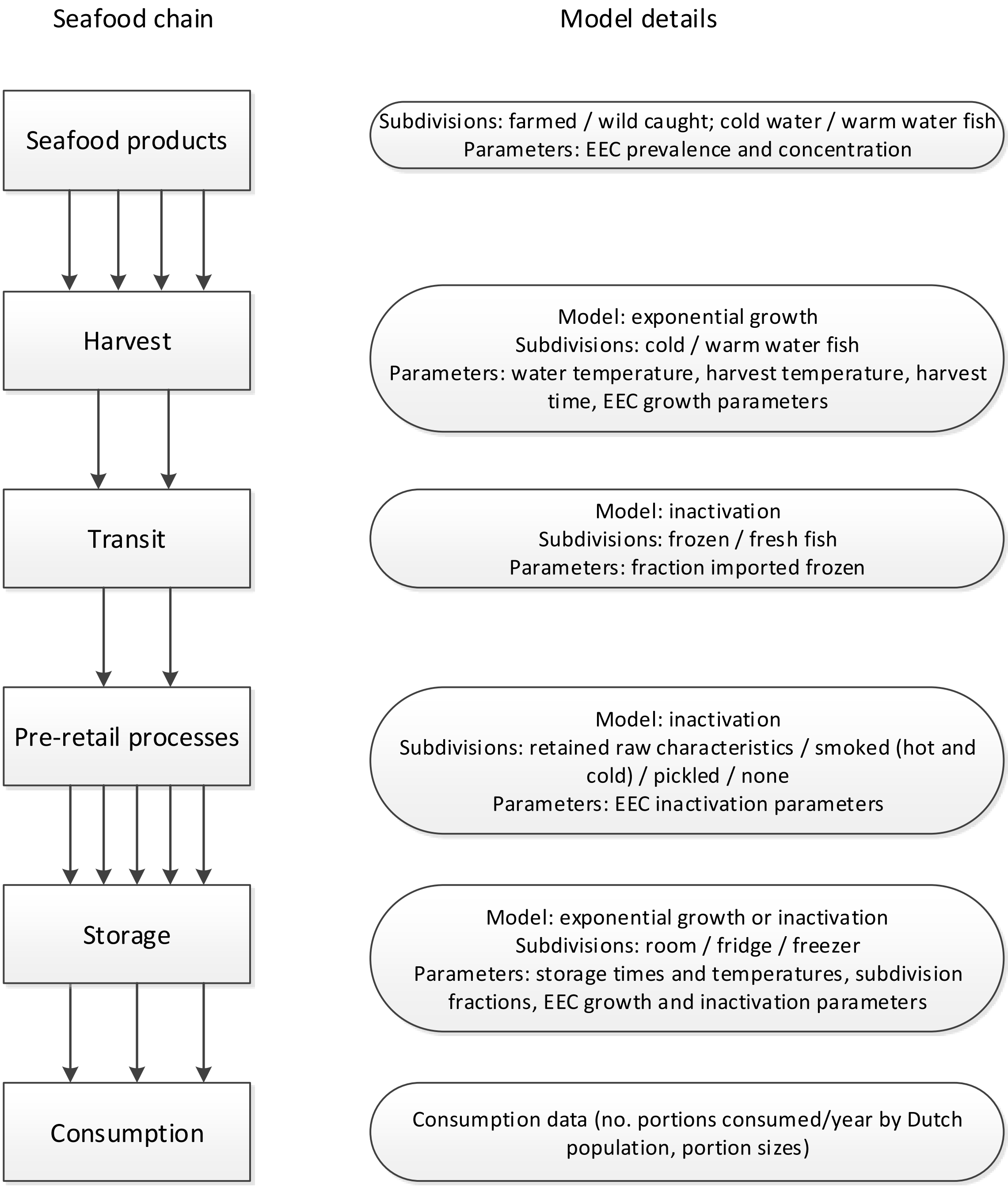

Fig. 1 Overview of QMRA model used for the calculations including details for each stage

Output was calculated at the population level. The number of microorganisms ingested per contaminated portion was calculated alongside the number of portions consumed in a year by the Dutch population and the fraction of contaminated portions at consumption. The total exposure was calculated in order to estimate the attribution from each seafood product.

Parameter values

Prevalence on raw fish product: The literature was searched for studies which described either the prevalence or concentration of EEC within fish products. Due to limitations in data availability, it was assumed that fish of similar geographical distributions and whether they were a farmed or wild caught species would share similarities in concentration and prevalence data. Therefore, fish were classed into one of four categories according to information from FishBase, a global database of fish species (Froese and Pauly, 2019, Table 2).

Table 2. Classification of fish types

Category	Description	Possible examples
1a	Cold water farmed fish	Salmon, eel, sturgeon, halibut
1b	Warm water farmed fish	Trout, pangasius, tilapia
2a	Cold water, wild caught fish	Herring
2b	Warm water, wild caught fish	Mackerel, anchovy, tuna, sardines

One example fish species was chosen per category and all fish types within that category were assumed to have the same prevalence. If more than one source of information was available, the study sampling fish at retail closest to the Netherlands was selected. The EEC prevalences were 3.6% for category 1a products (based on salmon in Spain; Vitas et al., 2018) and 9.5% for category 1b (based on tilapia in the Netherlands; MARAN, 2018). Data from wild caught fish (categories 2a and 2b) were unavailable and so the prevalence values were assumed based on values from farmed fish in the same geographical region (1a and 1b respectively).

Concentration on raw fish product: There were very little data on EEC concentrations on raw seafood products. Therefore, for each of the fish categories (Table 2), the literature was searched to find studies reporting non-ESBL *E.coli* concentrations. Non-ESBL *E.coli* concentrations from category 1a were estimated at 4.71 log CFU/g based on sushi in Italy (sushi products were assumed to be primarily produced from salmon; Muscolino et al., 2014), category 2a were estimated at 1.8 log CFU/g (based on herring in the Philippians; Gabriel and Alano-Budiao, 2015) and category 2b were estimated at 3.35 log CFU/g (based on sardines in Algeria: Dib et al., 2018). Note that no fish products consumed raw belonged to category 1b and so were not included in the analysis. Ryu et al. (2012) sampled 2,663 seafood products and found that 8.4% of *E.coli* isolates carried the ESBL-resistance gene, bla_{TEM}. Therefore EEC concentration data were estimated to be 3.63, 0.72 and 2.27 log CFU/g for category 1a, 2a and 2b respectively.

Growth and inactivation during harvest: The Fish and Fishery Products Hazards and Controls Guidance state that scrombotoxin-forming fish which are exposed to air or water temperatures >28.3^{o}C should have an internal temperature of ≤4.4^{o}C within 6 hours post-death or within 9 hours if exposed to air or water temperatures ≤28.3^{o}C (FDA, 2011). Fish were assumed to go from a starting temperature at the point of catch, T_{catch}, to a transit temperature, $T_{transit}$, of 4.4^{o}C within a specified harvest time, $t_{harvest}$, of 9 or 6 hours depending on whether they were within fish subcategory a (fish within colder oceans) or b (fish within warmer oceans) respectively. T_{catch} was assumed based on the average annual Atlantic Ocean temperatures within temperate regions (16.1^{o}C) and tropical regions (28.1 ^{o}C) respectively (Temperature, 2019).

Growth and inactivation during transit: A proportion of seafood products are transported fresh (assumed 4.4°C) and a proportion are transported frozen. The United Nation's Comtrade database was searched to calculate the net weight (kg) of imported frozen or fresh whole fish into the Netherlands from 2016-2018 for each fish type (United Nations, 2019). The proportions imported frozen were then calculated. In fresh products, no additional growth was assumed during transport as $T_{transit} < T_{min}$. Frozen products had an assumed 1 log reduction in EEC concentration during transit due to the freezing process (Evers et al., 2017).

Concentration reduction due to pre-retail processing: Log reductions were applied to the EEC concentrations after transit depending on whether pre-retail processes had been applied. Five categories of preretail processes were considered important (Table 3). The detailed pre-retail processes for each product were taken from the Fish and Fishery Products Hazards and Control Guidance (FDA, 2011).

The main effect for category 1 products was individual quick freezing (IQF) with extended storage or high hydrostatic pressure processing (FDA, 2011). Soused herring, and raw tuna must be frozen at a temperature of ≤-20oC for ≥24 hours to kill anisakiasis parasites according to Regulation 853/2004 (European Union, 2004) and 2074/2005 (European Union, 2005). Farmed salmon is exempt from the EU legislation requiring raw products to be rapidly frozen (European Union, 2004; European Union, 2005). These products are clearly listed in the food consumption surveys as "salmon farmed raw" and therefore, were assumed to have a zero log reduction (category 5). Caviar/cod roe is also eaten raw.

Growth and inactivation during storage at the consumer's home: Products were classed into three storage profiles depending on whether they were items intended for direct consumption, perishable or shelf stable. Each storage category had different proportions of products stored at room, refridgerator and freezer temperatures. The storage time at these temperatures was also category-dependent. Room temperature was assumed to have a mean temperature of 18°C (Evers et al., 2017), refridgerator temperature was assumed to have a mean temperature of 4°C (NACMCF, 2008) and frozen storage was assumed to be time (and therefore temperature) independent (Evers et al., 2017). In contrast to meat items, a larger portion of seafood products were considered likely to fall into a "direct consumption" category (Table 1). It was assumed that 50% of these products would be stored at room temperature and consumed within a mean of 3 hours and 50% stored in the refridgerator and consumed within a mean of 6 hours. For "perishable products", the proportions of portions stored at each temperature (9% room, 81% refridgerator, 10% freezer) was taken from a Dutch food consumption and handling survey using responses for smoked salmon (Chardon and Swart, 2016). Mean storage times in the refridgerator (79 hours) were taken from the mean value for smoked salmon (Chardon and Swart, 2016). Storage time at room temperature was taken as 1/5th of that in the refridgerator (Evers et al., 2017). Finally, values for "shelf stable" products were taken from Evers et al. (2017) (proportion of portions stored: 19% room, 80% refridgerator, 1% freezer; and storage time: 168 hours at room temperature, 149 hours in refridgerator).

Table 3. Pre-retail processes applied in model.

Pre-retail category		Description	Products	Log reduction	Reference
1.	Products designed to retain raw characteristics	Individual quick freezing (IQF) with extended frozen storage	Soused herring, raw tuna	3.52	(FDA, 2015; FDA, 2011)
2.	Cold smoked	Lightly brined then cold smoked at 32ºC.	Smoked salmon, salmon pate, bloater	3	(NACMCF, 2008; Sabanadesan *et al.*, 2016)
3.	Hot smoked	Smoked and heated to 62.8ºC for 30 minutes then salted at 2.5-3.5% water phase salt.	Smoked mackerel, kipper, smoked eel, smoked halibut, steamed mackerel	6	(NACMCF, 2008; FDA, 2011)
4.	Pickled	pH ≤5.0	Anchovy, pickled herring	4	(Glass *et al.*, 1992; FDA, 2011)
5.	No processing	-	Raw salmon, caviar/cod roe	0	(European Union, 2004; European Union, 2005)

Population level consumption: The latest Dutch National Food Consumption Survey from 2012 – 2016 was analysed (NEVO, 2019). These surveys included 4,313 respondents including children aged 1-18 years (n=2,235) and adults aged 19-79 years (n=2,078). Food intake data was collected by two diaries and/or two non-consecutive 24-hour dietary recalls. The frequency of consumption and quantity consumed per serving (g) was collected for all seafood products listed as being consumed raw.

Scenario analysis: A scenario analysis was performed on parameters deemed most uncertain to investigate the impact of this uncertainty on the model results. Namely temperature at point of catch, T_{catch}, is 5ºC cooler (scenario 1), pre-retail processes are 10-fold less efficient (scenario 2), pre-retail processes are 10-fold more efficient (scenario 3) and extended maximum storage time (scenario 4).

RESULTS

Smoked salmon, soused herring and raw salmon were the top three consumed products in the Netherlands from 2012 – 2016 (Table 4). The fraction of contaminated portions was highest for all category 2b products (mackerel, anchovy and tuna) at 9.5%. Exposure per contaminated portion (number of EEC per portion) was highest for smoked eel, caviar / cod roe and raw salmon and lowest for steamed mackerel, kipper and pickled herring. Total exposure (number EEC per year) was highest for raw salmon (48.1%), smoked eel (43.9%) and caviar / cod roe (4.6%).

An exposure assessment along the seafood chain can be seen for individual fish species (rather than products) from contamination at the start (stage 1), at the point of importation (stage 2), after pre-retail processing (stage 3) and at the point of consumption (stage 4) (Fig. 2). Harvest and transportation processes have a minimal effect on reducing the microbiological contamination levels, by an average of 0.5%. Pre-retail processes decrease the EEC concentrations on average by 35.1%. The largest reduction occurs in mackerel products 61.7%, halibut products 56.6% and herring products 53.5%. Storage by the consumer increases the EEC contamination in all products, by an average of 12.4%. This effect is largest in anchovy (50.2%), halibut (21.4%) and sturgeon (12.5%) products.

Results from the uncertainty analysis show that the effect of parameter values on the contribution from the five highest attributed products was generally limited. However, the attribution to smoked eel was reduced in all uncertainty analysis scenarios. Moreover, when maximum storage times were five-times average times, pickled herring obtained 60% of the total attribution in that simulation.

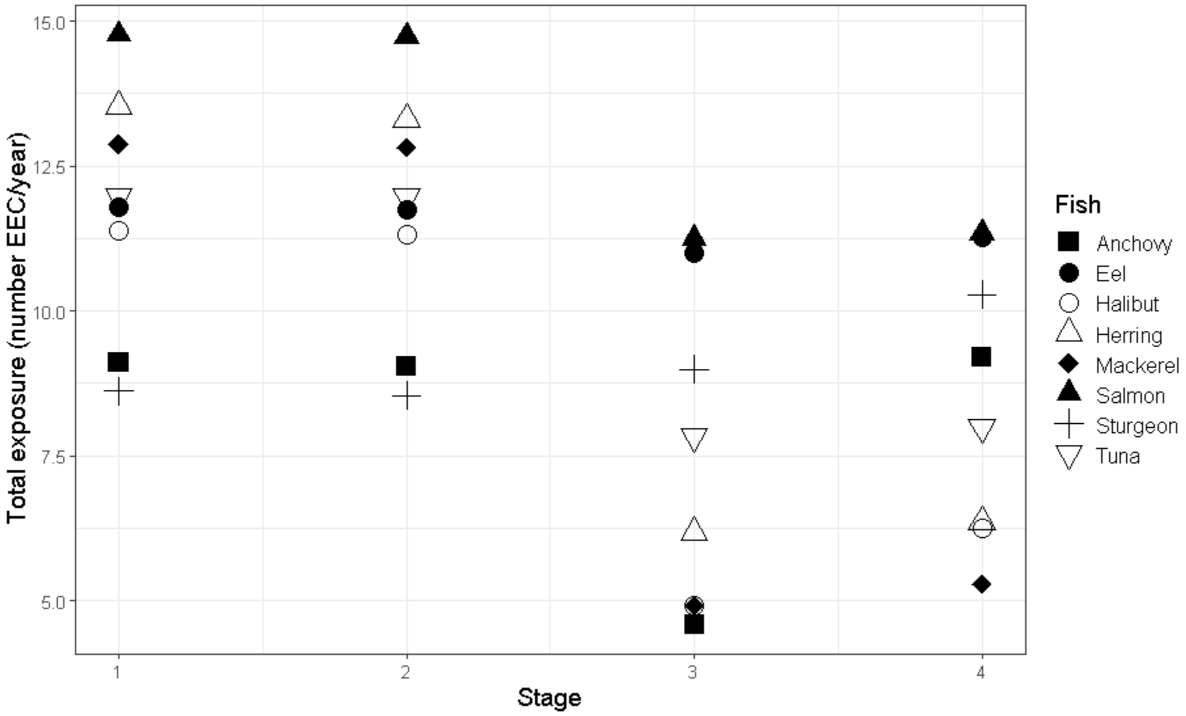

Fig. 2 ESBL-producing E.coli population-level exposure at each stage of the seafood chain (1 at catch, 2 at importation, 3 at retail, 4 at point of consumption)

DISCUSSION

The model presented here is an adaptation of a previous exposure assessment for EEC through consumption of meat products (Evers et al., 2017). It has been adapted to capture complexities along the seafood chain including the effect of harvest temperatures, probability of frozen produce at importation and pre-retail processes unique to seafood production. The two products with the highest EEC exposure (raw salmon and smoked eel) accounted for 92% of the exposure to the Dutch population. The three highest meat products for EEC exposure were filet americain (7.16E+8 EEC per year), chicken fillet (1.47E+8 EEC per year) and ossenworst sausage (1.01E+8 EEC per year) (Evers et al., 2017). The top five seafood products (raw salmon, smoked eel, caviar / cod roe, smoked salmon and anchovies) all had a higher annual exposure estimate then any meat product. This is in agreement with a recent source attribution study, which predicted that consumption of seafood (6.6% attribution) could have a higher risk than consumption of chicken meat (4.5% attribution) or bovine meat (3.6% attribution) (Mughini-Gras et al., 2019).

Table 4. Contribution of different seafood products to the exposure of humans to EEC through seafood at the moment of consumption

Product	Total number of portions consumed per year	Exposure per contaminated portion (EEC CFU/portion)	Total exposure (EEC CFU/year)	Total attribution (%)
Raw salmon	3.83E+07	1.52E+05	2.03E+11	48.1
Smoked eel	7.45E+06	1.02E+06	1.85E+11	43.9
Caviar / cod roe	1.35E+06	4.30E+05	1.93E+10	4.6
Smoked salmon	9.03E+07	3.73E+03	1.20E+10	2.8
Anchovy	2.71E+06	1.05E+05	1.60E+09	0.4
Salmon pate	2.71E+06	6.65E+03	6.40E+08	0.2
Raw tuna	1.35E+07	8.15E+01	9.92E+07	<0.1
Soused herring	3.99E+07	4.00E+00	2.26E+06	<0.1
Smoked halibut	6.77E+06	2.35E+01	1.80E+06	<0.1
Smoked mackerel	2.03E+07	2.50E+00	1.90E+05	<0.1
Bloater	6.77E+05	1.05E+01	8.99E+03	<0.1
Steamed mackerel	2.03E+06	5.00E-01	3.47E+03	<0.1
Kipper	2.71E+06	0.00E+00	0.00E+00	<0.1
Pickled herring	1.08E+07	0.00E+00	0.00E+00	<0.1

Four out of the top five fish products (raw salmon, smoked eel, caviar / cod roe and smoked salmon) were categorised into group 1a which had the highest EEC concentrations at baseline. This class was categorised as “farmed fish from cold waters”. Antibiotic use in salmon aquaculture ranges from ~0.02-0.39 g/tonne harvested biomass in Scotland and Norway to ~660g/tonne in Chile (Rodriquez and Pastor, 2015). It is clear that seafood products have the potential to pose antimicrobial resistance (AMR) risks to public health (Watts et al., 2017). Further work is needed to understand the risk of antimicrobial usage in aquaculture to human health.

Raw salmon (a product categorised as having no pre-retail processing) contributed 48.1% to EEC exposure by seafood whereas smoked salmon (a cold smoked product) had a contribution of only 2.8%. Farmed salmon is exempt from the EU legislation requiring raw products to be rapidly frozen (European Union, 2004; European Union, 2005). Conversely, raw tuna (ranked 7th in overall exposure) and soused herring (ranked 8th in overall exposure), both products intended to be consumed raw, must be frozen at a temperature of ≤-20°C for ≥24 hours to kill anisakiasis parasites. More research is needed to investigate whether emerging aquaculture hazards, like AMR, are adequately controlled by current EU legislation. Arguably, there should be a more risk-based approach to pre-retail processes, including the freezing of all farmed aquaculture products. The increasing popularity for consumption of raw salmon adds more weight to the need to thoroughly investigate the microbiological safety of this product.

Caviar / cod roe and anchovy were the third and fifth highest ranking products for EEC exposure. These products were both classed as “shelf-stable” in the consumer stage. Storage at the consumer phase increased EEC contamination by 12.4% on average and up to 50.2% in anchovy products. The storage of food products by consumers was taken from a Dutch food consumption and handling survey (Chardon and Swart, 2016) as well as similar QMRA models (Evers et al., 2017). These surveys are invaluable in understanding consumer habits and comparing the relative risks of different products. However, more research is needed to understand storage at a variety of food establishments including restaurants and fast-food establishments. This would enable a more reliable assessment of population-level exposure risks through routes other than those prepared by the consumer.

The data used in this study had several major uncertainties. EEC concentrations at the point of catch were based on one study reporting the prevalence of bla_{TEM} in *E.coli* samples from seafood products and several studies reporting *E.coli* concentrations from different geographical locations. Although the pre-retail processes were based on industry recommendations according to the Fish and Fishery Products Hazards and Control Guidance (FDA, 2011), it was not possible to establish how closely these processes were followed, nor where regular breaks in the guidance occurred. Many of the log reductions after the pre-retail processes were based on bacteria other than *E.coli*. Fewer studies were available because *E.coli* is not typically considered as a key indicator of contamination in fish products, which are typically more concerned with *Vibrio* and *Listeria* spp. It should therefore be assumed that absolute exposure values in this study are very uncertain. However, relative exposures are likely to have less uncertainty and so this study is useful in understanding which seafood products to target for surveillance or intervention studies. More research is needed to understand the processes involved in the seafood chain, including for example, harvest and transit practices, the maintenance of the cold-chain from harvest to retail and pre-retail processes, and their impact on *E.coli* (and EEC) concentrations. When compared to slaughterhouse practices and meat chain processes, seafood products and the fishing industry receive little research interest from Veterinary Public Health scientists. The General Food Law requires food legislation to be based on risk analysis. The lack of research interest in seafood products is an area which deserves further attention in order to ensure that producers of seafood products are adapting to emerging Public Health concerns.

This study highlighted that the risks of EEC exposure from seafood consumption are not inconsequential. Raw salmon, smoked eel and caviar / cod roe ranked top for EEC exposure. The model highlighted difficulties in understanding the complexity of the seafood industry and suggested areas where more research is needed.

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PRODUCTION CHAIN RISK FACTORS FOR BROILER SLAUGHTERHOUSE CONDEMNATION CATEGORIES

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SUMMARY

In this study we assess farm- and slaughterhouse-level data to explore risk factors that are associated with slaughterhouse condemnation of broiler chickens. This paper describes application of a novel combination of association rules analysis, regression tree and multivariable mixed-effect Poisson regression analyses to prioritise multiple outcomes and explanatory variables. Association rules analysis was used to address the heterogeneity of conditions that result in condemnation. Regression tree analysis and multivariable mixed-effect Poisson regression were applied to prioritise between large numbers of explanatory variables. Addressing these factors can improve the productivity, health and welfare of broiler chickens.

INTRODUCTION

In broiler chicken populations, the incidence of condemnation during slaughterhouse meat inspection can be used to provide a quantitative measure of some morbidities that may be associated with on-farm, in transit or processing level risk factors. Control strategies and improvements in husbandry to reduce morbidities may focus on individual factors, such as the occurrence of a pathogen, or be combined in a blanket approach to alleviate multiple interlinked morbidities (Dekich, 1998). The latter approach may be particularly amenable for broiler chickens reared within intensive agricultural systems since most are commonly produced in homogeneous units. At the end of rearing, each broiler flock is divided into batches to be transported and slaughtered as a unit. Gross abnormalities detected during processing are classified using a defined list of reasons, producing composite condemnation outcomes for batches of broiler chickens. This results in the generation of multimorbidity reports at the broiler batch-level. Detecting batch-level associations between multiple morbidities and identifying their common risk factors could highlight specific health concerns and point towards potential interventions.

Broiler slaughterhouse condemnation has been demonstrated to have a multifactorial nature (St-Hilaire and Sears, 2003; Haslam et al., 2007, 2008; Lupo et al., 2009). Previous studies on slaughterhouse condemnation during meat inspection have focused on (i) the frequency of individual, grouped, or total condemnation categories, and/or (ii) risk factors for individual or total condemnation categories (Haslam et al., 2007, 2008; Lupo et al., 2009; Lupo et al., 2010).

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Despite the link to broiler health and welfare, research into condemnation outcomes has been scarce (Stärk et al., 2014; Salines et al., 2017). Research on multimorbidity occurrence is a more recent approach to studying condemnation. For example, links between multimorbidity and population health status have been demonstrated with cattle used for meat production through the application of cluster and multiple factor methods (Dupuy et al., 2013). Recognising that most of the research in broiler chickens has focused on a practical understanding of individual condemnation categories, this study was intended to identify the most prevalent comorbidities diagnosed during broiler meat inspection and assess the occurrence of shared production chain risk factors.

MATERIALS AND METHODS

Data collection

The study population consisted of Ross 308 broiler chickens raised using conventional commercial methods by an integrator company located in England (Aviagen ROSS, 2018). The unit of analysis was a broiler batch, defined as a group of broiler chickens reared on the same farm, in the same shed, which was harvested and transported together on the same vehicle, and was slaughtered and inspected as a unit.

The study was carried out using a mix of retrospective and prospective data. Initially, meat inspection records on condemnation reasons were obtained retrospectively for all broiler batches raised and processed by the integrator between January 2015 and December 2017. Subsequently, a longitudinal study design was used to collect production chain information from farms supplying broilers to the integrator's slaughterhouse. All farms were approached quarterly, between April 2017 and March 2018, to collect information pertaining to the farm and a specified broiler flock via a postal questionnaire. Farm-level information regarding rearing, management and transport practices, broiler characteristics and health history, was collected in a standardised questionnaire and from the integrator's electronic records, linking them to their specific batch-level slaughterhouse outcomes. A total of 109 explanatory variables were included in the analysis.

For the longitudinal study, a sample of 389 broiler batches with a minimum size of 1,000 chickens was calculated to be sufficient to detect a variation of 20% from the baseline condemnation rate of 1% and, assuming a 1:1 ratio on the presence/absence of a risk factor, at 5% significance for Poisson regression with 85% power. Sample size accounted for the design effect using the intracluster correlation coefficient (ICC of 0.23), estimated in mixed-effect Poisson regression analysis conducted in a pilot study (author's unpublished data) (Signorini, 1991; Gelman and Hill, 2006).

Definition of outcome variables

For each broiler batch, outcomes were expressed as the rate of condemnation cases per 1,000 processed broilers for every condemnation category. Twenty-three health and welfare-related condemnation categories that were recorded as outcomes during the meat inspection process by the integrator slaughterhouse were used in the first analytical stage of the study (Table 1).

Table 1. Health and welfare-related condemnation categories recorded at slaughterhouse of the integrator company. Processing-related condemnation are not included.

Code	Category type	Description
ABN	1. Abnormal colour	Septicaemia, toxaemia
IR	2. Intake runts	Small birds identified and rejected at intake
EVR	3. Evisceration runts	Small birds identified and rejected at evisceration
AST	4. Ascites	Accumulation of fluid in the abdominal cavity as a result of circulatory system malfunction
BRU	5. Bruising and Fractures	Accumulation of blood. Broken bones
CEL	6. Cellulitis	Inflammation of connective tissue of the skin
SKN	7. Skin other	Other skin conditions not classified under “dermatitis”
DER	8. Dermatitis	Inflammation of the skin
FOL	9. Folliculitis	Inflammation of skin follicles, sub-type of dermatitis
DOA	10. Dead on Arrival	Dead birds found during shackling
EMA	11. Emaciation	Decreased muscle tissue, low weight
HEP	12. Hepatitis	Inflammation of the liver
JNT	13. Joint Lesions	Joint inflammation, bone deformities
HB	14. Hard breast	Hardening of breast muscle due to myopathy
JND	15. Jaundice	Yellow colour of the skin due to bilirubin accumulation
FOT	16. Oregon, myopathies, etc.	Oregon: green colour of breast muscle. White stripe: white muscle fibres
PCS	17. Pericarditis	Inflammation of heart membrane pericardium
Heart only	18. Partial rejection-heart	Rejection of the heart results from identification of pericarditis
PTS	19. Peritonitis	Inflammation within the peritoneal cavity
PHS	20. Perihepatitis	Inflammation of the liver capsule
Livers only	21. Partial rejection-liver	Rejection of the liver results from identification of perihepatitis
RES	22. Respiratory conditions	Inflammation of air sacs
TUM	23. Tumours and nodules	Abnormal tissue growth

Further description of individual conditions has been provided elsewhere (Part et al., 2016). Batch-level rates of condemnation cases were categorised based on median values for each of the 23 categories and coded as follows: 1 for values above the median; 0.5 for values equal to the median or below; and batches with no occurrence recorded for any given condition were coded 0.

At the conclusion of the initial analysis, condemnation categories for ascites, abnormal colour, perihepatitis, cellulitis, hard breast, tumours and dead on arrival, as well as partial condemnation of liver only (perihepatitis) and heart only (pericarditis), were prioritised as outcomes of interest. A composite outcome variable was included, representing the total rate of condemnation due to all nine of these condemnation categories combined. Condemnation records were linked to the corresponding flock and farm-level data obtained from company records and the postal survey for statistical analysis.

Statistical analysis

First, association rules analysis was applied to the retrospective data to identify the most prevalent associations between condemnation categories. Subsequently, production chain factors that were similarly linked to these associated condemnation categories were identified. Poisson regression tree analysis was used to assess associations of a high number of explanatory variables with each of the nine chosen and one combined condemnation outcomes. Then, associations between explanatory variables and the outcomes were explored in multivariable mixed-effect Poisson regression models.

Association rules analysis: Analysis was conducted using retrospective condemnation records to identify the most prevalent associations with confidence ≥80% (i.e. conditional probability between condemnation reasons) and support ≥10% (i.e. frequency of a rule in a dataset, where the 'rule' was a group of associated condemnation reasons that followed an "if this then that" expression). The generated rules were pruned using Bayardo improvement, where a rule was removed when a more general rule with the same consequent and the same or higher confidence was available (Bayardo et al., 1999). Association rules analysis was conducted in two successive steps. First, the whole dataset of broiler batch-level records from three years was mined for associations. Subsequently, the associations that were discovered were validated separately for the years 2015, 2016 and 2017 using the established thresholds for support and confidence, 10% and 80%, respectively. A detailed description of the Apriori algorithm-based association rule mining approach used in this study has been described elsewhere (Agrawal et al., 1993). Association rules analysis was conducted in the R arules package (v. 3.4.3) (Hahsler et al., 2007).

Poisson regression tree analysis: A non-parametric and non-linear regression tree modelling approach was used to identify explanatory variables associated with each of the ten outcome variables. A tree-based approach with recursive partitioning of the sample where categorisation was based on data homogeneity within the partition in regard to the outcome variable was used for identification of risk factors, as has been demonstrated in the context of public health (Morgan, 2014). Two Poisson regression tree models were fitted to each of the ten outcomes. Given the multilevel organisation of the data, the first regression tree model was generated including random effect cluster identifying variables (farm and shed identifiers). The second regression tree model was run omitting cluster identifying variables. The two regression tree models therefore aimed to select an inclusive list of explanatory variables both correlated and uncorrelated with hierarchical cluster identifiers. Explanatory variables whose inclusion had the lowest sum of squared error (SSE) were isolated as tree nodes and selected for the next stage of analysis. A detailed description of the method is available elsewhere (Breiman et al., 1984).

In regression tree analysis two-way interactions were suspected when branches subdividing a higher-level node had explanatory variables at the lower level (Camp and Slattery, 2002). Outcome variables were modelled using Poisson methods in the Rpart package in R software as condemnation counts per 1,000 slaughtered broilers, with the size of the broiler batch as an offset variable (Therneau and Atkinson, 2008). No pruning, stopping criteria or limits to the depth of the trees were specified in analyses in addition to default (Therneau and Atkinson, 2019).

Multivariable Poisson regression analysis: Explanatory variables were identified in multivariable poison regression models fitted separately for each of ten condemnation outcomes. Models were then compared to determine factors that were common to multiple condemnation categories. Poisson regression models were fitted in STATA version 13.0 (Stata, 2013) using a manual backwards elimination procedure (Wald's test p-value <0.05). Collinearity between explanatory variables was suspected (X2 test, p-value <0.05) and assessed using a 10% change in standard error as a cut-off value, and, if identified, a variable with a higher measure of effect estimate was retained in the analysis. The unit of analysis was the number of whole or partial carcass condemnations in a batch of broilers per 1,000 slaughtered birds, with the number of broilers in a batch as an offset. Random effect intercepts were introduced to account for a farm- and flock-level clustering of broiler batches. Interactions between explanatory variables selected from Poisson regression tree analysis were reported and retained in the final models if the p-value of the likelihood ratio test was <0.05. The fit of the final models was assessed by evaluating the difference between observed and predicted values, and by assessing R squared, a value ranged from 0 to 1, which was used to indicate the model fit. The higher the R-square, the better the model fitted the data.

RESULTS

Sampled population and descriptive statistics

Retrospectively collected condemnation data: 55,918 broiler batches were slaughtered between 2015-2017. The most prevalent condemnation categories were ascites and abnormal colour (Fig 1).

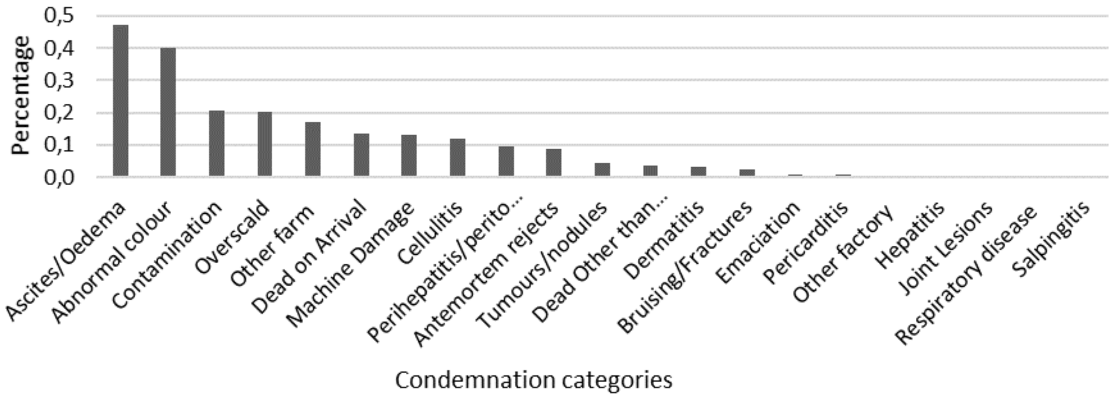

Fig. 1 Frequency of condemnation conditions detected in batches of broiler chickens in a commercial integrator slaughterhouse. The percentage of carcasses condemned was calculated for the corresponding conditions throughout the total period of 2015, 2016 and 2017.

Consideration of the historical condemnation records revealed that the median number of broilers per batch was 5,700, with a range of 700-11,000. 99.3% of batches had two or more conditions leading to condemnation, with only 0.6% (338) of batches free from diagnosed conditions. Condemnation due to a single condition was reported from just 0.1 % (83) of batches (Fig. 2). The most common number of co-morbidities within a batch was either ten or eleven conditions (Fig. 2).

Data from the prospective longitudinal study: These were collected from a total of 3,354,747 broiler chickens, produced in 115 sheds on 49 farms and processed in 539 batches. Thus, 49 farms completed the questionnaire at least once, with an overall response rate of 59%. Only 15 farms responded in all four data collection rounds. The study sampled circa 2.7% of all broilers processed by the company during the study period. The median size of a broiler batch in the study was 6,000 broilers, with the smallest batch found to include 146 and the largest 9,758. The median number of sheds on the sampled farms was four (minimum two and maximum 14 sheds).

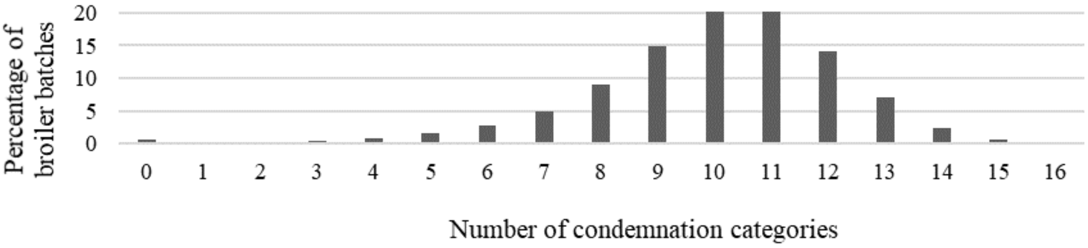

Fig. 2 Batch-level co-occurrence of condemnation categories using 55,918 batch integrator records for 2015, 2016 and 2017, where the occurrence of a condition was negative or positive (at least one condemnation case for a given category in a broiler batch).

The frequency of each of nine individual condemnation outcomes per thousand slaughtered broilers and the composite category for all nine is presented in Table 2. The composite incidence of any of the nine outcomes combined was 14.4 whole or partial condemnations per 1,000 slaughtered broilers. The three most frequent individual condemnation categories were ascites, abnormal colour and hard breast. Co-occurrence of condemnation outcomes was high, with 88% of broiler batches showing condemnation due to seven, eight or nine condemnation outcomes.

Table 2. The number and rates of nine individual and combined condemnation outcomes per 1,000 slaughtered broilers. Calculations are presented for 3,354,747 broiler chickens originated in 539 batches, whose data were collected between April 2017 and March 2018.

Condemnation conditions	Number of condemnations	Condemnation rate per 1,000	95% confidence interval
Ascites	15,167	4.5	2.7-7.4
Abnormal colour	11,954	3.6	2.0-6.3
Hard breast	4,979	1.5	0.5-3.6
Dead on Arrival	4,538	1.4	0.3-3.2
Cellulitis	4,293	1.3	0.3-3.2
Livers (partial condemnation)	3,077	0.9	0.2-2.8
Perihepatitis	2,084	0.6	0.1-2.3
Tumours	1,254	0.4	0.1-1.9
Hearts (partial condemnation)	1,007	0.3	0.1-1.9
All nine, combined	48,353	14.4	10.8-19.0

Associations between condemnation categories

Association rules analysis generated eighteen association rules. These were composed of nine condemnation categories: ascites, abnormal colour, perihepatitis, cellulitis, hard breast, tumours, dead on arrival, partial condemnation of liver only (perihepatitis) and heart only (pericarditis). Focused consideration of the nine morbidities identified a complex network of associations. These represented conditions that occurred in batches at a median frequency or below and at a higher frequency than the median formed two independent networks of associations, demonstrating complicated interactions between the categories and relevance of ascites and abnormal colour with links to multiple association rules (Fig. 3).

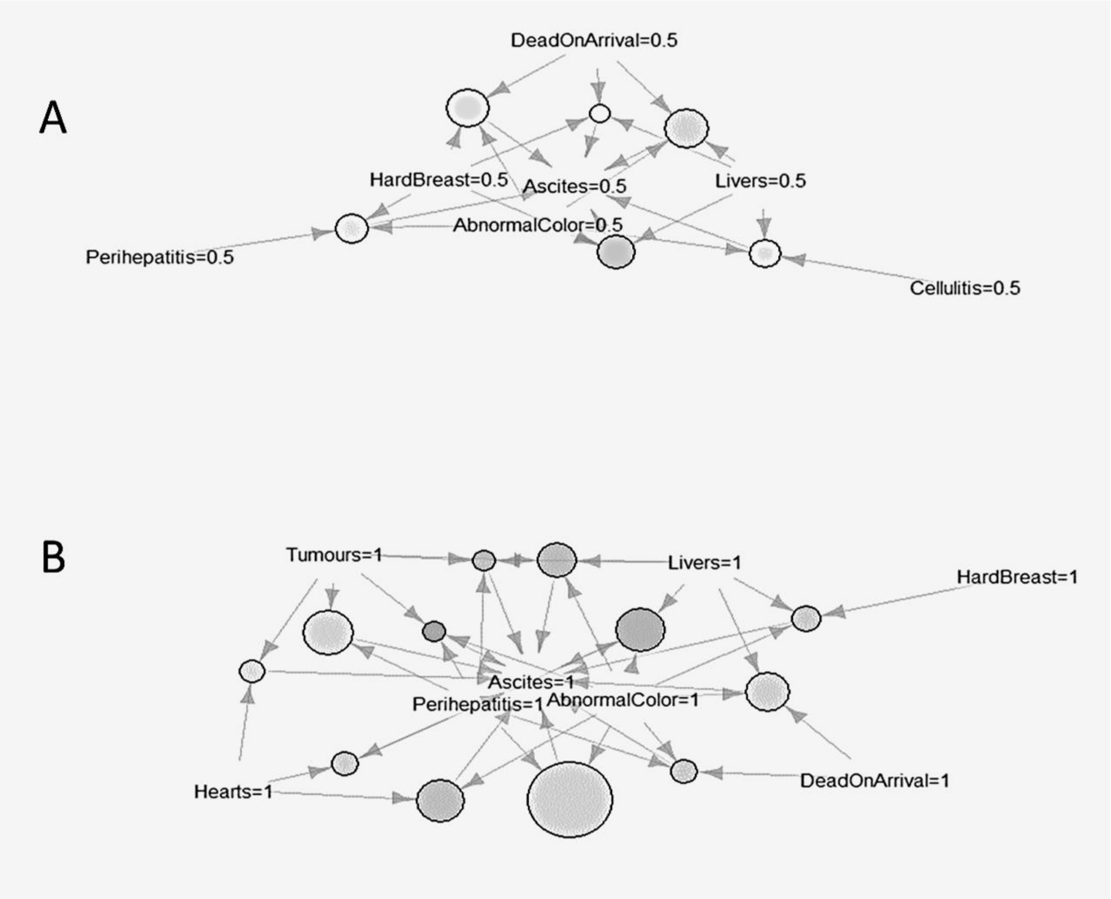

Fig. 3 Networks of morbidities associated in 18 rules generated in association rules analysis with support of 10% and confidence of 80%. The division between rules with conditions identified at below (coded as 0.5; A) and above (coded as 1; B) median values is presented by two distinct networks of associations. Node size: support (0.1-0.175). The intensity of node colour: lift (1.606-1.746)

The most prevalent combination of morbidities was observed in 17% of broiler batches and included abnormal colour, perihepatitis and ascites. The most strongly associated rule indicated that 86% of batches with condemnations for perihepatitis, tumours, and abnormal colour also had condemnations for ascites. When association rules analysis was carried out separately on data from three successive years (2015-2017), the composition of the rules and list of conditions remained stable, with only a minor change in support or confidence for each of the rules over the three years.

Factors associated with condemnation outcomes

A total of 61 explanatory variables were selected as predictive of the nine individual and one combined condemnation outcomes in regression tree analysis. The highest number of variables per outcome was 18 and the lowest was 8. Farm and shed effects, and the effect of catching team was associated with all ten outcomes. The next most commonly associated explanatory variable was the type of broiler removal (thinning and clearing). In total, 31 explanatory variables were found to be associated with two or more condemnation outcomes in Poisson regression tree analysis.

Nine explanatory variables were found to be independently associated with two or more individual condemnation outcomes after adjusting for the effect of other explanatory variables in multivariable Poisson regression models (Table 3). One variable was related to parental flock characteristics, three variables were related to broiler flock-level characteristics, three were relevant to depopulation and transport, and two were related to slaughterhouse practices. Five of the explanatory variables common for two or more condemnation outcomes displayed a unidirectional effect on condemnation outcomes (Table 3). Four of the variables displayed a contrasting effect, being both risk and protective factors for different condemnation outcomes. For example, parental flock age at the time of lay displayed an association with increased condemnation for cellulitis (Rate Ratio (RR) 1.18, ranging from 8% to 27%), but a decrease in liver only condemnation (RR 0.83; between 8% and 25%).

DISCUSSION

Farmed broiler chickens are raised and processed in largely homogeneous units. Pathologies acquired during production and processing that are relevant to food safety and quality are identified during meat inspection at the end of the production cycle, generating broiler batch-level multimorbidity reports. Control strategies that can influence several conditions are highly desirable for broiler production settings. An analytical approach to inform the development of such integrated strategies need to prioritise between multiple pathologies and can benefit from identification of relevant risk factors. Here, multiple morbidities were recorded in meat inspection data at the broiler batch-level. Associations were determined between condemnation categories that were co-diagnosed in broiler batches and their shared production chain factors were identified.

Common co-occurring morbidities are likely to incur high costs to broiler producers. Initially, nine of 23 meat inspection morbidities were found to co-occur in a high proportion of broiler batches. The network of associations included condemnations due to ascites, abnormal colour, dead on arrival, tumours, perihepatitis, hard breast, cellulitis, liver only and heart only morbidities (Fig. 3). The associations were dominated by ascites and abnormal colour, perhaps as a result of their high prevalence. In addition to their prevalence, the central role of ascites and abnormal colour could be explained biologically as both conditions can affect the general health status of broilers, predisposing them to other morbidities. Ascites is a metabolic condition that has been associated with genetic selection of broilers for high growth rate resulting in circulatory deficiencies, and has previously been linked to the presence of pericarditis (Olkowski et al., 2003; Baghbanzadeh and Decuypere, 2008). Abnormal colour, along with perihepatitis and pericarditis, could be a result of septicaemia associated with the presence of pathogens (Fisher et al., 1998). The high prevalence of ascites and abnormal colour detected in our study agreed with a study by Part et al. (2016). No previous research has identified groupings of morbidities around circulatory deficiency (ascites) or infection-related (abnormal colour) conditions, indicating a requirement for further investigation

Table 3. Incidence rate ratios for variables associated with two or more condemnation outcomes selected from ten multivariable Poisson regression models (p-value<=0.05) with 95% Confidence intervals in brackets.

Category	Major age of parental flock at time of lay	Level of *Campylobacter*	Weight at 7 days of age	Weight at slaughter	Type of bird removal	Identity of catcher team	Number of birds per crate	Slaughterhouse shift	Slaughterhouse line number
ABN			1.35 (1.23-1.48)				0.85 (0.82-0.87)		
AST				1.44 (1.36-1.53)			0.83 (0.80-0.91)		1.36 (1.25-1.48)
CEL	1.18 (1.18-1.27)							1.84 (1.67-2.02)	
DOA					0.88 (0.82-0.95)	0.997 (0.995-0.999)			
HB				1.78 (1.61-1.96)		1.004 (1.001-1.10)	0.86 (0.80-0.87)		
Heart only			1.41 (1.14-1.75)		2.93 (2.32-3.70)				
Liver only	0.83 (0.75-0.92)								
PHS					2.21 (1.92-2.57)				
TUM		1.09 (1.03-1.14)			1.88 (1.60-2.00)			1.74 (1.51-2.01)	
All nine		0.98 (0.96-0.99)		1.20 (1.16-1.23)	1.27 (1.20-1.33)				1.13 (1.08-1.18)

The second stage of analysis identified risk factors common to several meat inspection outcomes, focusing on nine associated condemnation categories. Although no risk factors were found to be consistent across all nine, some were associated with more than one category. Notable examples included weight at slaughter, identified as a risk factor in two of the nine final individual models (ascites and hard breast), as well as persisting in the model for composite condemnation inclusive of all nine categories combined. This negative effect of weight at slaughter has previously been linked with all-cause condemnation, as well as hock burns, ascites and dead on arrival (Haslam et al., 2007, 2008; Lupo et al., 2009). The association between weight and condemnation for ascites could be explained by the increased growth rates of broilers that reach a higher final weight. A high growth rate has been shown to induce a higher demand for oxygen, which in turn leads to the development of ascites through chronic hypoxia in fast-growing birds (Julian, 1998).

This study provided further evidence for the involvement of multiple broiler production stages in slaughterhouse condemnation outcomes. Multiple factors present in parental flocks, during rearing, transport and slaughter of broiler chickens were identified as associated with two or more condemnation outcomes. Slaughterhouse practices and broiler weight at different production stages were identified as risk factors for multiple outcomes. Slaughterhouse shift, a team of inspectors working either day or night shift pattern, and line characteristics were associated with increased ascites, cellulitis, tumours and composite condemnation outcomes. Broiler weight was a risk factor for abnormal colour, ascites, hard breast, heart only and composite condemnation outcomes. Further, some factors were found to associate with reduced condemnations such as an increased density of broilers in transport crates. Many of the risk factors identified here may be amenable to modification, supporting a whole production chain approach to studying factors that affect condemnation (Lupo et al., 2010).

Although this study did not provide evidence to explain associations discovered between all nine target condemnation categories, the information generated can be used to inform strategies to control multiple pathologies that result in condemnation. The work described here supports a whole production chain approach to study factors that affect slaughterhouse condemnation.

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STAKEHOLDER ENGAGEMENT

PIG DATA: HEALTH ANALYTICS FOR THE SWISS SWINE INDUSTRY

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SUMMARY

The Pig Data project uses a transdisciplinary approach to bring researchers and industry stakeholders together, with the common goal of fostering swine health and welfare in Switzerland.

We gathered a team of researchers from various domains to respond to the information needs of industry stakeholders. The latter were highly involved in all project stages and regularly provided data to be used in subsequent analysis. To assure the outcomes were of relevance, industry partners were prompted to list research questions or dream queries that they would like to see investigated. In this paper, we will present an example answered by our research team. This dream query related to the impact of several factors (weather, transport conditions, production type) on carcass quality.

By engaging stakeholders throughout the entire research process and combining data from multiple sources, we were able to generate valuable information for the Swiss swine industry.

INTRODUCTION

Big Data is a growing research field that promises to revolutionise public health and medical care (Raghupathi & Raghupathi, 2014). There are four main characteristics associated with the concept of Big Data, generally known as the 4Vs: velocity, variety, volume and veracity. Velocity implies that data streams are generated at a fast pace and accumulated in real-time. Variety relates to the fact that data vary in nature. To maximise the potential of the analysis, different data sources (including structured and unstructured data) need to be collated. A central characteristic of Big Data is its high-volume with data being generated at a large scale. Finally, veracity relates to data uncertainty, focusing mainly on three dimensions: truthfulness, credibility and objectivity (Camacho et al., 2014; Lukoianova & Rubin, 2014; Raghupathi & Raghupathi, 2014; Fosso Wamba et al., 2015).

Big Data approaches have been widely applied in several fields and their benefits are well described for many industries (Fosso Wamba et al., 2015). In livestock production, despite multiple reports promoting its benefit in animal health, the use of Big Data approaches has fallen short of expectations (Pfeiffer & Stevens, 2015; Barrett, 2017; McCue & McCoy, 2017).

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Integrating data from multiple sources might provide researchers the opportunity to better and more holistically understand production systems, which is of particular relevance in the face of poorly-structured or complex problems that require an overview of the whole system.

As highlighted by Faverjon et al. (2019), the poor implementation of Big Data approaches in livestock production might reflect the late adoption of computer and information technologies in the management of livestock production and health.

Another factor hampering broader use of these methods relates to data ownership and confidentiality. In public health data sharing has been described as a relevant issue in the current age of information, with multiple barriers impeding more fluid access to data (Van Panhuis et al., 2014).

In livestock production, access to data is further complicated by the fact that multiple public and private actors are part of a production chain and they collect their data independently. These data are most commonly collected for the purpose of controlling and managing individual business processes.

The Swiss pig industry is unique within the European context. Unlike most swine industries in developed countries, Swiss swine production is a decentralised and fragmented network encompassing a large number of small independent actors (Sterchi et al., 2019). These diverse actors collect a huge amount of heterogeneous data, which led to our overall research question: can we create new and useful information by combining many different data from the swine industry?

The complexity of the environment where we developed our project called for a transdisciplinary approach. As described by Pohl et al. (2017): “The transdisciplinary research process connects scientific knowledge production and societal problem handling”. We aimed at engaging multiple stakeholders from the Swiss swine industry. Their active participation would be crucial not only for accessing and integrating data, but also for the interpretation of results. Moreover, a team of researchers from various fields of expertise was put together, allowing this project to emerge in a truly transdisciplinary environment.

In this paper, we will provide some background information about the Pig Data project. We will then explore how transdisciplinary methods can sustain big data approaches by engaging stakeholders and scientists from different realms. For this purpose, we will use one specific example on how we answered a question posed by the stakeholders pertaining to the impact of several factors (weather, transport conditions, production type) on carcass quality.

MATERIALS AND METHODS

The Pig Data Project

The Pig Data project is a research project funded by the Swiss National Science Foundation. It started in June 2017 and it is planned to run until December 2020. By combining Big Data approaches and transdisciplinary methods, the Pig Data project aims at creating information from the complex network of the Swiss pig industry and providing sustainable solutions that meet the needs of the enrolled partners.

The research consortium consisted of a team of veterinary epidemiologists, porcine herd health management specialists, computer scientists and geographers. Its main objective was to analyse and respond to the information needs of industry stakeholders. The latter were involved in the project right from the start, participating in the pre-project discussions and contributing to the project design. Their output requirements were continuously identified and reassessed throughout the project. Industry partners included: veterinarians, marketers, transporters, slaughterhouses, feed mills, and the Swiss Federal Food Safety and Veterinary OfficeSwiss Veterinary Services. For confidentiality reasons, the names and exact number of stakeholders involved in the project cannot be revealed.

To accommodate the many data sources, a centralised data store (named the Pig Data Space) was developed. Jupyter (https://jupyter.org/), a browser-based interactive computational environment allowed the research consortium to access the stored the data and keep track of their provenance, as well as to interactively collaborate on the data management and analysis using different programming languages, such as Python and R. The Pig Data Space is regularly fed with new data from the industry partners. These data include information about health, animal transport, meat quality, farm information, fertility parameters and climate (Fig. 1).

The project includes several interlinked components: a) Data integration; b) Network description and modelling; c) Risk factor identification; d) Early event detection; and e) Dream queries. This paper will focus on the last of these components.

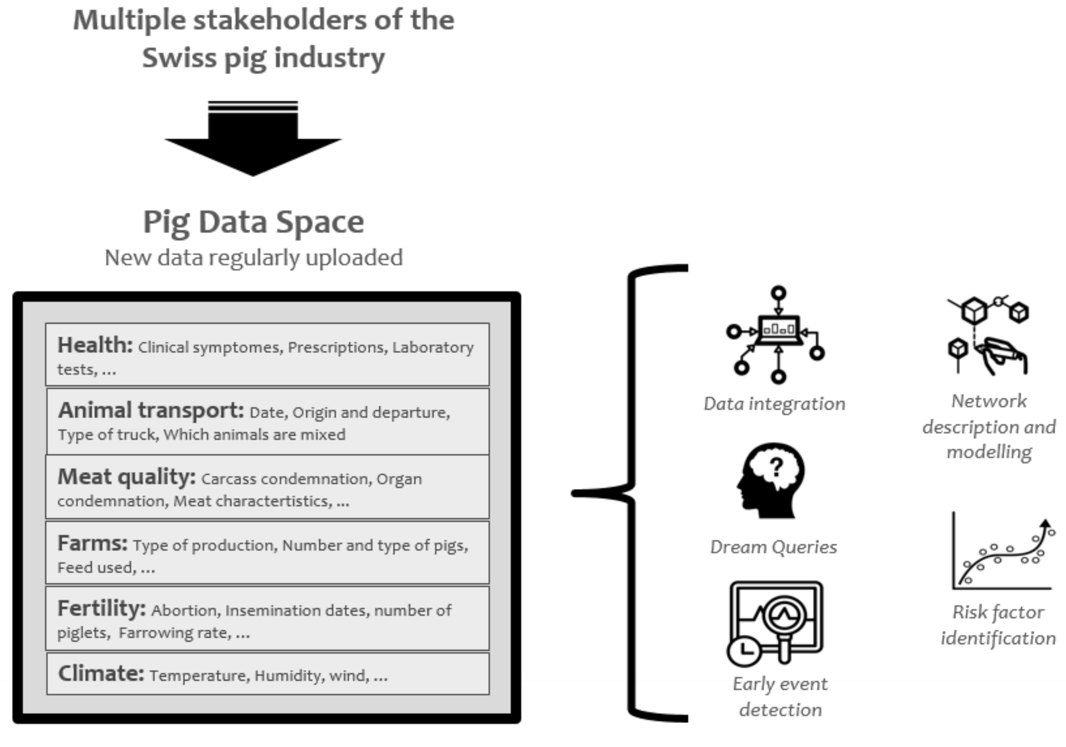

Fig.1 Schematic diagram of the centralised data store - Pig Data Space

All the stakeholders joined the project on a voluntary basis, without receiving any monetary incentive. Further details on the project organization and the transdisciplinary methods used can be found in the paper by Faverjon et al. (2019).

Dream Queries

To assure that project outcomes were of relevance and valuable information was generated, industry stakeholders were prompted to list research questions they would like to see investigated. These were named dream queries. With the purpose of highlighting the benefits of data sharing, we encouraged the selection of dream queries that required combining multiple datasets. Each dream query was approved by the entire research team and all industry partners. Every stakeholder had the right of veto the answering of any dream query. This meant that if a stakeholder did not agree that a certain analysis should be performed he/she had the right to anonymously stop the analysis for the dream query without providing any explanation.

In total, 18 dream queries were approved. Table 1 provides a selection of dream queries that demonstrates the scope of queries proposed by stakeholders, and illustrates that merging multiple data sources was needed to answer them.

Table 1. Example of dream queries

Number	Dream Query
1	What is the general relationship between genetics, breed, weather, transport condition (duration, loading time, etc.), and carcass quality (lean meat, condemnation rate, slaughter weight, weight pork chop calculated, ham weight calculated, and ham weight calculated without bones, fat, and tendons)?
6	Does the frequency of parturition depend on the phase of the moon?
8	Is there a difference in fattening performance and revenue between batches of fattening pigs originating from the same breeding herd compared to mixed batches, i.e., with pigs from different herds?
12	Is there a seasonal pattern or do weather conditions influence the occurrence of haemorrhagic intestinal syndrome and cannibalism?
16	How is the Swiss swine industry organised? In particular, what is the proportion of fatteners with assigned breeders vs. those enrolled in a free market scheme?

Industry partners were highly engaged throughout the analysis of dream queries. They actively contributed to improving data literacy and the discussion of results and their interpretation. In addition, stakeholders were frequently asked about their satisfaction with the progress of the project and were required to provide feedback on project activities.

In order to guarantee data confidentiality and build trust with the industry partners, guidelines were developed for sharing of analysis and results both within and outside the Pig Data consortium

Dream Query 1 - Relationship between weather, transport conditions, production type and carcass quality

Here we provide an example to illustrate the process used during the analyses to answer a dream query. The example pertains to the relationship between several production factors and carcass quality. All the analyses were conducted in Jupyter notebooks using R.

Data selection: The first step was to assess the feasibility of answering the dream query considering the data that was available in the Pig Data Space. Data about transport duration, lairage time, production type, weather and carcass quality were available. However, information about genetics and breed were not available and could not be included in the analysis. These limitations were communicated to the industry partners and the dream query was modified accordingly.

Slaughterhouse, transporter and weather data were used for this analysis. Slaughterhouse and transporter data were merged using the farm identification number, the "date of slaughter" and "date of arrival". If a farm used more than one transport on a given day to send pigs to the slaughterhouse, all the transports from this farm on this specific date were removed from the dataset. We were unable to join the multiple transports in the two datasets. The delivery time recorded at the slaughterhouse was often the same for all the pigs coming from the same farm on a given day, even if the pigs actually arrived at different times and on different trucks. Only animals classified as fattening pigs were included in the analysis; boars and sows were excluded given that they represented a limited number of animals within the datasets.

Data cleaning and pre-processing: Values deemed impossible by experts in the research team were excluded from the analysis. Due to the fact that data were collected at different levels (individual vs. batch) for certain parameters, two different datasets were created. Dataset 1 was used to assess factors affecting lean meat percentage (MFA), polyunsaturated fatty acid (PUFA), warm weight and iodine values. These parameters were recorded for batches with more than 10 pigs. Because PUFA and iodine values were only available at the batch level, the average lairage time for each batch was used. This dataset contained a total of 4'811 transports between 01-07-2014 and 31-03-2018. This included 12'655 batches and 554'503 pigs from 634 farms. Dataset 2 was used to assess factors affecting organ condemnation. These parameters were only available at the batch level. For this reason, the average lairage time for each batch was computed. This dataset contained information from 4'584 transports occurring between 01-07-2014 and 31-03-2018. These data pertained to 12'025 batches. Data from a total of 528'591 pigs from 630 farms were included in the analysis. A summary of the variables included in the analysis can be found in Table 2.

Statistical analysis: Linear Mixed Models (LMM) were used for continuous outcome variables. For binomial outcome variables logistic Generalised Linear Mixed Models (GLMM) were used. For both types of models, farm and batch were included as random effects, with the latter nested with the farm variable. The Likelihood Ratio Test (LRT) was used to compare each univariate model with the corresponding null model. The significance level of the LRT p-value was set at 0.25. Multivariate analyses included only those explanatory variables in which the LRT p-value was less than 0.25 in the univariate analysis. The two random effects (farm and batch) were included in these models. Multivariate model selection was performed using a backwards step selection procedure. Regarding the random effects analysis, the variance partitioning coefficient (VPC) and the intraclass correlation coefficient (ICC) were used for the models with a continuous outcome. The median odds ratio (MOR) was used instead for models with a binomial outcome.

Communication strategy: Acknowledging the relevance of the communication of results in transdisciplinary projects, we developed a communication strategy. Results were transmitted to the stakeholders via several channels: scientific report, newsletter and face-to-face meetings. Our partners were given the opportunity to comment on the results and help to interpret the results.

Table 2. Summary of variables included in the statistical analysis (part I)

Variable	Outcome/ explanatory variable	Type of variable	Further information
Lean meat percentage (MFA)	Outcome	Continuous	Collected at individual level
Warm carcass weight	Outcome	Continuous	Collected at individual level
Polyunsaturated fatty acids (PUFA)	Outcome	Continuous	Collected at batch level
Iodine value	Outcome	Continuous	Collected at batch level
Head condemnation	Outcome	Binomial	Collected at batch level
Cannibalism	Outcome	Binomial	Collected at batch level
Skin lesions	Outcome	Binomial	Collected at batch level
Transport time	Explanatory	Continuous	Transport time = pig drop time – pig load time
Lairage time	Explanatory	Continuous	Lairage time = delivery time – slaughter time
Production type	Explanatory	Categorical	3 categories: 1) BIO - pig from organic farms; 2) WELFARE+ - pigs from farms with higher standards in terms of animal welfare; 3) Standard - pigs from farms with standard production system
Time of the day	Explanatory	Categorical	3 categories: 1) morning; 2) afternoon; 3) night In summer (May to September), “morning” was from 5h to 10h, “afternoon” from 10h to 20h, and “night” from 20h to 5h. In winter (October to April), “morning” was from 7h to 11h, “afternoon” from 11h to 16h, and “night” from 16h to 7h.

Table 2. Summary of variables included in the statistical analysis (part II)

Variable	Outcome/ explanatory variable	Type of variable	Further information
Average temperature	Explanatory	Categorical	4 categories: 1) Very low temperatures were those with an average temperature bellow 0; 2) Low temperatures were those with an average temperature between 0 and 10 degrees; 3) Medium temperatures were those with an average temperature between 10 and 20 degrees; 4) High temperatures were those above 20 degrees
Temperature variation	Explanatory	Categorical	Temperature variation = Maximum temperature – Minimum temperature 3 categories: 1) Small temperature variation were days with variation between 0 and 5 degrees; 2) Medium temperature variation were days with variation between 5 and 10 degrees; 3) Large temperature variation were days with variation greater than 10 degrees
Average relative humidity	Explanatory	Categorical	3 categories: 1) Low Relative humidity were days with relative humidity bellow 50; 2) Medium Relative humidity were days with relative humidity between 50 and 70; 3) High Relative humidity were days when the relative humidity was above 70

RESULTS

Meat quality parameters - Dataset 1

Univariate models: The results obtained for each univariate model of dataset 1 and 2 are summarised in Table 3 and 4, respectively.

Multivariate models: To make it easier for the reader, the results of the multivariate models for datasets 1 and 2 (related to meat quality parameters) are summarised for each explanatory variable.

Table 3. P-values of the likelihood ratio test (LRT) obtained by comparing each univariate model with the null model for the outcome variables (MFA, warm weight, PUFA, iodine value) for dataset 1. * highlights the variables for which the p-values were below the 0.25 threshold and, thus, were kept for the multivariable analysis.

Explanatory variables	Outcome variables			
	MFA	Warm weight	PUFA	Iodine value
Transport time	0.254	0.041 *	0.456	0.486
Lairage time	0.190 *	<2.2x10^{-16} *	1.7x10^{-7} *	1.0x10^{-14} *
Average temperature	0.649	<2.2x10^{-16} *	1.0x10^{-11} *	<2.2x10^{-16} *
Temperature variation	8.3x10^{-9}*	<2.2x10^{-16} *	1.0x10^{-13} *	<2.2x10^{-16} *
Average relative humidity	0.001 *	0.001 *	0.356	0.494
Loading time	0.154 *	0.093 *	0.003 *	0.003 *
Drop time	0.029 *	2.2x10^{-7} *	0.0003 *	1.9x10^{-10} *
Production type	0.001 *	5.3x10^{-10} *	<2.2x10^{-16} *	<2.2x10*

Transport time was not associated with MFA, PUFA, warm carcass weight and iodine. It had a limited association with calculated pork chop weight, calculated ham weight, and ham weight calculated without bones, fat and tendons. The warm carcass weight decreased by 0.01kg/hour of transport. These results should be interpreted carefully as there are concerns about the quality of data for transport time. Lairage time had a negative association with all the meat quality parameters except for iodine values (+0.11 g/100g per hour). The largest impact of transport time was on the warm carcass weight: pigs lost on average 0.61 kg/hour of lairage.

The time of day when pigs were loaded or dropped off was associated with some meat quality parameters. Warm carcass weight was lower by 0.24 kg for pigs loaded during the night compared to pigs loaded in the morning. Iodine values were higher for animals loaded in the afternoon (+0.73 g/100g compared to animals loaded during the morning and +0.75 g/100g compared animals loaded at night).

Production type was associated with the entire set of meat parameters. Carcasses from BIO producers had higher PUFA (+2.22 compared to pigs from Standard production, and +2.37 compared to WELFARE+) and iodine (+2.50 g/100g compared to WELFARE+, and +2.00 g/100g compared to Standard). Carcasses from WELFARE+ producers had lower warm carcass weights (-1.18 kg compared to BIO and -1.42 kg compared to Standard). Finally, carcasses from Standard production had higher MFA (+0.28), warm weight (+1.42 kg), and iodine (+0.5 g/100g) than carcasses from WELFARE+ producers.

The lower the average temperature on transport days, the higher the warm weight, PUFA, and iodine value. The impact of temperature on PUFA was, however, very limited. Large daily variation in temperature during transport were associated with lower warm weight, PUFA, and iodine value. On the other hand, larger daily variations in temperature were associated with an increase in MFA.

Daily average humidity affected some meat quality parameters. High average relative humidity was associated with increased MFA.

Random effects: Differences between farms is much larger for PUFA (62.8%) and iodine values (58.1%) than for MFA (11.3%) and warm weight (10.7%). The ICC was only used for continuous variables for which data were recorded at batch level – MFA (23.2%) and warm weight (35.2%).

Organ condemnations - Dataset 2

Univariate models: When head condemnation was used as an outcome variable, the models with loading time and the production type were significantly better than the null model. On the other hand, all the variables were kept for the multivariate model with cannibalism as an outcome variable. For the univariate analysis of skin lesions, the p-value of the LRT was smaller than 0.25 when modelling transport time, average lairage time, average temperature, temperature variation and drop time.

Multivariate model: The multivariate model for skin lesions did not converge, probably due to the low number of cases (73 cases out of 528’591 pigs slaughtered). With respect to head condemnations, none of the selected variables were statistically significant. On the other hand, with the exception of average temperature and average relative humidity, all the variables selected in the univariate analysis for cannibalism were kept in the final model. The variable with the largest impact on cannibalism was the type of production system. Carcasses with signs of cannibalism had 1.55 times higher odds of coming from a standard production system than from a WELFARE+.

Random effects: Regarding the head condemnation, the difference between farms appears limited (MOR=1.49). However, differences are more important for cannibalism (MOR=4.26).

DISCUSSION

Before this project, individual stakeholders within the Swiss swine industry limited the use of their data to the control of their own business and production processes. By combining data from multiple varied sources, we were able to answer questions posed by industry partners that would not have been possible otherwise. The dream query presented in this paper provides an insight into the operationalization of a transdisciplinary approach and exemplifies how these approaches can be useful for generating information of value to stakeholders.

Based on our experience, transdisciplinary approaches can be useful in supporting the implementation of Big Data in two aspects. The first is data sharing. It has been reported that data sharing constitutes a barrier in the field of public health (Van Panhuis et al., 2014). In the private sector, this problem is exacerbated by potential competitive disadvantages caused by inadvertent sharing of sensitive data. Transdisciplinary methods build trust between stakeholders and researchers. Furthermore, it applies scientific knowledge to solve a societal issue, thus benefiting the data provider. The second is data integration. This has been highlighted as a major challenge for Big Data approaches (Chen et al., 2013). Data integration is a particular challenge when many independent actors are involved (as is the case in this project and livestock production in general) in data collection as data semantics and data syntax are more likely to differ between data collectors. Transdisciplinarity can facilitate data integration because close collaborations are established between analysts and data providers. Stakeholders play a significant role in understanding the different data sources, which is essential for integrating divergent data, as well as in the interpretation of results.

An almost sine qua non condition of transdisciplinary approaches is to produce outputs of relevance for the societal group. In the presented example, we answered specific and practical questions that were raised by the industry partners. The results we generated were of value to inform the stakeholders’ practices with respect to transport and lairage conditions and production methods. These results can help the Swiss swine industry to maximise economic benefits an improve animal welfare. The results of the random effect analysis constitute a good example of the value of the information generated. We calculated high VPC’s for PUFA and iodine values. This is relevant information for pig producers because it means that some farm characteristics or production practices (these could be nutrition, fattening performance, age of the pigs, barn conditions, or many other factors) had a consistent effect on these carcass quality parameters. Such farm characteristics and production practices can be further investigated and identified. Once they are known, producers who wanted to improve the PUFA and iodine of the carcasses they produce can implement them as part of their farm management.

The use of large datasets is not without significant challenges. It must be stressed that the data in our study were generated for a non-scientific purpose, mostly to manage the stakeholders' operations. The original purpose of the data affects the data format, which can affect data quality, the analysis that can be performed on the data and the generalisability of the results. The dream query example presented in this paper reflects those difficulties. Due to the way data were collected, we had to restrict the analysis to fattening farms that sent batches of more than 10 pigs and only one transport per day to the slaughterhouse. The level (pig vs. batch) at which we could perform the analysis was also influenced by the data characteristics. The concept of the dream query analysis put the emphasis on the needs of the industry partners; but it also circumvents the traditional hypothesis-driven rationale of most scientific questions. Both above mentioned factors present emerging challenges for researchers that will require new skillsets.

We have shown that Big Data approaches can be successfully implemented in livestock production systems using transdisciplinary methods. Throughout the Pig Data project, we were able to generate high-value information for both industry stakeholders and researchers.

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ASF IN WILD BOAR – GIVING A VOICE TO LATVIAN HUNTERS

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SUMMARY

African swine fever (ASF) is circulating in several parts of the world, including Europe. Control measures for ASF in wild boar include wild boar population management and passive surveillance. The willingness of key stakeholders to participate in these measures is of utmost importance. In Latvia, tools of participatory epidemiology were used to allow hunters to express their views about working with other stakeholders to control ASF. The results of this study provide new insights into the perception of Latvian hunters on different topics related to ASF, its control in wild boar and hunting in general. These perceptions can form the basis for adapting ASF control in the country. Involving participants may help to improve the willingness of hunters to support the fight against ASF.

INTRODUCTION

The spread of African swine fever (ASF) in the world, but also within the European Union (EU) is unrelenting. Since its introduction into the Baltic States and Poland in 2014, the disease has continued to spread. It reached Belgium in western Europe and East Asia including China in 2018 (EFSA, 2015, 2018b). Latvia was one of the first infected countries in the EU and there are still new cases appearing in wild boar today (EFSA, 2018b; Schulz et al., 2019).

The spread and manifestation of the disease with large geographical jumps, which are most probably due to human activities, go along with local spread caused by contact between wild boar and their movement (EFSA, 2015; Podgorski and Smietanka, 2017; EFSA, 2018b; Pejsak et al., 2018). Wild boar play an important role in the risk for introduction of ASF into domestic pigs (Olsevskis et al., 2016; Nurmoja et al., 2017a; Nurmoja et al., 2017b; Nurmoja et al., 2018). Therefore, several ASF control measures in Latvia have not only addressed wild boar hunting, but also the reporting, sampling and removal of wild boar carcasses from the environment.

Implementation of the measures, which focus on control of the wild boar population and on passive surveillance, depends mainly on the involvement of hunters. Their role in population management and their knowledge of the local situation contribute largely to the success of ASF control. Therefore, stakeholder involvement should be further prioritised in control measure planning and evaluation (Allepuz et al., 2017; EFSA, 2018a).

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Participatory epidemiology has proven to be a viable tool to include key stakeholders (particularly in developing countries) in different stages of decision making (Chambers, 1994; Calba et al., 2014; McKerr et al., 2015; Allepuz et al., 2017). Its rising reputation in developed countries may be based on the possibility of including the expertise of local stakeholders on daily routines and looking at complex issues from different viewpoints (Catley et al., 2012; Bronner et al., 2014; Calba et al., 2015b; Schulz et al., 2016; Allepuz et al., 2017; Bach et al., 2019).

Conducting focus group discussions is a suitable technique in this context. By applying this method in cooperation with Latvian hunters, including also ranking and visualization methods, this study assessed the acceptance of ASF control measures by Latvian hunters.

MATERIALS AND METHODS

Recruitment of participants

Hunters from five different counties in Latvia were recruited. Overall, the participating hunters came from five out of six statistical regions. (Fig. 1). In collaboration with the two largest Latvian hunters' associations, hunters were contacted by phone from April to June 2019. Using a list of potential regional/local participants provided by the associations, leading hunters of the regionals hunting clubs were also contacted.

During the initial contact, hunters were informed about principles and tasks of participatory epidemiology, the study aims and logistic details and rules regarding the meetings, including confidentiality and recording.

Using these instructions, the leading hunters suggested and contacted hunters to take part in the meetings.

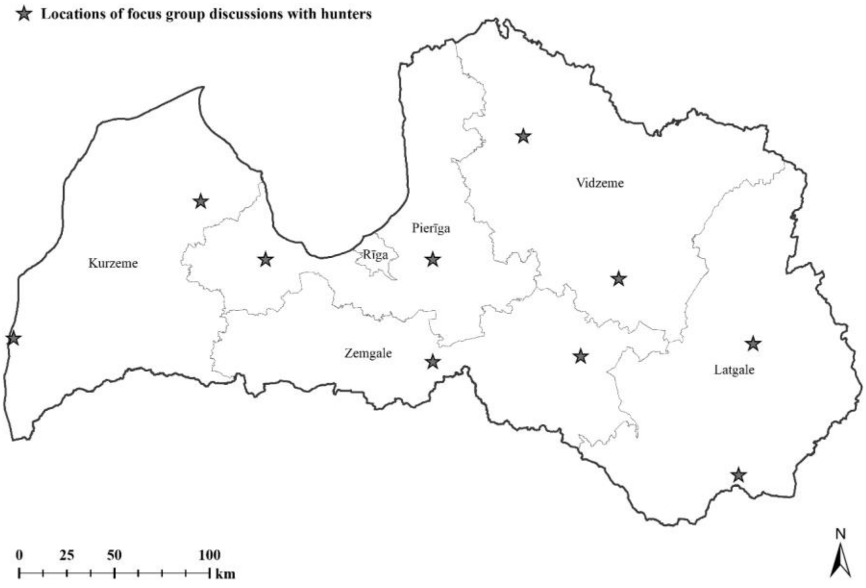


Fig. 1 Locations of the ten focus group discussions with hunters in Latvia

Focus group discussions

For the meetings, three to six hunters were recruited per focus group discussion. All meetings were moderated by the same scientist. All participants gave their permission for a written record of the meeting to be prepared by the scientist and for publication of the results under the limitation that the identity of the hunters was not disclosed. The entire discussions were transcribed. The discussions were held in Latvian and the transcripts later translated into English to avoid any language barriers.

Participatory methods were adapted from Schulz et al. (2016), Calba et al. (2015a) and data from a study conducted in Estonia (Urner et al., unpublished). All analyses were performed in a semi-quantitative manner and the discussion results were included descriptively.

Relation diagram

To evaluate the satisfaction of the hunters with their professional network, they were asked to list every stakeholder, they could think of, who is involved in planning and implementing control measures for ASF in wild boar. These stakeholders were recorded by the facilitator. At the same time the hunters were asked to describe the role of these stakeholder in relation to the control measures. Afterwards, the hunters, who were also stakeholders were added to the list. In the next step, the participants were asked to display the quantity of contact, they had with each individual stakeholder and to rate it from ‘no contact’ to ‘daily contact’ with arrows (Fig. 2). Consensus had to be reached in each group on this issue. Each contact was described in both directions, i.e. from the hunters to the stakeholder and vice versa. In a further step, the relationship was rated qualitatively. To this end, every participant rated each arrow individually using smileys (Fig. 2). In the last step, ‘proportional piling’ was used to assess the hunters’ trust in each individual stakeholder, including the hunters themselves, in the implementation of control measures (Catley et al., 2012). Based on consensus, the participants had to distribute 100 glass beans to the stakeholders relative to the trust, they had in the respective stakeholder to implement control measures.

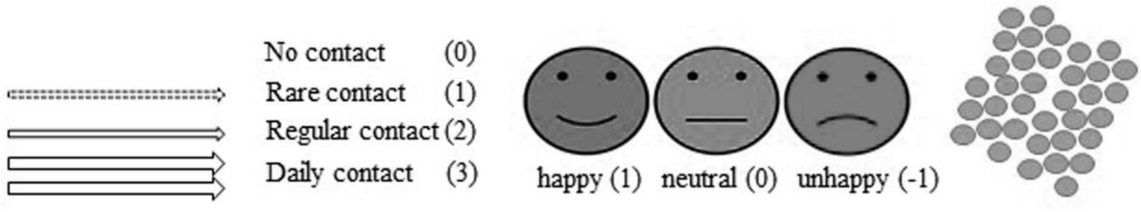


Fig. 2 Arrows, smileys and glass beans used in the participatory tools shown with the assigned ranks for the quantitative analyses

Acceptability of control measures

In order to evaluate the acceptability of control measures against ASF in wild boar, trust in the measures to control ASF and the satisfaction in implementing these measures was measured. The following six control measures for ASF in wild boar were presented to the hunters:

- Fencing
- Ban of hunting
- Including professionals for intensive hunting (police/ army)
- Increased hunting of females
- Incentives for hunting
- Increased carcass search and removal

After explaining the tasks to the hunters and ensuring a shared understanding of the listed measures, the participants were given the chance to add further control measures. With the use of the smileys, the hunters were asked to contribute their personal views on the listed control measures. Discussions were conducted until consensus for the hunters' trust in the measures had been reached within the group. Proportional piling was then applied to portray the hunters' trust in the effectivity of the measures (Fig. 2). Thereby, 100 glass beans were proportionally assigned to the listed control measures.

Quantitative analyses of focus group discussions

By assigning the arrows ('no', 'rare', 'regular', 'daily contact') to ranks (0, 1, 2, 3), the average for contacts with each individual stakeholder was calculated.

To illustrate satisfaction in implementing control measures, the ranks (1, 0, -1) were assigned according to the corresponding smileys. By calculating the average of the ranks assigned by each hunter, a single value for each control measure was obtained for every focus group. Subsequently, the means of all groups were calculated for each control measure.

The proportional distribution of the glass beans represented the trust of the participants in the measures to control ASF in comparison to the other control measures mentioned in the group. Several factors were included to calculate the weighted average for the perceived trust. Included factors were (1) the glass beans assigned to the respective control measure, (2) all control measures mentioned in the same group and (3) the number of all control measures mentioned in all groups.

RESULTS

Recruitment of participants and meetings

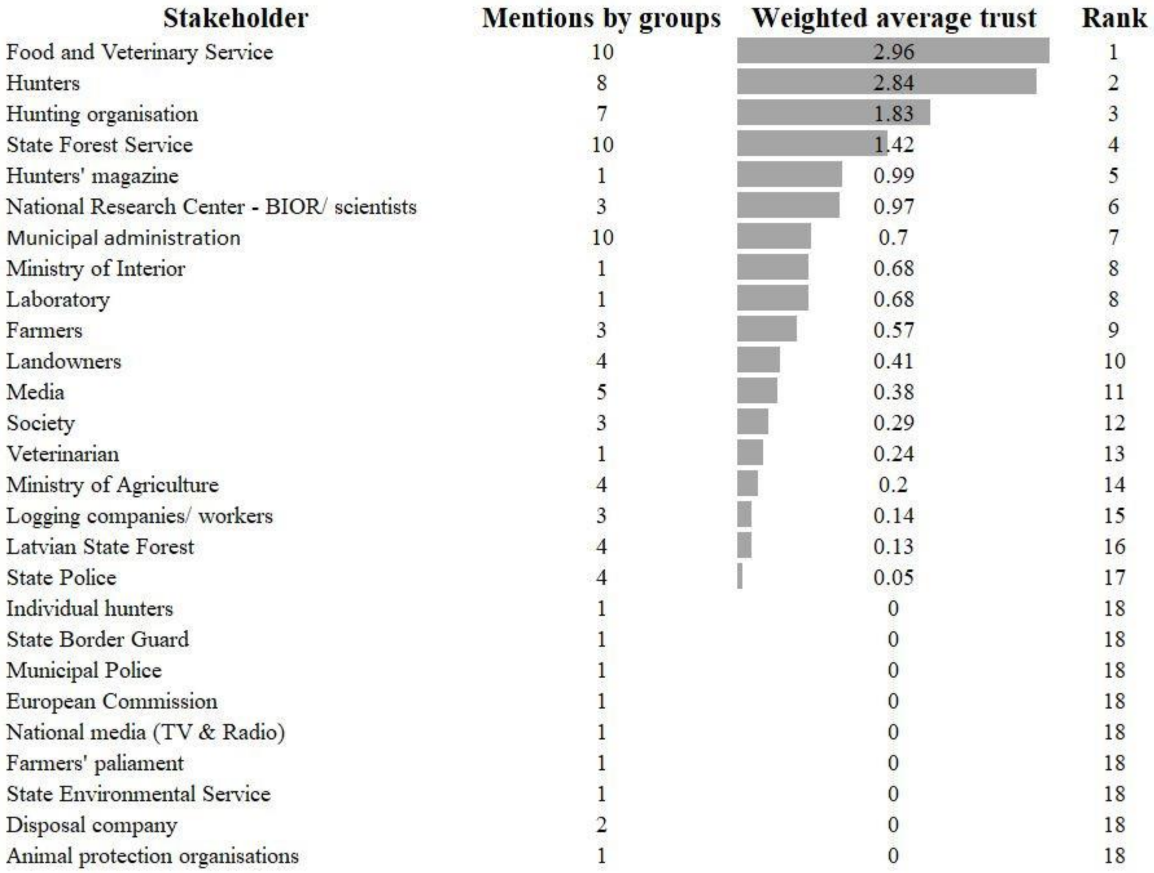
Ten focus group discussions in ten different locations across Latvia were conducted from May to July 2019 (Fig. 1). The hunters who attended the discussions were from the area around the meeting place. 50 hunters participated in total, 49 were male and one female. The average age was 50 years (Table 1).

Perceptions

A total of 27 stakeholders were named by the participating hunters. Food and Veterinary Service (FVS), the State Forest Service (SFS) and the Municipalities were mentioned by all groups, followed by hunters as stakeholders by eight groups and hunting organisation by seven groups. The media, landowners, Ministry of Agriculture (MoA), Latvian State Forest, State Police, Scientists of the National Research Centre (BIOR), farmers, society and logging companies were listed by three to five groups (Fig. 3). The participants had the highest trust in the correct implementation of control measures in the FVS followed by hunters, hunting organisation and the SFS (Fig. 3).

Table 1. Number of participants per city of the performed focus group discussions

City	No of participating hunters
Ropazi	6
Tukums	4
Valmiera	4
Madona	4
Barbele	7
Jekabpils	6
Talsi	5
Liepaja	5
Kraslava	5
Rezekne	4

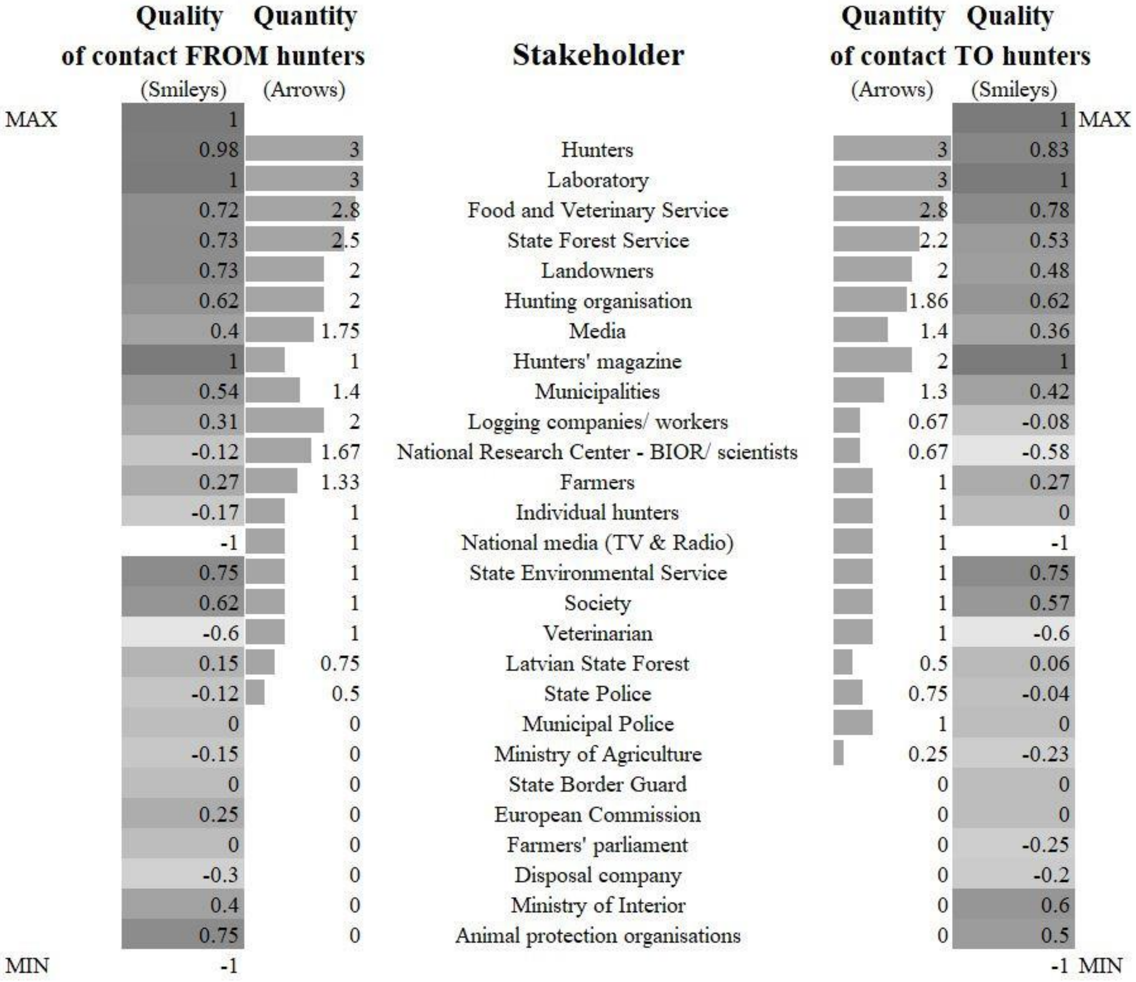


Stakeholder	Mentions by groups	Weighted average trust	Rank
Food and Veterinary Service	10	2.96	1
Hunters	8	2.84	2
Hunting organisation	7	1.83	3
State Forest Service	10	1.42	4
Hunters' magazine	1	0.99	5
National Research Center - BIOR/ scientists	3	0.97	6
Municipal administration	10	0.7	7
Ministry of Interior	1	0.68	8
Laboratory	1	0.68	8
Farmers	3	0.57	9
Landowners	4	0.41	10
Media	5	0.38	11
Society	3	0.29	12
Veterinarian	1	0.24	13
Ministry of Agriculture	4	0.2	14
Logging companies/ workers	3	0.14	15
Latvian State Forest	4	0.13	16
State Police	4	0.05	17
Individual hunters	1	0	18
State Border Guard	1	0	18
Municipal Police	1	0	18
European Commission	1	0	18
National media (TV & Radio)	1	0	18
Farmers' paliament	1	0	18
State Environmental Service	1	0	18
Disposal company	2	0	18
Animal protection organisations	1	0	18

Fig. 3 Stakeholders named by all groups ranked by their trust of the participants to implement control measures against ASF

The participants had most contact with other hunters followed by stakeholders at the local level, for example the laboratory, the FVS, the SFS, hunting organisations and municipalities. Contact with veterinarians, disposal companies, the police, the Ministry of Agriculture and the

European Commission was rare or missing (Fig. 4). The quality of contact with other hunters, the laboratory, the FVS, the SFS, the State Environmental Service, landowners and hunting organisations was high, whereas the quality of contact to the national research centre, national media, disposal companies, police and the Ministry of Agriculture was low (Fig. 4). The quality of contact with stakeholders with a high level of interaction was generally higher than the quality of contact with stakeholders who were less frequently contacted.



	Quality of contact FROM hunters (Smileys)	Quantity of contact FROM hunters (Arrows)	Stakeholder	Quantity of contact TO hunters (Arrows)	Quality of contact TO hunters (Smileys)	
MAX	1				1	MAX
	0.98	3	Hunters	3	0.83	
	1	3	Laboratory	3	1	
	0.72	2.8	Food and Veterinary Service	2.8	0.78	
	0.73	2.5	State Forest Service	2.2	0.53	
	0.73	2	Landowners	2	0.48	
	0.62	2	Hunting organisation	1.86	0.62	
	0.4	1.75	Media	1.4	0.36	
	1	1	Hunters' magazine	2	1	
	0.54	1.4	Municipalities	1.3	0.42	
	0.31	2	Logging companies/ workers	0.67	-0.08	
	-0.12	1.67	National Research Center - BIOR/ scientists	0.67	-0.58	
	0.27	1.33	Farmers	1	0.27	
	-0.17	1	Individual hunters	1	0	
	-1	1	National media (TV & Radio)	1	-1	
	0.75	1	State Environmental Service	1	0.75	
	0.62	1	Society	1	0.57	
	-0.6	1	Veterinarian	1	-0.6	
	0.15	0.75	Latvian State Forest	0.5	0.06	
	-0.12	0.5	State Police	0.75	-0.04	
	0	0	Municipal Police	1	0	
	-0.15	0	Ministry of Agriculture	0.25	-0.23	
	0	0	State Border Guard	0	0	
	0.25	0	European Commission	0	0	
	0	0	Farmers' parliament	0	-0.25	
	-0.3	0	Disposal company	0	-0.2	
	0.4	0	Ministry of Interior	0	0.6	
	0.75	0	Animal protection organisations	0	0.5	
MIN	-1				-1	MIN

Fig. 4 Stakeholders mentioned by the participants and ranked by the quantity of contact in total. In addition, the calculated average quality of contact is provided

In the discussions, the work with the FVS was described as positive. It was stated that there was sufficient feedback and that the employees of the FVS were considered as very responsive, understanding and helpful in solving problems. The SFS was mentioned to be helpful and straight forward in working towards controlling ASF. Nevertheless, a lack of conversation and information flow towards hunters was reported. The participants pointed out that there were only contacts with the municipalities and the police, if these stakeholders had a problem and the hunters could help to solve it. The solidarity of hunters among each other and with the hunting organisation was seen as satisfying and important, as the participants perceived the hunters themselves and their organisation as the real workers against ASF with the biggest burden to carry. Logging companies and workers were regarded as not interested in issues relevant to hunting an ASF control. The presumed important role of loggers in the local spread of ASF was mentioned, as they were believed to ignore biosecurity rules. Authorities like the

Ministry of Agriculture or the national research centre BIOR were perceived as focused exclusively on their office job, without knowledge on the daily business in the forest. Further communication, including a chance for the hunters the express their opinions, was desired.

Acceptability of control measures

The most desirable and the most trusted measure to control ASF was vaccination. Incentives for hunting, intensive hunting and biosafety measures were also trusted and regarded as important. Selective increased hunting of females was less trusted and less desirable. Increased carcass search and removal, fencing and involvement of the army or the police was not trusted at all and perceived as the least satisfactory measures (Fig. 5). All mentioned control measures were included in the calculation of the weighted average of trust and satisfaction regarding implementation.

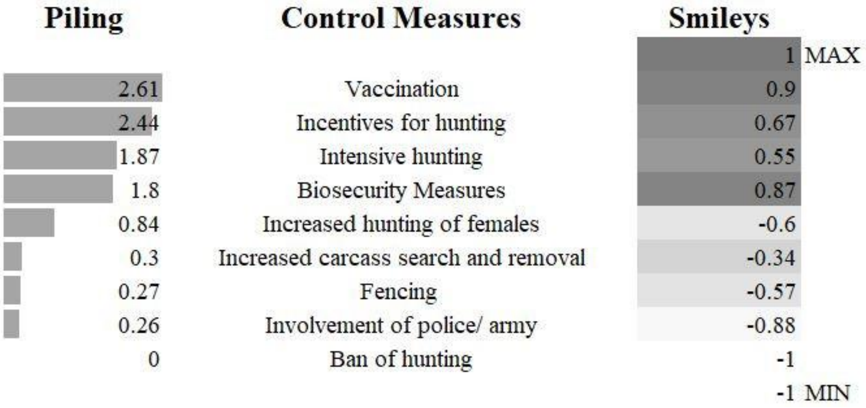


Fig. 5 Control measures mentioned by at least three focus groups, ranked by the perceived trust to control ASF and the correonding satisfaction of the participants to implement them

In the discussions, receiving money for hunting was mentioned to be obligatory to cover the costs of used materials. Nevertheless, it was seen as ethically questionable to go hunting only for money. Further controls and education on biosafety measures, especially for hunters, veterinarians and loggers, were regarded as beneficial.

Selective increased hunting of females was associated with producing orphans in the farrowing season and graded as unethical. However, this method was often perceived as the most effective for reducing the population size of wild boar.

The participating hunters believed that increased carcass search and the involvement of the army and police in implementing these measures would drive the spread of ASF forward. Furthermore, army and police were described as unfamiliar with hunting. It was feared that they might kill animals in an inappropriate or unethical way. The participants mentioned the negative effects of fencing on hindering the free movement other wild animals, but not the perceived vectors of ASF, like birds or small predators.

DISCUSSION

Although participatory approaches have huge value in involving key stakeholders, these methods have not been intensively use in veterinary epidemiology in developed countries (Catley et al., 2012; Calba et al., 2014; Calba et al., 2015a; Schulz et al., 2016). Using these methods, knowledge on special needs, daily routines and topic-related experience may become accessible. This information can then be included in decision making and framing directives. As any other method, participatory approaches have limitations. To reduce bias, the facilitator practiced the discussions and the meetings were transcribed afterwards.To evade local opinions and experiences, hunters across Latvia were involved in the study. Nevertheless, selection bias of participants, who were for example already in contact with authorities or eager to express their opinion, is a limitation of social studies and cannot be completely eliminated (Calba et al., 2015a; Schulz et al., 2016). The attendance of only a single female hunter represents a gender bias. Moreover, the number of only 50 participants may question the representativeness of the study for the Latvian hunting community, but theoretical saturation of new ideas and experience reports has been observed using this number, which may indicate that the sample size in our study war large enough (Glaser et al., 1967). Another limitation could be the loss of information. Despite our efforts to document each response of the participants, the translation process may have led to a loss of information and to misinterpretation.

Despite all of these limitations, the results may be used to adapt current control programmes for ASF in Latvia. The high quality of contact to the FVS and the State Forest Service is of high importance, especially in combination with the high contact rate. Office and field work regarding control measures against ASF in wild boar is done by these services and the hunters themselves, therefore the existing relationship, perceived as pleasant, can be seen as beneficial for controlling ASF. To provide a better communication and information flow from the SFS towards the hunters, joint meetings for discussing duties of every stakeholder and sharing latest information might improve the relationship. For logistical reasons and to take the hunters’ trust of their own association into account, representatives of the hunting organisations could be invited to attend these meetings and spread relevant information in the hunting community. As the participants perceived the FVS, the hunters, the local government and the police as the most important stakeholders, it would be advisable to include also representatives of the state police in these meetings. This may also help to mitigate the perceived lack of communication and interest of the police in tasks regarding ASF.

The low quality of contact with the national research centre and the Ministry of Agriculture was mostly due to the hunters’ interest in having more contact with the ministry. Information on new scientific data or the political situation regarding ASF could be provided through such meetings. Alternatively, hunting magazines may be included in the information flow, as a good interaction between hunters and magazines/media exists already. A faster and broader flow of information from the national research centre and the Ministry of Agriculture could be provided. Another suggestion was to post regular news on ASF via the media, which may provide continuous information to the society in general and raise awareness.As the contact with logging companies regarding biosecurity was seen as difficult, but perceived as important for controlling further spread, the communication of the authorities towards logging companies may have to be improved. Clear instructions from the authorities might be essential for the logging companies. Thereby, hunters may feel released regarding the burden they have to carry with their contribution controlling ASF.

Assuming that trust in effectiveness and satisfaction in implementing control measures are important to improve the acceptance of these measures, this study provides approaches to estimate the acceptance of ASF control measures by Latvian hunters.

Vaccination was mentioned by the participants as a measure for controlling ASF. However, the facilitator replied to these comments that no vaccine is available yet (Sanchez-Vizcaino et al., 2013; Barasona et al., 2019). Nevertheless, the hunters perceived a possible vaccine as the best solution, better than money and hunting, and the only way to control ASF completely. This mind-set could weaken the motivation to implement any of the currently available control measures. Therefore, further explanation and education on the effectiveness of other measures could lead to higher acceptance of these. Continuous education of hunters was also mentioned by the participants themselves on several occasions regarding knowledge on ASF and the understanding of control measures and their effects. As the individual mind-set of persons was perceived as more important than incentives to implement directives, further workshops or meetings were seen as helpful.

Hunting and every other mentioned measure supporting hunting were highly accepted. The need to cover the costs of materials combined with the attitude that money should not be the main reason to hunt, has to be taken into consideration, when providing monetary incentives. In order to assess the currently arising costs for hunters, they should be evaluated together with several hunters.The hunters saw an increased biosecurity problem in the already existing presence of loggers, veterinarians, citizens and hunters in the forest. However, through intensified carcass searches and the involvement of the army and police in hunting activities, this increased biosecurity problem was perceived to be even higher. Implementing discussions on biosecurity during work, transportation and presence in the forest especially for loggers, veterinarians and hunters could lead to a better understanding of its importance.

Some control measures were regarded as unethical (e.g. hunting sows) and thus poorly accepted. Therefore, altering such measures could lead to higher acceptance. Limiting the hunting of sows from all year round to the time outside of the farrowing season, as suggested by the participants, could lead to higher acceptance by all hunters. The involvement of army and police was considered as inappropriate as it may lead to uneducated and unethical “killing” of wild boar. Giving hunters a leading role in educating external forces and controlling the hunt jointly with these forces, may lead to a change in the attitude towards this measure. Fencing was seen as a waste of money and time, due to the fact, that it was perceived as ineffective. It may also hinder the movement of all wild animals. It seems therefore difficult to increase the acceptance of hunters for this method, even by limiting its use to small areas and including natural borders.The results of this study provide insights in hunters´ views on ASF control measures. Taking these views into account, may help to improve the control of ASF in Latvia. The findings may also be applicable in other countries.

ACKNOWLEDGEMENTS

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All hunters participated voluntarily in the group discussions and were informed about the objectives of the study. They approved the anonymous publication of the results. Formal consent or ethics approval was not required for this study, as it did neither include clinical trials in humans, for which ethical approval might have been required, nor animal experiments.

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DOES ‘SOFT’ DATA ADD VALUE TO THE DUTCH CATTLE HEALTH SURVEILLANCE SYSTEM?

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SUMMARY

In the Dutch cattle health surveillance system, routinely collected data are analysed to monitor trends in animal health. Changes in cattle health (e.g. increased cattle mortality), might also be explained by changes in cattle health management, which could be driven by changes in the farmers perceptions. Therefore, we created and conducted a questionnaire on some technical aspects and farmers perceptions regarding animal health (management) in Dutch dairy herds. The included farms were representative for the general Dutch dairy population in most herd characteristics but had a better herd health status. Many (26%) questionnaire variables were univariably associated with calf mortality in the study period, whereas none of the routinely collected factors, i.e. herd size, were. This indicates a relatively large attribution of ‘soft’ farm(er) characteristics to variation in calf mortality. Repetition of the questionnaire would enable us to investigate changes in perceptions and their association with trends in animal health.

INTRODUCTION

In 2002, a national cattle health surveillance system (CHSS) was introduced in the Netherlands that includes a quarterly data analysis. The aim of this data analysis, which is called the Trend Analysis Surveillance Component (TASC), is to monitor trends and developments in cattle health using routine census data (Santman-Berends et al., 2016). The routinely collected census data that are monitored include, for example: mortality, fertility, udder health, metabolic disorders, herd status for specific diseases, and antimicrobial usage. The associations between these key health indicators and characteristics such as herd size, purchase of cattle and region are investigated using population averaged regression models (Santman-Berends et al., 2016). However, several studies have shown that herd health (management) is highly influenced by “soft” subjective characteristics of the farmer, e.g. attitude and perceived control with regard to animal health (Ritter et al., 2017; Adler et al., 2019). Such data are not routinely registered and can only be obtained by interviewing a representative group of farmers about these factors.

The aim of this study was to develop and conduct a questionnaire among dairy farmers to deduce their perceptions, operationalised as their attitude and perceived efficacy of internal

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(e.g. management choices) and external (e.g. influence of the veterinarian) factors, related to animal health and welfare, with focus on management of calves. As such, we aimed to investigate the added value of including farmer characteristics in the TASC in explaining variation in animal health.

MATERIALS AND METHODS

Study population

A random selection of 5,000 Dutch dairy farmers was approached by e-mail in April 2019. The e-mail contained a link to the electronic questionnaire. One week after the first e-mail a reminder was send to farmers who did not start the questionnaire yet. Three weeks after the reminder, the questionnaire was closed.

Questionnaire

The questionnaire was kept short (maximum of 15 minutes) in order to maximise the response and prevent farmers' from prematurely ending the questionnaire. We also wanted to be able to repeat the questionnaire after 1-2 years to monitor changes in farmers perceptions.

Three brainstorm sessions with veterinary experts on dairy cattle health were organised to construct the questionnaire. The first session resulted in several themes that were ranked on their importance and expected variability between farmers in the second session. In the third session, a draft of the questionnaire topics was discussed. Then, the exact way to question farmers about these topics was discussed with a social psychologist (MP). The draft questionnaire was tested by eight farmers and adjusted based on their feedback. The final questionnaire in Dutch is available on request. The Royal GD legal department approved the questionnaire protocol and the questionnaire was carried out in accordance with the new EU privacy legislation. In the beginning of the questionnaire, participants were asked for their consent to use their questionnaire answers in this study. The questionnaire included questions on farmer characteristics (e.g. age), general farm management and themes linked to the key elements of the theory of planned behaviour: attitude, subjective norm and perceived behavioural control (Ajzen & Madden, 1986; Ajzen, 1991). With regard to attitude, farmers were asked about how they perceived animal health, which aspects of deceased calves bothered them the most, to what degree they enjoy farming and their view on future farming possibilities. With regard to the subjective norm, farmers were asked how they perceived the influence of their farm advisors on animal health (as a proxy for their influence on health management) and influence and support of the general public. With regard to perceived behavioural control, farmers were asked whether they thought they lacked knowledge on topics and how important they considered their own knowledge. Furthermore, questions were asked about available time and available financial resources for animal health and perceived influence of measures related to disease introduction and housing conditions on animal health (perceived behavioural control). Finally, famers were asked consent to combine their questionnaire results anonymously with data from the TASC.

Representativeness of participating farmers

It was possible to check the representativeness of participants for the Dutch dairy sector using data from TASC. Both populations were compared on TASC herd characteristics, such as herd size, milk production, growth in herd size, replacement rate, purchase of cattle, presence of an automatic milking system (AMS), location (province) and health statuses for BVD, IBR, paratuberculosis and salmonella. The participants were compared to the population of dairy herds in the first quarter of 2019 using either a linear regression or a test for equality of medians in case of continuous variables and logistic regression in case of dichotomous variables (Stata® 15.0).

The farmers that did not give consent could only be compared with the participants that did. Participants who prematurely ended the questionnaire were categorised as 'without consent to combine results with data from TASC'. Differences between the two groups were tested using a test for equality of medians (continuous variables) and chi-square tests (categorical variables).

Questionnaire analysis

In the questionnaire, single choice, multiple choice and likert scale questions (score 1-7) were included. The distribution of answers to the single/multiple choice questions were described using tables. The distribution of scores given to subjects and propositions of likert-scale questions were described visually per theme (R® 3.6.1).

To obtain an indication for how much variance the questionnaire results can explain, the association between questionnaire results and one of the key health indicators from the TASC: calf mortality rate between 15-56 days old, was investigated. The associations were investigated using a regression model with a Poisson distribution for the period in which the questionnaire was conducted (Stata® 15.0). In this case, the number of dead calves between 15-56 days was linked to the number of animals at risk to die (number of animal days at risk / the risk period of 41 days), the same method as used in the TASC (Santman-Berends et al., 2019). Likert-scale questions were included as continuous independent variables assuming a linear association with calf mortality rate between 15-56 days old. No full case analysis was performed as the questions were not obligatory and therefore some answers were missing. All questionnaire variables that were univariably associated with the calf mortality rate with a P-value ≤ 0.05 were subsequently investigated on their mutual correlations (Spearman). For highly correlated variables ($\rho > 0.5$), only the strongest associated variable was included during multivariable model selection. Using forward selection, a final model was obtained, taking into account confounding (>20% change in the regression coefficient).

The added value of the questionnaire results was determined by the increase in the approximate explained variance (pseudo R^2) when added to the regular TASC model for calf mortality rate. When this was not possible due to a limited amount of observations, all TASC herd characteristics univariably associated with a p-value ≤ 0.05 were taken into account during the forward model selection.

RESULTS

Description participating farm(er)s

In total, 592/5,000 farmers (11.8%) opened the questionnaire. In Fig. 1, a flow chart shows the number of farmers who filled in the questionnaire and how many of the farmers gave consent to link their questionnaire results to their data from the TASC. A proportion of farmers decided to end the questionnaire prematurely. Eventually, 387 farmers completed the questionnaire and 255 (65.9%) gave consent for linking their anonymised results to TASC data.

Due to new privacy legislation in the EU, farmers had to formally give consent for the usage of their personal data (i.e. their questionnaire results)

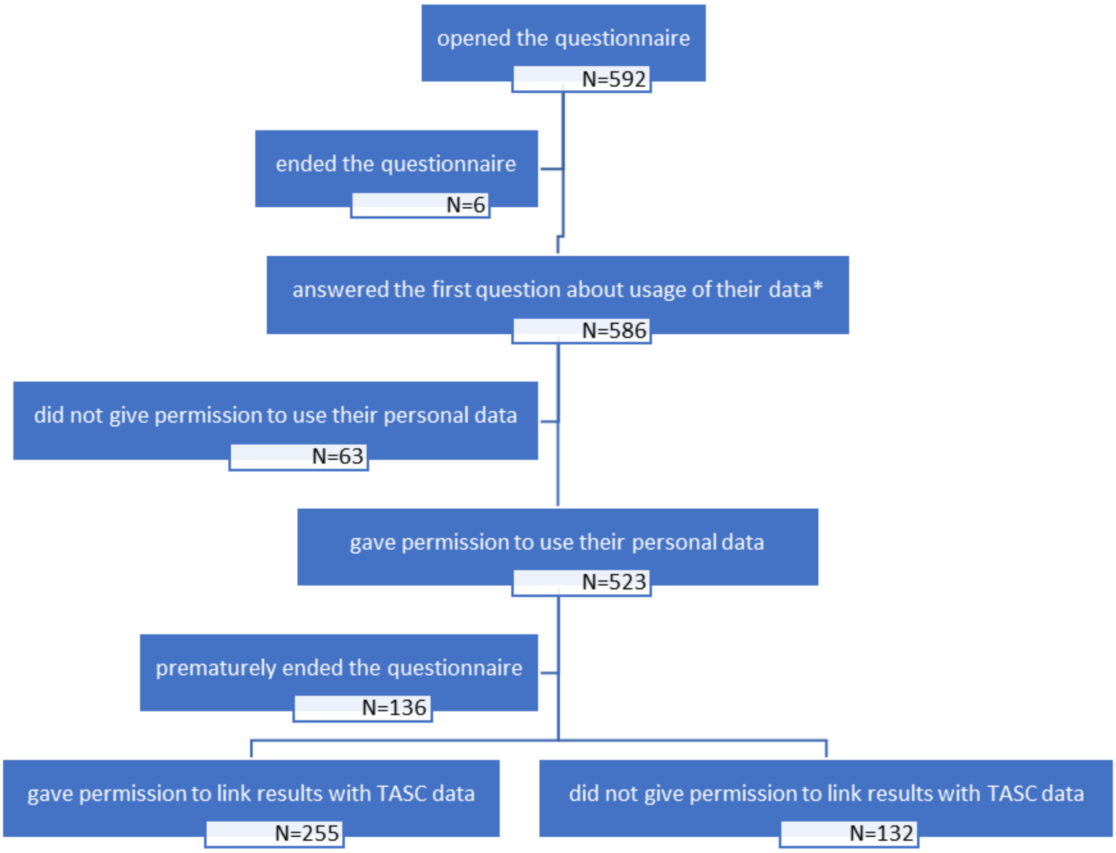

Fig. 1 Flowchart of farmers included in the study.

Representativeness of the participating herds

Study herds with TASC data had similar herd size and milk production compared to all Dutch dairy herds (Table 1). In addition, the number of farms with an AMS and the distribution over regions was similar. Study farmers less often purchased cattle and had a lower replacement rate compared to the average Dutch dairy farm. Furthermore, study herds were more often free of Bovine Viral Diarrhoea (BVD) and Infectious Bovine Rhinotracheitis (IBR), more often salmonella and/or paratuberculosis unsuspected than the average Dutch dairy herd.

With regard to participants with and without TASC data, it was possible to check the comparability of questionnaire results for 89 variables, of which 72 did not differ significantly between participating farmers with and without TASC data (80.1%).

Table 1. Comparison between study herds with TASC data and all Dutch dairy herds in the first quarter of 2019. Mean value including 95% confidence intervals (CI) and P-value for the test of difference.

Characteristic	Mean Dutch dairy sector	Mean study herds with TASC data	P-value
Herd size (number of cows (>2 years old))	103 (102 - 104)	101 (94 - 108)	0.67$^{\$}$
% young stock as opposed to the total number of cattle	14.3 (14.2 - 14.4)	14.5 (13. 8- 15.3)	0.95$^{\$}$
Net gains (indication of the milk production level)	2272 (2266 - 2277)	2304 (2263-2345)	0.14$^{\&}$
Standardised dairy production per cow in kg	42.2 (42.1 - 42.3)	42.9 (42.2 - 43.6)	0.07$^{\&}$
Percentage of growth in the number of cattle	1.8 (-0.9 - 4.6)	-2.4 (-3.5 - -1.3)	0.98$^{\$}$
Percentage replacement of cattle	27.5 (27.4 - 27.6)	26.2 (25.2 - 27.1)	0.01$^{\&}$
Percentage of herds not purchasing cattle	48.8 (48.1 - 49.6)	59.6 (53.5 - 65.7)	<0.01*
Percentage of herds with an AMS	27.0 (26.3 - 27.7)	30.1 (24.4 - 35.9)	0.27*
Percentage BVD free herds	65.5 (64.8 - 66.3)	76.1 (70.8 - 81.3)	<0.01*
Percentage IBR free herds	50.1 (49.3 - 50.8)	65.5 (71.4 - 59.6)	<0.01*
Percentage paratuberculosis unsuspected herds	80.9 (80.3 - 81.5)	89.0 (85.2 - 92.9)	0.01*
Percentage salmonella unsuspected herds	34.8 (34.1 - 35.6)	50.6 (44.4 - 56.8)	<0.01*

$^{\$}$ difference between the 2 groups is tested using a non-parametric equality of medians test

$^{\&}$ difference between the 2 groups is tested using linear regression

* difference between the 2 groups is tested using logistic regression

Description of questionnaire results

The mean age of the participating farmers was 49 years old (median of 51 years old). Almost all participants were owners of the farm and worked on the farm (94%). The mean amount of full-time employees on the farm was 2.1 (median 2). This included unpaid family work and paid employees. On half of the participating farms, (part of) the work was done by paid employees. The majority of the farmers (partly) outsourced work (73%). This concerned primarily land work (68%). Automatisation most often present was a rotating groom brush (71%). More than half of the participants used concentrate feed boxes (67%) and/or a manure robot (or comparable system; 56%). An AMS was used in 30% of the study herds. Different sorts of sensors, such as pedometers, were used in 29% of the herds.

Regarding calf management, the farmer him/herself was mostly responsible for the young calves (68%). In 19% of the herds this work was done by a family member and in 1% of the herds by employees. We also asked farmers which aspects of calf mortality bothered them the most. The primary issue among participating farmers was welfare and the issue of least importance was the opinion of other people (Fig. 2).

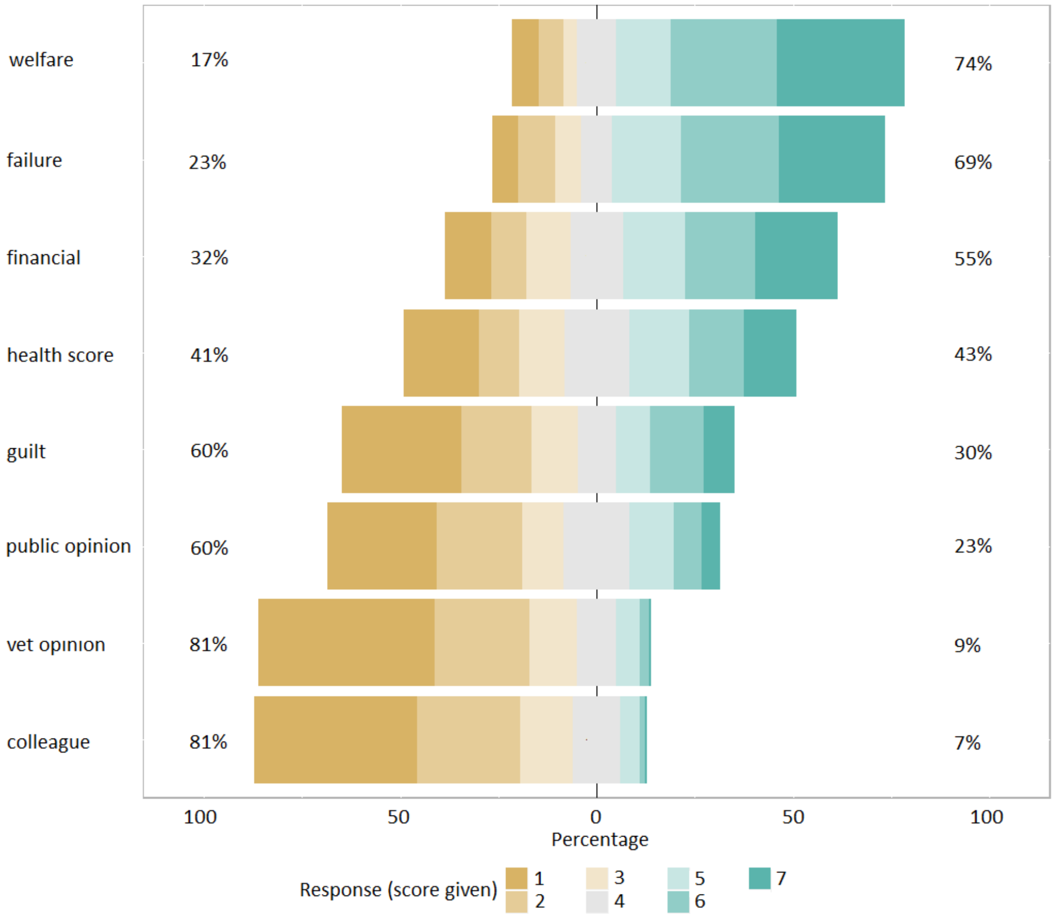

Fig. 2 Results for the question: "Please indicate on a scale of 1 (not applicable to me) to 7 (very applicable to me) to what degree the following statements are applicable to you: A deceased calf bothers me because: …". N=437-441. The percentages present the percentage of farmers' with a negative (score 1,2,3) or positive (score 5,6,7) score. Welfare=because of animal welfare, failure=because it feels like failure, financial=because of the financial consequences, health score=because my animal health/welfare score goes down, guilt=because I have the feeling that I did not do all I could to prevent it from happening, public opinion=because the publics' opinion about it, vet opinion=because of the veterinarians' opinion about it, colleague=because colleagues' opinion about it

Associations with young calf mortality rate

In total, 90 questionnaire items were checked for an univariable association with calf mortality rate between 15-56 days old and twenty-three were associated with a p-value ≤ 0.05 (significant; 25.6%).

After a check on highly correlated variables, 6 out of the 23 selected variables were excluded from the multivariable model selection. In addition, we excluded variables that concerned the farmers' perceived status of young stock raising, durability of the herd management and animal health as they are likely not to have a cause-consequence association with the calf mortality rate and might explain variance in the calf mortality rate that can also be explained by factors more indicative for farmer characteristics.

The number of observations did not allow for investigating the added value of the variables in addition the variables regularly included in the TASC model for calf mortality rate. The final multivariable model comprised of four variables, three variables from the questionnaire and one TASC variable which was included when, during model selection, TASC characteristics were taken into account (table 2). Firstly, farmers who considered their own knowledge and experience more valuable for animal health (higher score), had a lower calf mortality rate (Incidence Rate Ratio (IRR) 0.8; 95% CI: 0.6-0.9). Secondly, farmers who considered not using the calving pen for sick cattle as important in this context, also had a lower calf mortality rate (IRR 0.8; 95% CI: 0.7-0.9). Thirdly, farms with exclusively external labour (N=7) had a higher calf mortality rate than farms with family working on the farm (N=200), of which 106 were purely family farms and 94 farms also had some external employees. Finally, farmers who purchased >2 cows in the last 12 months had a higher calf mortality rate than farmers without purchase (IRR 2.4; 95 CI: 1.4-4.3).

Table 2. Poisson regression model for the calf mortality rate 15-56 days old. N=207 participants. Pseudo R2=14.3%.

Variable	IRR	95%CI	P-value
How important do you consider your own knowledge and experience in relation to animal health on your farm, on a scale of 1 (no influence) to 7 (much influence)?	0.8	0.6-0.9	0.001
How important do you consider absence of sick cattle in the calving pen in relation to animal health on your farm, on a scale of 1 (no influence) to 7 (much influence)?	0.8	0.7-0.9	<0.001
Percentage of family work of the total full-time equivalents			
0%	1		
100%	0.3	0.1-0.9	0.028
Partly	0.2	0.1-.0.5	0.002
Purchase of cattle			
Closed in the last 12 months	1		
Semi closed (1-2 cows in the last 12 months)	0.8	0.2-2.6	0.683
Open (>2 cows in the 12 months)	2.4	1.4-4.3	0.002

DISCUSSION

The aim of the study was to obtain subjective data from a large group of representative dairy farmers and to verify the added value of such data in the national surveillance programme. The added value can be both quantitative, i.e. as additional explanatory variables in the multivariable models in the TASC or qualitative, i.e. more insight in the drivers and barriers in animal health management experienced by dairy farmers.

The study herds were not different from the general Dutch dairy population in most herd characteristics such as region, herd size and milk production levels, but seemed to have higher herd health statuses. Despite these specific characteristics of the study population, there was enough variation in the questionnaire answers to relate them to the calf mortality rate 15-56 days old. This TASC health indicator was chosen because of the fairly high mortality in this age group of replacement heifers and the expected large role of management of the farmer in mortality in this age group. A quarter of the questions (25.6% of the items) were univariably associated (p-value ≤0.05) with the calf mortality rate in the study period. When univariable analyses were performed for the herd characteristics normally taken into account in the TASC, only one of the characteristics was univariably associated with the calf mortality rate in the study group with a P-value of 0.05. This indicates a relatively large attribution of subjective characteristics to the variation in the calf mortality rate.

Farmers who considered their own knowledge and experience more valuable to animal health had a lower calf mortality rate. These farmers may perceive more control over their herd health status, which links to ‘perceived control’ in the theory of planned behaviour (Ajzen & Madden, 1986; Ajzen, 1991). Farmers who consider their own knowledge and experience more important may also be more active in obtaining knowledge, which may result in management that leads to a lower calf mortality rate. The use of a calving pen for sick cattle is known to be associated with morbidity in calves. Farmers who consider using a calving pen for sick cattle a more important risk may be more likely to have a separate pen for sick cattle and thus have a lower calf mortality rate (Seppä-Lassila et al., 2016). Finally, farms where the work is partly or entirely done by family members instead of paid employees had a lower calf mortality. Continuity and regularity are important in calf rearing and possibly this is achieved more easily with only family involved. The variable ‘Who took care of the calves (farmer himself, family, employee, differs)’ did not end up in de the final multivariable model though.

It would be interesting to investigate if the farmers perceptions change with current changes in regulations and the public opinion regarding farming. Therefore in 1-2 years, the questionnaire should be repeated in a large, representative sample. The response to the questionnaire was not satisfactory though and the participating farmers seemed to have a better herd health status than the average Dutch dairy herd. In addition, 26% of the farmers who actually started the questionnaire, ended it prematurely. Personal or telephone interviews would have resulted in a higher response rate, less premature ending and maybe a better representation of the Dutch dairy herds. However, the costs would have been much higher as well.

To conclude, we were able to deduce farmers perceptions on animal health (management) and it was possible to explain additional variation in calf mortality 15-56 days old using the questionnaire. Nevertheless, the response was disappointing and, on some characteristics, the study herds differed from the average Dutch dairy herds. However, to investigate if trends in the perceptions are taking place and if these trends can be associated with trends in animal health, we would recommend that the same procedure and questionnaire is used.

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COMPANION ANIMAL EPIDEMIOLOGY

USE OF QUANTITATIVE AND QUALITATIVE METHODOLOGIES TO IDENTIFY A CORE OUTCOME SET (COS) FOR FELINE CHRONIC KIDNEY DISEASE RESEARCH

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SUMMARY

Chronic Kidney Disease (CKD) is a common cause of feline morbidity and mortality. To date, the existing evidence base for treatment efficacy for this condition has no consensus on which parameters are the most important to inform effective decision making for these patients. This study addressed this need, using systematic review, eDelphi and consensus meeting methodologies to create a proposed Core Outcome Set (COS) for feline CKD. The COS consists of 29 parameters, streamlined into 9 core themes. All parameters were rated as very important for inclusion in a COS (8 or 9 on a Likert scale 1-9) by > 80% of an international panel of stakeholders. The preliminary COS proposed in the eDelphi stage was discussed, confirmed, added to and streamlined in an international in-person stakeholder consensus meeting. This is the first feline COS ever created and only the second created in the veterinary field.

INTRODUCTION

In order to determine the most effective treatment and management strategies for a particular disease, it is imperative that during veterinary randomised controlled trials the most appropriate outcomes that matter to patients, clinicians and clients are measured. A Core Outcome Set (COS) can be defined as an agreed set of outcomes or outcome measures that should be measured and reported as a minimum in any trial conducted relating to a particular disease (www.comet-initiative.org). This concept has originated in human healthcare and has been used most notably in rheumatoid arthritis clinical trials, with a COS originating from the Outcome Measures for Rheumatology Clinical Trials (OMERACT) initiative. Since this COS was introduced, the consistency of measurement of the core outcomes proposed has been shown to improve (Kirkham et al., 2013). The importance of COSs is well established in human healthcare. It is recognised that without COSs, the outcomes reported in trials may not be reflective of endpoints that are meaningful for health service users (Williamson et al., 2012). In addition, where high quality COS are known to have been developed, their use in human healthcare trials is increasingly mandated by research funders and journal editors (Webbe et al., 2013). The Core Outcome Measures in Effectiveness Trials (COMET) Initiative brings COSs together. It has been created to foster methodological research for COSs, bring

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researchers together, develop resources, improve user engagement and to raise awareness of COSs. An internet based resource has been created where all COS developed and in development can be registered (Williamson et al., 2012).

The creation and use of COSs permits the robust comparison of results between studies, facilitating evidence-based clinical decision-making (Clarke & Williamson. 2018), and reduces unnecessary research waste (Hughes et al., 2019).

Chronic Kidney Disease (CKD) is defined as sustained structural or functional damage to the kidneys and is a common cause of morbidity in cats. It can affect cats of any age but is more common in older cats, affecting ⩾30–40% of cats over 10 years old (Sparkes et al., 2016). It causes clinical signs including: polydipsia, polyuria, weight loss, inappetence, hypertension, weakness, lethargy, vomiting, and anaemia (Sparkes et al., 2016). Treatment is symptomatic and supportive as it cannot be cured, aimed at slowing the progression of the disease, reducing the clinical signs and improving quality of life and life expectancy (Cannon, 2016). Cat owners and veterinary surgeons have to make important decisions about which treatments to give at the different stages as CKD is diagnosed and progresses. There are internationally recognised guidelines to support this decision making process, published by the International Renal Interest Society (www.iris-kidney.com). CKD can progress to the point where decisions have to be made around euthanasia, if the quality of life of the cat has become compromised. Research to date has not reached consensus on which parameters are the most useful to aid the treating veterinary surgeon or cat's owner in these decision making processes. Current published clinical trials research measures a wide variety of parameters, and information on all parameters is not available for all tested treatment options, limiting the evidence available to aid decision making.

The creation of COSs are commonplace in human healthcare. One has been created for veterinary healthcare, for canine atopic dermatitis (Olivry et al., 2018). The aim of this study was to create a COS for assessing treatment efficacy in cats with CKD.

MATERIALS AND METHODS

Systematic literature review

A systematic literature review was conducted which focused on identifying all parameters that had been measured and reported in published CKD treatment randomised controlled trials. A systematic review is a structured review that identifies published manuscripts which are relevant to a research question of interest, using inclusion and exclusion criteria. Information is then extracted and synthesized from the manuscripts in a pre-defined and structured manner (Jahan et al., 2016). Across the 20 publications which met the inclusion and exclusion criteria for the review, 104 individual parameters were extracted that assessed treatment efficacy for CKD. These parameters were then arranged into groups according to when and how the parameter might be measured. The groups were as follows: parameters the cat's owner might notice at home (e.g. exercise tolerance), parameters examined in the veterinary consultation (e.g. body condition score), urine test parameters (e.g. urine specific gravity), parameters related to CKD progression and lifespan (e.g. survival time), parameters related to being in a trial (e.g. occurrence of adverse events), blood test parameters (e.g. Packed Cell Volume) and more advanced testing (e.g. Plasma Renin Activity).

eDelphi

An eDelphi was designed to be carried out with an anonymous, international panel of stakeholders representing a number of different groups including clinical experts, journal editors, regulatory agencies and cat owners with experience of CKD. An eDelphi is an online form of a Delphi process, and is typically carried out using questionnaires or email (Hall et al., 2018). This process is a recognised and structured methodology for gathering opinions of experts and stakeholders and facilitating consensus (agreement) on decision making between them. It does this by presenting information or questions in a number of rounds to an anonymous panel. Initially the panel give their answers independently, but in subsequent rounds they are presented with the anonymised answers of the rest of the group and allowed to change their own answers in light of that information if they wish. This method helps to create a group consensus of opinion, without allowing any individuals to dominate or influence the decision making process (Sinha et al., 2011).

Panellists were selected from stakeholders who registered an interest in taking part after the study was advertised through known contacts and social media. The panel was selected from all those registered so that the final panel would be as international as possible, with all stakeholder groups represented, so that the veterinary surgeons selected varied as much as possible in date and University of qualification. The balance of stakeholder group relative sizes was decided in advance to be as close as possible to that used in the HOME group methodology (Schmitt et al., 2010) where of the whole panel size, 25% were owners (or patients in the HOME methodology), 60% were clinical experts and 15% were journal editors and regulatory agency representatives. These proportions also aimed for a balanced consensus across the stakeholder groups, in line with the *a priori* definition of consensus; for a parameter to reach over 80% of the whole panel rating it as 8 or 9, most of each stakeholder group had to rate it 8 or 9. The only way a parameter could reach consensus for inclusion without all stakeholder groups being in agreement was if all the owners and all the clinical experts rated it very highly, and it was thought that in that circumstance, the parameter would therefore be important enough for inclusion.

The panel in this study was tasked with completing a series of three iterative rounds of online questionnaires to build consensus on the most important parameters to measure when treating cats with CKD. Parameters extracted from the systematic review were presented to the panel, arranged into groups as described above. The definition of a COS was explained, and they were asked to rate the importance of including each parameter in the COS using Likert scales (1-9; 1 being not important to 9 being very important to include). Consensus was defined *a priori* as 80% of participants rating the parameter as 8 or 9 for inclusion and 1, 2 or 3 for exclusion. After each round, panellists were given the results of the whole panel's ratings (median and range) and their own previous rating. They were then given the opportunity to change their rating or keep it the same. In the early rounds of the eDelphi, panellists were encouraged to suggest new parameters they felt had not already been included. These were then presented to all panellists for rating in the later rounds of the eDelphi. It was decided in the study protocol that where >10% of panellists or a whole stakeholder group answered "I do not understand what this is" for a parameter, additional definitions would be given, and the parameter re-presented to panellists for rating. After all eDelphi rounds were completed, all parameters would have been rated by all panellists twice and a shortlist of parameters proposed for the final COS would be created. If panellists failed to complete a questionnaire, they were not included in the next questionnaires as the results of the eDelphi were cumulative.

Consensus meeting

An in-person one day consensus meeting was held consisting of an international panel of stakeholders, representing the same stakeholder groups as the eDelphi.

Borderline parameters: In this meeting, the first task was to identify any parameters which had not reached the consensus in the eDelphi for inclusion in the COS but had been the closest to reaching it. These were termed "borderline parameters". Within the borderline parameters, those for which there had been the greatest disagreement between stakeholder groups were addressed. Stakeholder responses to the eDelphi for the borderline parameters were separated into cat owner responses and all other stakeholders' (termed "healthcare professionals" or "HCPs") responses. This was to mirror the methods used in human healthcare COSs, where patient responses are compared to HCP responses, and the purpose of the consensus meeting is used to clarify and reach agreement on the ratings for parameters over which there has been the greatest disagreement on ratings in the eDelphi rounds (Thorlacius et al., 2018). This was designed to ensure that both patients and HCPs fully understood the definitions of each parameter and had the opportunity to understand and appreciate each other's perspectives. Then the final whole group ratings on each parameter were borne out of mutual understanding, and a shared agreement was reached.

Identifying the borderline parameters for which there was the greatest disagreement between groups was carried out using two different approaches. First, by extracting the parameters with the highest percentages of the whole panel rating them as 8 or 9 (excluding those which had already reached over 80% of panellists rating them as 8 or 9, and therefore already shortlisted for inclusion). The second approach examined the percentages of owners and HCPs who had rated each parameter 8 or 9, and the difference between the two groups. Those where it appeared there had been the greatest disparity between the two groups ratings were targeted for discussion. For example, parameters where over 80% of HCPs had rated it as 8 or 9, but only 50% of owners had rated it as 8 or 9. It was important for parameters to be fully discussed, defined and re-rated so that the interests and priorities of both groups could be understood by the whole panel, and the final rating fully represent true agreement of the whole panel.

Consensus for inclusion in the final COS after discussion and re-rating was pre-defined as over 80% of panellists as a whole group rating the parameter as 8 or 9 on a Likert scale (1-9; 1 being not important to 9 being very important to include). Consensus for exclusion from the final COS was pre-defined as over 80% of panellists as a whole group rating the parameter as 1, 2 or 3 on the Likert scale. Panellists were provided with a list of the parameters for discussion in advance, including the ratings of both owners, HCPs and the whole group. They were asked to think in advance about their opinion on the inclusion of the parameters in the COS, and whether there was anything about the parameter that they did not understand. The meeting was chaired by an impartial chair, who was experienced in chairing consensus meetings for human healthcare COSs. Rating was done anonymously using the online interactive presentation software Mentimeter (www.mentimeter.com). Panellists identified themselves as either a cat owner or HCP and then rated the parameters online when directed to do so by the chair, using their own tablet or smartphone device.

Streamlining the COS: In the second phase of the consensus meeting, the original COS shortlist from the eDelphi, and any parameters voted in after the additional borderline parameters had been discussed, were presented to the panellists as a list. A session of chaired discussion and voting to streamline the parameters into a more manageable list, grouping them into body systems or similar measurement methods was planned, so that the final COS would be as straightforward and transparent as possible. In advance of this meeting, panellists had been presented with the shortlist of parameters already proposed by the eDelphi, and were asked to consider how they might streamline the list.

RESULTS

eDelphi

Two hundred and ten people registered an interest in joining the study panel, via an online questionnaire distributed via contacts and social media. The size of the final invited panel for the eDelphi was 73. As only 11 panellists matching the stakeholder groups "Regulatory Agencies" and "Journal Editors" signed up to participate, the rest of the panel was altered accordingly to align with the percentages planned to be met (as described in the methods section), and the final invited panel size for the eDelphi was 73. The breakdown of panel numbers can be seen in Table 1, and further detail on the clinical expert group can be seen in Table 2.

Table 1. Number of panel participants in each stakeholder group invited to join the eDelphi process

Stakeholder group	Percentage of total panel size	Number of participants
Owners	25	18
Clinical experts	60	44
Regulatory agencies/Journal editors	15	11

Table 2. Clinical expert stakeholder group details

Clinical expert type	Number of participants
First opinion vets	14
Researchers or vets with additional qualifications	14
Pharmaceutical and food industry representatives	10
Veterinary nurses and clinical pathologists	6

The three rounds of the eDelphi were comprised of four questionnaires (the first two questionnaires formed the first round of the eDelphi as the number of outcomes identified from the systematic review were so numerous). In the first two questionnaires, the 104 parameters were presented to the panellists for rating for the first time. The first questionnaire was completed by 57/73 panellists and the second questionnaire by 54/57 panellists. After these two questionnaires were completed, 14 parameters had reached consensus for inclusion in the

COS, as can be seen in Table 3, and no parameters had reached consensus for exclusion from the COS.

Table 3. Parameters which had reached consensus for inclusion in the COS after the first two questionnaires (Round 1)

Parameter	Percentage of panellists rating the parameter 8 or 9
Urine protein: creatinine ratio	94.7
Creatinine	94.4
Phosphate	92.6
Urea	92.6
Quality of life	91.2
Urine specific gravity	89.5
End point for renal survival	86.0
Blood pressure	85.2
Biochemistry	85.1
Full clinical examination	84.2
Body condition score	84.2
IRIS stage/ stage of disease	82.5
Survival time	82.5
Packed Cell Volume (PCV)	81.5

In the third questionnaire, the 20 new parameters that had been suggested by panellists were presented for rating, alongside parameters for which >10% of panellists had said they did not understand what it was (n=3 parameters). The third questionnaire was completed by 44/57 panellists. After the third questionnaire was complete, 3 parameters had reached consensus for inclusion in the COS; hydration status, pain and discomfort, Symmetric dimethylarginine (SDMA). In the fourth questionnaire, the panellists were given the opportunity to re-rate the parameters or leave their rating the same, after seeing their own and the median and range of the whole groups responses. The fourth questionnaire was completed by 31/44 panellists. After the fourth questionnaire was completed, 10 additional parameters had reached consensus for inclusion in the COS; occurrence of adverse events, overall assessment of efficacy, owner not giving the treatments to the cat, time enrolled in study, cause of death, haematocrit, progression of renal dysfunction, appetite for food, muscle condition score, protein in urine.

In the first two questionnaires, there were 3 parameters which >10% of the panel did not understand. After the four questionnaires were all completed, none of these 3 parameters (semi quantitative urine albumin ELISA, fractional excretion of phosphorus in the urine, C-TEA clearance as a measure of effective renal plasma flow) had reached consensus for inclusion in the COS. No parameters had reached consensus for exclusion from the COS after all rounds of the eDelphi were completed.

After the three rounds of eDelphi had been completed, 27 parameters were proposed for inclusion in the COS, 20 were considered borderline and none had been excluded.

Table 4. Borderline parameters identified by the eDelphi process, for discussion and rating in the consensus meeting.

Parameter	Percentage of whole eDelphi panel rating the parameter 8 or 9 on a Likert Scale 1-9	Percentage of owners in eDelphi panel rating the parameter 8 or 9 on a Likert Scale 1-9	Percentage of HCPs in eDelphi panel rating the parameter 8 or 9 on a Likert Scale 1-9
Complete blood count	77.4	57.1	87.5
Bodyweight	77.4	71.4	83.3
Phosphorus	77.4	85.7	79.2
Change in demeanour	77.4	85.7	79.2
Thirst	77.4	100.0	75.0
Wellbeing	74.2	85.7	75.0
Haemoglobin	74.2	85.7	75.0
Overall history	74.2	85.7	70.8
Erythrocyte count	74.2	57.1	79.2
Difficulty administering/ giving treatments to cat	74.2	71.4	79.2
Potassium	71.0	71.4	75.0
Overall amount of food eaten	71.0	71.4	75.0
Mentation	67.7	42.9	79.2
Drinking behaviour	64.5	85.7	62.5
Ocular fundoscopic examination	61.3	28.6	75.0
Palpable size of kidneys	54.8	100.0	45.8
Weakness	38.7	71.4	33.3
Constipation	32.3	71.4	16.7
Decrease in creatinine clearance	32.3	71.4	25.0
H inulin clearance	29.0	71.4	20.8

Consensus Meeting

The consensus meeting was attended by an international group of 16 stakeholders, representing all stakeholder groups except journal editors (seven were invited, but none were able to attend). Of the 16 meeting panellists; six were cat owners, two represented regulatory agencies, three represented food or pharmaceutical companies and five were veterinary surgeons from either research or veterinary practice. Twenty borderline parameters were identified for discussion prior to the meeting and the results can be seen in Table 4.

Once all 20 had been discussed and rated independently by the panel, a further six parameters had reached the definition of consensus for inclusion in the COS sand five parameters had been excluded. These six parameters proposed for inclusion during the consensus meeting were discussed alongside the 27 parameters identified in the eDelphi. The use of flipcharts and lists created by the study team during the course of the meeting, helped as a visual aid as the streamlining progressed. The final shortlist of parameters decided upon was 29, which were streamlined into nine main outcome areas (Table 5).

Table 5. Final proposed COS outcome areas for feline CKD

Main outcome areas
Clinical examination
Total amount of food eaten
Serum biochemistry
Complete Blood Count
Urine tests
Quality of life
Survival time
Progression of CKD
Adverse events

DISCUSSION

This is the second veterinary COS to be created, and the first for the feline species. It represents the views of stakeholders from all stages of decision making for feline CKD, from owners and veterinary surgeons, through research, to industry and pharmaceutical regulation. Therefore, if used in future treatment efficacy randomised controlled trials for feline CKD, it will help to ensure the outcomes of the research are useful and relevant for all decision makers.

The first veterinary COS was published in 2018 for canine atopic dermatitis (Olivry et al., 2018). The COS was called COSCAD'18 and it contained three outcomes (veterinary assessment of skin lesions, owner assessment of pruritis and owner reported global assessment of treatment efficacy) (Olivry et al., 2018). The stakeholder groups included in COSCAD'18 were similar to those in the current study, however the methodology was different as no consensus meeting was included.

The threshold definition for consensus for inclusion in the COS used in the current study is much higher than that which has been used in other human COS studies previously and in COSCAD'18, where 70% was the threshold (Olivry et al., 2018). A systematic review of Delphi methodology found the most common definition of consensus used was percentage agreement, and the median threshold to define consensus as 75% of participants scoring an item 1, 2, 3 or 7, 8,9 (Diamond et al., 2014). The higher threshold used in our study suggests that all parameters included in this COS are very important to all panellists, who represent decision makers at all stages of feline CKD diagnosis and treatment.

Although the eDelphi and consensus meeting methodologies are well recognised for enabling the achievement of group agreement, a COS can only ever represent the views of those who have participated in its creation. It is possible that the outcomes may have been slightly different if the balance of stakeholder groups had been different or if the number of panellists had been larger. However, there is no agreed best sample size for the Delphi technique. It is recognised that more members will increase the reliability of group judgements (Murphy et al., 1998), and a minimum of 7 respondents per stakeholder group is suggested to be large enough for a consensus process (Mullen. 2003). Therefore, the panel size used in this study for the eDelphi is hoped to be adequate, despite the inevitable loss to follow up. Although panellists were lost to follow up from all stakeholder groups, every group was still represented at all stages of this COS development, except that no journal editors attended the consensus meeting stage. In addition, being prescriptive in relation to the number of participants included from each stakeholder group in the study design enabled opinions from a broad base to be gathered and ensured that no one stakeholder group could dominate. The panels for both the eDelphi and consensus meeting were international, however due to the geographical range of panellists registering for the study, the majority of panellists were from the United Kingdom and were all English speaking. It is possible that consensus on the final COS may have been different if the panels had been more diverse.

Many of the parameters in the COS proposed here are likely to be familiar to veterinary surgeons examining and treating cats with CKD, and many are likely to form part of the normal veterinary consultation, disease staging and management. Most already are objective parameters with established methods for measuring and assessing them (e.g. serum biochemistry, survival time), or should be straightforward to measure and record in clinical trials (e.g. adverse events, cause of death). The more subjective parameters (e.g. quality of life) may be more difficult to assess. However, the initial focus of the development of any COS is always to establish what to measure and deciding how to measure comes later in the process (Williamson et al., 2012). The next stage of this work will focus on establishing the most appropriate measurement tools for each parameter proposed in this COS.

The methodologies used here appeared to translate well from human healthcare to the veterinary field, and should be utilised to determine COSs for other veterinary diseases of importance. The patients in veterinary COSs are represented by their owners. If patients in human healthcare COSs are unable to advocate for themselves they are represented by carers (e.g. for COSs for neonatal care where parents can represent the patients; Webbe et al., 2019). It is not surprising therefore that methodologies which work well in human healthcare appear to work well in veterinary also. Further work is required to determine whether this approach works equally well for diseases in a range of other veterinary species.

This is the first COS for the feline species and includes the perspectives of an international range of stakeholders experienced in treatment efficacy decision making for feline CKD. Including this COS in future clinical trials relating to CKD will ensure results will be relevant to all stakeholders, strengthening the evidence base available for clinical decision making, and will reduce research waste.

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UNDERSTANDING VARIATION IN, AND DRIVERS FOR, ANTIMICROBIAL USE IN COMPANION ANIMALS: A NOVEL APPROACH COMBINING EPIDEMIOLOGY WITH ANTHROPOLOGY

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SUMMARY

Antimicrobial use in companion animals could be an overlooked contributor to antimicrobial resistance relevant to human health. This study harnessed the strengths of epidemiology and anthropology to investigate antimicrobial use in companion animals whilst considering the organisational context in which increasing numbers of veterinarians work. A hierarchical model examined Highest Priority Critically Important Antimicrobial (HPCIA) use in a VetCompass™ dataset composed of 468,665 antimicrobial dispensing events in 240,998 dogs. The odds of events comprising of HPCIAs varied between large veterinary groups (odds ratios 1.00 – 7.34; p<0.001) whilst intraclass correlation was more strongly clustered at dog than clinic level (0.710 vs 0.089). Fieldwork revealed how the structure of the companion animal veterinary sector was more fluid than that depicted in the statistical model, identifying opportunities and challenges regarding altering antimicrobial use. This unique, mixed-methods study demonstrates the importance of working across disciplinary silos to inform the design of antimicrobial stewardship interventions.

INTRODUCTION

Initiatives seeking to tackle the complex problem of antimicrobial resistance have largely overlooked antimicrobial use in companion animals. This is despite humans and companion animals sharing classes of antimicrobials and living spaces, circumstances that could drive the development and spread of antimicrobial resistance relevant to human health (Pomba et al., 2017). Therefore, it is important to include companion animal veterinary care within antimicrobial stewardship activities. The World Health Organisation focuses stewardship efforts on antimicrobials for which there is strongest evidence of the transmission of resistant microbes or resistance genes from animal sources to humans (World Health Organisation, 2019). These medicines, designated by the World Health Organisation as Highest Priority Critically Important Antimicrobials (HPCIAs), include third and fourth generation

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cephalosporins, fluoroquinolones and macrolides, which are available for use by companion animal veterinarians.

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Epidemiological programmes such as VetCompass™ (O'Neill, 2013) collate anonymised electronic patient records (EPRs) from primary-care veterinary clinics and have recently allowed the quantification of antimicrobial use. In the UK, antimicrobials are routinely used in companion animals with HPCIAs accounting for around five to six percent of antimicrobial prescribing events in dogs (Buckland et al.; 2016, Singleton et al., 2017). Unlike the livestock sector (O'Neill et al., 2016; Veterinary Medicines Directorate, 2019), there are no published target levels for appropriate use in companion animals; however, professional bodies such as the British Veterinary Association (2015) and the British Small Animal Veterinary Association (2018) advise that HPCIAs should not be routinely used as first line treatment. Variation in the use of HPCIAs could act as a surrogate measure for ‘appropriate’ use and offer potential opportunities to benchmark companion animal veterinary clinics in the future.

In addition to companion animal and veterinarian characteristics (Radford et al., 2011; Hughes et al., 2012), veterinary organisational structure is associated with antimicrobial use. For example, the proportion of companion animals receiving antimicrobials varies approximately twofold between UK practices (Radford et al., 2011). Singleton et al. (2017) investigated longitudinal changes in HPICA utilisation in veterinary consultations via a model that included practice (a single veterinary business) and premises (branches that form a practice) as random effects. They identified a similar amount of variance at practice (0.225) and premise level (0.175), however they did not explore the impact of belonging to different large veterinary groups. Across the UK companion animal veterinary sector, there has been increasing corporatisation in recent years with half of practices now belonging to large groups (Wedderburn, 2017). Understanding the context in which a growing number of companion animal veterinarians work may provide insights as to where to focus effective antimicrobial stewardship interventions. For example, identifying the organisational level at which antimicrobial use is most tightly clustered could indicate the most effective leverage point at which to intervene to change prescribing habits.

The social sciences are recognised to play a crucial role in understanding antimicrobial utilisation (Chandler et al., 2016). For example, the methods and theories of anthropology offer a means by which to ask, *“what makes common sense here, and why?”* (Denyer-Wills & Chandler, 2018), pertinent questions when tackling ‘irrational’ and ‘inappropriate’ antimicrobial use (O'Neill et al., 2016). A key approach is participant observation which enables the study of enacted practice – both conscious and subconscious. This provides additional insight over much of the existing research into antimicrobial use, especially in companion animals, which frequently relies on surveys that can only describe self-reported behaviour (Will, 2018). Furthermore, anthropologically informed studies seek to explore the messy complexities of everyday lives whilst situating them in their broader political, economic, historical and social contexts (Wolf, 2015). This is crucial if the structural factors influencing antimicrobial use, beyond the moment of prescribing, are to be understood. Such studies also address calls for exploration of issues of power, professional identity and reputation with respect to veterinary prescribing of antimicrobials that, to date, remain under-scrutinised (Wood, 2016).

This mixed-methods study harnesses the complementary strengths of epidemiology and anthropology. This enables the painting of a more complete picture of antimicrobial use in companion animals, one that is "*greater than the sum of the parts*" (O'Cathain et al., 2010). The aims of this paper are i) to quantitatively describe the variation in the percentage of antimicrobial events comprising of HPCIAs in companion animal dogs attending clinics belonging to large veterinary groups and, ii) to illustrate how the organisational structure of companion animal veterinary medicine influences antimicrobial use, based on insight gained from anthropological fieldwork. This study will help inform stewardship efforts.

MATERIALS AND METHODS

Epidemiological study

Design: A VetCompass™ dataset spanning June 2012 to June 2014 inclusive, previously used to quantify antimicrobial use in UK companion animals (Buckland et al., 2016), was analysed. Due to the time constraints of this current PhD project, the study population was limited to dogs, the most common UK companion animal species. The percentage of antimicrobial dispensing events comprising of HPCIAs was selected as the outcome measure, given the policy interest in these agents. In addition to the previously applied inclusion and exclusion criteria (Buckland et al., 2016), only data from large corporate veterinary groups with over five clinics was retained (Table 1; Fig. 1)

Table 1. The inclusion and exclusion criteria of the epidemiological study. Adapted from Buckland et al. (2016)

	Inclusion criteria	**Exclusion criteria**
Antimicrobial event	An electronic record of the dispensation and administration of medicines that destroy or inhibit the growth of bacterial microorganisms and authorised for systemic use (i.e. injectable, tablets/capsules and oral suspensions)	An electronic record for the dispensation and administration of other antimicrobial agents (e.g. antiviral, antifungal, biocides) or those be delivered topically (e.g. medicated creams, topical solutions for treatment of eye or ear infections).
Dog	Dogs with a unique patient identifier who had at least one electronic patient record entry.	Dogs without a unique identifier or groups of dogs included under a single unique identifier.
Clinic	First opinion clinics situated in the United Kingdom and participating in VetCompass™ during the study period.	Clinics engaged in the provision of solely referral and/or emergency care services. Clinics belonging to veterinary groups with five or fewer clinics

Data cleaning and processing: Buckland et al.'s (2016) definition of an antimicrobial agent and application to the dataset were re-used (Table 1). Additional HPCIA coding based on the

WHO’s (2019) definition was added. As per Buckland et al.’s approach, an antimicrobial event was defined as an independent record (line) in the treatment data field of the EPR-derived dataset and, consequently, multiple events could arise from a single consultation.

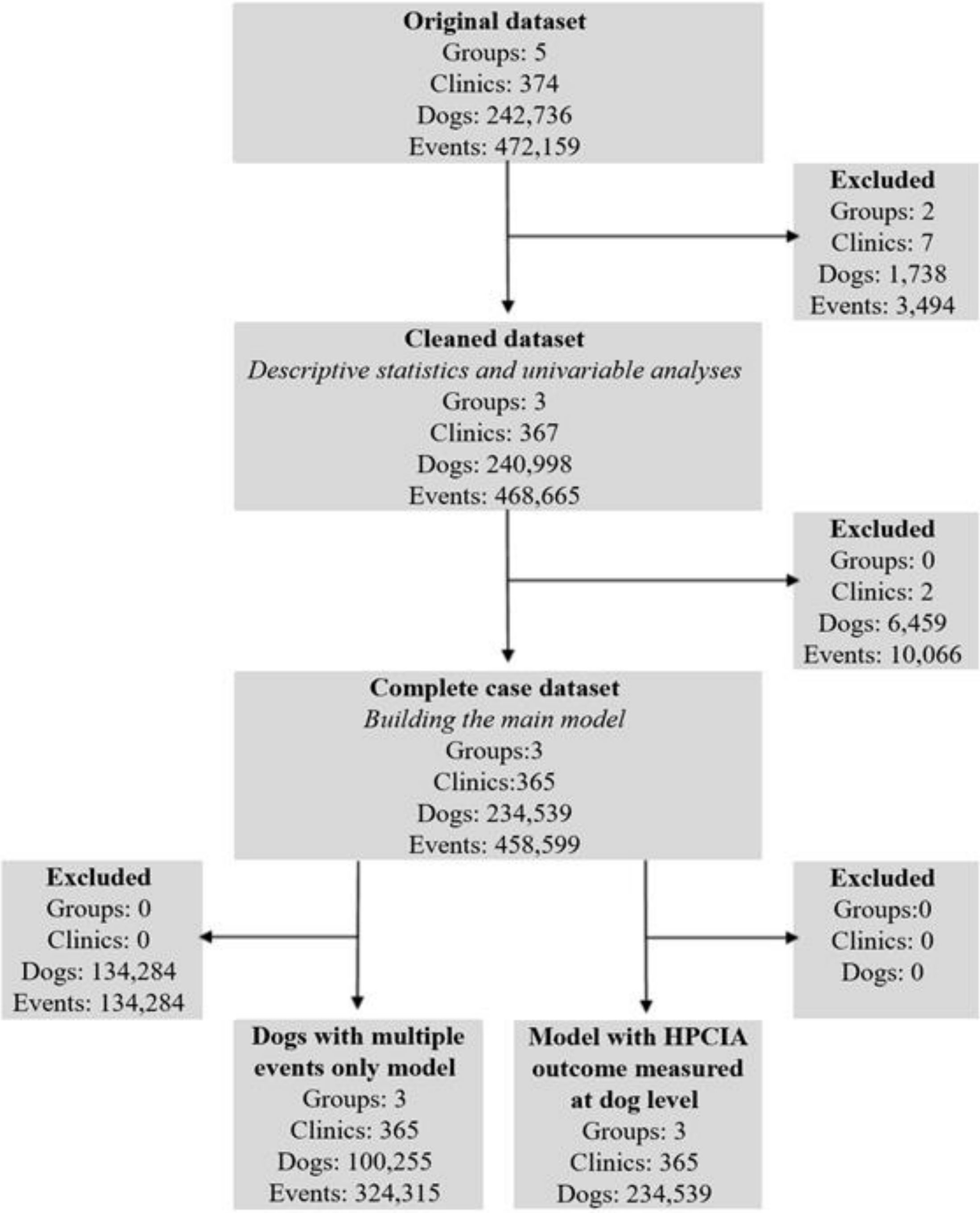

Fig. 1 The flow of data through the VetCompass™ epidemiological study including the hierarchical models

Dog age was calculated as the difference between the date of antimicrobial dispensing and dog birth date; results <0 or >24 years were coded as missing. Age was grouped *a priori* into quartiles to allow for non-linearity of effects and to facilitate interpretation. Dog sex was coded as male, female or missing. The 20 most prevalent dog breeds in the dataset were taken as categories, the remaining pure breeds were pooled together ('other purebreds') as were 'cross breeds'. The postcode of the clinic was used to derive the region in which it was situated.

Descriptive and univariable analyses: From the total number of antimicrobials events, the continuous outcome measure of the percentage of events compromising of HPCIAs was calculated at dog, clinic and corporate veterinary group levels along with 95% confidence intervals (95% CIs).

Counts and percentages were calculated for each categorical variable (dog sex, breed, clinic region). Dog age was summarised for each quartile using medians and interquartile ranges after reviewing its distribution. The Pearson Chi-square test and the Mann Whitney U test checked for differences between the sample characteristics of each veterinary group.

The linear test for trend was calculated for the percentage of antimicrobial events comprising of HPCIAs across dog age quartile. Univariable logistic regression investigated the possible association of each variable with the binary outcome of whether an antimicrobial event comprised of a HPCIA (yes versus no), in addition to calculating odds ratios (OR) and 95% CIs. Likelihood ratio tests were used to estimate each variable's overall association with HPCIA use.

Hierarchical modelling: A multilevel logistic regression model was built for the binary outcome of whether an antimicrobial event comprised of a HPCIA (yes versus no) using complete cases (antimicrobial events with full data on dog identification number, dog age, dog sex, dog breed, clinic identification number, clinic region, veterinary group identification number) in the dataset. This was with the aim of investigating the clustering of HPCIA use within dogs, clinics and veterinary groups. Dog and clinic were added as random effects whilst veterinary group was included as a fixed effect. A screening criterion of a univariable significance of a p-value <0.25 was applied when considering the inclusion of additional fixed effects (dog age, sex, breed, clinic region) (Hosmer & Lemeshow, 2004).

Following the fit of the multivariable model, a backward elimination approach was adopted to verify the inclusion of each variable. Likelihood ratio tests were used to compare the performance of the new, smaller model to the original. The estimated coefficients of the remaining variables were compared to those from the full model to check there was no sizable change in their magnitude (Hosmer & Lemeshow, 2004). ORs and 95% CIs were calculated for each fixed effect variable.

The model variables comprised of multiple categories making the interpretation of pairwise interactions between them problematic. The association between age quartile and percentage of HPCIA events was investigated in each veterinary group. However limited computational power prevented the inclusion of an interaction term in the final model. Different model structures were not considered as this would have prevented the investigation of HPCIA use at dog, clinic and veterinary group levels. Model performance was assessed using Receiver Operator Curve (ROC) statistics and Hosmer Lemeshow residuals (Hosmer & Lemeshow, 2004).

As dog identification number was included in the model as a random effect but most dogs had a single antimicrobial event, the analyses were re-run i) in the same model using a dataset limited to dogs with multiple antimicrobial events only and ii) a model with a binary outcome of whether a dog received any HPCIA (Fig.1). The results and performance of these models were compared to the main model.

The intraclass correlation coefficients (ICCs) at a dog and clinic level were calculated to assess the clustering of HPCIA use (Dohoo et al., 2003). Data analyses were conducted in Stata 16 (StataCorp, Texas, USA) and statistical significance was set at the 5% level.

These analyses were covered by the VetCompass™ research ethics approval given by the Royal Veterinary College's Ethics and Welfare Committee (SR2018-1652).

Anthropological study

Data collection: Fieldwork was undertaken by the lead author over nine months in 2019 at three UK companion animal clinic sites belonging to three large veterinary groups (two commercial and one charitable). The extended nature of placements enabled the researcher to become embedded in the clinic teams who became less conscious of being 'studied'. All aspects of daily clinic life were observed including consultations, surgical procedures, administrative and reception duties. The researcher's non-veterinary background facilitated a 'fresh pair of eyes' on taken-for-granted situations, illuminating the unwritten rules surrounding companion animal veterinary work. Within these observation periods, informal interviews were undertaken with veterinarians, support staff and owners to clarify arising issues. Detailed field notes describing relations, language, metaphors, and sense-making between those at the interface of antimicrobial use were made with attention paid to both verbal and non-verbal gestures. Additional data sources included clinic policies and media articles as well as formal, semi-structured interviews with veterinarians. The latter followed a topic guide but with flexibility to follow up issues raised by interviewees. The formal interviews were audio-recorded and transcribed.

Data analyses: The software NVivo 12 (QSR International Pty Ltd, USA) was used to organise the qualitative data and facilitate thematic coding. Initial, low level codes situated in the data were developed into more abstract themes (Ziebland & McPherson, 2006). Analysis involved comparing sites to draw out similarities and differences. Moving to a new space - and shifting between emic (insider) and etic (outsider) perspectives - rendered visible the enacted 'common sense' and supporting infrastructures (Chandler, 2019) in each location. The empirical fieldwork data was considered in response to - and building on - the existing theoretical literature. Analyses were conducted by the first author as part of their doctoral research. Interim findings were discussed amongst the multidisciplinary research team as a form of 'sense checking'.

All study participants gave informed consent. The anthropological study was approved by the research ethics committee of London School of Hygiene and Tropical Medicine (16126).

RESULTS

Epidemiological study

Descriptive results: The cleaned dataset contained 468,665 antimicrobial events with 294,016 (62.7%) of these arising from veterinary group C (Table 2). Of the total antimicrobial events, 29,984 comprised of HPCIAs (6.4%, 95% CI 6.3 - 6.5%): This percentage differed between veterinary groups ranging from 4.9 % (95% CI 4.8 - 5.0) in group B to 15.6% (95% CI 15.2% - 16.1%) in group A (p<0.001). However, the characteristics of the dogs and clinics in each group differed (data not presented due to space constraints). At a clinic level (n = 367), the median percentage of HPCIA events was 5.9 (IQR 3.4 – 10.4%) with a range of 0.0% (10 clinics) to 69.9% (1 clinic). The median number of antimicrobial events per dog was 2 (interquartile range (IQR) 1 - 4, range 1 - 60), whilst the median number of HPCIA events was 0 (IQR 0-0, 0 - 60).

Hierarchical modelling results: All variables (dog age quartile, sex, breed and clinic region) met the screening criterion for inclusion in multivariable model building. The removal of dog sex made no statistically significant difference in the model performance and this variable was excluded from the main model. Therefore, the main model comprised of clinic and dog as random effects, and corporate veterinary group, age quartile, breed and clinic region as fixed effects. The main model estimated the OR of an antimicrobial event comprising of a HPCIA as statistically significantly different between the veterinary groups ($p<0.001$). Compared with group B, an antimicrobial event in group C had 2.04 (95% CI 1.56 – 2.70) times the odds of comprising of a HPCIA whilst in group A the odds were 7.34 (95% CI 5.14 – 10.49). The area under the Receiver Operator Curve was 0.983 (95% CI: 0.983 - 0.984) and the Hosmer-Lemeshow test was non-significant ($p=0.314$) suggesting a good model fit.

Table 2. The distribution of antimicrobial and HPCIA events by corporate veterinary group in a VetCompass™ UK dataset from 2012 -2014. Distribution is reported in total and at a clinic level (No.: Number; HPCIA: Highest priority critical important antimicrobial; CI: confidence interval; IQR: interquartile range)

Veterinary Group	No. Antimicrobial events	No. HPCIA events	Percentage of antimicrobial events comprising of HPCIAs (95% CI)	No. Clinics	Median clinic percentage of antimicrobial events comprising of HPCIAs (IQR)
A	25,909	4,044	15.6 (15.2 – 16.1)	90	13.8 (10.9 – 19.9)
B	148,740	7,280	4.9 (4.8 – 5.0)	117	3.7 (2.1 – 6.1)
C	294,016	18,660	6.3 (6.3 – 6.4)	160	5.3 (3.6 – 7.7)
Total	468,665	29,984	6.4 (6.3- 6.5)	367	5.9 (3.4 – 10.4)

Table 3 reports the intraclass correlation (ICC) for the three versions of the hierarchical model, the results of which were broadly similar. Each suggests that HPCIA use within a dog is more strongly clustered than HPCIA use within a clinic: for example, 0.710 versus 0.089 in the main model.

Table 3. Intraclass correlation (ICC) estimates of an antimicrobial event comprising of an HPCIA within individual i) dogs and ii) clinics (CI: confidence interval)

Model	Anti-microbial events	Dogs nested within clinics		Clinic	
		ICC (95% CI)	Standard error	ICC (95% CI)	Standard error
Main model –all events	458,599	0.710 (0.701 - 0.719)	0.004	0.089 (0.076 -0.104)	0.007
Dogs with multiple events only	324,315	0.727 (0.718 - 0.735)	0.005	0.086 (0.073 - 0.101)	0.007
Any use of HPCIA measured at a dog level[a]	-	n.d.		0.105 (0.089 - 0.123)	0.009

[a]n = 234,539

Anthropological study

The statistical model presents a representation of the companion animal veterinary work in which a dog attends a single veterinary clinic and that each clinic is a neatly bounded entity under the umbrella of a corporate veterinary group. Time in the field revealed more fluid structures which are described below. They are arranged to reflect the levels of the statistical model.

“Highest priority what?”: HPCIA – the quantitative outcome classification used in the statistical model - had little meaning ‘on the ground’. Antibiotics were organised in clinic based on their formulation type (tablet, injectable) rather than other categorisations. They were referred to by their brand names, for example there was awareness regarding the pressure to restrict use of Convenia (Zoetis), a third-generation cephalosporin.

“He’s just not himself”: Whether a dog received antimicrobials was shaped by a complex interplay of canine and owner characteristics. Owners determined if - and when - their dog attended the veterinary clinic and therefore could potentially access antimicrobials. Some owners presented at the first sign of trouble whilst others had to make tricky decisions about when to seek help based on limited financial and time resources. The model did not investigate this entanglement of biological and social factors.

“Oh no – here comes the antibiotics police”: Due to the anonymization of information available in VetCompass™, it was not possible to quantitatively investigate variation in HPCIA use at an individual veterinarian level. Observations revealed that this is important with several younger veterinarians taking on the role of local antimicrobials champion. They advised and, in some cases, cajoled their co-workers regarding appropriate use. They revealed that they did not feel able to challenge all of their colleagues, in part due to their relative hierarchical positions.

“We’re like ships that pass in the night”: Modern ways of working challenge the notion of the veterinary clinic as a bounded unit with a stable workforce and shared practices. Shortages of qualified staff presented ongoing challenges in the fieldwork sites with rota gaps being filled with veterinarians from other clinics or locum staff. In some cases, out-of-hours work was contracted out to separate businesses. However, the flow of staff offered opportunities to share best practice between clinics.

“There’s not enough hours in the day”: Belonging to a large veterinary group presented the potential to share some of the workload associated with antimicrobial stewardship. It was difficult for frontline veterinarians to personally carve out time to undertake such activities because clinical and revenue generating activities had to take priority under existing business models. At one fieldwork clinic, the corporate headquarters distributed template stewardship materials for completion; however there was limited local capacity for this work. In another group, a single ‘top-down’, business-wide policy regarding ‘appropriate’ use was introduced but there was muted buy-in at a clinic level. The level of clinic autonomy – for example deciding which drugs to stock – varied between veterinary groups, the organisational ‘culture’ of which was found to differ.

DISCUSSION

This study is the first to combine epidemiological and anthropological approaches to explore the complex issue of antimicrobial use in the UK companion animal veterinary sector. Based on a large VetCompass™ dataset, the study quantified the variation in the percentage of antimicrobial events comprising of HPCIAs between three different veterinary groups. It also identified that HPCIA utilisation was more strongly clustered within dogs than within clinics. The anthropological fieldwork highlighted how the organisational structure of the companion animal veterinary sector was more fluid than that depicted in the statistical model, identifying opportunities and challenges when seeking to intervene regarding antimicrobial use.

The statistical model focussed on HPCIA use as it is an area of intense policy interest (Veterinary Medicines Directorate, 2019). However, the term HPCIA was rarely heard during observations of day-to-day practice. This highlights how stewardship schemes and researchers seeking to share their work beyond their fields should tailor their language to reflect that of their target, non-specialist audience (Wellcome Trust, 2019).

The quantitative study used data from 2012 to 2014 and it is unclear to what extent these patterns of antimicrobial use persist. A UK-based SAVSNET study found the number of HPCIA events increased slightly between 2014 and 2016 (Singleton et al., 2017). Meanwhile, in the Netherlands, a statistically significant decrease in HPCIA use was measured between 2012 to 2014; however inter-clinic variation became more pronounced (Hopman et al., 2019). Furthermore, this study is also unable to comment on the variation in total antimicrobial and HPICA use. For example, a clinic may have a high percentage of antimicrobial events comprising of HPCIAs despite a relatively small denominator (total antimicrobial events), thus masking a limited frequency of HPCIA events. Such complexities should be considered when developing antimicrobial stewardship benchmarking indices for the companion animal veterinary sector in the future.

The quantitative study revealed that the odds of an antimicrobial event comprising a HPCIA was more tightly clustered at a dog level, perhaps reflecting their deployment in dogs with ongoing conditions. Less clustering was observed at a clinic level suggesting that companion animal veterinarians working in the same clinic do not automatically share collective ways of working with antimicrobials. Subsequent to these quantitative data, the British Small Animal Veterinary Association (2018) introduced its stewardship campaign which included developing clinic level antimicrobial use policies. It would be interesting to assess whether the clinic level clustering of HPCIA use has subsequently changed.

During fieldwork, the ‘clinic’ was not found be the bounded, stable unit that is modelled in epidemiological studies. Designers of future stewardship interventions should reflect upon how to reach out-of-hours, part-time and locum veterinary staff. For example, the forum of the clinic meeting may no longer be sufficient to inform all staff. Conversely, the circulation of staff between clinics presents opportunities to spread appropriate antimicrobial use messages.

From the anonymised data deposited in VetCompass™, it was not possible to quantify the clustering of HPCIA use at an individual veterinarian level or include the influence of owner characteristics. However, time spent in clinic demonstrated that the decision to use an antimicrobial arose from complex interactions including those between the consulting veterinarian and the dog owner, highlighting the benefits of a mixed-methods approach. A previous qualitative study reported that companion animal veterinarians feel under pressure from owners to prescribe antimicrobials; however, owners reported that it was the veterinarians themselves who encouraged their use (Smith et al., 2018). Social scientists, meanwhile, have argued that focussing on who to blame overlooks the broader structural factors supporting the continued use of antimicrobials (Chandler, 2019). Future research should further investigate the entangled roles of these actors whilst considering the context in which they operate.

On-the-ground, antimicrobial stewardship activities have to be fitted around existing, income generating workloads. Large veterinary groups may be able to shoulder some of this burden – for example by centrally auditing local antimicrobial use – subject to commercial pressures. However, the fieldwork indicates that careful reflection should be given to how to ensure ‘buy-in’ by frontline veterinarians. Furthermore, the organisational culture of each veterinary group varied, suggesting an ‘off-the-shelf’ approach might have limited impact. Whilst recent graduates may be willing to act as local champions for appropriate antimicrobial use, consideration is required of how the hierarchies and gender roles at play in veterinary work (Knights & Clarke, 2019) may help or hinder this role.

To conclude, this unique, mixed-methods study has provided novel insights into antimicrobial use in the companion animal veterinary sector. In doing so, it demonstrates the strengths of working across traditional disciplinary silos to tackle to this complex problem. By using both quantitative and qualitative approaches, it has enabled a deeper understanding of the organisational structure in which an increasing number of companion animal work and how this can influence antimicrobial use. These findings will help inform the design of sustainable stewardship interventions for this setting.

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JOHNE’S CONTROL

USING SERIAL MILK ANTIBODY ELISAS TO PREDICT THE PROBABILITY OF INFECTION WITH JOHNE'S DISEASE IN DAIRY CATTLE: MODELLING THE EFFECTS OF SENSITIVITY, SPECIFICITY AND RECENT TB TESTS

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SUMMARY

Analysis of serial testing for Johne's disease (JD) using milk ELISAs from UK dairy herds demonstrated that shorter intervals between bovine tuberculosis and JD tests were associated with an increased risk of testing positive for JD. Additionally, the probability of a cow being truly infected with JD was calculated by first estimating the specificity and age-dependent sensitivity of the test, and then quantifying the posterior probability of infection following each test result using Bayes' theorem. The effects of test specificity and test result pattern were modelled to display the posterior probability of infection (PPI) with different prior probabilities. In addition to the importance of prior probability on the PPI, the results illustrate that the current case definition of a high-risk, infectious animal used on most UK dairy farms (two positive tests in the previous four) is reliant on an exceptionally high specificity to produce a PPI of greater than 95%.

INTRODUCTION

Johne's disease (JD) is a chronic wasting disease of cattle caused by *Mycobacterium avium subspecies paratuberculosis* (MAP). The estimated herd prevalence in the UK is 68%, based on the presence of antibodies in the bulk milk (Velasova et al., 2017). In addition to the welfare concerns of chronic wasting, JD has a significant economic impact on dairy farms and there is increasing concern about MAP contributing to the aetiology of Crohn's disease in humans (McAloon et al., 2019).

One diagnostic approach for JD is to identify the humoral immune response to MAP with an enzyme-linked immunosorbent assay (ELISA) which can detect antibodies in both milk and serum. The sensitivity and specificity of milk JD-ELISAs range from 0.21 to 0.61, and 0.83 to 1.00 respectively, relative to detectable MAP in their faeces (Nielsen et al., 2008) However, the sensitivity has been reported to reach 0.79 in older animals based on modelling which used subsequent milk JD-ELISA results to define truly infected and non-infected animals (Nielsen et al., 2013).

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Farms which are attempting to control JD require rapid identification of infectious animals before they shed the organism around susceptible animals. Neonatal calves are considered to be at the highest risk of infection (Windsor et al., 2010), so animals which are likely to be shedding MAP need to be identified prior to calving to manage this risk. Due to the convenience of testing milk, the poor sensitivity of a single JD-ELISA during the latent phase of infection and the importance of identification of high-risk animals at critical points (i.e. prior to calving or breeding), milk JD-ELISAs are often conducted repeatedly at regular intervals on dairy farms (Geraghty et al., 2014). In the UK, these high-risk cows are often termed “red cows” to highlight their shedding status. Nielsen (2008) demonstrated that 35% of cows with two consecutive positive tests, approximately one month apart, would start shedding within one year. This was incorporated into control schemes in the UK so that cows with two consecutive positive tests (2 in 2) were classified as “red cows” and treated as high-risk of being, or becoming, infectious.

The value of using quarterly milk JD-ELISAs is that the infection status of the animal after a test, the posterior probability of infection (PPI), is continually updated. A recent study in the UK (Meyer et al., 2018) explored the probability of infection following serial JD-ELISAs and concluded that the probability of infection after two consecutive positive tests was similar to the probability following two positive tests that were not consecutive. However, the reported standard deviations in this study were large which suggests a wide dispersion in the individual results. In 2018 the largest milk recording organisation in the UK, National Milk Records (NMR), adjusted their test interpretation and subsequent advice to farmers to classify all cows with two positive tests in the previous four (2 in 4) as “red cows”.

Bovine tuberculosis (bTB) is endemic in the UK and it is under statutory surveillance with the single intradermal comparative cervical tuberculin (SICCT) test. The SICCT test uses two purified protein derivatives (PPD) from *Mycobacterium bovis* and *Mycobacterium avium* injected into the cervical skin to detect delayed hypersensitivity to each PPD. The testing regime is government mandated and dependent on the regional prevalence and previous test results in the herd; test frequency ranges from four-yearly if no positive animals have been previously identified and the herd is in a low-risk area, to every 60 days if positive animals have been identified during the previous test. It is advised to delay using JD-ELISAs after a SICCT test due the immune priming nature of PPD inoculation. The advice regarding exact interval between the two tests varies; many countries advise 90 days but in the UK this is often impractical due to the frequency of bTB testing, therefore a 60 day interval is recommended. Although there is a rise in MAP antibodies following intradermal inoculation with avian and bovine PPD (Kennedy et al., 2014; Roupie et al., 2018), it is not clear whether this represents a false elevation due to antibody cross-reaction or an anamnestic response due to stimulation from the PPD inoculation in genuinely MAP-infected animals. In other words, the effect of a recent SICCT test could be to decrease the specificity of the JD-ELISA, to increase its sensitivity, or both.

The aims of this study were: to quantify the change in posterior probability of testing positive with JD-ELISAs based on the proximity to a SICCT test, and to determine the probability of infection following different test result patterns, including the different “red cow” definitions, and with different test specificities.MATERIALS and methods

The study was conducted following ethical approval from the University of Liverpool Veterinary Research Ethics Committee (VREC567). Farms were recruited if they supplied milk to the British supermarket Tesco during the period 2014 to 2017. These farms were contractually required to test for JD four times a year using milk ELISAs as an adjunct to their monthly milk recording. The JD-ELISA results with the accompanying milk records were requested directly from milk recording organisations: National Milk Recording group (NMR); Cattle Information Service (CIS) and Quality Milk Management Services (QMMS). Details requested included cow and herd identification, date of birth, milk recording date and the sample-to-positive ratio (S/P) of the JD-ELISA. All three milk recording organisations used the same indirect ELISA kit (IDEXX Laboratories, Maine, USA) and reported the same S/P cut-off for positive results (≥30). SICCT testing details were requested from the Animal Plant Health Agency (APHA) for all farms included in the dataset for the period 2012 to 2017. This extended period was used to include bTB tests for two years preceding the first JD-ELISA. The data from the APHA included cow identification, breed and date of SICCT test. These data were merged by cow ear tag with the milk recording data. The final dataset had one record for each JD-ELISA, with each cow having multiple records and the interval from the most recent SICCT test included for each record. Data was collated and formatted in Microsoft Excel and subsequently analysed in Stata (StataCorp, 2015) and R (R Core Team, 2019).

Effect of SICCT test to JD-ELISA interval

To explore the relationship between the time since SICCT test and JD-ELISA result, a logistic regression model was fitted to predict the JD-ELISA result as a binary outcome (S/P ratio ≥30) with SICCT test to JD-ELISA interval included as a fixed-effect. This interval was included as a categorical variable to reflect the specific time intervals of interest. Univariate analysis was performed between other potential confounders included in the milk recording data collected, such as age of cow and stage of lactation, and these were entered into the multivariate model if the univariate analysis was statistically significant at p-value <0.1. They were retained in the final model if they remained statistically significant at p-value <0.05. To avoid the effects of clustering within cow, and the difficulties of incorporating autocorrelation into modelling of repeated binary data, one test was selected randomly from each cow for inclusion in the final model which took the form in Eq.(1):

$$logit(y_{ij}) = \beta_0 + \boldsymbol{\beta_i} \boldsymbol{x_i^T} + \mu_j \qquad (1)$$

where $logit(y_{ij})$ is the natural log of the odds of cow i on farm j having a positive JD-ELISA, β_0 is constant, $\boldsymbol{x_i^T}$ is the vector of covariates for each cow and $\boldsymbol{\beta_i}$ is the coefficients for these covariates, μ_j is a random intercept for each farm. No interaction terms were included in the final model and the covariates were checked for collinearity which was not present.

Sensitivity and specificity estimation

Cows in the full dataset were classified as JD cases or non-cases based on the pattern of test results defined using the same criteria as Meyer et al. (2018). Cows with a minimum of three tests and where the final two test results were positive were classified as cases, and cows with a minimum of nine tests and where the final eight tests were negative were called non-cases.

Specificity and age-dependent sensitivity of the milk JD-ELISA were estimated using the approach described by Nielsen et al. (2013). To estimate specificity, a randomly selected test result was selected from each cow which was classified as a non-case and the specificity was calculated as the probability of the selected test being negative. To estimate sensitivity, a randomly selected test result was selected from each cow which was classified as a case and the sensitivity of the test at age t (*Se(t)*) was calculated as the probability of the selected test being positive test at a given age. $Se(t)$ was estimated using the regression model (Eq. 2) where a is the upper limit of the logit of sensitivity at maximum age (t), b is the scaling factor and c is the coefficient of decay as age (t) increases.

$$logit\left(Se(t)\right) = a - b * e^{-c*t} \tag{2}$$

Conditional probability using serial test results

Using the specificity and age-dependent sensitivity estimates, the PPI after different test results were modelled assuming quarterly testing. This allowed the effect of test pattern and age to be determined for different prior probabilities. Additionally, the PPI at the point of a cow being classified as a “red cow” using the 2 in 2 (+ +) and the 2 in 4 (+ - - +) criteria were modelled using different hypothetical test specificities. The 2 in 4 (+ - - +) test pattern was chosen because the combination of tests would produce the lowest PPI at the point of “red cow” classification compared to other 2 in 4 test patterns.

Before further analysis of the test patterns was conducted the dataset was standardised to increase the reliability of interpretation. The first criterium was each herd must have tested on at least three separate occasions in each year; this was intended to exclude herds that were not at least trying to test all cows quarterly, or which had to temporarily suspend JD testing during the study period. The second criterium was that a minimum of 50 or more cows must have been tested in each year; this was intended to exclude results that may have been from a targeted screen of suspicious animals rather than a whole herd test. With this restricted and standardised dataset, the apparent herd-level seroprevalence of JD was calculated for each year of study by dividing the number of cows with at least one positive JD-ELISA in that year by the total number of cows tested. The age of each cow at their first test in 2017 was used to calculate the age-dependent sensitivity (Eq. 2) for each cow at this timepoint. The mean of this age-dependent sensitivity, termed mean effective sensitivity (MES), for each herd was then calculated and, along with the estimated specificity (Sp), used to correct the apparent herd prevalence to account for the average test performance in that herd (Rogan et al., 1978; Eq. 3):

$$TP_{jy} = \frac{AP_{jy}+Sp-1}{MES_j+Sp-1} \tag{3}$$

where TP is the true seroprevalence, AP the apparent seroprevalence, j = herd and y = year.

To calculate the PPI after each test, a likelihood ratio given the test result was calculated as described by Dohoo et al. (2012) using Eq. 4 for positive (LR_+) and negative results (LR_-), where T is the test result and D is the true disease status of the cow.

$$LR_+ = \frac{P(T+|D+)}{P(T+|D-)} = \frac{P(T+|D+)}{1-P(T-|D-)} \ , \ LR_- = \frac{P(T-|D+)}{P(T-|D-)} = \frac{1-P(T+|D+)}{P(T-|D-)} \tag{4}$$

The calculated estimates for test specificity (Sp) and age-dependent sensitivity $(Se(t))$ were substituted into Eq. 4 to calculate the likelihood ratio for each test result given the age (t) of the animal at the test (Eq. 5):

$$Se(t) = P(T+|D+) \ , \ Sp = P(T-\mid D-) \tag{5}$$

$$LR_+(t) = \frac{Se(t)}{1-Sp} \ , \ LR_-(t) = \frac{1-Se(t)}{Sp}$$

The posterior odds of infection following each test result were calculated using Bayes' theorem as the product of the likelihood ratio and prior odds. The prior odds used were either the posterior odds from the previous test or, if no previous test results were available, the true seroprevalence of JD in the herd during the year of the cow's first test. Cows were categorised into groups (A-E) based on the total number of positive JD-ELISA results as described in Table 3a. Groups C (2 positive tests) and group D (3-7 positive tests) were sub-divided based on whether cows met the 2 in 2 or 2 in 4 definition of "red cows".

RESULTS

Effect of SICCT test to JD-ELISA interval

The dataset was collated from tests that were conducted 01/01/2014 – 31/12/2017 and consisted of 1,257,354 tests from 225,296 cows on 735 farms. In total, 3.3% of the test results were positive and 9.4% of cows had at least one positive test result. There were 928,472 JD-ELISAs that were matched to the most recent SICCT test, 20,599 (2.2%) of these were conducted 30 days or less after a SICCT test and a further 115,287 (12.5%) were conducted between 31 and 60 days after a SICCT test.

Univariate analysis of interval days since SICCT test, age, stage in lactation, breed, milk recording organisation, and farm (as a random effect) found a statistically significant (p-value <0.05) influence on the probability of a positive JD-ELISA for each variable. Consequently, these were all included and subsequently retained in the final multivariate model as displayed in Table 1. If the interval between SICCT test and JD-ELISA was 30 days or less, the odds of a positive JD-ELISA was 2.55 (95% CI: 2.16 – 3.00) higher than if the interval was greater than 90 days; this decreased for cows tested 31-60 days after a SICCT but the odds were still 1.24 (95% CI: 1.13 – 1.36) greater than if tested 30 days later i.e. >90 days after the SICCT test.

Sensitivity and specificity estimation

From the full dataset of 225,296 cows, 3,904 and 42,704 cows were classified as cases and non-cases respectively. The specificity was estimated to be 0.996 and age-dependent sensitivity was estimated from the non-linear logistic regression model (Eq. 2) with parameters a =1.396 (SE: 0.179), b = 5.915 (SE: 0.959) and c = 0.425 (SE: 0.084). It was therefore estimated that the test sensitivity was 0.24, 0.58 and 0.72 at two, four and six years old respectively.

Conditional probability using serial test results

A restricted dataset was created by imposing minimum requirements for testing frequency and absolute number of cows tested each year; this dataset contained 110,281 cows from 288 farms. The age profiles of these herds were calculated for 2017, the median age of cows tested was 5.1 years (IQR: 3.5 - 6.9 years), and subsequently the MES was calculated for each herd: the mean MES was 0.54 (SD: 0.05). The herd-level apparent and true seroprevalence are presented in Table 2. There were 4,821 cows (3.4%) in this dataset that would have been classed as "red cows" using the 2 in 4 definition, 4,217 of these (3.0% of total) would have also been classed as 'red' using the 2 in 2 definition. This is a 14.3% increase in "red cows".

Table 1. Mixed-effect model of the impact of days since SICCT test on JD-ELISA result in 663 UK dairy herds

	Odds ratio	Standard error	95% confidence interval	p-value
Age at recording (month)	1.02	0.001	1.01 – 1.02	<0.001
Milk yield (kg)	0.96	0.002	0.96 – 0.96	<0.001
SICCT test JD test interval (days)				
≤30	2.55	0.215	2.16 – 3.00	<0.001
31 – 60	1.24	0.059	1.13 – 1.36	<0.001
61 – 90	1.13	0.440	1.04 – 1.22	0.002
>90	Reference			
Days since calving				
≤30	Reference			
31 – 90	0.58	0.031	0.53 – 0.65	<0.001
91 – 150	0.49	0.028	0.44 – 0.55	<0.001
151 – 210	0.46	0.026	0.41 – 0.52	<0.001
211 – 270	0.48	0.027	0.43 – 0.53	<0.001
271 – 330	0.57	0.034	0.51 – 0.64	<0.001
>330	0.59	0.036	0.52 – 0.66	<0.001
Breed				
Holstein/Friesian	Reference			
Brown Swiss	0.72	0.150	0.47 – 1.08	0.11
Shorthorn	1.16	0.238	0.78 – 1.73	0.482
Ayrshire	0.67	0.130	0.46 – 0.98	0.040
Jersey	1.91	0.250	1.47 – 2.47	<0.001
Montbeliarde	1.46	0.231	1.07 – 1.99	0.018
Norwegian Red	1.81	0.317	1.28 – 2.55	0.001
Swedish Red	1.27	0.182	0.96 – 1.68	0.093
Other	1.46	0.205	1.11 – 1.92	0.007
Milk recorder organisation				
1	Reference			
2	0.92	0.111	0.73 – 1.16	0.477
3	0.68	0.050	0.59 – 0.79	<0.001
	Parameter			
Intercept	0.07	0.005	0.06 – 0.78	<0.001
	Intra-class correlation			
Random effect (farm)	0.13	0.010	0.11 – 0.15	

Table 2. Median and interquartile range (IQR) herd-level apparent seroprevalence of JD (proportion of herd with one or more positive JD-ELISAs in each year) and true seroprevalence of JD (adjusted for test sensitivity and specificity)

Year	Median apparent seroprevalence % (IQR)	Median true seroprevalence % (IQR)
2014	5.4 (3.2 – 8.2)	9.3 (5.4 – 14.3)
2015	5.9 (3.4 – 11.1)	10.4 (5.8 – 20.2)
2016	5.1 (2.9 – 7.8)	8.8 (4.7 – 14.0)
2017	5.1 (2.9 – 8.2)	9.0 (4.6 – 14.5)

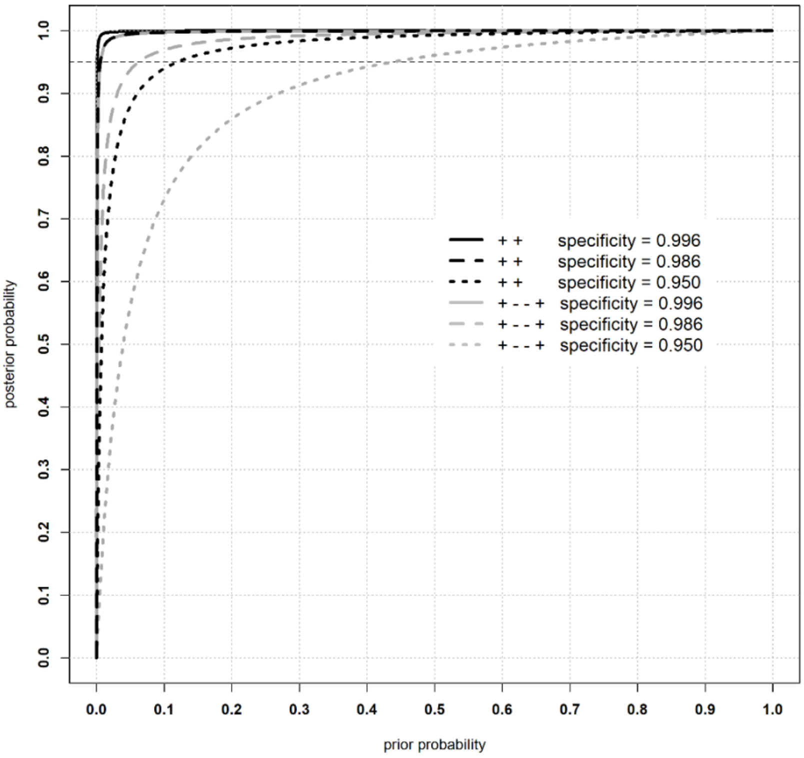

Fig. 1 The posterior probability of infection with JD with different prior probabilities after the second positive test in cows that would be classified as "red cows" using the 2 in 2 (+ +) or a 2 in 4 (+ - - +) definition. The effect of different test specificities is displayed using 0.996, 0.986 and 0.950 as examples.

The relationship between prior and posterior probability of infection with different test result patterns and with different test specificities were modelled, an example output is displayed in Fig. 1. The characteristics of each group based on the number of positive tests and "red cow" classification are displayed in Table 3a. Prior to any test results, the probability of infection is taken to be equal to the true seroprevalence in the herd, and this differs between the groups increasing as the number of positive tests in each group increases. This is also reflected in the PPI after a single positive test (Table 3b): the median PPI for group B (only one positive test) is 42.3% whereas the median PPI for cows in group E (more than 7 positive tests) is 91.8% despite cows in both groups having identical test results at this stage. After two positive tests, all applicable groups (C-E) have a median PPI of greater than 98% except group C2, cows which had two positive tests but not within four tests. In groups C1-3 there is a bimodal distribution to the PPI after the second positive test with some cows having a very low PPI.

Table 3a. Classification criteria and characteristics of each group (A-E) defined by the number positive test results (N = 110,281). Groups C and D are further divided based on whether cows would meet the 2 in 2 'red cow' definition and a third sub-group in group C (C3) for cows which would only meet the 2 in 4 'red cow' definition but not the 2 in 2 definition.

Group	Number of positive tests	Red cow	Number of cows (% of total)	Mean number of tests per cow
A	0	No	98,716 (89.5)	9
B	1	No	7,326 (6.7)	10
C1	2	Yes – 2 in 2	1,107 (1.0)	9
C2	2	No	443 (0.4)	12
C3	2	Yes – 2 in 4 only	369 (0.3)	11
D1	3 - 7	Yes – 2 in 2	1,953 (1.8)	11
D2	3 - 7	No	132 (0.1)	12
E	>7	Yes	235 (0.2)	13

Table 3b. Median and interquartile range (IQR) of first prior probability and subsequent posterior probabilities of infection (PPI) with JD following serial test results. Cows are split into groups based on the number of positive tests and whether cows meet the 2 in 2 or 2 in 4 "red cow" definitions (Table 3a).

Group	Median (IQR) first prior probability (%)	Median (IQR) PPI after first positive test (%)	Median (IQR) PPI after second positive test (%)	Median (IQR) PPI after final test (%)
A	8.5 (5.0 – 12.9)	-	-	0.0 (0.0 – 0.1)
B	11.9 (7.9 – 19.8)	43.2 (2.2 – 85.7)	-	0.2 (0.0 – 7.0)
C1	9.8 (7.3 – 17.0)	23.4 (1.2 – 75.6)	98.0 (67.1 – 99.8)	83.2 (5.9 – 98.8)
C2	13.4 (8.9 – 20.3)	75.1 (34.2 – 91.7)	74.8 (11.6 – 96.9)	8.7 (0.6 – 58.1)
C3	13.2 (8.4 – 22.3)	64.6 (8.4 – 89.7)	98.3 (73.3 – 99.8)	26.6 (1.6 – 91.2)
D1	12.5 (8.7 – 19.8)	65.0 (14.8 – 89.6)	99.4 (92.8 – 99.9)	100.0 (99.9 – 100)
D2	15.1 (9.6 – 27.3)	85.6 (52.5 – 93.9)	98.9 (84.5 – 99.7)	98.5 (85.2 – 99.9)
E	17.0 (10.1 – 24.7)	91.8 (80.2 – 96.3)	99.9 (99.8 – 100)	100.0 (100 – 100)

DISCUSSION

Despite advice not to perform a JD-ELISA within 60 days of a SICCT test, 14.7% of JD-ELISAs in this dataset were within this time period and this was associated with increased odds of a positive result. Picasso-Risso et al., (2019) recently showed increased odds of a JD positive test if the interval between bTB (using bovine PPD only) and JD test was less than 90 days; but only 3.6% of the JD-ELISAs were <90 days after the bTB so this group could not be split any further to examine the effects of even shorter intervals. Unfortunately, due to the current situation in the UK it can be challenging to avoid a short interval between the two tests as many herds are often required to have frequent SICCT tests on top of regular JD testing. As the greatest effect on JD-ELISA was present when bTB to JD test interval was 30 days or less, every effort should be made to avoid testing in this window if it not possible to wait the recommended interval of 60 days.

It is only possible to speculate whether the increased risk of a positive JD-ELISA represents an increase in false positive results or improved test sensitivity. Casal et al., (2014) found improved performance of an *M. bovis* antibody ELISA 15 days following intradermal inoculation with bovine PPD, suggesting that an anamnestic response could be triggered by intradermal PPD inoculation. Roupie et al., (2018) demonstrated that prior testing with intradermal bovine PPD resulted in increased MAP antibodies and the authors suggest this could be due to a cross-reactive immune response. These animals were experimentally infected with *M. bovis* and seronegative for MAP antibodies prior to infection, although there was evidence of MAP exposure on post-mortem examinations. Interestingly, in control animals not infected with *M. bovis* there was very little change in MAP antibodies following the intradermal bovine PPD inoculation.

In a Brazilian study, 2/17 cows in a herd considered free from bTB and MAP demonstrated an increase in MAP antibodies (using an in-house ELISA) following a SICCT test with avian and bovine PPD. Although in this case the S/P ratio for MAP antibodies did not exceed the threshold for positive results until 60 days or more after the SICCT test (Varges et al., 2009). In a 139-cow Irish dairy herd, which were all seronegative for MAP antibodies prior to a SICCT test, 30% were seropositive ten days following the test and this dropped to 12% after 42 days. This herd was bTB free, but JD had been previously diagnosed and most cows were expected to be exposed to MAP. Two years after this study 70 of the cows that remained were tested for MAP shedding using a faecal PCR, 3/70 were positive only one of which had been JD-ELISA positive in the original study (Kennedy et al., 2017). Authors of both these studies concluded that their results most likely suggested a decrease in JD-ELISA specificity following a SICCT test.

JD management on many dairy farms relies on culling “red cows” to limit spread of the disease. This requires confidence that the animal is infected and truly represents a risk to other stock, the PPI calculated in this study is the probability of the animal truly being infected and possibly infectious. Although there is no absolute figure for how high this PPI would need to be to warrant culling the animal, and culling decisions are rarely based on a single factor, a 95% probability of infection is a reasonable threshold to use for the purposes of discussion. The PPI at the point of “red cow” classification, and the changes with different combinations of tests were modelled and displayed in Fig. 1. The specificity estimate (0.996) was so high that 2 in 2 positive tests in a four-year-old animal result in a PPI of over 99% in all cases unless the prior probability was virtually zero. The PPI following 2 in 4 (+ - - +) positive tests is over 95% unless the prior probability is less than approximately 1%. This line overlays the plot of PPI following 2 in 2 positive tests but with a specificity of 0.986 and suggests this change in definition is analogous to a 1% reduction in specificity from 0.996. The reduced specificities that were modelled showed greater effects with the 2 in 4 (+ - - +) result patterns than with the 2 in 2 patterns. To generate a PPI of at least 95% with 2 in 4 (+ - - +) positive tests and a test specificity of 0.986, the prior probability needs to be greater than approximately 6%. If this prior probability is based on the true seroprevalence within the herd, rather than previous test results, then this would not be high enough to produce a PPI of greater than 95% for more than 25% of herds in this dataset (Table 2). With the specificity reduced to 0.95, the PPI is less than

95% if the prior probability is less than 11% with 2 in 2 positive results or less than 42% with 2 in 4 (+ - - +) positive tests. These results demonstrate that a high degree of confidence in infection status is reliant on an exceptionally high specificity and should anything reduce this, even by a small proportion, the probability of infection is substantially reduced, particularly if the 2 in 4 “red cow” definition is used.

The median PPI after the second positive test in cows which were classified as “red cows” using the 2 in 2 (group C1) or 2 in 4 (group C3) classifications were 98.0% and 98.3% respectively. This suggests that at this stage there is little difference in PPI with either “red cow” definition for the median cow. Crucially, however, the bimodal distribution of PPI means that for many cows the PPI would still be too low to cull many of these cows with a high confidence of infection.

Once cows are classified “red cows” they are treated as high risk regardless of future results and this is based partly on a longitudinal study in Denmark that showed these cows have a higher probability of shedding in the future (Nielsen, 2008). Following their final test, the median PPI in group C1 was 82.3% and in group C3 it was 26.6% because some cows subsequently test negative following their “red cow” classification. As the 2 in 4 definition is a relatively new change, it is likely that cows in group C3 were retained on farms longer than cows classified as “red cows” using the original definition (group C1). The number of tests required to reduce the PPI to a level with a high confidence of the cow being uninfected, such as a 5% PPI (95% probability of being uninfected), depends largely on the age of the animal. The modelling of PPI after quarterly, consecutively negative tests showed how significantly the age of the animal influences the value of a negative test. In a two-year-old animal the test sensitivity is so poor (0.24) that a negative JD-ELISA barely alters the PPI from the prior probability. For example, if the prior probability was 95%, after a single negative test at two years old the PPI would be 93.5%. If the starting prior probability is 95%, the number of consecutive negative tests needed to produce a PPI of below 5% is ten tests for an animal that was two years old at the first test but only five tests if the animals was six years old. Most cows that become “red cows” are not retained in herds because of the risk they represent and therefore it is not known what further tests in these cows would reveal. Meyer et al., (2018) reported 30% of “red cows” had no further tests once classified as such, but of those that did approximately 53% had only positive tests and 16% had only negative tests.

A major driver of the PPI is the prior probability which, when there is no testing history, can be substituted with the true herd seroprevalence at the time. Therefore, an important consideration in the interpretation of serial JD-ELISA results should be the expected seroprevalence within the herd. The apparent seroprevalence is generally easy to obtain from previous test results but should be adjusted to account for the poor test sensitivity (Rogan et al., 1978). In most herds this means approximately doubling the apparent seroprevalence to calculate an approximate true seroprevalence (Table 2). The probability of infection can be estimated based on the pattern of test results (Fig. 1) but in order to maintain high PPIs in “red cows” the test specificity needs to be exceptionally high, and this is particularly true if the 2 in 4 “red cow” definition is used. It is therefore advisable to ensure these decisions are not based on test results conducted within 60 days, and particularly 30 days, of a SICCT test where the specificity of a JD-ELISA may be reduced.

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FARMER AND VETERINARIAN OPINIONS ON DRIVERS FOR JOHNE'S DISEASE CONTROL IN ENGLISH DAIRY HERDS

P.A. ROBINSON[*] AND H. BOWN

SUMMARY

To successfully and sustainably control production animal disease, there must be an understanding of the reasons why key stakeholders engage in disease control efforts. These 'people factors' are increasingly being recognised within veterinary epidemiology and policy making as important influences on the success or otherwise of animal disease control programmes. Research methodologies from the social sciences offer ways to better understand this important dimension. The study reported here, based on interview research, investigated the opinions of dairy farmers and farm veterinarians on the drivers for controlling Johne's disease in England. The findings demonstrate the varied influences of veterinary advice and encouragement, a voluntary national control plan, and fear of a future food scare as important reasons to engage in Johne's disease control on dairy farms.

INTRODUCTION

Johne's disease (paratuberculosis) in cattle is a major global animal health challenge, and a particular problem for dairy industries worldwide (Whittington et al., 2019). In addition to having a significant impact on dairy cow health and herd productivity as an endemic disease in many countries, there are longstanding and increasing concerns about a possible association between the causative organism – *Mycobacterium avium ssp. paratuberculosis* (MAP) – and Crohn's disease in humans (Atreya et al., 2014; Kuenstner et al., 2017). This risk is mainly linked to the consumption of contaminated food products derived from cattle, direct contact with infected animals, and contact with sources of environmental contamination including water supplies (Waddell et al., 2015). In light of the animal health and welfare implications of this endemic infection, and the possible zoonotic impact in humans, there is an onus on achieving more effective control in cattle at farm, national and international levels.

A recent review of the knowledge gaps hampering the prevention and control of Johne's disease has highlighted the need for social science approaches to better understand the motivations of both farmers and veterinarians affecting participation in Johne's control (Barkema et al., 2017). There has been a lack of qualitative social science research in this area to date, and although two individual interview-based studies have been conducted in Canada and Ireland investigating farmer attitudes and opinions (Ritter et al., 2016; McAloon

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et al., 2017), to the authors' knowledge there has been only one similar qualitative study also involving veterinarians on this topic (Roche et al., 2019), and none in the United Kingdom (UK).

The aim of this study was therefore to investigate the views and experiences of both dairy farmers and veterinarians on Johne's disease control in England using an interview research methodology. The data presented here focus specifically on the drivers for participation in active Johne's control efforts.

MATERIALS AND METHODS

The study was conducted in two dairy-intensive regions of England in the South-West (Group A) and West Midlands (Group B). A purposive sampling strategy (Marshall, 1996) was used to select a range of dairy farmers with different herd sizes and involving a mix of those already engaged in active Johne's surveillance and control measures, and others who had minimal engagement. All of the farmers interviewed were responsible for the herd management decisions on their farm. The veterinarians all spent a considerable proportion (or all) of their time employed on dairy farms, and represented six different private veterinary practices. The interviews were conducted in two phases – between December 2016 and March 2017 (Group A), and between November 2017 and November 2018 (Group B).

In total, 17 dairy farmers and seven cattle veterinarians were engaged across 20 semi-structured interviews conducted by the authors. Most of the interviews were one-to-one, but there were also several one-to-two person interviews with farmers. The study received ethical approval from the research ethics committee of Harper Adams University before commencement. Interview participants were assured of anonymity, and the interviews were audio-recorded with written consent. The interviews were fully transcribed, and the transcripts were analysed thematically using *NVivo 11* software (QSR International Ltd, Australia). Multiple themes were identified, but this paper focuses specifically on the theme of drivers for the control of Johne's disease.

RESULTS

The 20 interviews lasted between 30 and 90 minutes each, and overall produced 122,854 words of transcript for subsequent analysis. The farmers interviewed managed dairy herds containing between 165 and 1000 cows (mean herd size = 397 cows, n=13). The following subsections present extracts of the transcripts of the interviews based on three main sub-themes: the impact of veterinary advice; the national voluntary Johne's control programme; and the fear of a food scare in the future.

The impact of veterinary advice

The veterinarians spoke of the direct influence they had on their farm clients as they tried to persuade them to actively test for Johne's disease and implement control measures. For some, this influence was more impactful than for others. Some of the veterinarians were exasperated by a lack of knowledge, interest or action on the part of some of their clients, despite clinical cases occurring on the farm:

> ‘We did have one guy [with 250 cows] and he had literally no idea what Johne’s was! … And it took 12 months of repeatedly having discussions with him for him to get it, and to appreciate that he needed to do something. He didn't milk record, so we got him to test all the milking cows at one point, but that's not enough - he wouldn't repeat it.’ (Int B01, veterinarian)

> ‘I can think of one herd in particular where we regularly turn up clinical cases, but they still don't seem to have the desire to find it in their herd at all; they’re just seeing it as an individual cow disease.’ (Int B09, veterinarian)

> ‘I can think of a great example: this man has a [supermarket] contract, so he has to do Johne’s testing - it's obligatory for him. He loses clinical Johne’s cows hand over fist, and every single time I go there to look at a sick cow that's dropped its milk [I say] … “Is it on the Johne’s list?” [He says]:“I haven't looked.” So he does the recording because his milk buyer tells him he has to, but he's making no effort, even though he's losing … 5% of the herd a year to clinical Johne’s.’ (Int B04, veterinarian)

Despite such examples of an apparent lack of desire to act to control Johne’s disease, the veterinarians also spoke of encouragements, where farmers were actively engaging in testing and control measures:

> ‘If you get people started, my experience would show that once they start they will see the benefit of it, and I have never seen anybody stop Johne’s control measures. If anything, they just ratchet them up and up as they go on further as they see more and more benefit from it.’ (Int A06, veterinarian)

> ‘I did a Johne’s meeting in late 2013 … and about six people at the end of that said “We should probably do something about this”. And year on year every September I do a Johne’s meeting … and more and more people come every year, and it's different people, and each time I get a couple more people interested.’ (Int B11, veterinarian)

> ‘From a Johne’s point of view I've had quite a lot of input on certain farms, instigating plans, or shall we say tweaking, control plans to get the best of it.’ (Int B10, veterinarian)

A few of the veterinarians spoke of their own personal ambition to engage their clients on Johne’s control, with some making it a priority disease to advise their farmers on:

> ‘Yeah, it's just something I feel passionate about, and it's something I can see being a real issue. I also like my job, and I want my clients to be there in ten years’ time, and this could be a make or break for a lot of people.’ (Int B11, veterinarian)

> ‘The vast majority of our herds are actively testing for Johne’s … A big focus for us at present is more on the moving the guys who [are] at that reclusive-traditionalist boundary, and you then get into a debate about whether you are better focusing your attention on improving the control of the ones who have already engaged, or continuing to hammer away at the ones who are difficult to engage with, and we're probably in the former of those buckets.’ (Int B04, veterinarian)

> ‘You do have personal crusades as a vet, don't you? And I have personal crusades to do with lameness, and transition health, and cow handling, and cow happiness and comfort; very

> much so. So does Johne’s rate in there for me personally? No it doesn't, but it does come in just below there. So I do feel embarrassed when I haven't got on top of it in certain herds, or I haven't managed to get their engagement.’ (Int B09, veterinarian)

While the veterinarians mentioned their influence on their farm clients, this was spoken of more infrequently by the farmers, but a few did speak positively about their veterinarian’s influence. For example, these farmers, who were early adopters of quarterly individual milk testing on their whole herd, spoke of the direct impact of their veterinarians on their efforts to control the disease:

> 1: ‘We're on a testing scheme, and we're moving towards an eradication scheme working with our vets.’
>
> 2: ‘Our vets are very keen on it.’ (Int B03, dairy farmers)

But other farmers believed that what their veterinarians were asking them to do on Johne’s was unrealistic and impractical, leading to inaction or a negative attitude towards Johne’s control:

> ‘They'd like to see me monitoring it. They'd like to see me reacting and isolating cows that are Johne’s positive and being more pro-active. I guess the problem is from a veterinary side it's all very well and good saying [that, but] …’ (Int B07, dairy farmer)

> ‘There’s a whole world of what [veterinarians] would want us to do ... like the idea you should only a give a calf its mother's milk - you know, it's just not possible, it's just not possible in a system like ours.’ (Int B02, dairy farmer)

Whether initially prompted by their veterinarian, or based on knowledge acquired from other sources such as the farming press, some farmers were clearly very motivated themselves to try to reduce the Johne’s prevalence in their herd, without having to be ‘forced’ into action. This ‘self-motivation’ was mentioned by a few of the veterinarians, particularly clearly demonstrated in this interview excerpt:

> Interviewer: ‘What are the main incentives for farmers to increase Johne’s control?’
>
> Veterinarian: ‘Self-motivation. There are financial incentives, but you’ve got to look at the long-term, haven’t you? … I think maybe as an industry we need to be a bit more progressive in taking control ourselves – gone are the days of subsidised incentives.’ (Int A07, veterinarian)

In the opinion of two veterinarians, a useful tactic to prompt engagement from late adopters was to apply pressure through asking their clients why they were not engaged when many other farmers were:

> Interviewer: ‘Do you think more could be done to encourage farmers to include more control strategies?’
>
> Veterinarian: ‘Yeah, I think it’s just chipping away at it constantly. And as time goes on more and more people get interested in it and get involved in it, and it’s getting to the critical mass of client base once you get more than 30-40% of your farms doing something then it’s

> easy to go out to the rest of them and say "Why aren't you doing this? Everyone else is doing it – you're behind the curve; you're losing out!" But to get to that point is just a lot of hard work and talking to individual farms and chipping away at it.' (Int A06, veterinarian)

> 'I think you have to wait for your moment, and I think there is usually a moment, when you're diagnosing the 17th Johne's cow of the year with them: "Do you know that you're the only farm in a 15-mile radius not testing for Johne's?" I don't think we're far from the tipping point for the message changing into "Everybody else is doing this, why aren't you?" That's the point they'll start asking questions, because that's when they'll go to the pub on a Friday night and they'll say "Are you doing anything about Johne's?' And their mate will turn round and say "Yeah, we've been testing the herd for 4 years! Have you not?" [*Laughs*] (Int B04, veterinarian)

The national voluntary Johne's control programme

A National Johne's Management Plan (NJMP) has been developed by the *Action Group on Johne's* – a forum of industry stakeholders interested in reducing Johne's disease incidence on dairy farms in Great Britain (Action Johne's, 2019). This forum is jointly funded by a national levy board (Agriculture and Horticulture Development Board - AHDB) and milk purchasers, and was first launched in 2015. It includes representation from organisations and companies such as milk purchasers and processors, farmer unions, veterinary associations, academic institutions and cattle companies. It is estimated that 80% of the total milk volume in Great Britain is covered by members of this scheme (Action Johne's, 2019).

Phase 1 of the NJMP, which ran from 1st April 2015 - 31st December 2017, was primarily focused on education and engagement with producers and training of veterinarians according to a standardised training course. Phase 2 of the scheme was launched in 2018 and involves members of the NJMP to require their associated farmers to obtain a signed declaration by an accredited Johne's veterinary adviser that they will implement one of the control strategies specified by the scheme (Action Johne's, 2019).

The scheme is not a statutory scheme, but enrolment has become a part of milk contracts between many milk purchasers and their milk suppliers. The roll-out of the scheme was reported to be having a very notable impact on engagement between veterinarians and their clients on Johne's, and between farmers and their milk purchasing companies:

> 'As of five months ago, just over half our herds were milk testing, and now it would be more than that. The [NJMP] has made my life easier in terms of getting people engaged, because there's that wall that you just push people towards, and they see what's coming, and they jump before they're pushed.' (Int B11, veterinarian)

> 'The [NJMP] has helped in that they have to do it [milk testing], and we won't sign something unless they are doing it.' (Int B09, veterinarian)

> 'There is the national Johne's control plan which is being rolled out and increasingly taken up by, and supported by, [milk] buyers. So more and more of our clients are going under that umbrella … The national control plan I think is a really practical tool and it allows you to do different things on different farms and still be under that umbrella, and still be compliant.' (Int A06, veterinarian)

Awareness of the NJMP as the primary driver for more active national Johne’s control efforts seemed to be low amongst the farmers interviewed, but all of them were aware that the disease had become more high profile. Their more immediate focus was specifically on what their milk buyer was requiring of them in terms of Johne’s control in their herd:

> Interviewer: ‘Did you first start testing because of the contract you were on?’
>
> Farmer: ‘Yes, pressure from milk buyers in the last year has been towards doing this. They haven’t dictated that we have to do it … I think there will be more pressure from the buyers to do it. So that’s why we do it.’ (Int A05, dairy farmer)
>
> Interviewer: ‘Are you getting any pressure from your [milk] processor?’
>
> Farmer: ‘Yes, we are now - just started.’
>
> Interviewer: ‘So that will force you into doing something?’
>
> Farmer: ‘Yes. There you go [*shows letter*]. So yes, it's a road - we're on a road, and hopefully it's factually-led. You think it's an issue, and government institutions think it's an issue, then I'll do whatever is required. But until that happens, that's where I'm at.’ (Int B07, dairy farmer)
>
> ‘A concern might be that when the milk price drops again like it did two or three years ago, if there’s any reason for your milk not to be as good as the person up the road they might take theirs and not yours. I guess I wouldn't want to give my milk purchaser any reason not to buy my milk.’ (Int B08, dairy farmer)

Despite this pressure from milk buyers through the NJMP, other farmers spoke of how their milk buyer [a supermarket retailer] had been encouraging routine testing of their milk for a number of years before the NJMP was launched. This meant that they had been actively engaged in Johne’s control for longer than most through identifying infected animals on milk serology:

> ‘We’re on a [supermarket retailer] contract and we’ve been doing Johne's ever since [the supermarket] wanted us to really.’ (Int A01, dairy farmer)
>
> ‘There have been others out there that are on [supermarket retailer] contracts, and other contracts as well, who have been testing for a [lot] longer than we have – four, five, six years.’ (Int A04, dairy farmer)
>
> Farmer 1: ‘[The supermarket retailer] actually made us test seven years ago. They paid us … to test.’
>
> Farmer 2: ‘But they don't do that any more.’
>
> Farmer 1: ‘No, but for six years they did … So full marks to [them] for getting right on board.’ (Int B05, dairy farmers)

When the veterinarians were asked about how influential the pressure from milk buyers and retailers was having on farmers' engagement with Johne's control, there was unanimous agreement that this was a very significant factor, as typified by these quotes:

> Interviewer: 'How much of a role do the milk buyers play in the farmers' willingness to manage the disease?'
>
> Veterinarian: 'Huge. Yeah, 'cos I think that's where a lot of the drive comes from now. I think they're being beaten with a financial stick from the milk buyers that if they are implementing a plan there is a potential for them to get a few more pence per litre. So yeah, I won't deny I think that's a massive part of it.' (Int A07, veterinarian)

> 'Most of our farmers that are coming on board now are doing it because they are told they have to by the supplier, if I'm honest.' (Int A06, veterinarian)

Despite this increasing engagement, there was concern that some farmers still viewed engagement with the NJMP as a bureaucratic 'tick-the-box' exercise, rather than being convinced that reducing the disease prevalence was in the interests of their herd profitability and sustainability:

> 'The NJMP has helped push things forward, but I do still find I'm sitting down for a drink of tea after a routine visit … and they say "You just need to sign this", and they pull out the declaration. And to some of them, it still is just a box-ticking exercise, and we've been incredibly resolute in saying "It isn't just a case of signing that - we're going to do you a Johne's risk plan, and we're going to do you a Johne's health plan, and we're going to make sure this is done properly, because this is a great opportunity to do it."'(Int B09, veterinarian)

This veterinarian's view appeared to be justified by what these farmers said in response to being asked about why they were starting to test routinely for Johne's; they demonstrated some degree of reluctance, and a failure to see a wider objective apart from meeting a contractual requirement:

> Farmer 1: 'Our milk company asks us to sign a declaration that we're doing something about it.'
>
> Farmer 2: 'But that's all - we're not being driven to do something - we're being asked to test.'
>
> Farmer 1: 'And we're not being incentivised to do anything about it either.'
>
> Farmer 2: 'We're not being paid to go Johne's disease clear; the milk companies aren't saying "We need you clear within 10 years." To tick the boxes we just need to say that we test, and we do test, so we tick the box.' (Int B02, dairy farmers)

> 'I wouldn't probably have done anything about it, because you read about it in the farming press, and the vets' newsletters - this is being honest - and you think, "Well, it's not really affecting me." But then two years ago [the milk buyer] sent us a Johne's form saying that we had to be seen to be doing something about it, so because of that I did a bit more reading, and spoke to our vet, and just said "What should we be doing?", because he agrees that we

> haven't really got a problem. So since then I decided to be proactive just in case there was something hidden.’ (Int B06, dairy farmer)

Fear of a food scare in the future

In addition to encouragement from veterinarians, and the pressure being exerted by milk buyers and retailers through the national management plan, there was also an important line of argument on why Johne’s disease needed to be more effectively controlled – fear of a potential zoonotic infection risk, and future food scare. This driver for control was mentioned by both farmers and veterinarians, but was particularly emphasised by the latter.

Some of the farmers spoke of their own fears of Johne’s potentially being a zoonotic disease linked to Crohn’s disease in humans:

> Farmer: ‘That does concern me. Yeah.’
>
> Interviewer: ‘Why?’
>
> Farmer: ‘Because it's a public health issue, and if that's proved to be correct, then we're all going to have to react, aren't we? Yeah, that is an issue. And I know a person with Crohn’s who seems to think it's my cows that created it.’ (Int B07, dairy farmer)
>
> ‘It was quite striking when I went to Ireland about ten years ago - when I'd only started dairy farming - that I went to a farm with a friend, and we were sat at the kitchen table and I asked him why he didn't drink his own milk, and he said “Oh, I've got Johne’s in the herd, and I wouldn't ever drink it - I don't want to get Crohn’s”. (Int B08, dairy farmer)

These views were echoed by some of the veterinarians, who were also similarly concerned about a zoonotic threat:

> ‘My assumption - the stance that I have taken - is that I'm fairly happy there is a link there to some degree. And I think if I adopt that stance and try to encourage my clients to work towards control and eradication policies, then I think that's the best approach - the sort of gold standard approach. Whereas I think if I reassured myself there's not a particularly significant link, then I think that would potentially induce you to take your eye off the ball a bit, and your foot off the gas.’ (Int B09, veterinarian)
>
> ‘It is a grey area scientifically but I think for me as a consumer or a mother feeding children, its enough of a unclear issue to say “No, I wouldn’t want to take that risk.” At one of my Johne’s [courses] I found it horrendously scary that they find it in infant formula. And the lecturer there was from the States and particularly in the States, the consumption of ground meat, minced meat - all these Johne’s cull cows go for mince meat don’t they, and they do find it. We think of it as just in the milk or blood or whatever, but in ground minced meat they were finding MAP in samples. And I find that really scary.’ (Int A07, veterinarian)
>
> ‘It’s been talked about for probably 20 years or more and nobody has really come up with any conclusive proof. So I think it’s one of those things that increasingly now farmers see as bit of a scaremongering tactic. It’s the thing that’s brought out of the drawer whenever you want to try and scare people into doing anything … It’s difficult – there’s no clear data

> that farmers have massively higher Crohn’s levels, and it is a difficult one to pin down but obviously, the same bug is found in both syndromes, so there is something there. (Int A06)

Certainly not all veterinarians were focused on a possible zoonotic risk. These veterinarians were not convinced that MAP was zoonotic, and were not using that specific argument to convince farmers to engage in Johne’s control:

> ‘I don’t know enough about that. I know that some farmers are aware there might be a link, and that’s one of the reasons why the milk buyers are very keen on people controlling it.’ (Int B01, veterinarian)

> ‘I've never had that [opinion], no. I think the reason is because our farmers tend to come at it from an animal health point of view, and we tend to come at it from an animal health point of view … Because there hasn't been any strong animal health-human health links drawn up for the disease, I think that's why I don't talk about it as if it's a zoonotic disease.’ (Int B10, veterinarian)

Ultimately, if a zoonotic risk from MAP and Johne’s disease was more irrefutably proven, there were fears about a future food scare which may affect the retail of milk and meat products from the dairy industry due to consumer concerns around product safety. This view was echoed by both farmers and veterinarians, and comparisons were made with previous food scares linked to animal diseases in the UK in the past, as exemplified in these quotes:

> ‘Everybody has got to have a Johne’s plan - they've got to be testing, and doing something about it, otherwise they'll be out of business … You’ve got responsibilities if you're going to keep cattle. And particularly as a milk producer, if Johne’s blows up like BSE (bovine spongiform encephalopathy), it could ruin us. You know what happened with … the eggs - all the British eggs were infected with *Salmonella*. So there is a political message - the milk buyers and the government just need to get everybody up to speed.’ (Int B05, dairy farmer)

> ‘The *Salmonella* in eggs scandal is where I could see this going if it's not managed well. I think as a country we are managing the situation better than anyone else has done before. So if we can get our farmers engaged, and get control measures in place, and we can say ... “If you have a herd that is a low risk Johne’s herd, you can sell your milk and sell your products in a world market.”’ (Int B11, veterinarian)

DISCUSSION

It is increasingly being recognised within veterinary epidemiology and policy making that the stakeholders involved in controlling a disease in livestock have an important influence on the success of disease control efforts (Garforth, 2015; Robinson, 2017). While studies have demonstrated that veterinarians are regarded by many farmers as trusted disease control advisers (e.g. Richens et al., 2015; Marier et al., 2016), veterinary advice is not always implemented by farmers, perhaps because of perceived feasibility or other on-farm priorities (Svensson et al., 2019).

As pointed out by Barkema et al. (2017), most Johne’s disease control programmes globally are voluntary, and their success depends ultimately on either the self-motivation of farmers to be involved, or the active encouragement of veterinarians, to promote enrolment over the long-term. Attempting to control this challenging endemic disease requires sustained effort, and it is particularly important to understand what the main drivers for Johne's control might be.

Veterinarians in this study were very keen to educate and engage their farm clients in Johne's control efforts, with some seeing it almost as a personal crusade. They were finding more willingness to test for, and prevent, the disease than in the past, but they also reported struggling to persuade a minority of farmers who had clinical Johne's disease in their herd to actively seek to control the disease. Even those who were testing for the infection may not be fully convinced of the benefits. Similar challenges in convincing all farmers on the importance of Johne's control were reported by Ritter et al. (2016) and Roche et al. (2019) in Canada, and McAloon et al. (2017) in Ireland. As McAloon et al. suggest, convincing farmers to voluntarily control the disease on the basis of animal and herd health arguments alone may not be sufficient to achieve engagement, particularly when farmers perceive the proposed solutions as impractical.

In the absence of statutory control, a national Johne's programme (NJMP) led by an industry stakeholder forum was found in this study to be having a significant impact on farmer participation, as reported by both the farmers and veterinarians interviewed. Although having a national control programme is certainly not unique, and other countries across the world have attempted similar co-ordinated efforts (Geraghty et al., 2014), what is notable here is the impact that contractual requirements from milk buyers were having on farmer uptake. This was reported to be facilitating the pathway towards deeper veterinary engagement in the disease enabled by the vehicle of the NJMP. The driver of food industry demand and the market setting the standards for disease control, rather than government, is more clearly elucidated in this study than in previous qualitative studies on Johne's.

Previous qualitative studies have also found levels of concern among farmers (particularly McAloon et al., 2017 and Roche et al., 2019) about the potential for a zoonotic risk and link to Crohn's disease in humans, and future negative economic impact. This appeared to be a powerful driver for several of the farmers and the majority of the veterinarians interviewed in this study. Both groups compared how a food scare could develop in the future centred on dairy products to what had happened in the past with BSE in cattle and *Salmonella* in chickens. As Atkins (2008) shows, negative consumer reactions to zoonotic food hazards have a long history in the UK.

The study therefore found that Johne's disease control in English dairy herds is about more than the economic benefits of improving dairy herd health at the individual farm level, but would appear to be strongly influenced by wider industry and market concerns around the possible health implications of an association between MAP infection and Crohn's disease in humans. The sample of interviewees is not statistically representative of all dairy producers and veterinarians in England, and nor was it meant to be, but assured by the recurring themes which were raised across the interviews, the findings are likely to be indicative of the wider population. The study raises interesting questions about the inter-relationships between the drivers and responsibilities for the control of endemic disease in food animals, food safety, and public and private goods in a global marketplace.

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MODELLING WITHIN-HERD TRANSMISSION OF PARATUBERCULOSIS IN AN IRISH DAIRY HERD: INFECTION DYNAMICS AND THE EFFECT OF TESTING AND CULLING

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SUMMARY

Johne's disease is a chronic bacterial infection of cattle caused by *Mycobacterium avium* subspecies *paratuberculosis* (*Map*), inducing large economic losses to farmers. We simulated *Map* transmission in Irish dairy herds with a seasonal pasture-based milk production system and compact spring calving. We used this stochastic model to investigate *Map* persistence, relative importance of transmission routes, and effectiveness of control measures. Mean within herd prevalence in absence of control measures 15 years after *Map* introduction was 63.5%. The main transmission routes were a contaminated general environment and in utero infection. The most effective control measures were culling all high shedding test positive animals within 4 weeks after testing (28% absolute decrease in prevalence after 15 years) and managing calf exposure by separating calves from cows on pasture (15% absolute decrease in prevalence). Both control measures effectively reduce persistence and slow the rate with which prevalence increases over time.

INTRODUCTION

Johne's disease (JD) is a chronic bacterial infection of the intestine in cattle caused by *Mycobacterium avium* subspecies *paratuberculosis* (*Map*). JD is endemic within the dairy sector worldwide (McAloon et al., 2019). It has an economic impact due to milk losses, early culling, and increased mortality (Garcia & Shalloo, 2015).

There are many challenges with JD, such as those related to diagnosis and control (Barkema et al., 2018). Susceptibility reduces with age, and older animals are assumed to be resistant (Windsor & Whittington, 2010). Clinical signs (decreased milk production, weight loss, diarrhoea) usually do not appear until later in life (after first calving), or are sometimes never observed (Mitchell et al., 2015). Infected animals without clinical signs are difficult to diagnose because diagnostic tests have a poor sensitivity (Barkema et al., 2018). Thus, animals can go unnoticed in a herd while contributing to *Map* transmission within the herd. Transmission occurs in utero or via ingestion of faeces or milk containing *Map*. However, the relative

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importance of the main transmission pathways at farm level and their drivers associated to the farming system are not yet fully understood.

Simulation models are useful tools to study *Map* transmission and evaluate control options (Marcé et al., 2010). Previous modelling results show a large effect of farm management on *Map* transmission for French herds with all-year-round calving (Marcé et al., 2011a). In these herds, the level of exposure of calves to a contaminated environment and the age of calves during the period of exposure to adults were especially important for transmission (Marcé et al., 2011a). In Ireland, the dairy system is designed to optimally use grazed grass as the primary feed source for lactating cattle (Ramsbottom et al., 2015). The milk production system is, therefore, pasture based and more than 90% of herds adhere to a compact spring calving period (Butler et al., 2010; Ramsbottom et al., 2015). Because of these seasonal herd demographics, existing results of modelling a year-round production system cannot be directly extrapolated to the Irish pasture based dairy system.

To better understand *Map* transmission in Irish dairy herds, we developed a stochastic individual-based epidemiological model that accounts for seasonal herd demographics in Ireland. We used this model to investigate the relative importance of different transmission pathways, the probability of *Map* extinction following introduction, and the relative effectiveness of different control strategies in herds with a pasture based dairy system.

MATERIALS AND METHODS

Transmission model

To simulate *Map* transmission within an Irish dairy herd and to assess the relative impact of different control strategies, we used an adaptation of the stochastic and individual-based model that was developed to simulate *Map* transmission in a French dairy herd (Marcé et al., 2011b; Camanes et al., 2018). Herd demographics and infection dynamics are considered in the model. The transmission model is fully described in Marcé et al. (2011b), Beaunée et al. (2015), and Camanes et al. (2018). In brief, animals in a herd are divided into six age groups: newborn calves, unweaned calves, weaned calves, young heifers, bred heifers, and cows. An animal moves to the next group at a defined age or moment in the year. Six health states are modelled: susceptible (S), resistant (R), transiently infectious (I_T), latently infected (I_L), moderately infectious (I_M), and highly infectious and possibly clinically affected (I_H). Calves up to one year of age are susceptible to infection, with susceptibility decreasing exponentially with age. Uninfected calves are assumed to become resistant to infection after one year. Animals in health states I_T, I_M, and I_H are infectious and can shed *Map* in their colostrum/milk and faeces. The quantity of *Map* shed depends on the health state. *Map* is transmitted via ingestion of contaminated colostrum/milk, via an environment contaminated with faeces, or in utero (which includes transmission during parturition) (Fig.1). Two environment types were modelled, the local and general environment. Transmission via the local environment is defined as the risk posed by other animals held in the same place but not necessarily at the same time. Transmission via the general farm environment is defined as the risk posed by animals of other ages (other management groups) kept indoors at the same time but not necessarily at the same place. The total amount of *Map* present in the general environment is the sum of the amount of *Map* present in all local indoor environments, thus all infectious animals that reside indoors contribute to the contamination of the general farm environment (Marcé et al., 2011a,b). If testing and culling was implemented, a serum ELISA was performed on all animals older than

2 years of age. The specificity of the test was 0.985 and the sensitivity was 0.15 for I_T and I_L, 0.47 for I_M and 0.71 for I_H animals (More et al., 2015). In the absence of precise knowledge, I_H cows detected as positive were assumed to be highly positive (Camanes et al., 2018).

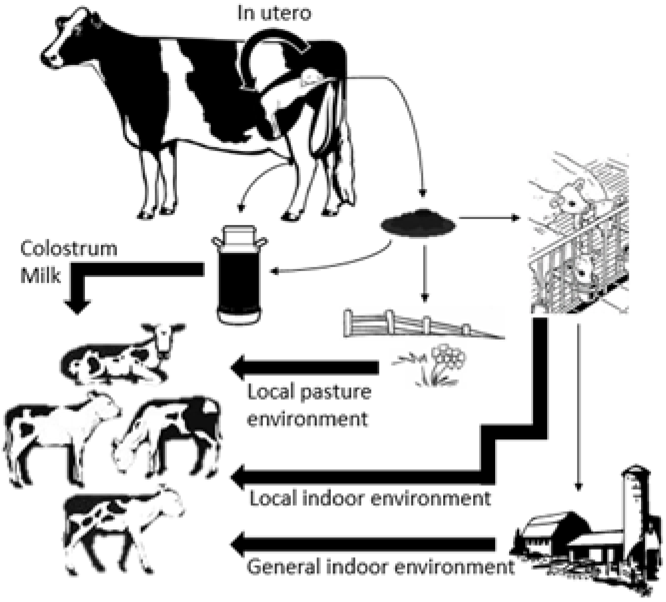

Fig. 1 Transmission routes considered in the model. Susceptible calves can become infected in utero, via colostrum or milk contaminated with *Mycobacterium avium* subspecies *paratuberculosis* (*Map*), or via a faeces-contaminated environment. The local environment is shared with other animals within the same management group of similar age at the same place but not necessarily at the same time, a local environment can be indoors or on pasture. The amount of *Map* in the general environment is the sum of *Map* present at a given time in the indoor local environments.

Adaptations were made for the model to represent a typical Irish dairy farm with associated management. The Irish dairy system is designed to optimally use grass as the primary feed source for lactating cattle. To match the supply of grass with the intake demand of cattle, more than 90% of the Irish dairy herds adhere to a spring-calving seasonal production system (Butler et al., 2010; Ramsbottom et al., 2015). Herd demographics are seasonal, with cows calving early in the year (February – March) and then being turned out to pasture directly after calving (Ramsbottom et al., 2015; Bloemhoff et al., 2014). Milk production increases after calving and reaches its peak at about the eighth week of lactation (Quinn, 2005). Cows are dried off and housed again at the end of November (Bloemhoff et al., 2014; Ramsbottom et al., 2015). Cows that are not in-calf will be culled at the end of the grazing period together with other cows destined for culling (Maher et al., 2008). Male calves and surplus female calves are usually sold within the first month after birth (Shalloo et al., 2004). The majority of calves were born between week number 4 and 13, including 50% of these calves in the first three of these weeks. The male calves and all calves born after the first three weeks were assumed to be calved from a dam bred to a beef bull and were sold at four weeks of age. Calves were weaned at 14 weeks of age. The pasture period started in week number 9 for cows, 11 for heifers, and 16 for calves. The pasture period ended in weenumber 45. An overview of model parameters for processes related to herd dynamics and management is given in Table 1.

Table 1. Model parameters for processes related to herd dynamics and management

Parameter definition	Value	Reference
Average number of lactating cows	82	(Bloemhoff et al., 2014; Ramsbottom et al., 2015)
Weaning age (weeks)	14	[a]
Age becoming young heifer (weeks)	52	[b]
Age at first calving (weeks)	104	(Kennedy, 2016)
Proportion of calves born dead	0.043	(Mee et al., 2008; Mee, 2013)
Proportion of newborns sold	0.5	[a]
Exit rate calves 2 to 4 weeks old (week^{-1}) [c]	0.206	(Shalloo et al., 2004)
Exit rate week 1 and 2 (week^{-1})	0.018	(Ben Romdhane, 2017)
Exit rate week 3 to weaning (week^{-1})	0.0025	(Ben Romdhane, 2017)
Exit rate weaning to first calving (week^{-1})	0.00024	(Ben Romdhane, 2017)
Exit rate parity 1 cows (week^{-1})	0.00047	(Maher et al., 2008)
Exit rate parity 2 and 3 cows (week^{-1})	0.00043	(Maher et al., 2008)
Exit rate parity 4 cows (week^{-1})	0.00044	(Maher et al., 2008)
Exit rate parity 5(+) cows (week^{-1})	0.00229	(Maher et al., 2008)
Culled fraction bred heifers (year^{-1})	0.1800	[a]
Culled fraction parity 1 cows (year^{-1})	0.1120	[a]
Culled fraction parity 2 cows (year^{-1})	0.0588	[a]
Culled fraction parity 3 cows (year^{-1})	0.0700	[a]
Culled fraction parity 4 cows (year^{-1})	0.2184	[a]
Culled fraction parity 5(+) cows (year^{-1})	0.5796	[a]
Replacement rate	0.22	[a]
Sell all calves after week number	11	[a]
Cull bred heifers and cows in week number	47	(Maher et al., 2008; Butler et al., 2010)
Start grazing calves (week number)	16	(Bloemhoff et al., 2014)
Start grazing young heifers (week number)	11	(Bloemhoff et al., 2014)
Start grazing cows (week number)	9	(Bloemhoff et al., 2014; Ramsbottom et al., 2015)
End grazing (week number)	45	(Bloemhoff et al., 2014; Ramsbottom et al., 2015)
Colostrum fed to calf (L/day)	5.0	(Ben Romdhane, 2017)
Milk fed to calf (L/calf)	7.0	(Ben Romdhane, 2017)
Faeces produced non-weaned calf (kg/day)	0.4	(Ben Romdhane, 2017)
Faeces produced weaned calf (kg/day)	4.1	(Ben Romdhane, 2017)
Faeces produced young heifer (kg/day)	7.5	(Ben Romdhane, 2017)
Faeces produced bred heifer or cow (kg/day)	22.5	(Ben Romdhane, 2017)

[a] Expert opinion

[b] Model assumption

[c] After three weeks, 50% of the calves (*i.e.*, all male calves) have exited the herd

Scenarios

We investigated the impact of three factors on *Map* persistence and transmission. Calf exposure (calves grazed on pasture in contact with cows, or calves grazed on pasture completely separate from cows), test and cull (I_H cows that were test positive were culled within 3 months, or these cows were culled within 1 month after detection), and calf disposal (keeping all calves from dams that were highly test positive, or removing these calves). When there was contact between cows and calves on pasture, they were on adjacent pastures and while physically separated, exchange of bacteria between their environments was possible. The transmission rate parameter for cows to calves on adjacent pastures was assumed to be 6.65×10^{-7} per week. After an I_H dam tested positive, her offspring was culled at 21 weeks (calf) or 90 weeks of age (heifer). All control strategy combinations were explored.

We simulated 1000 herds for each scenario. Infection was introduced in the fully naive herds through the purchase of a moderately infectious (I_M) first-parity cow. Except for this purchase, herds did not introduce animals. Weekly output data, *e.g.*, on individual disease status, was obtained over 15 years. *Map* persistence, prevalence, incidence, age at infection, and the main transmission routes were assessed. Infection was considered to persist when at least one infected animal was present in the herd or when *Map* was present in the environment. The model was developed in C++ Standard 14 (Stroustrup, 2000). Output was analysed in R version 3.6.1 (R Core Team, 2018).

RESULTS

Persistence of *Map* infection

Extinction (*i.e.*, the opposite of persistence) within 15 years after *Map* introduction occurred in 57.3-67.8% of the herds where calves and cow could have contact on pasture, and in 70.6-81.1% of the herds where calves and cows were separated on pasture (Fig.2). Early extinction (within the first two years) occurred in ~20% of the herds with contact on pasture and in ~30% of the herds with separated pastures. In herds where early extinction occurred, infection persisted in the first two years mainly because the environment was still contaminated in herds where infectious animals were no longer present (results not shown). Control strategies did not affect the probability of extinction within the first five years after *Map* introduction. Thereafter, culling dams that were detected as high positive increased the probability of extinction in a herd. In herds where highly test positive dams were culled within four weeks of detection, the infection became extinct more frequently compared to herds in which such dams were culled within thirteen weeks. Whether the offspring of dams that tested highly positive was kept or sold had no appreciable effect.

Map spread within persistently infected herds

For herds in which infection persisted for at least 15 years, mean prevalence at the end of the simulated period (15 years) was highest for herds where calves and cows could have contact on pasture (63.5% infected (I_T, I_L, I_M, and I_H), 27.6% infectious (I_T, I_M, and I_H), and 4.4% highly infectious (I_H) animals; Fig.3). Five years after the introduction of *Map* into the herd, a positive effect of control strategies on the prevalence was observed. After fifteen years, compared to herds with no control strategy, the average prevalence was ~15% lower in herds that culled I_H dams within 13 weeks after a positive test result and ~28% lower in herds that culled I_H dams within 4 weeks after a positive test result, irrespective of the pasture strategy. Whether the offspring of dams that tested highly positive was kept or sold had no effect.

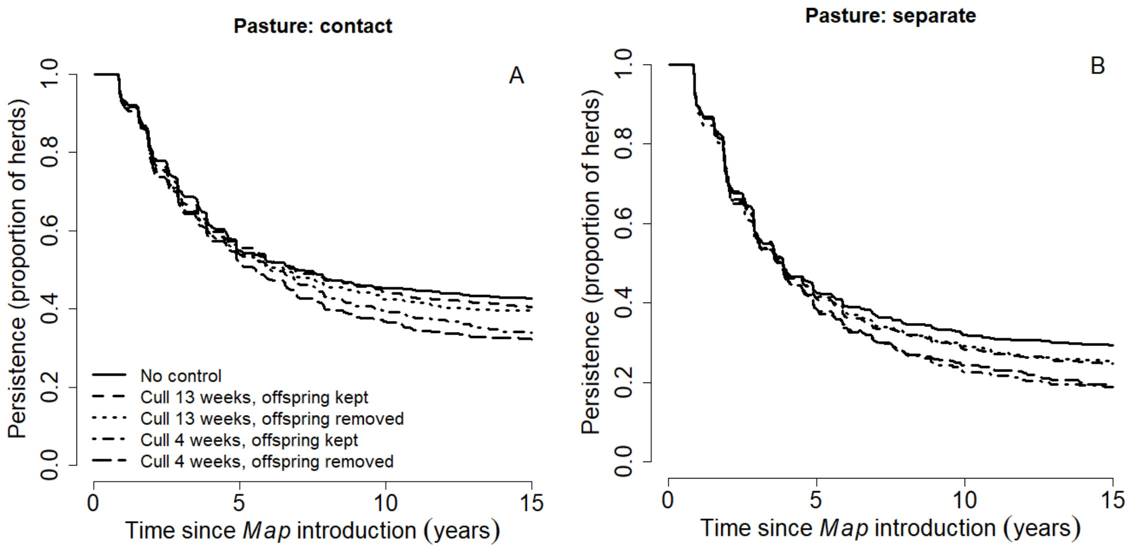

Fig. 2 Proportion of herds in which Mycobacterium avium subspecies paratuberculosis (Map) infection persisted over time, i.e. when at least one infected animal was present in the herd or when Map was present in the environment. Cows and calves could have contact (A) or were separated (B) on pasture. Cows that were high shedding and tested positive were culled within 13 or 4 weeks and offspring of these cows was either kept or sold.

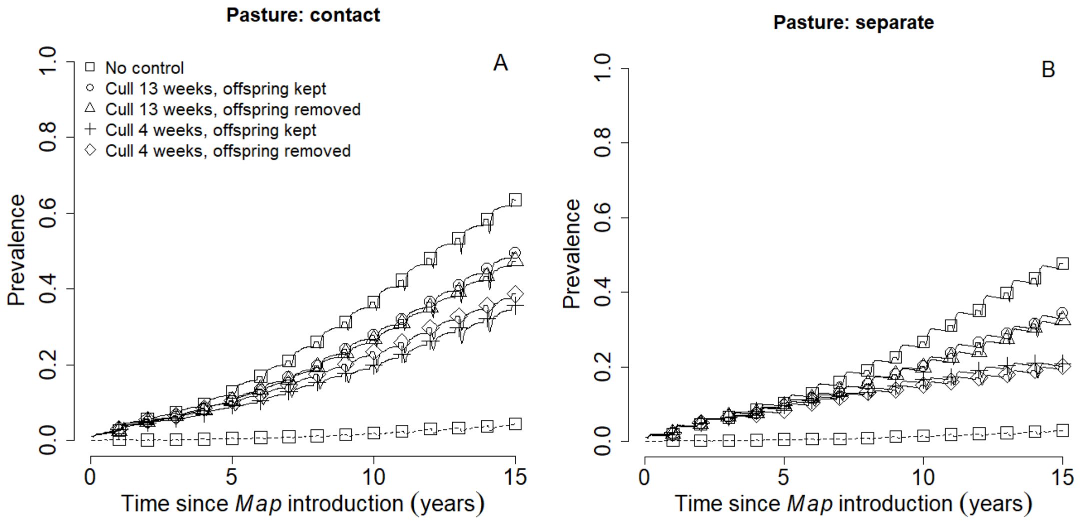

Fig. 3 Mean prevalence of all animals (I_T, I_L, I_M, and I_H; solid line) and only highly infectious animals (I_H; dotted line, other scenarios showed similar patterns) over time in those herds where *Mycobacterium avium* subspecies *paratuberculosis* (*Map*) infection persisted for at least 15 years. Cows and calves could have contact (A) or were separated (B) on pasture. Cows that tested highly positive were culled within 13 or 4 weeks and the offspring of these cows were either kept or sold.

The main transmission route was by far the general farm environment (Fig.4). For herds where calves and cows could have contact on pasture, the second most important transmission route in the first four years after *Map* introduction was the local environment, thereafter, in utero transmission became the second most important transmission route (Fig.4A). The local environment did not contribute to transmission in herds where calves and cows were separated on pasture (Fig.4B). For all herds, colostrum and milk were minor transmission routes. The relative contribution of transmission routes over time in herds with control strategies showed similar patterns (results not shown).

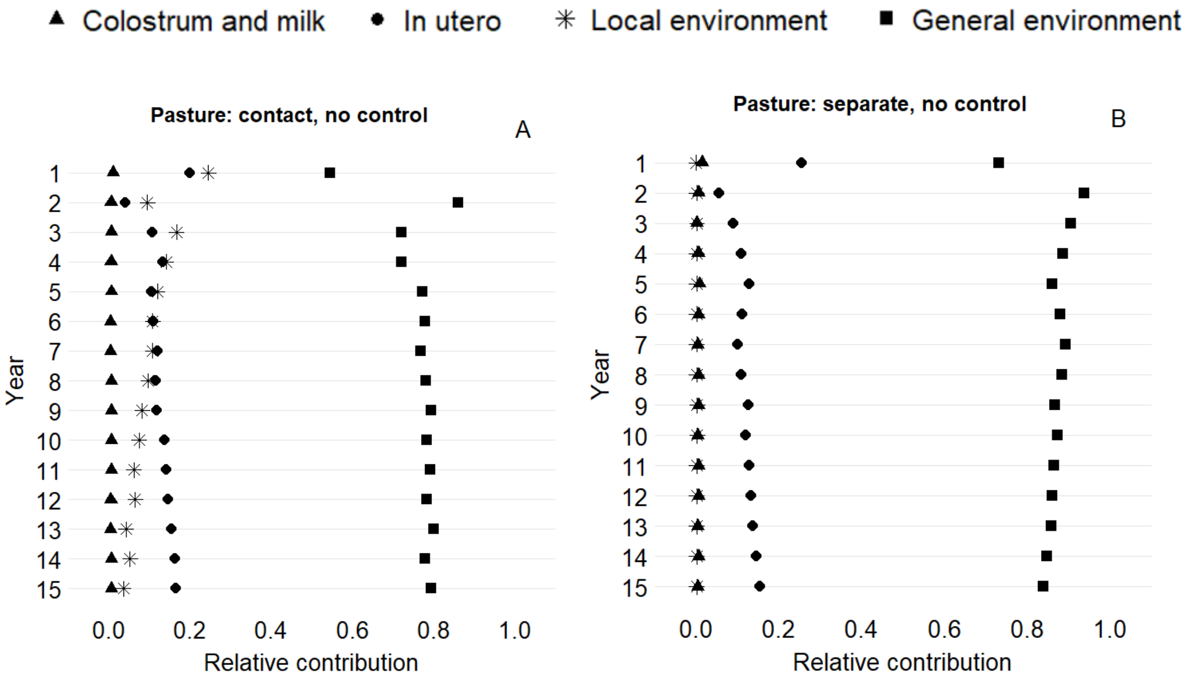

Fig. 4 Relative contribution of *Mycobacterium avium* subspecies *paratuberculosis* (*Map*) transmission routes over time and in the absence of control strategies (contributions per year sum to one). Calves and cow could have contact on pasture (A) or were separated on pasture (B).

Figure 5 shows the incidence over the course of an average year with transmission routes specified. Highest incidences were observed between week numbers 4 and 14, when calves were infected in utero or via the general environment. A drop in incidence was observed between week numbers 8 and 9, noting at this point that cows go to pasture and do not subsequently contribute to the general environment. For herds where calves and cows could have contact on pasture, calves could become infected via the local pasture environment during the pasture period (Fig.5A). At the end of the pasture period (week number 45), there was a slight increase in incidence because of transmission via the general environment. The incidence over the course of an average year in herds with control strategies showed similar patterns (results not shown).

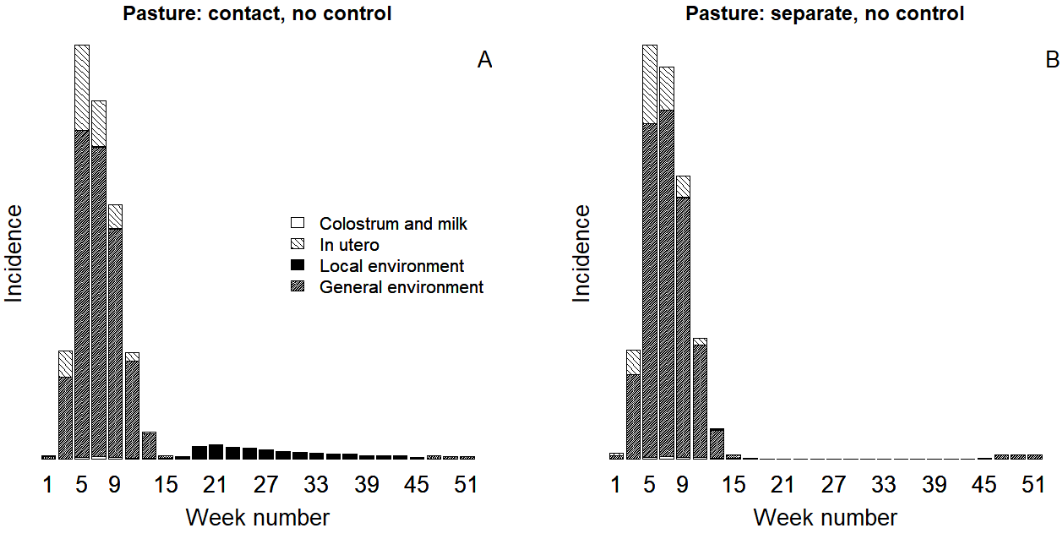

Fig. 5 *Mycobacterium avium* subspecies *paratuberculosis* (*Map*) incidence per two weeks of an average year with contribution of transmission routes specified for herds in the absence of control strategies. Calves and cow could have contact on pasture (A) or were separated on pasture (B).

When looking at the age at infection (Fig.6), most of the infected animals were newborns infected in utero or via the general environment. As calves aged, the probability to be infected decreased rapidly. When animals were weaned and came in contact with cows on pasture at 14 weeks of age, there was a slight increase in the incidence (Fig.6A), these calves were infected via the local pasture environment. The age of infection in herds with control strategies showed similar patterns (results not shown).

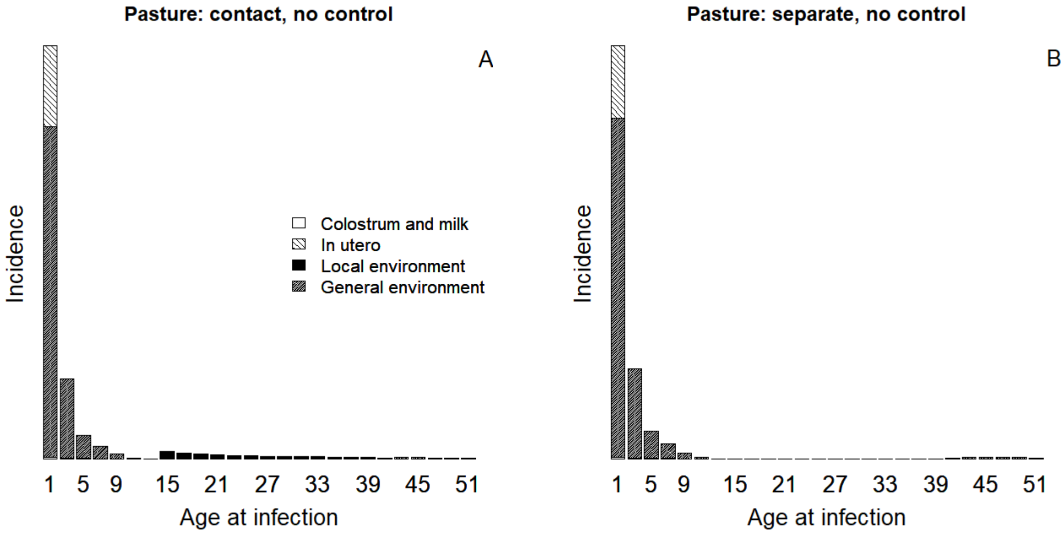

Fig. 6 Incidence per age in two weeks with contribution of transmission routes specified for herds in the absence of control strategies. Calves and cow could have contact on pasture (A) or were separated on pasture (B).

DISCUSSION

To better understand *Map* transmission in Irish dairy herds, we adapted a stochastic individual-based epidemiological model (Marcé et al., 2011b; Camanes et al., 2018) so that it accounted for seasonal herd demographics in Ireland. We used this model to investigate the probability of extinction following *Map* introduction, the relative importance of different transmission pathways, and the effectiveness of control strategies.

In herds with no control strategy, *Map* extinction occurred within 15 years in 57.3% of the herds where calves and cow could have contact on pasture, and in 70.6% of the herds where calves and cows were separated on pasture. These numbers are similar to the 66% probability of extinction in France dairy herds with all-year-round calving (Marcé et al., 2011b). However, the probability of early extinction (within two years) was higher in France, at 43% (Marcé et al., 2011b), than the 20% and 30% observed in this study. A possible explanation for this difference might be the way in which cows exit a herd. In Ireland, as presented in the model, cows are usually culled once a year, after dry-off at the end of the pasture period (week number 47). At this time, 22% of the cows are culled to be replaced by in-calf heifers in the beginning of following year. Culling rates of cows during the rest of the year are low, which means that the first infectious cow that was introduced into the herd has a high probability to remain in the herd until culling. Even if the cow is removed from the herd at this time, it already contaminated the environment for some weeks. In France, cows are culled all-year-round and have therefore a higher chance to be removed from the herd over the course of a year and before heavily contaminating the environment, leading to a higher probability of early extinction compared to Ireland. Thus, in herds with seasonal demographics the probability of early extinction is lower but over a longer period of time, seasonal demographics do not give rise to a different probability of extinction compared to herds without seasonal demographics.

For herds in which infection persisted for at least 15 years, mean prevalence of infected animals (I_T, I_L, I_M, and I_H) increased over time, up to over 60% in 15 years, while the mean prevalence of highly infectious cows (I_H) remained relatively low (4.4% maximum). So, regardless of the mean prevalence of infected cows, only one to three cows will be highly infectious and possibly affected. Therefore, the number of highly infectious cows in a herd is not a good indicator of the actual number of infected cows. The prevalence within a herd could be much higher than a farmer is expecting based on the number of clinical cases, the so-called "Iceberg effect" (Magombedze et al., 2013).

A contaminated general farm environment and in utero infection were the most important transmission routes, while colostrum and milk were minor transmission routes. These results are in contrast with farmer's perceptions that transmission via milk, not the environment, is the most important transmission route (McAloon et al., 2017). However, in this model, transmission via this route is only possible via ingestion of colostrum or milk that is contaminated directly or via faecal contamination of the liquid (Marcé et al., 2011b). For example, infection via faecally contaminated buckets used to feed calves is considered to be transmission via the general farm environment (Marcé et al., 2011b). Also, incidences dropped between week numbers 8 and 9, when cows go to pasture and stopped contributing to the general environment. Subsequently, when calves were sent to pasture and came into contact with cows, transmission via the local (pasture) environment was observed. This highlights the importance, irrespective of the transmission route, of avoiding exposure of calves to an environment contaminated with faeces from adult cows (Marcé et al., 2011b).

The most effective control measures were found to be calf management to ensure separation from cows on pasture and culling all highly positive animals within 4 weeks after testing. Similar results were observed with all-year-round calving herds in France where a combination of both of these control strategies was required for the prevalence not to increase (Camanes et al., 2018). The removal of offspring of I_H dams that tested positive had no effect in Irish herds on the probability of persistence and on the mean prevalence compared to culling I_H dams only. Also in France, removing offspring had no effect when the within herd prevalence was higher than 7% (Camanes et al., 2018).

In conclusion, the general farm environment is a major transmission route for JD in Ireland. It emphasizes the importance of limiting calf exposure to contaminated cow environments, especially in the first weeks of life when calves are the most susceptible. Furthermore, testing and culling of highly infectious animals is an effective control measure to reduce persistence and, in herds in which infection persists, to slow the rate with which prevalence increases over time.

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AVIAN INFLUENZA

BIOSECURITY RISK FACTORS FOR HIGHLY PATHOGENIC AVIAN INFLUENZA (H5N8) VIRUS INFECTION IN DUCK FARMS, FRANCE

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J.L. GUÉRIN AND M.C. PAUL

SUMMARY

Highly Pathogenic Avian Influenza (HPAI) subtype H5N8 outbreaks occurred in poultry farms in France in 2016-17. A retrospective matched case-control study was undertaken to investigate the inter-relationships between on-farm biosecurity practices and H5N8 infection status. Data were collected on 133 case and 133 control duck farms (i.e. the most affected species) located in one area of the country that was mostly affected by the disease and were analysed using Additive Bayesian Networks. Factors indirectly and directly positively associated with farm infection were inadequate management of vehicle movements (odds ratio [OR] 9.3, 95% credible interval [CI] 4.0-22.8) and inadequate delimitation of farm and units (OR 3.0, 95% CI 1.6-5.8), respectively. The findings highlight that reinforcing farm access control systems and reducing the number of visitors are key biosecurity measures to control farm vulnerability to H5N8 infection and could help setting priorities in biosecurity practices to prevent outbreaks re-occurrence.

INTRODUCTION

Highly Pathogenic Avian Influenza (HPAI) viruses continue to pose a serious threat to the poultry industry and public health by consistently causing emergent outbreaks. In 2014, a novel reassortant virus, HPAI H5N8, emerged and caused large outbreaks in East Asia (Kim et al., 2014). The virus spread intercontinentally and severely hit Europe during winter 2016-17 (Napp et al., 2018). The number of reported outbreaks in poultry, the diversity of affected wild birds and the geographic dissemination of the virus far exceeded those combined from all past HPAI outbreaks in Europe.

With a total of 484 poultry outbreaks within a 4-month period, France was the most severely affected European country (Guinat et al., 2018). Outbreaks were mainly reported in duck flocks (81.6%) and located in south-west France. H5N8 incursion into the country was attributed to long-distance flights of infected migratory birds from northern Eurasia (Sims et al., 2017; Alarcon et al., 2018). Most of the geographic spread was linked to local farm-to-farm transmission events followed by long-range jumps in the disease dispersal towards the end, which was likely explained by the reinforcement of control measures (Guinat et al., 2018). The

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epidemic was finally contained by end of March 2017 in the country, through the application of severe control measures, including pre-emptive culling and stringent movement restrictions that ultimately led to the eradication of H5N8 (DGAl, 2017a, 2017b).

Following the emergence of H5N8 in the country, on-farm biosecurity measures have received considerable attention, with the implementation of national biosecurity audits in poultry farms conducted by the veterinary authorities (DGAl, 2016). Previous research using standard statistical approaches has shown that compliance with certain biosecurity measures could influence the risk of farms becoming infected with HPAI viruses, including carcass disposal management (McQuiston et al., 2005; Garber et al., 2016), hygiene measures before entering premises (Nishiguchi et al., 2007; Metras et al., 2013), shared equipment or visitors between farms (McQuiston et al., 2005; Henning et al., 2009; Fasina et al., 2011). However, traditional statistical approaches used in these studies do not provide a complete and holistic picture of how risk factors are related to each other, as opposed to Additive Bayesian Networks (ABN). ABN have the potential to discriminate direct and indirect associations between variables and have been fruitful for unravelling far more about key features of complex disease systems, including HIV (Poon et al., 2007), avian influenza (Lycett et al., 2009) and antimicrobial resistance research (Ludwig et al., 2013; Hidano et al., 2015; Hartnack et al., 2019), with the view of designing better disease control and prevention programmes.

Given the important socio-economic impact of H5N8 outbreaks to the poultry sector, the objective of this study was to further investigate the inter-relationships between on-farm biosecurity factors and H5N8 infection status using ABN, to provide new insights regarding promising targets for intervention.

MATERIALS AND METHODS

Study design

The study was conducted on duck farms from one French department (i.e. Landes). Among all duck farms that were assessed for their biosecurity level in this department (n=346), case farms were defined as duck farms which had experienced a confirmed H5N8 outbreak between 2016 and 2017 (n=133). Data on the H5N8 infected poultry holdings that were reported in France during winter 2016-17 were obtained from the Direction Générale de l'Alimentation (DGAl) of the French Ministry of Agriculture, Paris, France. For each case farm, one control farm was randomly selected among duck farms, (i) with the same type of production: rearing (1-day old ducklings reared for around 3 weeks), breeding (1-day to 3-week old ducks bred for around 9-12 weeks), force-feeding (12-week old ducks force-fed for around 12 days) and mixed, (ii) located within 10km of an outbreak and (iii) which had not reported a H5N8 outbreak between 2016 and 2017.

Explanatory variables

Data on biosecurity practices were derived from the national database recording results from the official biosecurity audits conducted by the French veterinary authorities following the H5N8 epidemic in France. Out of the 28 variables initially present in the database, a selection of the most relevant variables was made based on removing variables with too numerous missing data and that were redundant. The final list of nine variables considered in this study is given in Table 1. Association between pairs of variables was investigated using Goodman and Kruskal's lambda coefficients and considered statistically significant if their value was close to 1 (Goodman & Kruskal, 1979).

Additive Bayesian Network (ABN) analysis

Identifying a globally optimal model: The globally optimal graphical model was identified using the order based exact search approach (Koivisto & Sood, 2004). This model is a directed acyclic graph (DAG) which is a graphical representation of the structure of the associations between all variables of the model. First, an exact search was run using a parent limit constraint of 1 (a maximum of one arc pointing to each node). This process was then repeated until increasing the parent limit did not result in a model with an improved goodness of fit metric (log marginal likelihood). A uniform prior distribution was used for the network structure meaning that all eligible model structures were assumed equally plausible regardless of their complexity. Uninformative prior parameters were used at each node to fit the models.

Adjustment for overfitting: The globally optimal model was then adjusted for overfitting using a parametric bootstrapping approach (Friedman et al., 1999). First, the globally optimal model was used to generate 5,000 bootstrap datasets of equal size to the original data. A globally optimal model was then identified for each bootstrap exactly as described before, resulting in 5,000 different bootstrap models. Finally, the final pruned model was obtained by removing any links in the model from the original data that were not supported by the majority (>50%) of the bootstrap models (Poon et al., 2007).

Estimation of parameters: The odd ratios (OR) for each explanatory variable were estimated along with their posterior 95% credible intervals (CI) from the marginal posterior distribution expressed by the final pruned model.

The analysis was implemented in R using the *abn* package (Kratzer et al., 2017). Bootstrap datasets were simulated with Markov chain Monte Carlo (MCMC) implemented in JAGS (http://mcmc-jags.sourceforge.net/).

RESULTS

Variables description

The majority of farms exclusively raised ducks (89.8%) and included an outdoor area for grazing (78.2%) (Table 1). Inadequate use of anteroom was reported in almost half of farms (46.6%). Inadequate protection of feed and facilities, inadequate delimitation of farm and units and inadequate cleaning of facilities and surroundings were reported in about one-quarter of farms (28.2%, 25.2% and 19.5%, respectively). Lower proportions of farms showed unsatisfactory scores related to pest control programmes (15.0%), management of vehicle movements (10.9%) and management of dead birds (6.0%). No strong pair-wise associations amongst the nine variables (lambda values close to zero) were identified, resulting in the inclusion of the nine variables in the analysis.

Table 1. Description of variables investigated for associations with highly pathogenic avian influenza H5N8 infection of duck farms, France 2016-17

Variable group	Description	Farms with risk factor:		
Presence of chickens	Presence of production unit with chickens	% of total (n=266)	# cases (n=133)	# controls (n=133)
Presence of outdoor area	Presence of outdoor area for grazing	10.2	17	10
Inadequate delimitation of farm and units	Inadequate physical separation of professional and public zones	78.2	106	102
Inadequate management of vehicle movements	Traffic map not adequate with separation of professional and public zones (incoming and outgoing movements can intersect, either in time or space, possible entry of the rendering truck onto the farm area, etc.)	25.2	44	23
Inadequate use of anteroom	- Absence of anteroom (no physical separation between clean and dirty areas), - Anteroom not restrictive (access to unit not compulsory through anteroom), - Anteroom not operational (no access to soap and water)	10.9	17	12
Inadequate cleaning of facilities and surroundings	- No record of cleaning and disinfection events, - No access to air ventilation, watering, feeding and manure disposal systems, - Presence of waste around the building	46.6	73	51
Inadequate protection of feed and facilities	- Absence of means to protect access to food storage by wild birds, - Absence of means to protect access to buildings by wild birds	19.5	34	18
Inadequate pest control programme	- Absence of baits, - No record of pest control events	28.2	35	40
Inadequate management of dead birds	- Absence of rendering bin on the production site, - Not registered to a rendering company	15.0	22	18
		6.0	2	14

ABN outcome

After adjustment for overfitting, the final globally optimal model included 9 links with a log marginal likelihood of -1306 (Fig.1). Two variables were directly associated with the infection status. “Inadequate delimitation of farm and units” was positively associated with “Infected by H5N8” (OR=3.0) while “Inadequate management of dead birds” was negatively associated with this outcome (OR=0.1). A positive association was observed between “Inadequate delimitation of farm and units” and “Inadequate cleaning of facilities and surroundings” (OR=6.6). “Inadequate management of vehicles and people movements” also showed a positive association to “Inadequate delimitation of farm and units” (OR=9.3), which in turn, was positively associated to “Inadequate use of anteroom” (OR=6.5). “Presence of outdoor area” was positively associated with “Inadequate use of anteroom” (OR=3.8), which in turn was linked to “Inadequate protection of feed and facilities” (OR=3.5) and “Inadequate pest control programme” (OR=5.1). “Presence of chickens” was not linked to any of the variables in the model.

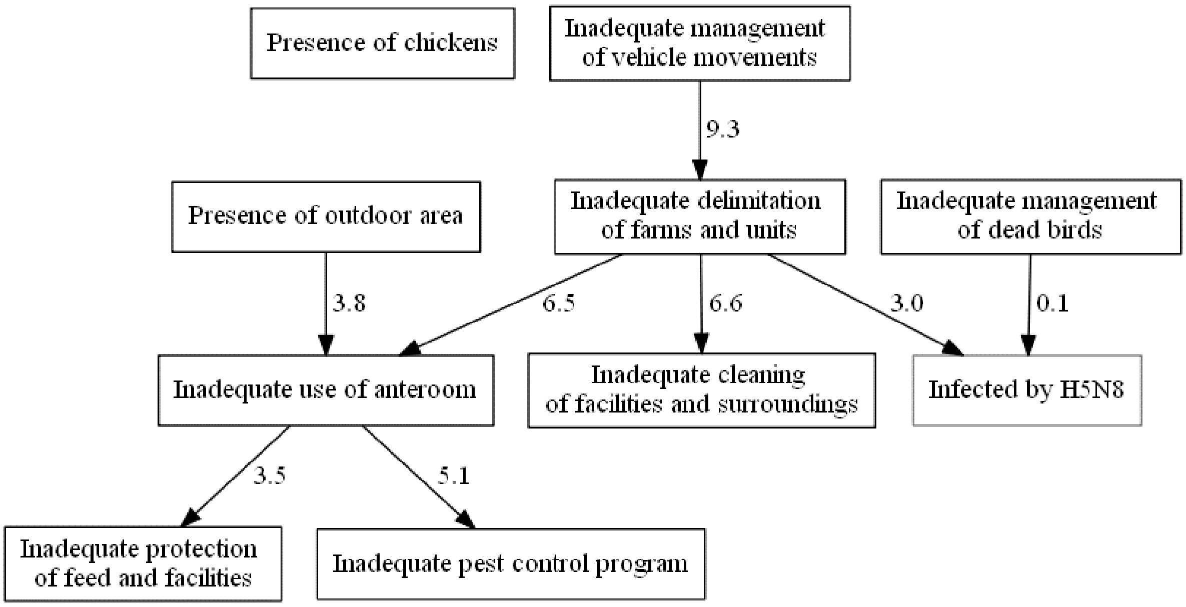

Fig. 1 Final globally optimal model of biosecurity variables associated with highly pathogenic avian influenza H5N8 infection of duck farms, France 2016-17. Numbers represent marginal posterior odd ratios (median)

DISCUSSION

This study provides insight into the underlying relationships amongst biosecurity measures in the face of the 2016-17 H5N8 epidemic in France. Two variables were found directly associated with the farm infection status. Premises for which farm and units delimitation was unsatisfactory were more likely to have been infected by H5N8 than the others. This could reflect vehicles and people entering the farm (via transport of birds, feed delivery, disposal of dead birds, movement of crews, use of shared employees or equipment, etc.) by a location that does not minimise the risk of virus introduction and may inadvertently transmit the virus into the farm by coming close to the production units, especially when coming from a newly infected farm where clinical signs are not yet fully displayed (McQuiston et al., 2005; Fasina et al., 2011). Thus, minimising the number of visitors or restricting access of vehicles to the perimeter of the farm could be advised. For example, ensuring that the rendering container is located at the farm perimeter would reduce the risk of virus introduction by eliminating the need to come into close proximity to the poultry barns.

H5N8 infection was also more likely to have occurred in farms where the management of dead birds was satisfactory. This negative association could be interpreted as farmers having experienced H5N8 outbreaks, were more likely to dispose of dead birds via an optimal rendering system (adequate size, location and sealing of the rendering container, as well as its cleaning procedures), since this was required by the authorities following the course of an outbreak.

Four variables representing the spatial and structural organisation of the farm were all strongly associated with each other. Therefore, if vehicle and people movements were not adequately managed, then it was more likely that the farm and unit delimitation was unsatisfactory, which in turn might not help the use of anteroom and the cleaning of facilities and surroundings. The results also showed that the presence of outdoor areas was positively associated with the inadequate use of the anteroom. This is consistent with what has been reported from the field, with farmers lacking the motivation and time to implement an anteroom or failing to address the importance of the anteroom when accessing the outdoor area (Delpont et al., 2018). Interestingly, the inadequate use of the anteroom was not found to be associated with H5N8 infection, even if the presence of functional anterooms on poultry farms became mandatory as biosecurity requirements by the French veterinary authorities following the H5N8 outbreaks in winter 2016-2017 (DGAl, 2016). This is probably explained by the fact that anterooms did not provide sufficient protection against virus introduction when the farm surroundings were already contaminated thus, highlighting that the prevention of farm contamination proved crucial.

In line with previous results (Guinat et al., 2019), the presence of production units with chickens on the farm was not linked to the farm infection status, which may be explained by the hypothesis that H5N8 would be less adapted to chickens than ducks (Bertran et al., 2016). However, no detailed information was available on the type of chicken production, preventing further hypotheses to be tested. Two variables “Presence of outdoor area” and “Inadequate protection of feed and facilities” were used as proxy of potential contact with wild birds. Wild birds are frequently observed on outdoor areas around drinkers and feeders but also close to silo stockers (Delpont et al., 2018; Scott et al., 2018), providing opportunities for interspecies transmission.

Among the limitations of this study is the fact that the biosecurity scores were collected on average 6 months after the detection of H5N8 on farms. Thus, one could argue that biosecurity practices could have changed in the meantime. However, it is very unlikely considering the important financial input and organisational efforts they generally require. Since having only one interviewer to perform all biosecurity audits was impractical, potential variations in biosecurity scores among interviewers were reduced by training interviewers to administer the questionnaire in a similar standardised way. A larger sample size could have also increased the ability of this study to detect associations between biosecurity variables with a low proportion of unsatisfactory scores and disease occurrence.

Recently used in the field of animal health epidemiology (Lewis et al., 2011; Firestone et al., 2014; Comin et al., 2019), ABN have the advantage over traditional statistical approaches of being able to discriminate direct and indirect associations. The approach provides visual and intuitive outputs through statistical inference, such as the graphical visualisation of complex observational study data. However, practical challenges still exist in ABN approaches. While adjustment for overfitting is conceptually straightforward, the procedure is highly computationally demanding. The exact search algorithm used in this study is only feasible for small datasets (less than 25 variables with strong modelling assumptions) (Koivisto & Sood, 2004). Alternative heuristic algorithms exist to handle up to hundreds of variables but they remain computationally demanding and lead to approximate inferences (Lewis & McCormick, 2012; Lewis & Ward, 2013). The search can however be guided by incorporating causal knowledge to limit the amount of computations (de Campos & Castellano, 2007).

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OPTIMISING THE EARLY DETECTION OF AVIAN INFLUENZA H7N9 VIRUSES IN LIVE BIRD MARKETS

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SUMMARY

Identified as high-risk environments for avian influenza H7N9 transmission, live bird markets (LBMs) are key sites to target surveillance for early detection, implement rapid response and prevent human infections. Here, a within-LBM H7N9 meta-population model with demography was fitted to different Vietnamese LBMs at risk of H7N9 introduction. The model was used to determine optimal sampling strategies and to assess the cost-effectiveness of portable PCR devices for real-time virus detection in LBMs. Results show that utilising portable PCR devices was more expensive but reduced substantially the number of infected birds leaving the LBM before the first detection, as compared to a lab-based surveillance strategy in which samples are sent to official diagnostic laboratories to be processed. Findings also suggest that banning birds staying overnight would represent an effective intervention to reduce the risk of H7N9 spread but would increase the risk of not detecting the virus before it disappears.

INTRODUCTION

New influenza A virus strains continue to pose serious clinical and economic challenges to global public and animal health. Recently, a novel avian-origin H7N9 virus has emerged in Eastern China, causing severe respiratory disease and fatalities in humans (Gao et al., 2013; Kageyama et al., 2013). Since the first report in early 2013, China experienced five epidemic waves, during which the number of reported cases has increased significantly, reaching over 1600 human infections. During the fifth wave in 2017, a highly pathogenic (HP) H7N9 avian influenza virus (AIV) variant emerged (Qi et al., 2018).

In Asia, live-bird markets (LBMs) are known as high-risk environments for the transmission, evolution and maintenance of AIV (Indriani et al., 2010; Zhou et al., 2016). In China, most human H7N9 cases have been associated with previous exposure to poultry at live bird markets (LBMs) (Chen et al., 2013). In LBMs, H7N9 virus has been extensively detected in chickens (Wu et al., 2015), which are considered as the primary source of H7N9 infection in humans (Husain, 2014). Frequent interactions among different poultry species and humans

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at LBMs provide an ideal interface for transmission of viruses and emergence of new variants by mixing viruses from different sources (Bao et al., 2013). Long distance transportation of asymptomatically infected poultry to gathering sites, such as LBMs, also enables rapid dissemination of AIVs beyond the local scale.

In response to the H7N9 outbreaks, major efforts were undertaken to temporarily close and sanitise LBMs during epidemics. These interventions aim at temporarily decreasing H7N9 environmental contamination and at reducing the risk of H7N9 infection in humans (Kang et al., 2015). Systematic surveillance at the LBMs also remains essential for detecting novel H7N9 viruses and reducing the risk of human infections (Yu et al., 2014). Optimising surveillance strategies in LBMs has mainly been addressed by identifying the most effective sample types for AIV detection (Indriani et al., 2010; Vergne et al., 2019) and by designing more sensitive diagnostic tools (Luan et al., 2016). However, lack of knowledge remain on how the sampling design (i.e. sampling time and sample size) could be optimised to maximise the detectability of AIV, given the dynamic nature of LBMs and the rapid turn-over of their poultry populations.

Given the sharp increase in the number of H7N9 outbreaks in China and regular cross-border trade of birds originating from China, the rapid detection of emerging AIVs is crucial for food safety, food security, farmers' well-being and public health in Vietnam. Current surveillance programme for H7N9 in this country involves the biweekly random sampling of chickens in LBMs considered at high risk of virus introduction. According to the protocols approved by the Vietnamese Department of Animal Health (DAH), all samples are transported to an official diagnostic laboratory where they are consecutively screened for M, H7 and N9 genes using RT-PCR. As an alternative to this traditional surveillance strategy, a portable PCR device has been developed recently to improve H7N9 preparedness and response capacities in Vietnam (Inui, In Press). This device can be directly deployed in LBMs and allows virus detection within a few hours after sampling, while the traditional lab-based surveillance protocol takes on average 72 hours due to logistic reasons.

In the light of the development of this innovative diagnostic tool, the general objective of this study was to identify the optimal surveillance strategy required to improve the timeliness and effectiveness of the detection of low pathogenic (LP) H7N9 viruses at LBMs, while considering the surveillance costs and the key dynamic features of LBMs. To do this, a within-LBM LP H7N9 transmission model, accounting for chicken and duck population demography as well as the environment, was developed and calibrated to fit different LBMs in Vietnam at high-risk of H7N9 introduction and used to compare the cost-effectiveness of different surveillance strategies.

MATERIALS AND METHODS

Within-LBM H7N9 transmission model with demography

A baseline within-LBM demography model was first developed to simulate the population dynamics of birds in LBMs. It was assumed that chickens and ducks are introduced into the LBM at an entry rate *e(t)* that depends on the time of the day, and moved out for slaughter and trade at a time-varying exit rate $l_1(t)$, *and* $l_2(t)$, respectively. The moving out for trade represents the high-risk route for secondary spread outside the LBM. The model formulation also allows birds to stay overnight in the LBM and therefore potentially contribute to the maintenance and amplification of the virus. The model considers separately chickens and ducks to allow for different population structures in the LBM but assumes that the dynamic of entry and exit of these two bird species are similar.

Once the virus is introduced into a LBM, chickens and ducks can pass through four infection stages: susceptible (S), infected but not infectious (E), infectious (I) and recovered (R). All birds that are introduced into the LBM are assumed to enter as susceptible. Viral persistence of H7N9 in water has been recently reported (Vidaña et al., 2018), suggesting that the environment may play an important role for H7N9 virus transmission. Therefore, it was assumed that infectious ducks and chickens can contaminate the environment (F) by excreting infectious doses at an excretion rate ξ_d and ξ_c, respectively. The amount of infectious doses in the environment are assumed to decrease at an inactivation rate γ. The force of infection, determining the rate at which susceptible birds move from S to E, was defined as the sum of the force of infection due to contacts with infectious birds and of the force of infection due to environmental contamination. The force of infection due to environmental contamination (λ_{env}) was adapted from (Codeço, 2001; Paroissin et al., 2005) using the Hill-type function (Eq.(1)):

$$\lambda_{env} = w * F\theta + F \tag{1}$$

with w being the contact rate with one infective dose in the environment, θ being the number of infective doses that are necessary to infect a bird and F being the number of infective doses present in the environment. With this functional form, the force of infection exerted by the environment increases sublinearly with the level of environmental contamination and addresses more realistically the situation in which not all birds become necessarily infected despite heavily contaminated LBMs. Upon infection, birds enter the infected compartment (E) in which they stay during a period averaging the latent period duration (l_d and l_c for ducks and chickens, respectively) after which they enter the infectious compartment (I). Infectious birds stay in the I compartment during a period averaging the infectious period duration (i_d and i_c) after which they enter the recovered compartment (R) where they remain until the end of the study period. Note that all birds (whatever the compartment S, E, I or R) can leave the LBM at a time-dependent exit rate $l_1(t)$, and $l_2(t)$.

Model calibration

Most of the parameters related to LP H7N9 virus transmission were derived from the published literature. Values and references are summarised in Table 1. Traders reported that birds could remain for a maximum of 5 days within the LBM, which is much smaller than the natural life expectancy of the birds. Consequently, the natural mortality rate (attributable to other causes than H7N9 infection) for both species (m_c and m_d) was assumed to be negligible. Based on the H7N9 bird-to-bird infection rate estimated at 0.4 per day (Hsieh et al., 2014), the infection rate for chickens (β_c) was assumed to be 0.02 per hour. The infection rate for ducks (β_d) was considered smaller than that for chickens since ducks were reported to be less susceptible to H7N9 infection than chickens (Pantin-Jackwood et al., 2014). Therefore it was assumed an infection rate for ducks $\beta_d = \beta_c * K$ with $K<1$. To the authors' knowledge, no information was available on the duration of the latent period following H7N9 infection. Therefore, the latent period in chickens (α_c) and ducks (α_d) was assumed to be 0.62 days as estimated for H5N1 (Bouma et al., 2009), i.e. 14.9 hours. There is limited evidence that infected birds would die following infection by LP H7N9 virus, hence, the mortality rate due to H7N9 infection was assumed to be negligible for both species (v_c and v_d) (Spackman et al., 2015; Vidaña et al., 2018). In general, chickens and ducks infected by LP H7N9 virus have mild symptoms, but they can remain infectious for around 8 and 5 days, respectively (Pantin-Jackwood et al., 2014). Consequently, the duration of the infectious period for chickens (i_c) and ducks (i_d) was assumed to be 192 and 120 hours, respectively. The inactivation rate of 12 different influenza virus strains (other than H7N9) was reported at around 0.5 per day (i.e. 0.02 per hour) at 25°C, a pH of 7.2 and salinity of 0 (Brown et al., 2007; Handel et al., 2013). Hence, the inactivation rate for H7N9 (γ) was assumed to be 0.02 per hour. The number of infective doses excreted by ducks per hour (ξ_d) was considered lower than that for chickens (ξ_c) by a coefficient Q<1 since ducks were reported excreting less H7N9 virus than chickens (Pantin-Jackwood et al., 2014). To the authors' knowledge, no information is available to estimate the contact rate for birds with one infective dose (w). However, knowing that the H7N9 bird-to-bird R_0 in LBMs has been estimated around 4.1 (Hsieh et al., 2014) w was assumed to be 0.01. A sensitivity analysis related to Q, ξ_c, ξ_c, and w parameter assumptions was undertaken.

Table 1. Parameter values related to H7N9 virus transmission

Parameter	Description	Value (unit)
dt	step-time	hour
m_c	Natural mortality rate for chickens attributable to other causes than H7N9 infection (per hour)	0.001
m_d	Natural mortality rate for ducks attributable to other causes than H7N9 infection (per hour)	0.001
β_c	Infection rate for chickens (per hour)	0.02
β_d	Infection rate for ducks (per hour)	$\beta_c * K$
K	Ratio of infection rate for ducks vs. chickens	0.8
α_c	Latent period for chickens (hours)	14.9
α_d	Latent period for ducks (hours)	14.9
v_c	Mortality rate for chickens due to infection (per hour)	0
v_d	Mortality rate for ducks due to infection (per hour)	0
i_c	Infectious period for chickens (hours)	192
i_d	Infectious period for ducks (hours)	120
γ	Inactivation rate (per hour)	0.02
ξ_c	Number of infective doses excreted by chickens (per hour)	1
ξ_d	Number of infective doses excreted by ducks (per hour)	$\xi_c * Q$
Q	Ratio of excretion rate for ducks vs. chickens	0.8
w	Contact rate with one infective dose (per hour)	0.01
θ	Number of infective dose to infect chickens and ducks	1

The parameters related to the population dynamics of birds were derived from field observations conducted at four LBMs in northern Vietnam. A questionnaire survey was developed to collect information from LBM managers and traders on population dynamics of birds. The questionnaire included three components (LBM characteristics, management and biosecurity). Interviews were conducted in Vietnamese, by a native Vietnamese speaker proficient in English and were facilitated by representatives from District Veterinary Station (DVS) and sub-Department of Animal Health (SDAH). Direct observations were made during the visits (including the estimation of the number of poultry present early at the beginning of the day and the number of poultry left unsold at the end of the day) and used to cross-check interviewees’ answers.

The model formulation was embedded within a deterministic framework. It assumed homogeneous mixing, i.e. birds uniformly and randomly contact each other, be they ducks or chickens. The model was initialised by introducing one infectious chicken at the moment of the day with the highest entry rate in the LBM. The model was implemented in the R programming language (R Development Core Team, 2011).

Cost-effectiveness analysis of alternative surveillance strategies

The following surveillance strategies were incorporated in the model to assess their cost-effectiveness:

1. the *traditional lab-based surveillance strategy* (strategy 1): this baseline strategy, corresponding to the current surveillance programme for H7N9 in LBMs that are considered at high risk of H7N9 introduction in Vietnam, involves the random sampling of 40 oropharyngeal swabs on chickens only. Chickens are usually sampled twice a week between 8 and 10 am. All samples are then transported to an official diagnostic laboratory where they are all screened for the M gene using RT-PCR; those positive for the M gene are subsequently tested for the H7 gene. H7 viruses have not yet been detected in Vietnam so the test for N9 gene was not included in the calculation of surveillance costs. On average, the delay between sampling and diagnostic test result communication is around 72 hours.

2. the *current portable PCR surveillance strategy* (strategy 2): this first alternative strategy is similar to strategy 1 except that samples are directly analysed on site for the H7 gene using the portable PCR device (Inui, In Press; Schar et al., 2019), assuming an average delay of 7 hours between sampling and diagnostic test result communication.

3. the *optimised portable PCR surveillance strategy* (strategy 3): this second alternative strategy is similar to strategy 2 except that samples are collected at the time of the day that maximises the probability of sampling at least one infectious bird, i.e. at the time of the highest within-LBM prevalence of infection, as predicted by the model.

These three different surveillance strategies were combined with three different sampling frequencies: once a week, twice a week and every day. The surveillance strategies were also assessed assuming that the LBM policy could change and forbid the presence of birds staying overnight, as this intervention strategy has been shown to be an effective control option for avian influenza viruses, such as H9N2 and H5N1 (Fournié et al., 2010).

For a given combination of surveillance strategy, sampling frequency and overnight staying policy, the effectiveness was assessed by estimating the median detection delay from introduction and the average expected number of infected or infectious birds sold to farms or other LBMs at the median time of detection. The weekly costs of H7N9 surveillance were also estimated for each strategy, with cost parameters provided by the Sub-Department of Animal Health (SDAH).

RESULTS

Within-LBM H7N9 infection dynamic

Assuming an overnight stay of birds, the daily dynamic of infection was expected to reach a regular pattern around three to four days after virus introduction. At night, when the overall population within the LBM is closed (no entry, no exit), the virus spreads between the overnight-staying birds, resulting in the decrease and increase of the number of susceptible and infected or infectious birds, respectively. Given the model parameters, the virus was found to be able to persist in the environment long term, likely due to the presence of birds staying overnight in the LBM allowing virus amplification. The level of environmental contamination was expected to reach a plateau after 5 to 10 days. When implementing a ban on the presence of birds staying overnight, the expected number of infected birds and the environmental contamination level remained very limited, resulting in the fade-out of the epidemic within the first few days.

Cost-effectiveness of surveillance strategies

Overall, as compared to the traditional lab-based surveillance strategy (strategy 1), using the current portable PCR surveillance strategy (strategy 2) decreased the average most likely detection delay by three days and the average expected number of infected birds that have been sold to farms or other LBMs at the time of virus detection by several hundred days (depending on the sampling frequency). As an illustration, assuming weekly sampling, strategy 1 would most likely detect the virus introduction with a median of a 10-day delay, at a moment when close to 3,330 infected or infectious birds would have been sold to farms or other LBMs. Alternatively, strategy 2 would most likely detect the virus introduction with a median of a seven-day delay, at a moment when around 1,702 infected or infectious birds would have been sold to farms or other LBMs.

Based on the model simulations, the time of sampling that maximises the probability of sampling at least one infectious bird in the studied LBM was estimated at 1 am. This corresponds to the moment when the within-LBM prevalence is maximised, i.e. just before new susceptible birds are introduced into the LBM at the beginning of a working day. As compared to the current portable PCR surveillance strategy (strategy 2), using the optimised portable PCR surveillance strategy (strategy 3) allows decreasing the most likely detection delay by one to two days and the expected number of infected birds that have been sold to farms or other LBMs at the time of virus detection by several hundreds (depending on the sampling frequency). Assuming weekly sampling, strategy 3 would most likely detect the virus introduction two days earlier than strategy 2, averting the movement of 536 infected or infectious birds to farms or other LBMs.

Figure 1 illustrates the cost-effectiveness of the different surveillance strategies. Note that the traditional lab-based surveillance strategy involving the sampling of chickens twice a week (blue triangle), corresponds to the strategy that is usually implemented for the surveillance of H7N9 in LBMs in Vietnam. Figure 1 suggests that the optimised portable PCR surveillance strategy, involving the sampling of chickens once a week (green circle) or twice a week (green triangle) would be the most cost-effective option as compared to all other possible combinations of surveillance strategies. Compared to sampling once a week (green circle), sampling twice a week (green triangle) is expected to improve substantially the effectiveness of the optimised portable PCR (averting the movement of 808 infected or infectious birds to farms or other LBMs before virus detection) at a weekly cost twice as high (from 765 to 1,635 USD). Consequently, moving from weekly sampling to biweekly sampling using the optimised portable PCR strategy would allow averting 0.9 infected or infectious birds being sold to other farms or LBM per dollar invested weekly in the surveillance. However, weekly surveillance costs were expected to almost triple when increasing the sampling frequency to every day (from 1,635 to 4,681 USD) without a substantial improvement in the effectiveness (from 358 to 117 birds).

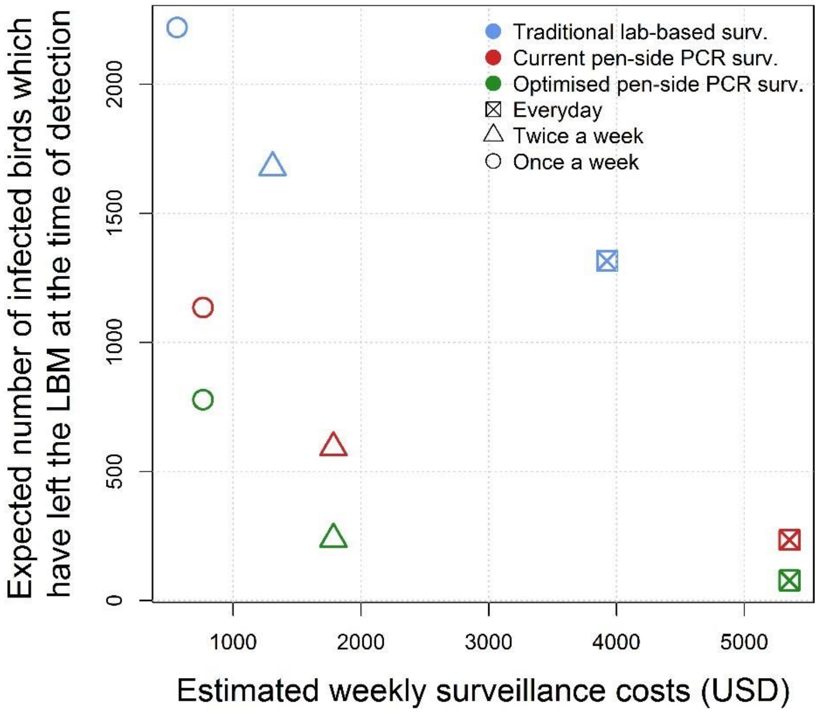

Fig. 1 Cost-effectiveness of surveillance strategies at Giếng Vuông market, Lang Son province, Vietnam

DISCUSSION

Model outputs suggest that H7N9 transmission could be sustained for weeks following the introduction of a single infectious bird through virus amplification and transmission within the LBM among birds staying overnight, which is consistent with results from a previous study on H5N1 (Fournié et al., 2010). The LBM system thus acted as a reservoir of infection for newly introduced susceptible birds. The model predicted that, using the traditional lab-based surveillance strategy for H7N9 in LBMs (strategy 1: sampling twice a week between 8 and 10 am, with an average delay of 72 hours between sampling and diagnostic test result communication), hundreds of infected birds would have already been sold to farms or other LBMs by the time the virus is expected to be detected in the LBM. The recent development of a portable PCR method to detect H7N9 virus at the LBM definitely allowed to reduce the time delay between sampling and diagnostic test result communication (with a minimum of 2 hours). Using this alternative surveillance strategy, model outputs showed that the median detection delay would be substantially shortened leading to a reduction in the average number of infected or infectious birds that would be sold to farms or other LBMs by the time the virus is detected in the LBM. This was shown to be true whatever the sampling frequency used as alternative to twice a week (once a week and every day). However, the study showed that adopting the portable PCR surveillance strategy would be costlier than using the traditional lab-based surveillance strategy, mostly related to higher diagnostic testing costs.

This study pointed out that the portable PCR surveillance strategy could be further optimised by sampling the birds staying at night in the LBM just before new susceptible birds are introduced at the beginning of an opening LBM day. However, this strategy would involve sampling and testing birds very early in the morning. This should be discussed with local communities and stakeholders as it may not be feasible in certain settings. Another limitation to consider is that scaling up surveillance systems involving a portable PCR device could be challenging due to constraints on the maximum number of samples that can be tested at once.

Birds infected with LP H7N9 generally show mild symptoms but they can excrete the virus for 5 to 8 days (Pantin-Jackwood et al., 2014). Traders reported keeping birds for a few days within the LBM until being sold, housing them in cages overnight. These birds staying for longer time at LBMs are more likely to get infected and play a role in the maintenance and amplification of H7N9 within the LBM. Expectedly, using a ban on keeping birds overnight at LBM in the model had a strong impact on the virus transmission dynamics and was actually expected to prevent virus amplification and maintenance within the LBM. Importantly, this intervention strategy is also likely to reduce the chance of virus detection. This is likely explained by the fact that birds that are unsold by the end of the day would be slaughtered and sold as carcasses, preventing the virus to be replicated within its host. More importantly, this could represent a less disruptive and more sustainable measure than LBM closure that could also be effective in reducing the risk of H7N9 spread beyond the LBM (Wu et al., 2014; Yu et al., 2014). Furthermore, LBM closure has been associated with substantial costs for the poultry industry and illegal trade in some LBMs or neighborhood, making disease monitoring and control more difficult (Peiris et al., 2016; Wu et al., 2016).

The Giếng Vuông LBM is one of the 17 LBMs of northern Vietnam that have been identified as high-risk of becoming H7N9 infected due to potential cross-border trade of birds from China. This is a unique LBM with its proper structure and functioning. Thus, its bird population dynamics differs from those of other LBMs, resulting in different optimised times of sampling. To address this limitation and allow stakeholders to apply this analytical framework to other LBM systems, a web application was developed using the shiny package (Chang et al., 2019) and made publicly available with a graphical user interface in html format at: https://envt-inra.shinyapps.io/optimia/. The online tool consists of an interactive interface whereby users input the key dynamic features of the LBMs of interest, including species and number of birds sold over time, and the details regarding the surveillance strategy protocol, including sampling time, sample size and associated surveillance costs. Outcomes of the application provides model simulation outputs adapted to the studied LBM, including graphical representations of the dynamics of the number of birds in each infection stage, the time of sampling for which the probability of virus detection is maximised, and summary tables of the cost-effectiveness for each surveillance strategy and sampling frequency.

Another limitation of the model was the lack of information regarding H7N9 virus survival in the environment. Environmental contamination by H7N9 in LBMs has been documented with positive samples retrieved from faeces, cages and floors, but also in de-feathering machines and chopping tools (Kang et al., 2015). However, little is known of the virus inactivation rate across time according to various environmental factors, such as temperature and humidity. While no information was available on the number of infective doses excreted by birds and on the contact rate with one infective dose, the sensitivity analysis showed that the assumptions related to these parameters did not impact the general outcomes of this study. Seasonality in population dynamics and infection patterns were also not explored while trade patterns might differ according to seasonal events, including Lunar New Year Festival (Magalhães et al., 2012; Delabouglise et al., 2017).

In conclusion, this study highlights key control strategies that should receive high priority in Vietnam and other Asian countries which are at risk of H7N9 introduction through bird trade across borders. Using the optimised portable PCR surveillance strategy would not only decrease the number of infected birds that have been sold to farms or other LBMs at the time of virus detection but also reduce the time delay for virus detection. A ban on keeping birds overnight was also shown to increase the likelihood of epidemic fade-out. These strategies applied within LBMs could help reducing zoonotic and pandemic risks posed by such emerging avian viruses. Further information would be needed on bird population dynamics and environmental H7N9 virus survival to refine optimal sampling time.

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EVALUATION OF VACCINATION STRATEGIES AGAINST AVIAN INFLUENZA IN FRENCH POULTRY PRODUCTION NETWORKS: APPLICATION OF THE EVACS® TOOL

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SUMMARY

Vaccination against highly pathogenic avian influenza (HPAI) is currently not authorised in France but has been discussed following recurring epizootic events. The objective of this work was to evaluate the potential benefit of vaccination strategies against avian influenza (AI) in France. EVACS®, a simulation tool previously developed to evaluate vaccination strategies, was applied in the major French poultry production sectors. Vaccination strategies tested at the national level combined the best vaccination scenarios identified at the individual sector level (i.e. hatchery vaccination in all hatcheries for broiler, layer and turkey sectors and farm vaccination in all integrated farms for fattening duck, meat duck and guinea fowl sectors) with the risk level of each production type. Vaccination strategies including the highest number of species and production types were the most efficient in terms of cost-benefits. Such study can support decision making on potential national vaccination strategies to control HPAI in France.

INTRODUCTION

France was hit with two epizootic waves of HPAI during the winter 2015-16 and the winter 2016-17. In both outbreaks, the viruses mainly circulated within the duck production network, the majority producing fatty liver (Le Bouquin et al., 2016; Bronner et al., 2017). The duck production processes were identified as the main reason for the spread of HPAI viruses in the south-eastern region of France (Guinat et al., 2019). To control the spread of the disease, surveillance was increased and birds in infected farms were systematically culled. During the second outbreak, given the rapid and extensive spread of the disease, preventive culling was also performed in areas around confirmed outbreaks. In 2016-17, about 6.8 million birds were culled (Guinat et al., 2018). Culling caused huge economic losses not only for farmers but also for the whole French poultry industry. Total French and European compensation reached 137 million euros in 2015-16 and 123 million euros in 2016-17 (partial estimation) (Lalaurette & Hercule, 2019). The psychological impact on the farmers was also very high (CIFOG, 2017). Vaccination against AI is currently not authorised in France, mainly due to the economic restriction on exports. During the second outbreak (2016-17), because of the very large number of birds culled, some farmers and the media raised the question of vaccination.

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Two main types of vaccines exist: inactivated whole AI virus vaccine and live recombinant vaccines (Peyre et al., 2009). Inactivated vaccines can be homologous (based on strains with the same haemagglutinin (HA) and neuraminidase (NA) as the circulating filed virus) or heterogeneous (based on strains with the same HA but different NA from the circulating field virus). Homologous are often more effective than heterogeneous vaccines but only heterogeneous vaccines can be used for differentiation of infected animals from vaccinated animals (Differentiating Infected from Vaccinated Animals (DIVA) strategy).

Recombinant vaccines are based on the insertion of an AI gene of interest into a carrier vector (non-pathogenic virus). Different types of recombinant vaccines exist: fowlpox recombinant vaccine (Swayne et al., 2000), reverse genetics recombinant vaccine (Subbarao & Katz, 2004), Newcastle disease recombinant vaccine (Veits et al., 2006) and Herpes virus of turkey’s recombinant vaccine (Kapczynski et al., 2015). Inactivated vaccine require several applications (boosters) to provide long-term protection while recombinant vaccines provide long term protection with a single application, mostly at the hatchery (Peyre et al., 2009). However, to date no study has compared the efficiency of vaccination strategies using these two different types of vaccines in French poultry production networks.

EVACS® is one of the few existing decision support tools that has been developed to compare vaccination strategies (Peyre et al., 2016). The objective of this study was thus to apply EVACS® to identify the most effective and efficient vaccination strategy, using inactivated farm vaccines and/or recombinant hatchery vaccines to protect French poultry production networks from another HPAI epizootic wave. These results will support future decision making on the use of vaccination to prevent and control HPAI in France.

MATERIALS AND METHODS

The EVACS® tool allows testing the performance of different vaccination strategies on the modelled animal production network (Fig.1). Strategies are compared in terms of vaccination coverage, immunity level and spatial distribution of immunity according to the different sectors and types of production adapted to the context of the country studied. A cost-benefit analysis also makes it possible to compare the added value of each strategies according to different sectors and types of production.

The network analysis was conducted for each poultry production sector, to define different vaccination scenarios for each sector. Based on the simulated performances provided by the immunity model of EVACS®, the best vaccination scenarios for each sector were used to define vaccination strategies at the national level. The immunity model of EVACS® was also applied on these vaccination strategies to identify the most efficient one.

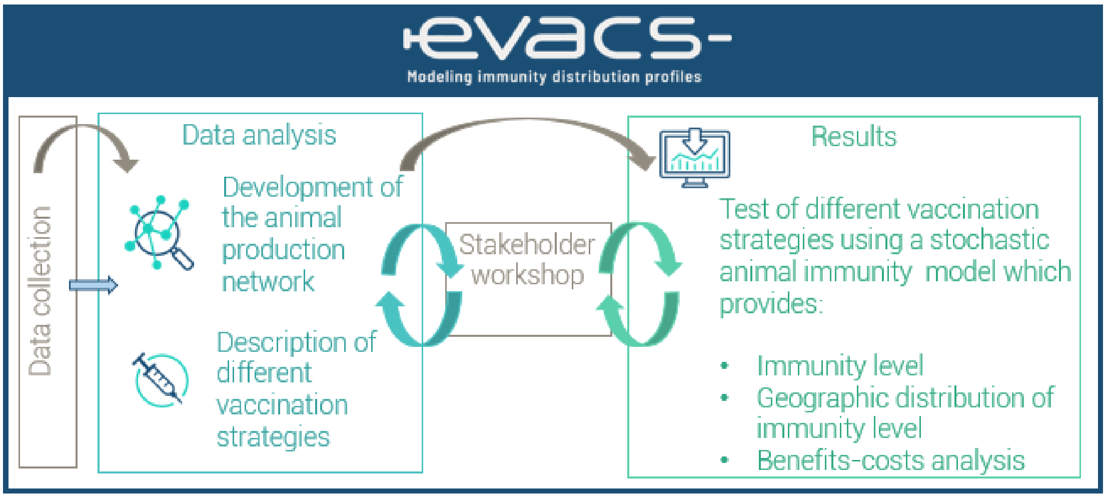

Fig. 1 Schematic representation of how the EVACS® tool works

Poultry production network organization and data collection

Data on the poultry network organisation were collected from public database (Agreste, 2018) and private database (Ceva Poultry database). In addition, interviews with key actors of the different French poultry production sectors were performed.

Vaccine efficacy data collection

As no AI vaccination is currently performed in France, data on vaccination efficacy were collected from the literature.

Network construction

Production networks models were developed for each of the major French poultry production sectors in France (i.e. broiler, layer, fattening duck, meat duck, turkey and guinea fowl). This model is based on the different production type (i.e. grandparents (GP), breeders (BR), free-range production, indoor production) but also on the integration level. A primary model was developed based on literature and information gathered from public and private databases. This model was then completed and validated with data obtained from the interviews of key actors of the different sectors. Each node represented a type of poultry production; each link represented either the movement of eggs (between BR farms and hatcheries) or the movement of day old chicks (DOC), day old turkey (DOT), day old duckling (DOD) or day old guinea fowl (DOGF) between hatcheries and farms. All the links were directed; that is, the links between the nodes showed the direction in which DOC/DOT/DOD/DOGF were moved from one node to another. Furthermore, directed-weighted matrices were developed based on the direction and volume of exchange of DOC/DOT/DOD/DOGF between nodes. For each production sectors, attribute tables were generated to provide information on the type of production (GP, BR, free-range production, indoor production) for farms and hatcheries, integration level (integrated with hatchery, integrated without hatchery, independent), and the number of birds (heads) on the farms. The final structures of the networks were validated during a stakeholder workshop.

Vaccination strategies

The first step of this work was to test several vaccination scenarios per sector to identify which vaccine type (hatchery or farm) and which application (full network or some nodes) offered the most efficient scenario. Vaccination scenarios were defined following the network organisation for each sectors. Most scenarios combined hatchery vaccination with a recombinant vaccine and/or farm vaccination with inactivated vaccine. Only scenarios using inactivated vaccines were tested for duck and guinea fowl productions, as no effective recombinant vaccine are available yet for these species.

The second step was to combined the characteristics (vaccine type and application) of the best vaccination scenario previously identified with the risk level of the production type (Table 1). The first vaccination strategy concerned only the most at risk population, i.e. layer and free-range production of all sectors (S1). The second focused on all production sectors except indoor broiler production (S2) and the third concerned only indoor broiler production (S3). The fourth concerned broiler, layer and turkey sectors (S4). The fifth added duck sectors (S5) and the sixth added guinea fowl sector (S6). The seventh included all farms of duck and guinea fowl sectors (including independent farms) (S7) to compare the added value provided by a strategy including all farms. GP and BR of all sectors were considered vaccinated for all strategies with inactivated farm vaccines applied correctly.

Table 1. Vaccination strategies tested at the national level

Vaccination strategy [b]	Sectors [a]					
	GP and BR (all sectors)	Broiler	Layer	Turkey	Duck (meat and fattening)	Guinea fowl
S 1	FV all	HV all free-range	HV all	HV all free-range	FV free-range integrated	FV free-range integrated
S 2	FV all	HV all free-range	HV all	HV all	FV all integrated	FV all integrated
S 3	FV all	HV all indoor	-	-	-	-
S 4	FV all	HV all	HV all	HV all	-	-
S 5	FV all	HV all	HV all	HV all	FV all integrated	-
S 6	FV all	HV all	HV all	HV all	FV all integrated	FV all integrated
S 7	FV all	HV all	HV all	HV all	FV all	FV all

[a] GP: grandparent, BR: breeder, [b] S: Strategy, HV: hatchery vaccination, FV: farm vaccination, - : no vaccination

Modelling the immunity distribution

The EVACS® immunity module previously developed was used to estimate the efficacy of vaccination strategies (Peyre et al., 2016). Briefly, for each vaccination scenario (sector level) and for each node of the network, the model calculated 1) the proportion of vaccinated birds (vaccination coverage) and 2) the proportion of sero-protected birds (immunity level). These values were calculated for each node of the network according to the vaccination protocol applied both in the node and its adjacent nodes and to the strength of links between the nodes (volume of DOC/DOT/DOD/DOGF exchange and immune status of DOC/DOT/DOD/DOGF).

The parameters used for the model are described in Table 2. As the vaccination would be mandatory if applied in France, the coverage at farm level was considered to be perfect (100%). Due to practical aspects, the coverage was considered to be better with hatchery vaccines (mean=98%, IC 95%=[95, 99]%) than with farm vaccines (mean = 95%, IC 95%=[90, 98]%) (Peyre et al., 2016). The same vaccine efficacy was used for both vaccination types based on literature data (Peyre et al., 2016).

Table 2. Input parameters

Vaccine type	Production type[a]	Vaccination coverage		Vaccination efficacy (% of seroconversion)
		% of farms vaccinated	% of birds vaccinated	
Inactivated vaccines (farm)	GP and BR	100%	98% [95-99]	92% [90 – 95]
	Layers, broilers and turkeys		95% [90 – 98]	
Recombinant vaccines (hatchery)	GP and BR, layers, broilers, turkeys, ducks and guinea fowls	100%	98% [95-99]	92% [90 – 95]

[a] GP: grandparent, BR: breeder

Spatial analysis

EVACS® spatial analysis module was used to assess the distribution of the immunity according to the different vaccination strategies (Peyre et al., 2016). Poultry census data at the region level were used for the spatial analysis (Agreste, 2018). Data were aggregated according to the production types (GP, BR, indoor production, free-range production and production sectors.

Benefits-costs analysis and break-even analysis

Benefits (B)-costs (C) analysis was conducted with the use of partial budget analysis to estimate the B/C ratio for each vaccination scenario and strategies as described before (Peyre et al., 2016). The benefits were limited to the value of the avoided production losses in the vaccinated population and calculated for a disease cumulated incidence (Ic) of 2.5% (level observed in France during the 2016-17 H5N6 epizooty from surveillance data). The costs were defined as the vaccination costs and the value of the losses in the non-vaccinated population.

As there is no vaccination against HPAI in France, the costs of vaccines applied against Newcastle disease in France were used to estimate the vaccination costs. The production losses due to AI infection were a function of the risk of infection at a certain point of time (Ic) and the vaccine efficacy in terms of sero-protection rate and duration of protection. The B/C analysis was applied only on the vaccination scenarios, which allowed reaching a national sero-protection level above 60% of the studied bird population. A break-even analysis was then performed to estimate the threshold level of disease Ic where vaccination would no longer be cost effective (B/C ratio <1).

Data analysis

All data collected were entered into an Excel spreadsheet (Microsoft Excel® 2007). EVACS® modules analysis was performed using the "RStudio®" software version 1.1. Cost-benefit data were analysed with the use of an Excel spreadsheet (Microsoft Excel® 2007).

RESULTS

Network analysis of the French poultry value chain

In 2018, almost 810 million commercial broilers (680 million indoor and 130 million free-range), 47 million layers, 42 million turkeys, 40 million meat ducks, 35 million fattening ducks and 30 million guinea fowl were produced in France. In France, most farms are integrated in a farmer association, which often includes a feed producer as a horizontal integration system. Some farmer associations include one or several BR hatcheries in a vertical integration system. In the layer, turkey, duck and guinea fowl production sectors, some BR hatcheries are integrated with selection, i.e. GP hatchery (vertical integration system with selection), but some hatcheries are independent. In France, no sector is fully vertically integrated i.e. GP farms integrated with BR farms and with production farms. Moreover, some farms do not belong to a farmer association and are considered as independent. These farms are mostly small farms with on-farm sales of their products (on-farm slaughter or with an individual contract with a slaughterhouse). Based on these observations, the level of integration makes it possible to divide production farms into three groups: farms integrated in a farmer association with a hatchery, farms integrated in a farmer association with no hatchery, and independent farms.

The level of integration concerns all production sectors except layers (no hatcheries are integrated with production farms). As requested by key actors of the turkey, meat duck and guinea fowl sectors, no distinction was made between farms integrated in a farmer association including a hatchery and farms integrated in a farmer association with no hatchery for these sectors. Only the broiler sector network analysis is presented here as an example (Fig.2). Similar analyses were conducted of the other production sectors.

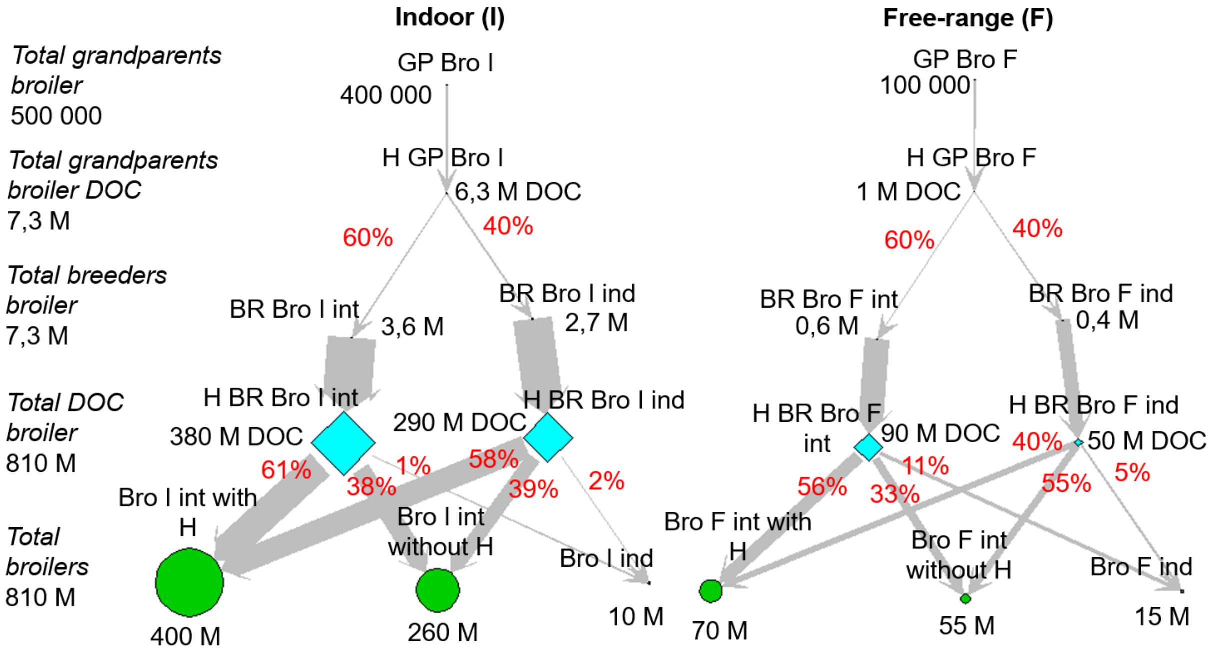

Fig. 2 French broiler production network. The type of nodes represents the different types of production (indoor (I) or free-range (F); integrated (int) or independent (ind)): grand-parents (GP) and breeders (BR) (point), hatcheries (H) (diamond), commercial broilers (Bro) (circle). (DOC: day-old chick)

Vaccination strategies at the national level

Vaccination strategies tested at the national poultry production level combined the best vaccination scenarios identified at the individual sector level (Hatchery vaccination in all hatcheries for broiler, layer and turkey sectors and farm vaccination in all integrated farms for duck and guinea fowl sectors) with the risk level of each production type (Table 1).

Immunity distribution profile

The vaccination of only GP, BR, layer and free-range productions (S1) did not allowed reaching a sufficient coverage and immunity level for the entire poultry population (more than 80 and 60% respectively) (Fig.3, S1). Interestingly, the vaccination of all sectors except indoor broiler would not be enough to reach a sero-protection level above 60% (Fig.3, S2), while the vaccination of indoor broiler production only would be enough to reach this level (Fig.3, S3). The vaccination including all farms in layer, broiler and turkey sectors, without duck and guinea fowl sectors, would be sufficient to get a coverage and a sero-protection level above 80% and a sero-protection level above 80% of the entire poultry population (Fig.3, S4, 5, 6 and 7).

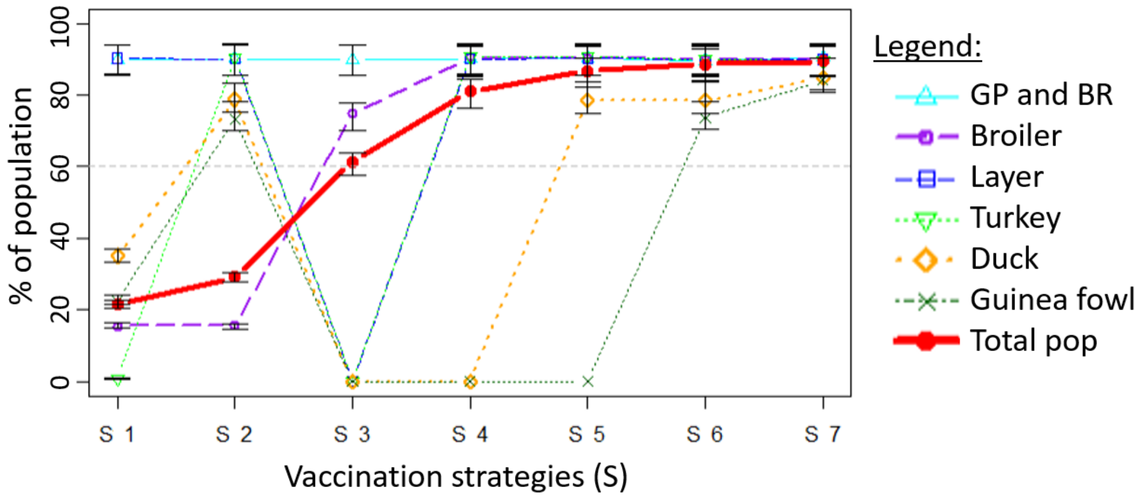

Fig. 3 Overall immunity level per production type according to the different vaccination strategies at the national level (S1 to S7) (GP: grandparent, BR: breeder, total pop: total population)

Spatial distribution of the immunity level

A sufficient immunity level (> 60%) was reached in the most at risk area (highest population density in West of France) when vaccination strategies included at least layers, broilers and turkeys sectors (Fig.4, S4 to S7). Vaccination strategies also including at least integrated duck farms allowed reaching a very good immunity level (> 80%) that was spatially uniform at the national level (Fig.4, S5, 6 and 7). Indeed, indoor meat poultry productions (broiler, turkey, meat duck) are localised in West of France and fattening duck production is mainly localised in South West of France while free-range productions are mainly localised in South of France. A vaccination strategy focused on high risk populations (layer and free-range productions) did not provide a sufficient immunity level (> 60%) (Fig.4, S1).

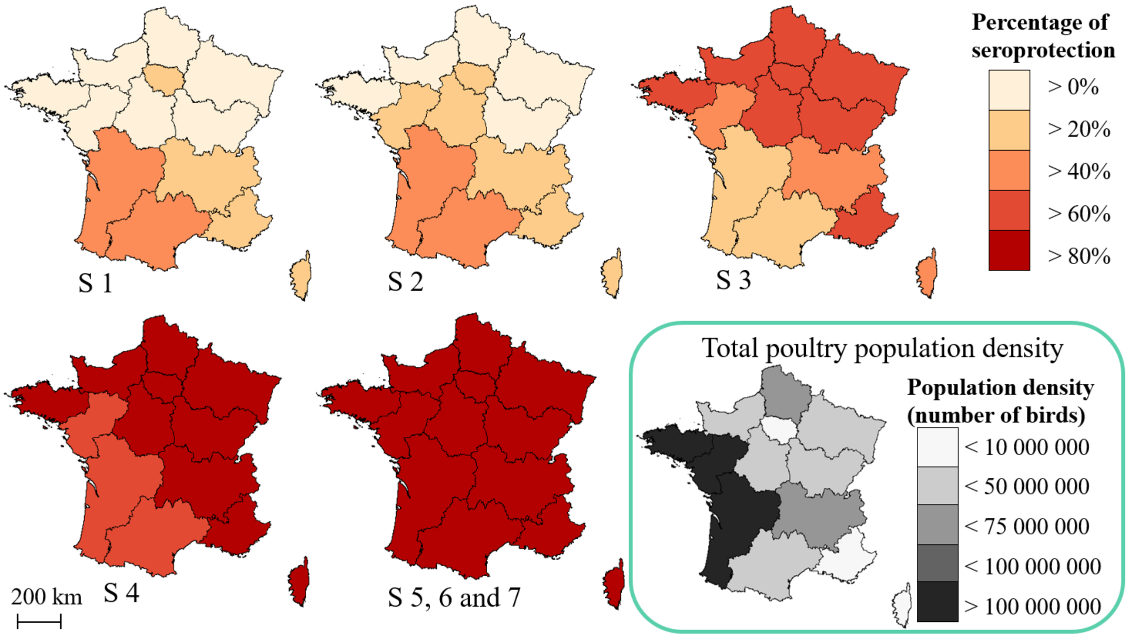

Fig. 4 Spatial distribution of the poultry population immunity against AI according to the different vaccination strategies (S) tested in the model

Benefits-costs analysis

All tested vaccination strategies had good cost-benefit ratios (B/C > 1) at the Ic level of the previous epizootic event (2.5%) except the strategy including only indoor broiler (Table 3, S3). Vaccination strategy including all farms in all sectors offered the best B/C ratio (Table 3, S7) but vaccination strategies including at least integrated duck farms (Table 2, S5 and 6) offered also a good B/C ratio.

Table 3. Benefits-costs analysis of the different vaccination strategies (S)

Vaccination strategy	Costs		Benefits (million euros)	Benefits/costs ratio
	Vaccination costs (million euros)	Losses costs (million euros)		
S 3	57	101	91	0.6
S 4	48	50	117	1.2
S 5	54	34	125	1.4
S 6	56	32	126	1.4
S 7	57	29	127	1.5

DISCUSSION

This study demonstrated the interest to apply the EVACS® tool to compare potential vaccination strategies against HPAI in French poultry production networks. Vaccination strategies which applied hatchery recombinant vaccine in all hatcheries for species for which such vaccines were available (i.e. broiler, layer and turkey) and inactivated farm vaccine in all integrated farms for the other species (i.e. duck and guinea fowl) were the most efficient. A vaccination strategy limited to the most at risk populations (i.e. layer and free-range production of all sectors) did not induce protective immunity for the whole population as indoor production is the major production type in France. Even if the vaccination of indoor broiler is necessary to induce protective immunity for the whole population, the vaccination strategy including only indoor broiler was not efficient, as noticed in previous studies (Peyre et al., 2016).

As AI vaccination is not currently authorised in France, no data are available on AI vaccine application in the French context. To avoid this lack of information, two hypothesis were made: 1) the vaccination coverage would be optimal and 2) the vaccines applied would be effective in ducks. If an AI vaccination strategy was applied in France, it would be mandatory and a sufficient coverage would be applied. However, the impact of AI vaccination on international trade, and especially on exports, would be important due to export ban. As exports represent a large part of the French poultry production, this decision would be taken only in the case of a large uncontrolled spread, even with culling, and with an effective vaccine. Effectiveness of AI vaccines in duck is questionable (Pfeiffer et al., 2010; Cha et al., 2013; Pantin-Jackwood et al., 2015) and limited studies have been conducted in guinea fowl (Bertelsen et al., 2007). This work showed that the vaccination strategy including broiler, layer and turkey was sufficient to induce protective immunity in all poultry production. As the previous AI epizootic waves concerned principally ducks farms, the development of an effective vaccine in this species to protect these two important production sectors in France is needed.

During the stakeholder workshop, the participants considered that if a vaccination strategy was applied in France, it would be only in an emergency context with a focus on a limited geographical area where the outbreaks occurred. Some results of this work could be applied also in case of an emergency vaccination strategy, e.g. vaccination of free-range production would not provide a sufficient immunity level for the whole production. In case of an HPAI outbreak concerning only a geographically limited production sector such as fattening duck production sector, the use of vaccination as a complementary tool to culling remains interesting to protect the network while limiting the economical and psychological impact of culling for the farmers.

The vaccination strategies could be tested using the EVACS tool according to the need of decision makers to compare the efficiency of these strategies. At this stage, the tool informs on the best strategies outputs in terms of coverage, immunity level and spatial distribution of this immunity level. There is a need to combine it with an epidemiological model to conclude on the effectiveness on the strategies tested to control HPAI.

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MODELLING INTERVENTIONS

THE EFFECT ON RANGING BEHAVIOUR AFTER SELECTIVE REMOVAL OF BOVINE TUBERCULOSIS TEST POSITIVE BADGERS (*MELES MELES*) USING A TEST AND VACCINATE OR REMOVE INTERVENTION IN NORTHERN IRELAND

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SUMMARY

Pro-active culling of European badgers (*Meles meles*) can potentially lead to social perturbation with a subsequent effect on levels of bovine tuberculosis (bTB) in cattle. A five-year wildlife research intervention project commenced in 2014 in a 100 km^2 area in Northern Ireland, based on selective removal of bTB test positive badgers and vaccination of bTB test negative badgers (Test and Vaccinate or Release; TVR). Monitoring the effects of TVR intervention on badger ranging behaviour was one of the study aims. This behaviour was analysed based on 149 home range estimates from 105 individual badgers over 21,200 Global Positioning System (GPS) collar-nights. The TVR intervention led to an estimated 2.0-10.2% of badgers being removed annually (compared to the majority of badgers in non-selective cull trials). Multi-variable linear mixed model analyses indicated no statistically significant difference in badger ranging behaviour after TVR intervention.

INTRODUCTION

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is the most important endemic cattle disease in the UK and Ireland and badgers are known to play a role in its transmission (Allen et al., 2011). When a wildlife population is persistently infected, reduction of spread can be difficult to achieve both within and between species (Morris et al., 1994).

Non-selective badger culling, which is used in England and Ireland with the aim of reduction of bTB in cattle, has been suggested to cause increased movement of badgers between social groups (Woodroffe et al., 1995; Riordan et al., 2010; Ham et al., 2019), which in turn has been suggested to increase the possibility of contact and bTB transmission between badgers and between badgers and cattle (Woodroffe et al., 2006). This so called 'perturbation effect' has been suggested to be induced by even low levels of culling (Bielby et al., 2014) and can be a major limitation when considering badger culling as a bTB control tool. However, contradictory findings suggest that a reduction in badger numbers can contribute to the control

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of bTB in cattle (Eves, 1999; Olea-Popelka et al., 2009; Downs et al., 2019). The reason for these recorded differences is still unclear (Downs et al., 2019), but could potentially relate to geographical differences such as the sizes of badger social groups (Smith et al., 2016).

An alternative badger intervention option to culling is vaccination, which has shown to reduce the severity and progression of disease and to indirectly reduce the incidence of disease in unvaccinated cubs (Carter et al., 2012). A previous study conducted in Northern Ireland indicated that the majority of farmers would allow badger vaccination and culling on their land with vaccination being the preferred option (O'Hagan et al., 2016).

The badger intervention option evaluated in the current study is based on a novel approach combining culling and vaccination in the form of selective removal of bTB test positive badgers and vaccination of the remainder (bTB test negative badgers). One of the main advantages of this 'Test and Vaccinate or Remove (TVR)' approach is that it involves a lower level of badger removal than non-selective culling making it therefore more publicly acceptable. Furthermore, it was anticipated that it could potentially lower the risk of inducing social perturbation (Carter et al., 2007). Considering these factors, a five year TVR research project was initiated in a 100 km^2 area of County Down, Northern Ireland in 2014. As this form of badger intervention is novel, the exact effect of this form of selective culling of badgers on badger movement was unknown. As a previous modelling study concluded that the likely benefit of selective culling will be dependent on social responses of badgers (Smith et al., 2016), the main aim of this study was therefore to research the effect on badgers' home range sizes after selective culling of bTB test positive badgers by analysing location data transmitted by Global Positioning System (GPS) collars on a subset of badgers within the TVR project.

MATERIALS AND METHODS

Study design

The 100 km^2 study area was selected as it had a high confirmed bTB cattle herd prevalence during 2011 and 2012 as well as having high cattle and active main badger sett densities (Smith et al., 2013). Furthermore the area had relative hard boundaries in the form of a busy dual-carriage way to the west and a river to its north-east, which reduced the migration of badgers into and out of the study area. The study area mainly consisted of grass land (67 km^2) and some arable land (13 km^2) with the remainder being woodland, residential areas and farm-yards. A preliminary badger sett survey was conducted prior to commencement of the study (Department of Agriculture, Environment and Rural Affairs, 2014).

The ranging behaviour in the study area, based on calculation of home ranges (the area an animal commonly uses for normal activities (Dürr & Ward, 2014)) was measured using GPS collars applied to a cohort of badgers every year. Badger capture involved saturation trapping with individual cage traps taking place from the start of July until mid/end October each year as this was the time period granted under licence by the Northern Ireland Environmental Agency (NIEA). Once trapped, badgers were anaesthetised and individually identified by implanting a microchip subcutaneously on their first capture. All captured animals received a detailed physical examination and blood samples were taken to determine their bTB status using a cage side test (DualPath Platform (DPP) VetTB test, Chembio Diagnostics Systems Inc., Medford, NY 11763 USA). A Tellus GPS collar (Followit Wildlife, Lindesberg, Sweden) was placed on suitable animals (≥ 8 kg; neck circumference having to be at least 1 cm less compared to the head circumference; collar able to turn freely; similar to Gaughran et al., 2018). GPS collars were not fitted if there was skin irritation around the neck or on badgers that were caught less than 1 km from the study area boundary. GPS collars were applied during the first four years of the TVR project as the fifth year focussed on collar removal. The aim was to deploy 40 GPS collars during each of the four years (two badgers (preferably a male and a female) per social group). If badgers were recaptured in the same year, they were identified, recorded and released. Captured badgers that were fitted with GPS collars from previous years had their GPS collars replaced provided the above criteria were met. The GPS collars were programmed to record eight locations per night (every hour from 21.00-04.00 hrs (similar to the settings used by O'Mahony (2014) and Gaughran et al., (2014)). Location data were collected until the GPS collar stopped transmitting. During the first year of the project no DPP test positive badgers were removed. In years 2-4 DPP test positive badgers were euthanized. The badger ranging behaviour in Year 1 (control year/no culling) were used as a baseline ('normal' badger home range sizes) for home range sizes in the follow up years 2-4 (selective culling).

Data analyses

A population estimate within the study area was collected based on results from previous research (Reid et al., 2012) and the Lincoln-Peterson Method (Seber, 1982). Distances between active main setts were calculated using the 'Nearest Neighbour Tool' in ArcGIS 10.3.1 (ESRI systems, USA).

Position data transmitted by GPS collars were used to determine home ranges of the collared badgers. These data were downloaded from the Followit website (https://www.followit.se/) and stored and manipulated in Microsoft Excel (version 2013; Microsoft Corporation, Redmond, WA, USA). After checking and cleaning, the data were transferred into ArcGIS (version 10.3.1; ESRI systems, USA) for further manipulation and analyses.

Home range sizes were based on 95% and 50% fixed kernels (FK) that were constructed for every collared badger for each year (Kauhala and Auttilla, 2010). Kernel density calculates a magnitude per unit area from a point or polyline feature using a kernel function to fit a smoothly tapered surface to each point or polyline. A FK refers to a kernel where the bandwidth remains the same for all calculations. The bandwidth used for the kernels in the current study was 0.0025 metres. After applying kernel smoothing of 95% and 50% to the data points separately for every individual badger each year, probability contours were applied to create polygons enclosing 95% or 50% of the probability distribution. As the home range sizes were not normally distributed (based on the Shapiro-Wilk Normality test), annual median values were provided.

Four analyses were performed. In the first analysis, the outcome modelled was the size of the home ranges based on 95% FK whereas in the second analyses, the outcome modelled was the size of the home ranges based on 50% FK. As social perturbation is anticipated to occur shortly after the removal of any badgers (King, 2007), analyses were repeated based on home range sizes using only the data collected in the first month of collaring (minus the first 4 days to allow badgers to recover from any effect of anesthesia (Wilson et al., 2016)).

Potential explanatory variables that were examined in all four analyses were TVR year (the year that a GPS collar was applied to the badger), number of days from application of each GPS collar to last GPS location transmission, number of location points collected per badger per year, sex and weight of the badger, cull intensity (low, medium, high) and main sett density (low, medium, high).

The analyses were conducted using GenStat (version 18; VSNI, Hemel Hempstead, UK). For each response variable, a list of candidate variables was supplied as possible explanatory variables. In each case a stepwise regression (forward selection with backward elimination) analysis approach was implemented. Each of the models generated at this stage was then refitted using a linear mixed model methodology with individual badger fitted as a random effect linear model (Searle, 1971; Patterson and Thompson, 1971). A second stepwise method (backward elimination) was then applied to each model so that only explanatory variables that were significant ($P<0.05$) were left in the final model in each case (Katz, 2011).

RESULTS

Permissions for trapping were obtained from 96% (n=831) of farmers equating to 93% of the fields (n=6,006) in the study area. The vast majority (89.9%) of GPS collars were recovered/accounted for after they stopped transmitting. The population density estimate for the study area was 550 badgers (5.5 badgers/km^2). The number of trapped badgers removed ranged from 11 to 56 during years 2-4 which represented between 2.0 – 10.2% of the estimated population (Table 1). The mean distance between adjacent active main setts in the study area was calculated to be 820 metres.

Table 1. Number and percentage of badgers trapped and removed during the Test and Vaccinate or Remove project

Year	Badgers trapped	Badgers removed	% of trapped badgers removed	% of estimated population removed
2014	280	0	0	0
2015	341	56	16.4	10.2
2016	271	11	4.1	2.0
2017	287	22	7.7	4.0
2018	341	19	5.6	3.5

The descriptive results of the badgers collared from 2014 to 2017 are displayed in Table 2. The number of badgers that received a GPS collar and transmitted their location points successfully varied from 35 - 41 per year. Over the TVR study period fewer female badgers were collared than male badgers (overall ratio 1:1.44). The median weight of the collared badgers varied from 9.1 - 9.5 kg, whereas the median number of days reading varied from 104 - 176 days and the location points from 724 - 909.

Table 2. Descriptive results of badgers collared in the Test and Vaccinate or Remove area (2014-2017)

		2014	2015	2016	2017
Total number of badgers collared		37	36	41	35
Number of females		17	17	13	14
Number of males		20	19	28	21
Ratio females : males		1:1.18	1:1.12	1:2.15	1.1:1.50
Weight of badger (kg)	Median	9.1	9.3	9.1	9.5
	Range	8.0-11.8	7.4-11.8	5.1-11.4	8.0-12.3
	Q^1-Q^3 [a]	8.3-9.9	8.6-10.3	8.6-9.8	8.8-10.5
Number of days of recording per badger	Median	176	132	152	104
	Range	20-337	11-303	26-302	18-151
	Q^1-Q^3 [a]	99-241	81-217	118-182	62-131
Number of locations recorded per badger	Median	909	753	887	724
	Range	108-2132	76-1947	101-1921	130-1050
	Q^1-Q^3 [a]	667-1363	548-973	709-1094	399-898

[a] Q^1-Q^3 25th percentile – 75th percentile

Badger ranging behaviour was analysed based on 149 home range estimates from 105 individual badgers over 21200 collar-nights. One badger was collared all 4 years, ten badgers were collared 3 out of 4 years, 21 badgers were collared 2 out of 4 years and 73 badgers were only collared once during the study period.

Descriptive summaries of the home ranges for each badger for each year based on the previously defined FKs are presented in Table 3. Based on the baseline data collected in 2014 from 95% FK home ranges, male badgers ranged over a larger area than female badgers although this was not statistically significant ($p=0.148$). As one would expect, the number of locations recorded per badger was strongly positively correlated to the number of days readings per badger ($r = 0.919$). There was a weak positive correlation between the number of locations and the size of the home ranges ($r = 0.381$ for 95% FK; $r = 0.278$ for 50% FK).

The results for the multivariable analyses showed that home ranges were significantly associated with the number of locations for three of the four models while sex was only a significant factor for the home range sizes based on 95% FK using all location points (Table 4).

Table 3. Home ranges based on 95% Fixed Kernels (FK) and 50% FK of badgers collared during the Test and Vaccinate or Remove project (2014-2017)

	2014 Median (Q^1-Q^3 [a])	2015 Median (Q^1-Q^3 [a])	2016 Median (Q^1-Q^3 [a])	2017 Median (Q^1-Q^3 [a])
95% FK[b] (km^2) Based on all location points				
	1.41 (1.02 – 2.06)	1.15 (0.83 – 1.50)	1.36 (0.84 – 1.61)	1.22 (0.79-1.55)
50% FK[c] (km^2) Based on all location points				
	0.15 (0.11 – 0.22)	0.10 (0.07 – 0.16)	0.14 (0.09 – 0.19)	0.13 (0.08-0.17)
95% FK[b] (km^2) Based on location points in first month				
	1.02 (0.79-1.20)	94.65 (0.59-1.21)	1.05 (0.65-1.28)	0.88 (0.71-1.40)
50% FK[c] (km^2) Based on location points in first month				
	0.12 (0.08-0.16)	0.10 (0.07-0.16)	0.09 (0.06-0.16)	0.08 (0.05-0.12)

[a] Q^1-Q^3 25th percentile – 75th percentile; [b] 95% Fixed Kernel; [c] 50% Fixed Kernel

Table 4. Multivariable analyses results based on the response variable home range size

	Variable		Estimate	S.E.[a]	P
95% FK[b] Based on all location points	Constant		64.99	13.318	
	Number of locations		0.07	0.011	<0.001
	Sex	Female	0.00	13.04	0.016
		Male	32.01		
50% FK[c] Based on all location points	Constant		8.68	1.499	
	Number of locations		0.007	0.002	<0.001
95% FK[b] Based on location points in first month	Constant		87.00	8.738	
	Number of locations		0.021	0.009	0.026
50% FK[c] Based on location points in first month	N/A[d]		N/A[d]	N/A[d]	N/A[d]

Standard Error; [b] 95% Fixed Kernel; [c] 50% Fixed Kernel; [d] Not applicable

DISCUSSION

When wildlife acts as a potential reservoir for infectious diseases, their home range sizes are important in relation to transmission dynamics (Woodroffe et al., 2006). In the case of badgers, which are widely accepted as a reservoir for bTB infection in the British Isles (as reviewed by Allen et al., 2011), knowledge of home ranges is especially of importance as previous research conducted in England has suggested that badger pro-active culling interventions could potentially lead to social perturbation with a subsequent perturbation effect of increased levels of bTB in cattle (Woodroffe et al., 1995; Woodroffe et al., 2006). However, these findings have been contradicted by other research conducted in Ireland (Eves, 1999; Griffin et al., 2005; Olea-Popelka et al., 2009).

The badger intervention described in the current study based on testing badgers for bTB and removing the positive ones and vaccinating the negative ones, is an approach that has never been conducted elsewhere. Therefore the effect of this approach on badger ranging behaviour is unknown. It is important to have an understanding of this effect as it can impact on the disease control benefits of the TVR approach (Smith et al., 2016) along with other factors such as penside test performance and trapping efficiency.

The most straightforward method to estimate a home range is the construction of the smallest possible convex polygon (100% Minimum Convex Polygon (100% MCP)) but it has important shortcomings such as the fact that the data point density is not taken into account (Dürr and Ward, 2014) and they can lead to an overestimation of the home range size (Burgman and Fox, 2003). For these reasons, 100% MCPs were not considered for the purpose of this study and the focus was on home ranges based on fixed kernels as they are probably the most accurate estimator of the area that animal uses during its normal activities (Worthon, 1989) and they exclude outlier locations. The 95% fixed kernel is often simply referred to as ‘home range’ whereas the 50% fixed kernel is often referred to as the ‘core area’ (Kauhalla and Autilla, 2010).

Badger ranging behaviour was analysed based on 149 home range estimates from 105 individual badgers over 21200 collar-nights. As the badger density in our study area was estimated to be in the band 3.0-9.99 individuals/km^2, Gaughran et al. (2018) considers this density to be classified as high. The current study showed that the number of locations measured (which was found to be positively correlated to the number of days reading) was found to be positively associated to the size of the home range.

In the multivariable analyses, the year of study was used as a proxy for the effect of the badger intervention as badger intervention took place in the last 3 years (2015-2017) of the study. In all of the four analyses conducted, *Year* was not an explanatory variable that had a significant influence on the outcome variable suggesting that the home ranges did not significantly differ in size between years. Therefore home range sizes were not significantly altered by the badger intervention/removal that was applied in the study area. This finding also applied when only the location points in the first month of collaring were taken into account. It has to be kept in mind that in the current study only a small proportion of badgers (2.0-10.2%) were removed every year. Currently it is unknown whether there is a threshold, in terms of social group members removed (Bielby et al., 2014; Smith et al., 2016). However, in the described selective culling approach based on TVR intervention, the proportion of badgers being removed has been substantially reduced compared to a non-selective culling approach, without inducing an increased ranging behaviour. Moreover, there were thought to be more badgers removed from the area by road traffic collision fatalities (Gallagher and Nelson, 1979) than through the intervention in this study. Indeed, during 2016, 29 road traffic fatalities were reported in the TVR area while only 11 badgers were removed through the intervention.

The number of locations was a significant explanatory variable in relation to the home range size, with an increase in the number of locations relating to an increase in the home range size. These findings are in line with previous research (Wauters et al., 2007). Furthermore, male badgers tended to have larger home range sizes and sex of badgers was a significant explanatory variable in the multivariable analyses based on the outcome variable 95% FK based on all location points. This outcome concurred with previous research findings (Morris et al., 1994; Gaughran et al., 2018).

There are some limitations to this study. Firstly, badgers were selected for suitability for collaring based on criteria relating to neck size and weight. This creates a bias in selecting larger badgers in the population (i.e. bias towards adult male badgers) for collaring. Moreover, it has been previously described that badger home ranges are largest in the summer months and smallest in the winter months (Kowalczyk et al., 2003). As all collars were applied in the time period July-October for licensing reasons and the median length of time the collars transmitted was 104-176 days, there was little opportunity to assess the influence of season on the size of the home ranges. Nevertheless further analyses based on calculation of monthly home range sizes will attempt to address this. It is worthwhile to note that in 2017 a drop in recordings was noticed due to apparent premature failure of refurbished collars. The adjustment for number of locations in the analyses should however have taken account of this bias as much as possible.

CONCLUSION

This paper describes the first research into the potential changes in home range sizes after badger intervention based on a test and vaccinate or remove approach. Based on a sample size of 149 home range estimates over four years, no significant alteration of ranging behaviour was found. This finding is important for informing on the development of future badger intervention strategies.

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EFFECTS OF CHANGES IN HERDS' STRUCTURE AND ANIMAL MOVEMENT ON THE SPREAD AND CONTROL OF FOOT-AND-MOUTH DISEASE

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SUMMARY

Foot-and-mouth disease (FMD) is caused by a highly contagious virus that affects cloven-hoofed animals. We compared herd and animal movement data from 2006/2007 to data from 2018 to identify changes to herd structure and animal movments. Thereafter, we used a simulation model to study the spread and control of FMD using the 2006/2007 and 2018 data under different control strategies. We found that the numbers of herds has generally decreased over timer, while herd sizes have generally increased. Model predictions suggest that an incursion of FMD virus in 2018 would result in fewer affected herds and shorter epidemic duration compared to an incursion in 2006/2007. However, an FMD incursion in 2018 would cause greater economic damage. Furthermore, the optimal control strategy in 2018 depends on the herd type where epidemics are initiated, and was in some situations not the same as in 2006/2007.

INTRODUCTION

Foot-and-mouth disease (FMD) is caused by a highly contagious virus that affects cloven-hoofed animals (Grubmann and Baxt, 2004). Due to the export ban on live animals and livestock products following confirmation of an FMD incursion in a previously FMD-free country, countries with large production and export of livestock and their products are highly vulnerable to the subsequent economic damage. The faster the disease is detected and controlled, the less the economic damage (Carpenter et al., 2011). Livestock farming has gone through drastic changes during the past two to three decades in several European Union (EU) countries. Livestock production has changed from mostly family farming to large and industrialized production systems, with many employees per farm. This specialization has reformed the structure of farming resulting in fewer but larger herds, potentially affecting not only the size of the farms, but also the contact structure between herds (e.g. Schulz et al., 2017).

The spread and control of infectious diseases [Symbol] such as FMD [Symbol] can be affected by these structural changes, because animal movements and contacts between herds play a major role in the spread of the disease (Gibbens et al., 2001). To maintain up-to-date contingency plans, it is important to understand how such changes affect the spread of

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infectious diseases such as FMD. This would ensure rapid disease control, following incursion of the disease in a country.

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The objectives of this study were two-fold: 1) to describe changes to herd structure and animal movements by comparing herd data and animal movement data from 2006/2007 and data from 2018 in Denmark, and 2) to study the spread and control of FMD using the 2006/2007 and 2018 data in Denmark under different strategies to control the disease.

MATERIALS AND METHODS

A stochastic and spatio-temporal simulation model (DTU-DADS; version 0.15) was used to simulate the spread and control of FMD in Denmark (Halasa et al., 2015). Three major processes are simulated in the model: 1. disease initiation and spread; 2. disease detection; and 3. disease control. These processes are briefly described below, while more details can be found in Boklund et al. (2013) and Halasa et al. (2015).

Herd data

Data from the Central Husbandry Register was obtained for swine, cattle, sheep and goat herds in Denmark for two time periods, and included information about cattle markets. The first period spanned from October 2006 to September 2007, while the second period spanned from January 2018 to December 2018. Because of a lack of contiguity, herds on the Island of Bornholm were excluded from both datasets. Data included the following information: herd ID, number of animals per age group (cattle and swine herds), the herd geographic coordinates, herd type, herd-specific daily frequency of outgoing animal movements, herd-specific daily frequency of outgoing weaner movements (swine herds), and herd-specific daily frequency of outgoing animal movements to a slaughterhouse.

Disease spread

At the start of each iteration, a herd that was pre-selected to initiate the infection process (as described below) was infected. Thereafter, infection could spread to other herds via: 1) animal movements between herds; 2) movements of animals to slaughterhouses; 3) medium risk movements (those with direct contact to animals e.g. veterinarians and artificial inseminators); 4) low risk contacts (those without direct contact to animals e.g. trucks and visitors); 5) trucks collecting milk; 6) markets; and 7) local spread (includes spread via rodents, flies and limited airborne spread).

Disease detection

Detection of infection occured via one of three processes: 1) first detection within the country, whose delay was simulated via a random PERT distribution with minimum, most likely and maximum of 18, 21 and 23 days, respectively; 2) disease detection via passive surveillance, e.g. the farmer and/or veterinarian notify clinical symptoms; and 3) detection via surveillance of herds within the protection and surveillance zones (Boklund et al., 2013; Halasa et al., 2015).

Basic control measures (Basic)

When the disease was detected, a set of control measures was applied in the model representing the basic control scenario. This scenario was based on the EU legislation (Council Directive 2003/85/EC) and on the Danish contingency plan and included (1) depopulation, cleaning and disinfection of detected herds; (2) a 3-day national standstill on animal movements; (3) implementation of a 3-km protection and a 10-km surveillance zone around detected herds, in which movements between herds and movements in and out of the zones were restricted and herds were surveyed at least once (those in surveillance zone) or twice (those in protection zone) before lifting the restrictions; and (4) backward and forward tracing of animal movements and contacts to and from detected herds.

Alternative control measures

The model simulates several control measures other than the basic strategy. These are: 1) ring-depopulation (**Dep**), which represents the basic control plus depopulation of herds within a defined radius around detected herds; 2) vaccine-to-kill (**VTK**), which represents the basic control plus vaccination of herds within a defined radius around detected herds, these herds being culled by the end of the epidemic; and 3) vaccine-to-live (**VTL**), which represents the basic control plus vaccination of herds within a defined radius around detected herds, these herds being allowed to proceed in production after the end of the epidemic. Within the model, 10 herds must be detected before preemptive depopulation or vaccination are initiated.

Disease initiation

In each iteration, the disease was initiated in one pre-selected herd. The selected herds represented three different initiation criteria: 1) 1,000 cattle herds randomly selected; 2) 1,000 sow herds, that have at least 200 sows, randomly selected; and 3) 1,000 sheep herds randomly selected. Each model run consisted of 1,000 iterations, initiated with one of the 3 above mentioned initiation criteria and one of the four different control strategies, resulting in 12 different model runs. Furthermore, the 12 different runs were run using the 2006/2007 and 2018 datasets, resulting in a total of 24 different model runs. For each iteration, the model was run for a maximum of one year.

Model outputs

The outputs of the model were: 1) epidemic duration (the time between first detection and the depopulation of the last detected herd); 2) number of detected herds; number of depopulated herds; number of vaccinated herds; and 3) the total costs (sum of the direct costs and export losses). The direct costs included costs of depopulation, cleaning and disinfection, welfare slaughter, compensation, empty stable, national standstill and vaccination (in case of vaccination). Export losses included the loss due to export ban on live animals and livestock products to both the EU and non-EU countries (Halasa et al., 2015).

RESULTS

Analysis of herd data from 2007/2006 and 2018 showed that the number of herds decreased from about 50,000 herds to about 33,000 herds. Furthermore, the average herds size for cattle increased from 69 to 96 animals (1.4-fold increase), for swine from 530 to 1648 animals (3.1-fold increase) and for sheep from 14 to 29 animals (2.1-fold increase). The total number of animals in Denmark decreased slightly for cattle, while the number of pigs and sheep increased by about 14% and 17%, respectively. The frequency of animal movements increased for cattle, swine and sheep herds by about 60, 26 and 38%, respectively.

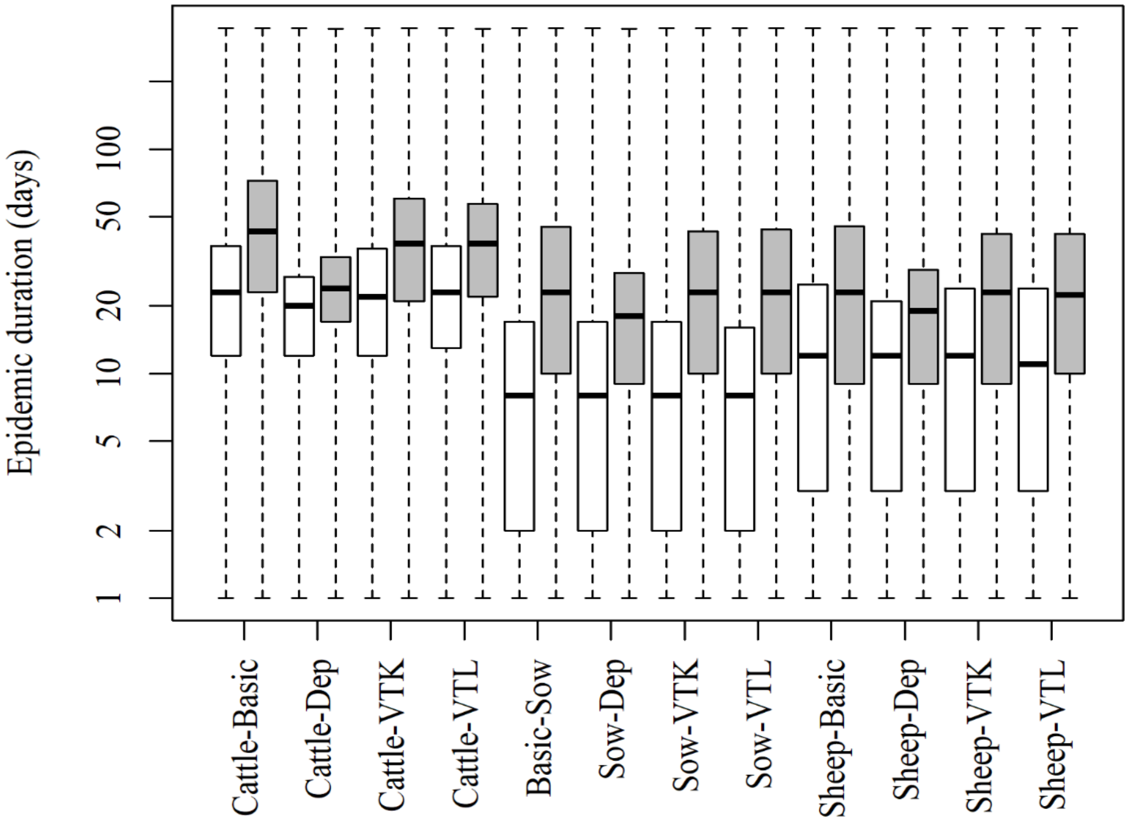

Fig. 1 Box plots of the predicted epidemic duration in simulated FMD-epidemics. Cattle, Sow or Sheep/goats indicate the herd type in which epidemics were initiated, gray and white boxes indicate use of 2006/2007 or 2018 data, respectively. The thick line in the middle of the boxes is the median value, the box limits are the 25th and 75th percentiles and the whiskers are the minimum and maximum values. Control strategies are indicated by the acronyms explained in the methods section

The model predicted that epidemics initiated in cattle herds would last longer (Fig. 1), involve larger numbers of affected herds (Fig. 2) and result in greater economic damage (Fig. 3) compared to epidemics initiated in swine or sheep herds using both 2006/2007 and 2018 data. Epidemics that were initiated using the 2006/2007 data were predicted to last longer and involve larger numbers of infected herds compared to epidemics that where initiated using 2018 data (Fig. 1 and Fig. 2). Nonetheless, epidemics initiated using 2018 data were predicted to cause larger economic damage compared to epidemics initiated using 2006/2007 data (Fig. 3).

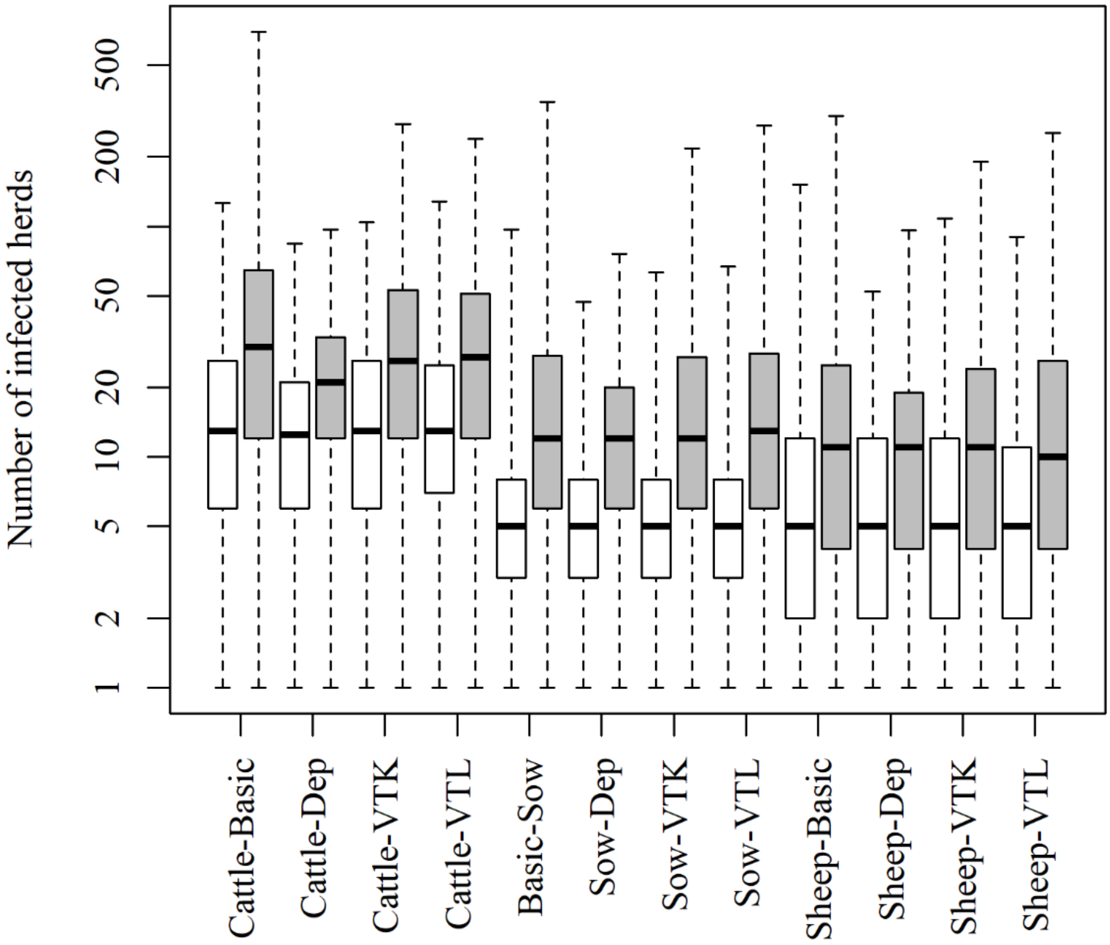

Fig. 2 Box plots of the predicted number of infected herds in simulated FMD-epidemics. Cattle, Sow or Sheep/goats indicate the herd type in which epidemics were initiated, gray and white boxes indicate use of 2006/2007 or 2018 data, respectively. The thick line in the middle of the boxes is the median value, the box limits are the 25th and 75th percentiles and the whiskers are the minimum and maximum values. Control strategies are indicated by the acronyms explained in the methods section

When comparing control strategies, the preemptive depopulation (Dep) strategy was predicted to provide the shortest epidemic duration (Fig. 1) and smallest total losses (Fig. 3), when epidemics were initiated in cattle herds, using either the 2006/2007 or 2018 datasets. Nevertheless, when epidemics were initiated in swine or sheep herds, the basic strategy was as good as the Dep or VTK strategy, from an economic perspective (Fig. 3). VTL was always more expensive than the other control strategies (Fig. 3).

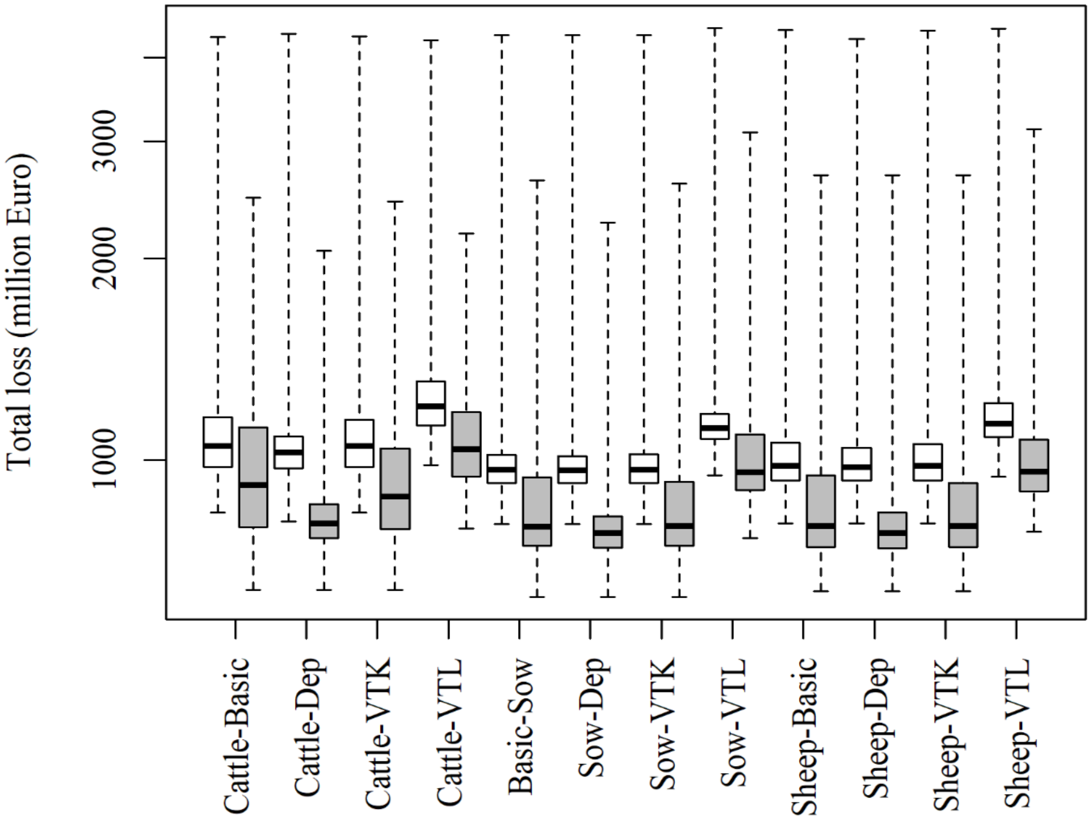

Fig. 3 Box plots of the predicted total loss in simulated FMD-epidemics. Cattle, Sow or Sheep/goats indicate the herd type in which epidemics were initiated, gray and white boxes indicate use of 2006/2007 or 2018 data, respectively. The thick line in the middle of the boxes is the median value, the box limits are the 25th and 75th percentiles and the whiskers are the minimum and maximum values. Control strategies are indicated by the acronyms explained in the methods section

DISCUSSION

With this study, we showed that the Danish livestock production has undergone drastic changes within the past decade, resulting in fewer but larger herds. Delgado et al., (2001) predicted that livestock production in developed countries would become more industrialized to satisfy the increasing global demand for food; and specifically animal protein. As these changes affect the contact pattern between herds, and because these contacts are a driving force for the spread of infectious diseases, the changes are expected to affect the spread of infectious disease that are of global concern, such as FMD. This emphasises the need for regular evaluation of strategies to control such diseases to mitigate their economic and social impacts.

The disease spread model predicted that the herd type in which epidemics are initiated would affect the consequences of the epidemics, regardless of the year of initiation. For instance, epidemics starting in cattle herds were predicted to be larger and result in greater economic damage than epidemics initiated in swine and sheep herds, whether 2006/2007 or 2018 data was used (Fig.3). This is because cattle herds have higher frequency of contacts than the other herd types, combined with the fact that these herds have substantially lower biosecurity than swine herds (see details model inputs in Boklund et al., 2013 and Halasa et al., 2015).

The model predicted that the epidemiological consequences of an FMD incursion into Denmark, using the 2018 data, would be substantially smaller than an incursion using the 2006/2007 data (Fig. 1 and Fig. 2). Nevertheless, the economic effects were predicted to be substantially larger when epidemics were initiated in 2018 (Fig. 3). These losses are driven by export losses (results not shown). This indicates that export patterns that perhaps are affected by politics and trade affect the economic consequences of an epidemic; such drivers should be considered in real-life decision-making, but are probably harder to predict within a modelling study.

Based on the 2006/2007 data that was used to initialize epidemics, the Dep strategy was the optimal strategy, in terms of number of infected herds, epidemic duration and economic losses, compared to all of the simulated strategies, regardless of the herd type in which the epidemics were initiated. Nevertheless, based on the 2018 data, the best strategy for controlling epidemics differed, depending on the herd type in which the epidemics were initiated (Fig. 3). For instance, when epidemics were initiated in cattle herds, the Dep strategy was predicted optimal, whereas when the epidemics were initiated in swine or sheep herds, no difference was predicted between basic, Dep and VTK. This demonstrates that the optimal control strategy might be affected by the changes in the livestock industry, which in turn emphasizes the need to regularly update contingency plans.

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THE ROLE OF LIVE BIRD TRADING NETWORKS IN SHAPING AVIAN INFLUENZA TRANSMISSION DYNAMICS IN BANGLADESH

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SUMMARY

Avian influenza viruses (AIVs) are endemic in Bangladesh, where poultry are traded alive in live bird markets (LBMs). Yet the country's poultry trading networks, within which LBMs are embedded, have never been described and their role in AIV spread and maintenance scarcely studied. A cross-sectional study, involving 2,000 poultry traders was conducted in the two largest cities of Bangladesh to assess the potential for poultry trading practices to promote AIV maintenance and dissemination, and to identify tailored surveillance and control measures. As a result of the management of marketed chicken populations and of the configuration of the networks shaped by transactions, AIV amplification likely starts as poultry are transported from farms to LBMs while viral reassortment most likely occurs at LBMs. This detailed understanding of a livestock population dynamics allowed the design of risk mitigation interventions to be truly tailored to the networks' characteristics.

INTRODUCTION

The first outbreaks caused by HPAIV H5N1 in Bangladesh occurred in 2007 (Biswas et al., 2008), The different species of poultry produced in Bangladesh are chickens, ducks, geese, and pigeons. The main chicken types produced are (i) exotic broiler chicken breeds, such as Hybro-PN, Hubbard classic, Ross, Cobb 500 (Dolberg, 2008), (ii) sonali chickens, a crossbreed between a Fayoumi female and a Red Island Red male (FAO, 2015), and (iii) deshi, or local, chicken breeds (Bhuiyan et al., 2005). Poultry farming systems are each associated with specific types of poultry: while broiler and sonali chickens are essentially raised in commercial farms, ducks, geese, pigeons and deshi chickens are almost exclusively raised as backyard poultry in semi-scavenging, subsistence farming systems (Dolberg, 2008). In addition, poultry types and their corresponding farming systems are spatially segregated. Because they are the most traded poultry types, the results presented here focused on broiler, sonali and deshi chickens.

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MATERIALS AND METHODS

Data collection

The study area consisted of the two largest cities of Bangladesh, namely Dhaka city corporation (DCC) and Chattogram City Corporation (CCC). Together, CCC and DCC accounted for 9% of the country's total human population (BBS, 2014). Dhaka and Chattogram districts were respectively the first and second largest commercial chicken producing districts and the second and third largest backyard chicken producing districts in Bangladesh (Dolberg, 2008).

Using a snowball sampling approach, we identified 55 LBMs in CCC and 124 in DCC. In CCC, all LBM vendors operating in all the identified LBMs were recruited, while in DCC that would have been impractical given the much larger number of vendors. As a result, 30% of the small LBMs, with less than 10 vendors (n=39), 50% of the medium size LBMs, with 10 to 30 vendors (n=15), and all large LBMs, with more than 30 vendors (n=10), were included in the study. All vendors operating in small and medium size LBMs were recruited. In large LBMs, the number of LBM vendors to be randomly recruited and interviewed was determined in order to be 95% confident of identifying 90% of the connections between the LBM and the origins of poultry sold at the LBM (Fournie et al., 2016). Mobile vendors (or middlemen) were identified during LBM vendors' interviews. Indeed, interviewed LBM vendors provided, as part of their interview, the contact details of the mobile traders (or middlemen) who supplied them. These mobile traders were subsequently interviewed and, using the same mechanism, so were their suppliers until the identified sources of poultry were farms rather than traders.

Structured questionnaires were developed for mobile traders and LBM vendors, and a cross-sectional study was conducted from March to September 2015. Questions covered the following topics: buying and selling frequency, numbers of poultry traded according to their origins and destinations, surplus management and prices. Questions were asked for each type of poultry traded, and related to trading patterns in the past 7 days.

The study was approved by the Royal Veterinary College Ethics and Welfare Committee and the Chattogram Veterinary and Animal Science University (CVASU) Ethics Committee. Each questionnaire was double-entered and checked using EpiData 3.1. Data was then imported into R 3.5.1 (RCoreteam, 2013) for analysis.

Data analysis

Based on the data, we estimated the chains of transaction through which chickens transited from farms to the surveyed LBMs.

The number of poultry purchased by end-users was first estimated. To do so, the number of chickens that LBM vendors reported selling to end-users was used as a reference. For each LBM vendor, a value was randomly chosen between the minimum and maximum number of chickens they reported selling to end-users. Secondly, the number of chickens purchased by those vendors from any possible origin was estimated, ensuring that the total number of chickens purchased was equal to the number sold. The number of chickens sold and purchased by upstream actors were thus sequentially defined until reaching the producing farms. The average proportions of chickens supplied by each upazila (i.e. subdistrict) were mapped using R.3.5.1 (RCoreteam, 2013) and its libraries maptools (Bivand and Lewin-Koch, 2017), maps and mapdata (Richard et al., 2016).

Marketing time, defined as the time elapsed between the moment chickens were sold at the farm gate and the moment they were purchased by an end-user at a LBM located in the study area, was estimated as the sum of the lengths of time that chickens spent with each successive actor involved in chicken trade. It was estimated in order to assess the potential for viral amplification to take place between the end of the production cycle and chickens' purchase by an end-user. The marketing time was therefore defined as the sum of:

- The transport period, defined as the length of time required to transport chicken from the farm gate to the place where it was offered for sale for the last time, in DCC or CCC. This was exclusive of the time for technical stops, (un)loading chickens, etc.
- The selling period, defined as the length of time during which a chicken was offered for sale, by each LBM vendor and mobile trader.
- The storing period, defined as the length of time during which a chicken was stored before being offered for sale, by each vendor.

A catchment area was defined as the proportions of chickens sold in a location (i.e. a given LBM or city), which were sourced in each upazila of the country. As defined in Eq. (1), the level of overlap between two catchment areas p and q was assessed using the niche overlap index, as defined by Pianka (Pianka, 1974):

$$\frac{\sum_n^i p_i q_i}{\sqrt{\sum_n^i p_i^2 \sum_n^i q_i^2}} \tag{1}$$

where p_i and q_i are the proportions of chickens supplied by farms located in upazila i for two different catchment areas. If the index is equal to 0, the two catchment areas did not overlap at all, whereas an index of 1 meant that they were identical. In order to determine if the level of overlap observed between two catchment areas was greater than what would be expected by chance, it was compared to indices obtained by random permutations. If less than 5% of the indices generated through the permutation procedure were as high as, or higher than, the observed index, the two compared catchment areas overlapped more than what would be expected by chance. R package EcosimR (Gotelli et al., 2015) was used to calculate Pianka's niche overlap indexes and to carry out the permutation test.

Pairwise comparison of catchment areas was conducted between CCC and DCC to assess whether different types of chickens supplied to these two cities were sourced from the same geographical areas. Pairwise comparison of catchment areas was also conducted between LBMs located within the same city in order to assess whether LBMs in a given city were supplied by the same geographic areas, or whether different LBMs sourced their chickens from different geographic areas.

In order to assess the role of LBM networks in the spread of AIVs, networks of contacts between LBMs within DCC and CCC were constructed. Networks were built for each chicken type and city. As mobile traders and vendors visited several LBMs without applying any bio-security measures as they left or entered a LBM, their vehicles, equipment or themselves could act as fomites, spreading pathogens as they moved between LBMs. To account for this, any two LBMs were assumed connected if they were visited by a given vendor or mobile trader within the previous 7 days. The networks were undirected and unweighted. Node-level centrality measures and network-level characteristics were computed for each city- and chicken type-specific network. This analysis was carried out using the packages “sna” (Carter, 2008) and “igraph” (Csardi and Nepusz, 2006) in R (RCoreteam, 2013).

RESULTS

There were 8.4 times more chickens sold to end-users in DCC (n≈3.6 million) than in CCC (n≈400 000). While 62.2% of chickens sold in DCC were sonalis, with broilers and deshis only accounting for 18.7% and 13.7% of the sales, most chickens sold in CCC were broilers (63.6%).

The number of actors involved in chicken trade varied according to the type of chickens traded, from an average of two actors for broilers and sunalis, to three for deshis. However, this difference was not significant (p>0.1, Kruskal-Wallis test). In DCC, chickens were more likely to transit through more actors than in CCC, as wholesalers, defined as LBM vendors who sold at least some chickens to other LBM vendors, played a bigger role in poultry trade. Indeed, the proportion of chickens handled by wholesalers in DCC was at least three times higher than the proportion of chickens handled by wholesalers in CCC, where LBM vendors usually purchased chickens directly from mobile traders, thus by-passing wholesalers.

All the interviewed LBM vendors sold their chickens in one single LBM, but some bought their chickens from several origins. For any chicken type, almost all (>96.2%) LBM vendors operating in CCC purchased chickens from mobile traders who visited their own LBM. Very few of them bought chickens in other LBMs (<0.6%), or from other LBM vendors (<3.8%). In contrast, in DCC, several LBM vendors purchased chickens from other LBM vendors operating in the same LBM as theirs (>19.3% for sonalis and deshis and >4.6% for broilers), and/or visited other LBMs than their own (>40%, for any chicken type). LBM vendors from both cities hardly ever purchased chickens directly from farmers. Finally, the networks through which each specific type of chicken was traded remained mostly separated until they intermingled in LBMs. Indeed, two-thirds of LBM vendors sold at least two chicken types daily, whereas almost all (96%) mobile traders traded only one type of chicken per day.

Depending on the type of chickens, 58% to 100% of LBM vendors reported having surplus at least once a week. In DCC fewer mobile traders had surplus at least once a week (2% to 19% depending on the type of chickens) than in CCC (56% to 83%, according to chicken type). When chickens were left unsold at the end of the day, the median number of chickens left unsold and offered for sale the following day varied from 6% to 26% according to the type of actor, chicken, and city. In both cities, almost all (>97%) traders purchased chickens daily, except mobile traders selling deshis in CCC, a third of them purchased their chickens less frequently.

The complementary cumulative distributions of the number of days elapsed between the moment chickens left their farms and were purchased by end-users at DCC or CCC LBMs are presented in Fig. 1. Half of the broilers and sonalis sold in DCC and CCC LBMs were purchased by end-users within 19 to 21 hours after having left their farm. Total marketing time of sonalis and broilers did not vary significantly according to the city where they were sold (Mann-Whitney U, p=0.21 (broilers) and p=0.24 (sonalis)). Deshis were marketed for significantly longer hours than sonalis and broilers, regardless of the city where they were sold (Mann-Whitney U, $p<10^{-15}$ for each pairwise comparison). All chicken types sold in DCC were transported over significantly greater distances than chickens sold in CCC (Mann-Whitney U,

$p<10^{-15}$). For both cities, broilers were transported over significantly shorter distances than sonalis and deshis (Mann-Whitney U, $p<10^{-15}$), and deshis were transported over significantly greater distances than sonalis (Mann-Whitney U, $p<10^{-7}$). Most broilers were sourced from less than 50km and 80km away from CCC and DCC, respectively, whereas most deshis supplied to DCC and CCC were transported over more than 260km and 150km, respectively.

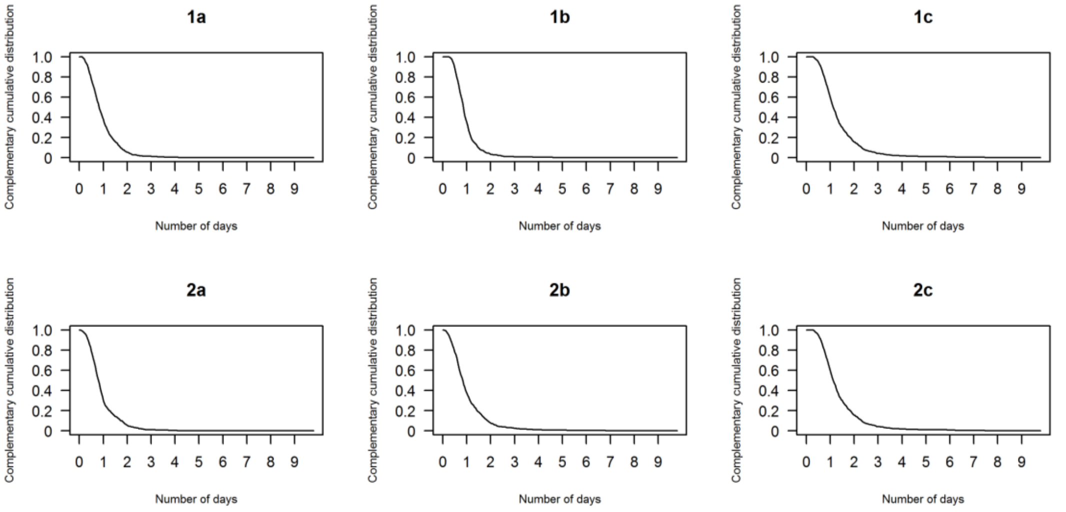

Fig. 1 Complementary cumulative distribution of the number of days between the moment broilers (a), sonalis (b) and deshis (c) were sold at the farm gate and the moment they were purchased by a customer at a LBM in Dhaka city (row 1) or Chittagong city (row 2).

Also, the geographical locations of supplying farms varied according to chicken type and city. Maps presenting catchment areas for each chicken type and city are presented in Fig. 2. CCC's catchment areas greatly overlapped for all three chicken types. Most chickens supplied to CCC originated from Chattogram district (59.5% of broilers, 24.4% of sonalis and 13.6% of deshis) or neighbouring districts (40.1% of sonalis and 53.9% of deshis). There was no – or limited – overlap between DCC's and CCC's catchment areas, as most of the chickens supplied to DCC originated from the centre and north-west of Bangladesh. In contrast to CCC, none of the chickens supplied to DCC were sourced from Dhaka district, and different areas supplied (i) broilers, and (ii) sonalis and deshis. Three districts located in the north or east of DCC (Gazipur, Mymensingh and Narshingdi) supplied 75.8% of broilers sold in the capital city's markets and three north-western districts (Bogra, Dinajpur and Joypurhat) supplied 81.2% of the sonalis. Origins of deshis were more scattered over northern and north-western districts, with no district accounting for more than 13% of deshis sold in DCC. The catchment areas of both sonalis and deshis supplied to DCC significantly overlapped.

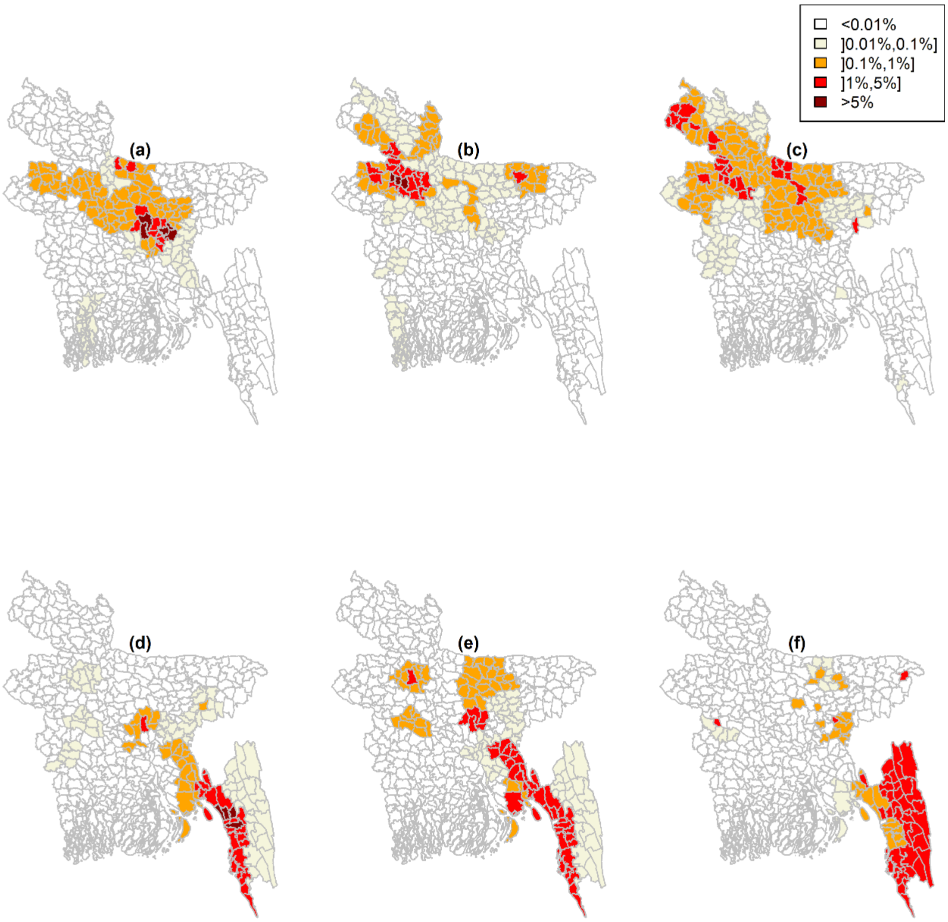

Fig. 2 Catchment areas of Dhaka city (a, b, c) and Chittagong city LBMs (d, e, f), for broiler (a, d), sonali (b, e) deshi (c, f) chickens. Upazilas were coloured according to the proportion of chickens they supplied: the darker the upazila, the more chickens it had supplied to city LBMs.

Within DCC, there was a high level of overlap between the catchment areas of most LBMs, for all types of chickens. Similarly, in CCC, the catchment areas of most LBMs greatly overlapped for broilers and sonalis, but the geographic origins of deshis seemed to differ according to LBMs. The movement of poultry traders between LBMs and farms could explain the high overlap of catchment areas observed here.

In CCC, most mobile traders sold chickens in several LBMs each week, contrary to DCC where most mobile traders sold chickens in one single LBM each week. For example, CCC mobile traders delivered broilers to 3 to 15 LBMs per week, with 55.8% of them supplying broilers to 6 LBMs/week. In DCC, mobile traders sold broilers to up to 4 LBMs/week, but 56.6% only supplied broilers to one LBM/week.

In DCC, networks included only one weak component, which included at least 71% of the LBMs – 78.6% (n=55) of the LBMs for the broiler network, 71.2% (n=42) of the LBMs for the sonali network and 75.5% (n=40) of the LBMs for the deshi network. In CCC, the sonali network (48 LBMs) included 2 weak components, one of 31 (64.6%) LBMs and one of 4 (8.3%) LBMs. In broiler and deshis networks, all connected LBMs were included in a single weak component of 51 (92.7%) and 23 (50%) LBMs respectively. For all DCC networks and sonali and deshi networks in CCC, distributions of degrees were right-skewed while for the broiler network in CCC, the degree distribution was approximately symmetric. In all chicken type-specific networks built in DCC, average path lengths were always significantly shorter than in simulated random networks and the clustering coefficients were significantly greater in the observed networks than in simulated random networks. These characteristics are typical of small-world networks. In all three observed chicken type-specific weakly connected networks built in CCC, average path lengths and clustering coefficients were significantly greater than those obtained in the simulated random networks.

DISCUSSION

According to our findings, the networks through which each specific type of chicken was traded remained mostly separated until they intermingled in LBMs, where chickens rarely spent more than 48 hours. Chickens offered for sale at DCC LBMs mostly came from different geographic areas than those sold at CCC LBMs but, within a given city, all LBMs shared similar catchment areas due to the high connectedness and small-world properties of within-city trading networks.

Several studies have hypothesised that higher prevalence of AIVs in poultry in Bangladeshi LBMs than in farms (Negovetich et al., 2011; Turner et al., 2017; Hassan et al., 2018; Kim et al., 2018) resulted from viral amplification taking place in LBMs, where poultry from diverse origins would be kept at high density for enough time to become infected and shed virus at the LBM, thus resulting in poultry populations offered for sale becoming viral reservoirs (Webster, 2004; Cardona et al., 2009; Fournié et al., 2011). However, according to results presented here, 50% of broilers, sonalis and deshis were sold by LBM vendors located in DCC and CCC in less than 17 hours, and almost all were sold within 2 days. Additionally, most studies have reported viral shedding of AIVs one to two days post inoculation (Spickler et al., 2008; Gonzales et al., 2012; Kang et al., 2018), and one study estimated that latent periods for H5N1 could be as short as 6 hours in unvaccinated chickens (95% confidence interval: 2-12 hours) (Bouma et al., 2009). Given such short latent periods, and the time we estimated chickens spent at LBMs, although viral amplification may occur in LBMs it is unlikely sufficient to explain the large increase in AIV prevalence observed between poultry in farms and offered for sale at LBMs. Our findings suggest, as did previous studies (Pepin et al., 2013; Fournie et al., 2016), that viral amplification may be initiated before poultry introduction into LBMs - at the very end of the production cycle, during transport and storage, either at the farm, or when mobile traders transport chickens to the LBMs.

As high levels of AIV prevalence (Kim et al., 2018) and strain diversity (Gerloff et al., 2016; Turner et al., 2017) in Bangladeshi LBMs may result from viral amplification occurring during poultry collection and transportation, and not only in LBMs, control measures should be implemented at all stages of the value chain: before poultry's arrival at LBMs in order to prevent viral amplification, and at LBMs to limit the consequences of viral amplification that occurred before poultry's arrival. For example, mobile traders should not enter farm premises, vehicles should be sanitised between each transport, and flocks from different farms should not be transported simultaneously. Then, at LBM level, several control measures could be implemented to limit the consequences of viral amplification which occurred previously. Since AIV strains can be maintained for over four days in humid environments such as LBMs (Stallknecht et al., 1990), daily introductions of new poultry shedding AIVs in the absence of daily sanitisation may quickly result in highly contaminated LBM environments which then become a public health threat for customers and workers, and a possible source of contamination for farms as traders and their vehicles may act as fomites as they move between LBMs and farms without applying biosafety measures (Nasreen et al., 2013; Ma et al., 2014; Chen et al., 2015; Nasreen et al., 2015). Measures such as wearing personal protective equipment, designating slaughter areas, and weekly closure days coupled with thorough disinfection, in the absence of poultry, as previously recommended (Lau et al., 2007; Fournié et al., 2011), could reduce human exposure to AIVs and/or reduce the levels of environmental contamination.

If, as suggested by our findings, (i) viral amplification mainly takes place before poultry's arrival at LBMs, (ii) poultry type-specific value chains remain isolated until poultry's arrival at LBMs, where poultry of different types are caged in close contact, and, as suggested by the literature (iii) different viral strains circulate, at different prevalence, in different production systems and geographic areas (Kim et al., 2018) then, at LBMs, poultry arrive excreting viruses specific of their geographic area and production system. Consequently, viral reassortment between strains circulating in distinct geographical areas and production systems is more likely to occur at LBMs than at farms or during transport to LBMs. Gerloff et al. (2016) identified a great diversity of AIV strains circulating in Bangladeshi LBMs , which is likely a result of the diverse origins of poultry offered for sale at LBMs, and a risk factor for viral reassortment. The specialisation of LBMs or LBM stalls according to poultry type would decrease the amount of contact between poultry from different geographic origins and production systems, and, consequently, the likelihood of viral reassortment. Indeed, although many poultry may not stay long enough at the LBM to (1) become infected with different viral strains and (2) shed viruses within the LBM, certain poultry, which stay longer at LBMs, could play a role in viral reassortment.

To the best of our knowledge, this study represents the first quantitative estimation of catchment areas of broilers, sonalis and deshis sold at LBMs located in DCC and CCC. These maps revealed that the production areas of chickens (i.e. catchment areas) sold in DCC LBMs hardly overlapped with the production areas of chickens sold in CCC LBMs. Since AIV strains circulating in Bangladesh appear to have different, although partially overlapping, geographic distributions (Gerloff et al., 2016; Khan et al., 2018), and under the hypothesis that viral strains identified at a city's LBMs provide information on the strains circulating in the city's catchment area, the population of viruses circulating in LBMs in DCC and in LBMs in CCC may be different in terms of viral strains, and prevalence. Therefore, if surveillance is aimed at capturing the diversity of viral strains currently circulating in the country then it should be carried out in DCC, CCC, and in other cities that have different, yet complementary, catchment areas to increase the likelihood of identifying as many different strains that circulate in the country as possible. Furthermore, this study showed that within each city, LBMs' catchment areas were, overall, not very heterogeneous and, within each city, LBMs were shown to be highly connected through the movements of traders. In such highly connected networks, which even expressed small-world properties in DCC, pathogens are expected to spread rapidly. As a result, all the LBMs within a city are expected to harbour very similar viral populations. It would therefore not be necessary to sample large proportions of LBMs in each city to gain a full overview of viral strains circulating in the city, and their catchment areas.

This study had some limitations. In the absence of a registry for mobile traders, our selection method relied on LBM vendors naming their suppliers. This method could have been subject to memory bias and we could not compare non-interviewed traders' practices with those of interviewed traders to qualify the consequences of our selection method. As a result of this memory bias, the sample may have been biased towards the mobile vendors who sold the largest numbers of chickens, to the largest number of LBM vendors. Consequently, the number of actors involved in poultry trade may have been underestimated, practices of occasional mobile traders may have been missed, network connectivity may have been underestimated and catchment area maps might have been biased towards an under-representation or over-representation of some upazilas depending on whether the non-interviewed mobile traders purchased from different or similar upazilas than the interviewed mobile traders. Secondly, catchment area maps were influenced by traders' knowledge of poultry origins. Indeed, interviewed traders were only able to provide the names of the upazilas where they purchased poultry, rather than the names of smaller administrative divisions. Additionally, up-to-date, accurate, and chicken type specific farm density maps were not available. As a result, catchment area maps could only be built using information provided by traders and their level of resolution could not be greater than the upazila level. Finally, in DCC, unlike in CCC, some small and medium LBMs were not included in the study. Exclusion of such LBMs may have led to the underestimation of LBMs' centrality measures. However, given the network structure, the high proportion of LBMs included in the study, and the homogeneity of trading patterns observed in such small and medium LBMs, the conclusions are expected to be a reliable representation of the main trading practices and network's characteristics.

To conclude, our findings suggest that the observed pattern of viral amplification from farms to LBMs, mostly resulted from viral amplification occurring before chickens reached LBMs. Moreover, the networks through which each specific type of chicken was traded remained mostly separated until they intermingled in LBMs. Also, as the geographical locations of supplying farms varied according to chicken types each chicken type delivered to LBMs may be associated with contrasting viral populations circulating in the specific farming systems and geographical areas from which they originate. Shortening and sanitising poultry value chains, and separating poultry types in LBMs, could, therefore, reduce AIV prevalence in LBMs, the likelihood of viral reassortment, and their zoonotic threat. Although catchment areas varied between DCC and CCC, all LBMs within a given city shared similar catchment areas due to the high connectedness and small-world properties of within city trading networks. Consequently, the population of viral strains circulating across a given city's LBMs are expected to be homogeneous, and effective monitoring of the genetic diversity of viral strains circulating in the country should target a few LBMs in several cities.

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DISEASE CONTROL PROGRAMS

GENERATING EVIDENCE FOR A BRUCELLOSIS CONTROL PROGRAM FOR INDIA

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SUMMARY

Brucellosis is a neglected zoonotic disease prevalent in several developing countries. This study was conducted to investigate the feasibility and acceptability of various brucellosis control strategies in India. Focus group discussions and key-informant interviews were conducted with 119 individuals including veterinarians, para-veterinarians, veterinary academics, farmers and some other stakeholders. Vaccination with *Brucella* strain 19 vaccine was considered feasible and acceptable, but the stakeholders were concerned about its non-availability, the risk of self-inoculation, the inability to vaccinate pregnant and male animals, the difficulty to differentiate vaccinated from diseased animals and the challenges of maintaining the vaccine cold chain in India. The test-and-cull and the test-and-segregation approaches were not considered feasible but improving personal protection and farm biosecurity were likely to be acceptable. A 10-point control program for brucellosis suiting Indian culture and norms was developed to improve livestock and human health using a One Health approach.

INTRODUCTION

Bovine brucellosis is a neglected zoonotic disease caused by *Brucella abortus* resulting in infertility and abortions in domestic animals and causing fever, chills, back-ache, fatigue and muscle pain in humans. Although some developed countries have successfully controlled or eradicated the disease (Lehane, 1996; Davidson, 2002), it is still prevalent in most developing countries (Zhang et al., 2018) including India (Renukaradhya et al., 2002). The successful control programs that used test-and-cull strategies in the West cannot be implemented in India because of various cultural and religious issues, including a ban on cow slaughter in most states of the country as they are considered sacred by Hindus.

We conducted several studies in the past five years in India to understand the epidemiology of bovine brucellosis with the overall aim of developing a context-based brucellosis control program that respects local culture and norms. Specifically, we identified that the disease is highly prevalent among veterinary personnel and that para-veterinarians are at a greater risk of contracting the infection (Proch et al., 2018). To strengthen the case for establishing a brucellosis control program, we estimated the economic loss due to the disease to the livestock

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industries (Singh et al., 2015) and the health and socio-economic impact of the disease in the human population (Singh et al., 2018a).

Further, we conducted a cost-benefit analysis of various control strategies to investigate whether the disease could be controlled without culling and whether such a program would be economically viable (Singh et al., 2018b). Some approaches were found to be credible under the investigated modelling scenarios but their suitability to the stakeholders was unknown.

This study was conducted to identify brucellosis control strategies suitable for Indian society. In this study, we conducted focus group discussions (FGDs) with field veterinarians, veterinary academics and farmers and key informant interviews (KIIs) with some other stakeholders. It is anticipated that besides helping control the disease in India, the systematic approach used to investigate disease epidemiology and control strategies in India could also be used in other countries to develop control programs suiting local cultures rather than trying to transplant programs successful in the West.

MATERIALS AND METHODS

Study area

The study was conducted in the Punjab state of India, located in the north-west of the country, on the border of Pakistan. The state is traditionally divided into three regions (named *Majha*, *Malwa* and *Doaba*) based on river boundaries and dialects spoken in these areas. It is predominantly an agricultural state, with rice and wheat cropping as the primary occupation. Dairy farming is mostly conducted as a secondary occupation to produce milk for personal consumption or to supplement agricultural income. Nearly 80% of dairy farming is undertaken by smallholders as a backyard enterprise (NSSO, 2013), although professionally managed larger dairy farms are also being established lately.

Selection of study participants

The ethics approval to conduct this study was obtained from the Human Ethical Research Committee, Dayanand Medical College and Hospital, Ludhiana (approval number DMCH/R&D/2018/273).

The field veterinary workforce was selected from three sub-districts – one from each of the three regions of the state – to capture any differences of opinions in these regions. Government Veterinary Officers in charge of the selected districts/sub-districts were contacted and requested to participate in the study. They invited other government veterinarians and para-veterinarians to the venues selected for FGDs, in conjunction with their regular monthly meetings. Veterinary academics were selected from the only veterinary university in the state (GADVASU) and included experts from the departments of Veterinary Medicine, Public Health and Zoonoses, Veterinary Microbiology, Animal Disease Research Centre, Veterinary Extension and Dairy Technology. Farmers were selected from three GADVASU farmer training workshops. GADVASU routinely organises such workshops that are attended by dairy farmers from across the state. KIIs were conducted with five purposively selected key informants to obtain additional insights about disease control and into the functioning of the dairy farming system. The participants included a commercial cattle farmer, a commercial buffalo farmer, a milk processor making value-added products and two milkmen. Milkmen are workers who collect raw fresh milk from farmers and sell it to consumers daily, usually by visiting pre-identified households.

Data collection

FGDs and KIIs were conducted in a comfortable environment in the local language (*Punjabi*). The participants were briefed about the study objectives and asked to provide written consent to participate in this study. All the participants were required to be more than 18 years old, working/living in Punjab and be able to understand the *Punjabi* language.

After an initial introduction about the study, the participants were asked to identify various value chains and risk nodes for transmission of infectious diseases, with a focus on brucellosis (data not presented in this manuscript). This was followed by a discussion about potential control options for brucellosis. The issues raised by the participants about each of the potential control options were noted and the FGDs were audio recorded. Additional data about demographics and the participants' perceptions about various control strategies were collected using a custom-designed questionnaire (available from the authors on request).

Data management and analysis

Written notes of FGDs were transcribed and translated into English. Flip-charts and audio-recordings were carefully reviewed to include any uncaptured data. The issues identified by the participants were reviewed by two co-authors (JS and HS) and refined after further discussion among co-authors. The audio recordings were listened to again if required during the preparation of this manuscript. Questionnaire data were transferred to excel for further quantitative analysis. Frequency tables were prepared for categorical variables, and frequencies and relative frequencies calculated. Graphs were prepared in the R software environment (R Core Team, 2013).

RESULTS

Seven FGDs with 114 participants were conducted, including (a) three FGDs involving 41 veterinarians and 15 para-veterinarians, (b) one FGD involving 12 veterinary academics, and (c) three FGDs involving 46 farmers. In addition, KIIs were conducted with five personnel. Majority of the participants were male (111/119) and middle-aged (>41 years old, 38%). The young adults (>18 to 30-year-old, 31%) and adults (>30 to 40 years old, 31%) were equally represented. Majority of the participants (89/119, 75%) worked in rural areas but only about two-thirds (81/119; 68%) lived there.

The veterinary personnel were well qualified. Of the 68 veterinary personnel participating in FGDs, 42% were graduates, 18% had Master degrees, 18% had PhDs and 22% had Diplomas. They were also quite experienced with 17 years since graduation and 15 years in employment on average. Of the farmers, about half had an experience of <10 years (22/43, 51%), 3 (7%) between 11 and 20 years and 18 (42%) of >20 years. They owned a median of 5 cows (Q1 – Q3: 3 – 9) and 6 buffaloes (3 – 12) and 71% of them had attended at least one other training.

Information about the feasibility, effectiveness and acceptability of control options obtained from FGDs and KIIs is discussed below and the results from self-completed questionnaires are summarised in Fig.1. The findings from the two approaches broadly agreed, but not always.

Vaccination

The feasibility, acceptability and effectiveness of vaccination with *Brucella* strain 19 vaccine were considered better than other control strategies, but concerns were raised about its non-availability, the risk of self-inoculation, the inability to vaccinate pregnant and male animals, the difficulty to differentiate vaccinated from infected animals and the challenges of maintaining the vaccine cold chain in India. Veterinary personnel reported that only a small number of doses of the vaccine were supplied to government animal health centres/dispensaries for subsidised vaccination. Although the vaccine was also available at some pharmacies, the awareness about its use amongst farmers was considered quite low.

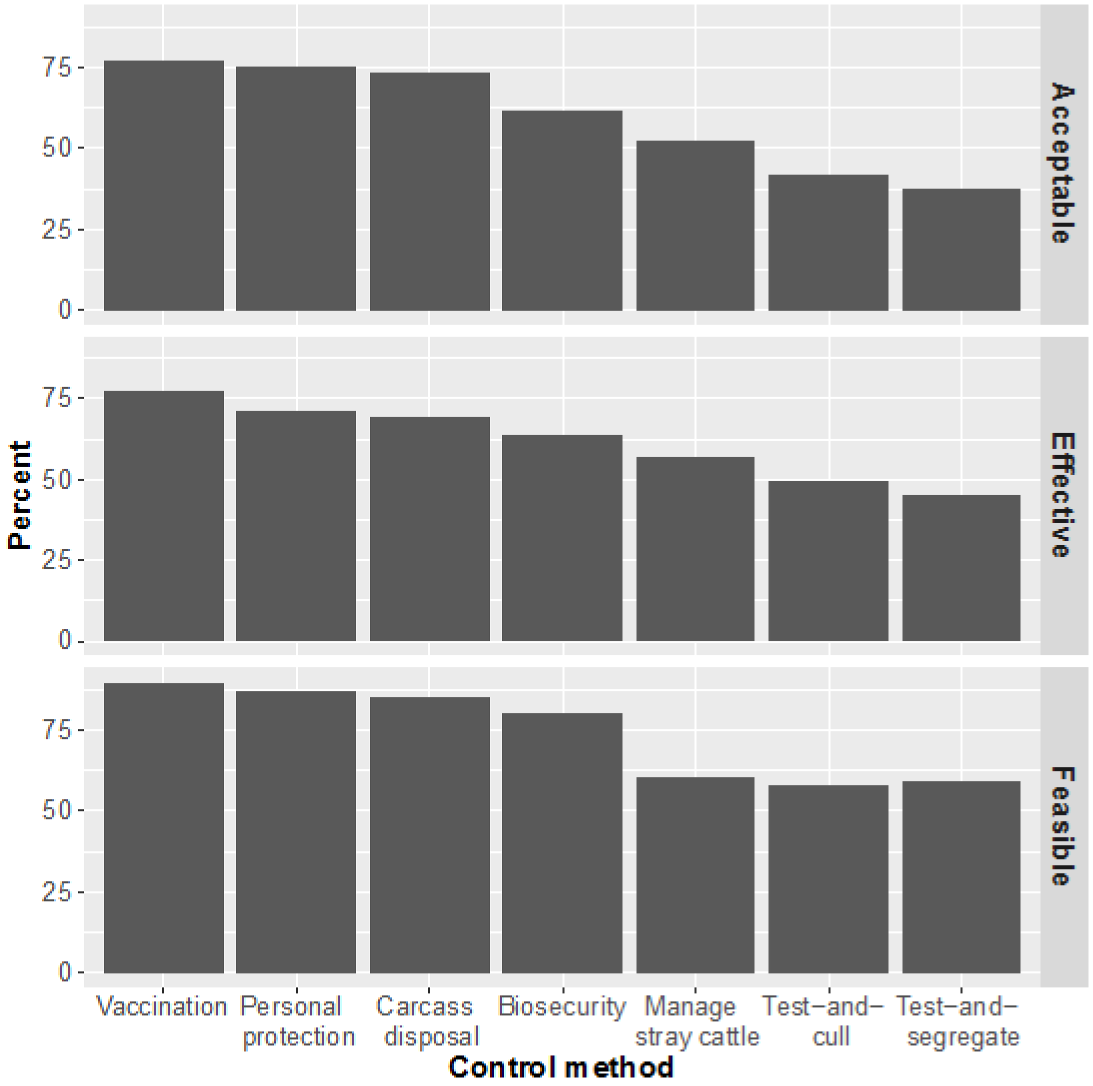


Fig. 1 Suitability of brucellosis control strategies as reported in seven focus group discussions and five key-informant interviews conducted with 119 stakeholders in India, 2018-19.

Even if the vaccine was available, some veterinary personnel were reluctant to vaccinate animals due to the risk of self-inoculation with the live vaccine. They were also concerned that the vaccine can potentially cause abortions if used in pregnant animals and the secretion of organisms in the semen if used in bulls, so effective coverage cannot be achieved. Further, some veterinarians mentioned that it was difficult to differentiate vaccinated from infected animals, so they were not sure what advice to give to the farmer whose vaccinated animals tested positive. One FGD group also reported the occurrence of fever in vaccinated calves. Another major concern was raised about the cold chain maintenance, which is essential for the live vaccine, but is challenging to implement under field conditions due to the non-availability of the refrigerators and the frequent power blackouts. However, despite these concerns, vaccination was still considered one of the most acceptable and effective approaches for brucellosis control.

Test-and-cull

The suitability of this approach was considered to be quite low. The major concerns about this approach were due to religious and cultural reasons: Hindus consider cows to be sacred and their slaughter is banned in most states. Many veterinarians/para-veterinarians were also not sure about the legal issues – i.e. whether they could legally recommend culling of a brucellosis positive cow. Regardless, they were not willing to do so due to the risk of conflict with the ultra-right-wing Hindu militant groups. The participants also observed that the uptake of the policy would be extremely low in the absence of any compensation for culling brucellosis positive cows, irrespective of the religious and legal issues.

Test-and-segregation

The suitability of this approach was not better than the test-and-cull approach. Its feasibility was low due to non-availability of places for segregation. Cow shelters (*gau shalla*) are operating in most towns and cities to keep senile and non-productive cows culled by farmers. However, these shelters are struggling to cope up with the number of cows brought in. The low acceptability of the approach was due to a lack of compensation for farmers as they would be reluctant to give away their animals for segregation without any compensation. Stakeholders were also concerned about the safety and well-being of animal handlers working in those shelters as they would be at high risk of contracting brucellosis. It was also considered to be logistically difficult to segregate animals on backyard farms due to physical area constraints, as livestock and the farmer's family share the same area.

Biosecurity

Biosecurity was defined in this study as measures taken to prevent the entry and spread of pathogens into a farm. The following biosecurity issues were discussed.

Animal introductions: Animal introductions were reportedly done without any testing, except in some large farms. However, veterinarians were of the view that all farmers should be encouraged to get their animals tested before purchasing them, or sellers should be required to declare the animal test status before selling them. This approach was considered feasible, but farmer education would be required to make it acceptable.

Insemination: Despite the wider adoption of artificial insemination, many farmers still prefer natural insemination, particularly for buffaloes. Bulls producing semen for artificial insemination in large corporative and government farms were required to be regularly tested for brucellosis, but such testing was not regularly done by small semen producing businesses or for the bulls used in villages for natural insemination. Some veterinarians were also concerned about the possible transmission of bacteria via insemination guns. Although disposable plastic sheaths are used to cover the metallic guns, veterinarians were concerned that vaginal/uterine fluids could potentially seep under the plastic sheath, and thus, contaminate the metallic gun, which was not regularly disinfected.

Movement between farms: As most of the farms are run in backyards and are managed by smallholders, there are virtually no restrictions on movements between them. Neighbours and friends – who are also backyard farmers – freely move between backyard farms, potentially transmitting pathogens between them. In particular, assistance from neighbours is sought for parturitions (which could include abortions besides normal deliveries) that could facilitate the transmission of brucellosis pathogens between farms.

Several movements between farms also occur due to the movement of service providers including milkmen, farriers, para-veterinarians and veterinarians. For example, veterinary personnel move between farms for pregnancy diagnosis, artificial insemination, treatment of sick animals and vaccination. It was not considered feasible to stop these movements, but the participants agreed that the use of hygienic practices among professionals and paraprofessionals could be improved.

Hygienic practices: The use of personal protective equipment (PPE) was reported to be negligible among professional and paraprofessionals. Handwashing with or without soap and the use of disposable arm sleeves are the only approaches commonly practised for pregnancy testing, artificial insemination and the removal of retained placenta. Similarly, hand washing is the only method routinely practised after completing a procedure at a farm/household and before moving to the next farm/household: the cleaning of shoes is rarely undertaken; overalls/gowns are rarely worn and thus not changed between farms; vehicle tyres (which are predominantly motorcycles/scooters) are seldom cleaned. All this facilitates the transmission of pathogens between farms/households.

Although the use of PPE is quite low, most of the participants believed that it was feasible to include hygienic practices in the mix of approaches for controlling brucellosis. However, they indicated that the PPE would have to be suitable for the hot weather, should not require a lot of effort or time to use and should not be expensive to be acceptable to the veterinary workforce. Availability of PPE and training in its use were considered as important issues reducing their uptake.

Carcass/foetus/placenta disposal: Veterinary personnel were concerned about poor practices of disposal used for the carcass, aborted foetus and placenta. Dead animals were reported to be taken to a knacker yard for deskinning and deboning and then left for the scavengers, mostly stray dogs, to eat. Many farmers were reported to dispose of the foetus/placenta on a rubbish dump (instead of burying/burning them), which could also end up as food for stray dogs. Dogs could potentially spread the pathogens to other farms/households they visit. The use of PPE by the knackermen was non-existent.

Stray cattle: All groups of stakeholders considered the stray cattle population as a serious issue and agreed that it was challenging to control this population in the presence of a ban on cow slaughter. Further, backyard farms were reportedly not properly fenced, which also enabled the contact of stray cattle/dogs with the farm animals.

Community grazing: Community grazing and watering increase the risk of transmission of infectious diseases, including brucellosis, but both of these practices were becoming less common in the study area, except among an ethnic pastoral community (*Gujjars*).

Other issues

Consensus could not be reached on milk pasteurisation. Many participants argued that milk pasteurisation should be compulsory and strictly implemented, whereas others thought that such regulation was unnecessary, given that most people boil milk before consumption.

Some participants observed that there was little coordination in public health, animal health and agricultural departments. No regular official meetings at the sub-district level were conducted, although some reported making efforts to do so at their personal level.

DISCUSSION

This study was undertaken to investigate the suitability of various brucellosis control options in India. FGDs and KIIs conducted with 119 individuals in 2018-19 enabled the identification of control strategies that could be implemented in the country. The discussions also highlighted the practices potentially increasing the risk of disease transmission and would have to be managed for better control of bovine brucellosis. The study also enabled us to identify ancillary practices that could be recommended for improving the success of a brucellosis control program. Based on the findings of this work (and our previous research) we propose a 10-point brucellosis control program for India (Box 1).

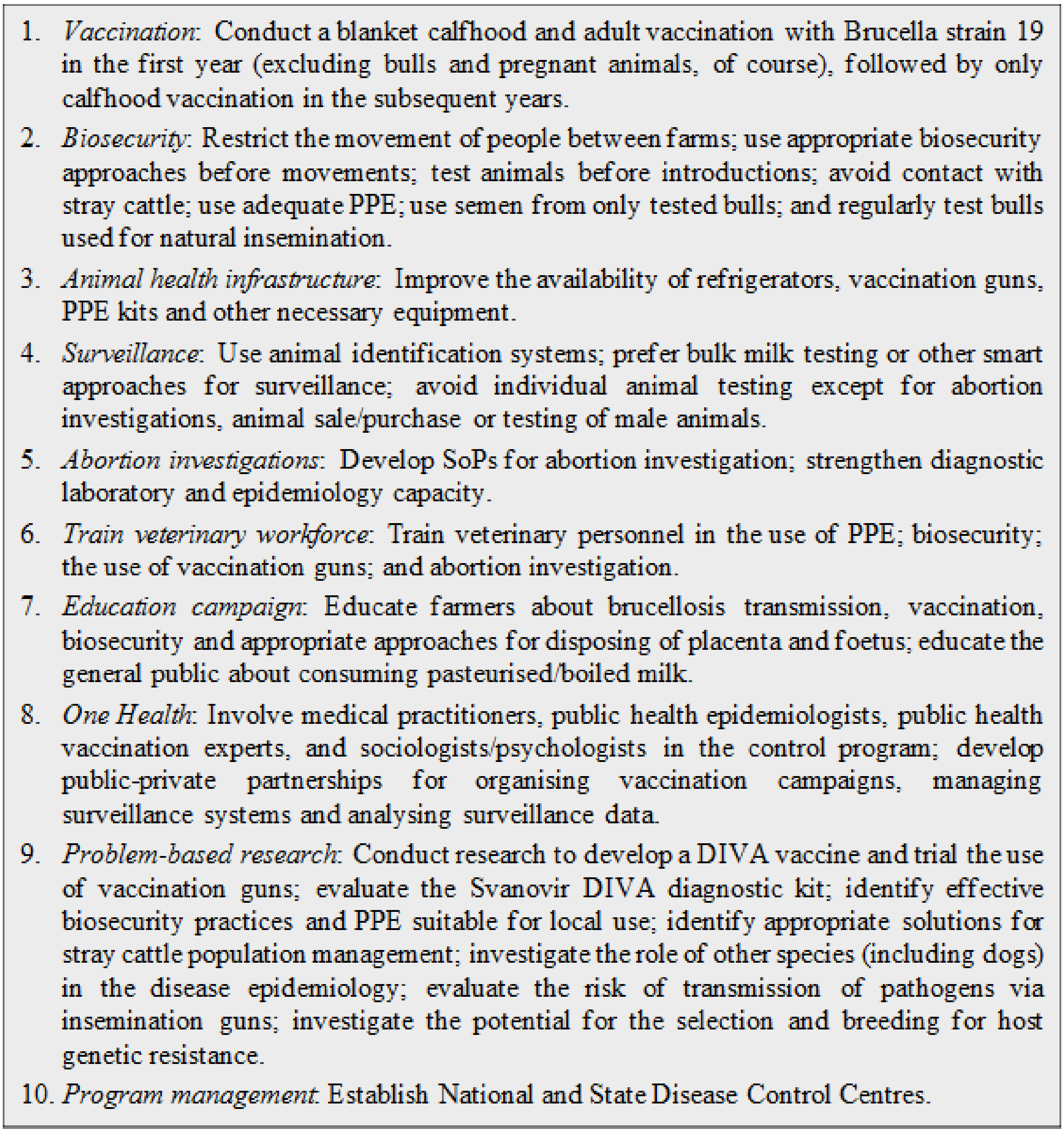
Brucellosis vaccination was identified as one of the most feasible and acceptable solutions for brucellosis control, although the participants did raise concerns about vaccine availability. This issue could soon be resolved as the re-elected Indian government decided in its first cabinet meeting to fund a nationwide brucellosis vaccination program (PTI, 2019). Vaccine availability, however, does not resolve other issues such as the risk of self-inoculation, the inability to vaccinate pregnant and male animals, the interference with diagnostics and the need to maintain the cold chain.

The risk of self-inoculation with the vaccine can be reduced by using vaccination guns and by producing larger vaccine vials. A similar issue of self-inoculation with the Gudair® vaccine for ovine Johne’s disease was resolved in Australia using vaccination guns, which significantly reduced the risk of self-inoculation. However, the guns will have to be trialled under Indian conditions to investigate if they work well in buffaloes with thick skins. A larger vial would reduce the need for repeated filling of the syringe, thus reducing the chances of self-inoculation and production of aerosols.

The concern of veterinarians and veterinary academics that it was difficult to differentiate vaccinated from infected animals could be resolved using a high specificity competitive ELISA that can potentially differentiate between vaccination and infection antibodies (Nielsen et al., 1995) (https://www.svanova.com/products/bovine/bp09.html). The test should be evaluated under real-life conditions in India but could have major benefits for the control program in alleviating concerns of animal health policymakers and veterinarians regarding the differentiation of infected from vaccinated animals.

A campaign launched by various state governments in the last few years to eartag animals will also complement the brucellosis control program by enabling the identification of vaccinated animals.

Box 1. The proposed 10-point brucellosis control program for India



1. *Vaccination*: Conduct a blanket calfhood and adult vaccination with Brucella strain 19 in the first year (excluding bulls and pregnant animals, of course), followed by only calfhood vaccination in the subsequent years.
2. *Biosecurity*: Restrict the movement of people between farms; use appropriate biosecurity approaches before movements; test animals before introductions; avoid contact with stray cattle; use adequate PPE; use semen from only tested bulls; and regularly test bulls used for natural insemination.
3. *Animal health infrastructure*: Improve the availability of refrigerators, vaccination guns, PPE kits and other necessary equipment.
4. *Surveillance*: Use animal identification systems; prefer bulk milk testing or other smart approaches for surveillance; avoid individual animal testing except for abortion investigations, animal sale/purchase or testing of male animals.
5. *Abortion investigations*: Develop SoPs for abortion investigation; strengthen diagnostic laboratory and epidemiology capacity.
6. *Train veterinary workforce*: Train veterinary personnel in the use of PPE; biosecurity; the use of vaccination guns; and abortion investigation.
7. *Education campaign*: Educate farmers about brucellosis transmission, vaccination, biosecurity and appropriate approaches for disposing of placenta and foetus; educate the general public about consuming pasteurised/boiled milk.
8. *One Health*: Involve medical practitioners, public health epidemiologists, public health vaccination experts, and sociologists/psychologists in the control program; develop public-private partnerships for organising vaccination campaigns, managing surveillance systems and analysing surveillance data.
9. *Problem-based research*: Conduct research to develop a DIVA vaccine and trial the use of vaccination guns; evaluate the Svanovir DIVA diagnostic kit; identify effective biosecurity practices and PPE suitable for local use; identify appropriate solutions for stray cattle population management; investigate the role of other species (including dogs) in the disease epidemiology; evaluate the risk of transmission of pathogens via insemination guns; investigate the potential for the selection and breeding for host genetic resistance.
10. *Program management*: Establish National and State Disease Control Centres.

PPE: Personal protective equipment; SoPs: Standard operating procedures; DIVA: Differentiation of infected from vaccinated animals.

Besides these improvements, the government will have to ensure that infrastructure is available for the maintenance of a cold chain. In the interim, it would be sensible to use the public health infrastructure which is in relatively better shape as India has successfully run various public health vaccination campaigns, such as for poliovirus (Arora et al., 2010). Administrative and field experience gained in these campaigns would also be beneficial for managing the brucellosis vaccination campaign. This would also increase collaboration and coordination between animal and public health, which was considered welcome in our FGDs.

Vaccination should be supported by the use of biosecurity approaches, many of which were considered to be feasible and acceptable during our discussions. In particular, animals should be tested before introduction to herd, movements between farms should be restricted, farmers/professionals should be educated about using biosecurity practices for movement between farms and the PPE use should be encouraged. Although it is easier said than done, these ancillary approaches are important to ensure the success of the control program, given that the test-and-cull and the test-and-segregation approaches were not found to be feasible or acceptable.

The ban on cow slaughter due to religious reasons has had several consequences. Although most farmers are smallholders, they are still running a business, and therefore, it is not economically viable for them to feed cows that become senile, unproductive or develop untreatable mastitis or infertility. They send out these animals to cow shelters, which are running above their capacity and often decline to take more animals. As a result, farmers are forced to abandon the animals which wander off on streets and roads, causing accidents and other hazards. Male calves that are not of any use or older male bulls that do not remain useful anymore because of infertility, also face the same consequences. These ‘stray’ cattle also routinely enter other farms for feed, water and shelter. They pose a risk of spread of infection to healthy animals given that many of these animals become stray in the first place because they are infertile, and could potentially be brucellosis positive. Stray animals would have to be controlled as part of any brucellosis control program. Research should be conducted to thoroughly evaluate the reasons and to identify the solutions for reducing stray cattle population. Farmers could also be encouraged to have good fences/barriers to prevent the entry of animals on to their properties, but it would require substantial investment. Note that this is not a problem for buffaloes, as unproductive and male buffaloes are sent for slaughter for meat purposes, as the slaughter of buffaloes is not banned in India.

Farmers should be educated about the proper disposal of foetus and placenta by burying or burning to prevent the spread of infection by dogs and other scavengers. We are not aware of any study conducted in India to investigate the role of dogs in the epidemiology of the disease, but hunting dogs have been reported to be infected with *Brucella suis* from infected feral pigs in Australia (Mor et al., 2016; James et al., 2017). Therefore, further studies should be conducted to clarify the role of stray dogs in spreading bovine brucellosis in India. Similarly, it is also possible that other species like sheep, goat and pigs may play a role in brucellosis epidemiology. Further studies are required to clarify this.

As expected, the test-and-cull approach was not favoured. Although according to the Prevention and Control of Infectious and Contagious Diseases in Animal Act, 2009 (GOI, 2009), state governments have powers to slaughter infected animals (and brucellosis is included in the diseases listed in the act), these powers are not often used due to religious and personal safety concerns as several people transporting cows for slaughter have been lynched by vigilante mobs in the past few years (Biswas, 2015).

In the absence of the test-and-cull approach, the test-and-segregation approach has been considered as an alternative in India for some time (Kollannur et al., 2007; Singh, 2010). Animal health policymakers have pondered if brucellosis positive animals can be segregated to cow shelters to reduce the risk of disease transmission (Lindahl et al., 2019). Although this approach makes sense at least theoretically, our results suggest that it is no different from the test-and-cull approach in terms of feasibility and acceptability. To make things worse, it increases the risk of brucellosis transmission among other animals in the cow shelter and among animal carers. Thus, this approach may not yield the desired results.

Without a clear policy and in the absence of compensation, individual animal testing would not be beneficial and could be detrimental because farmers could sell test-positive animals to unsuspecting farmers, actually resulting in the spread of infection. Therefore, a surveillance approach based on testing of bulk milk samples (Renukaradhya et al., 2002) or using veterinary workforce/animal handlers as sentinels (Dhand et al., 2018) should be preferred. This will allow the government to identify high prevalence regions or villages which can be targeted for brucellosis control in the initial stages. However, individual animals would have to be tested in the event of abortion or for confirming their status before sale/purchase. Legal issues concerning the culling of a brucellosis positive animal should be clarified. Policy decisions should be made at the central or state government level about the actions to be taken if an animal or a farm tests positive. Further, bulls should also be regularly tested for brucellosis and culled if found to be positive.

Preparedness work is required to develop standard operating procedures for abortion investigation. Currently, there is no standard list of differential diagnoses to follow or the samples to test in the event of an abortion. The availability of such lists and guidelines for sample collection for abortion investigation will help thorough epidemiological and laboratory investigations of abortions, which besides identifying the reasons for abortions, will also enable the development of an evidence-base of the reasons for abortions. This would require strengthening of the laboratory and the field epidemiology capacity in the country. A brucellosis control program would also require the setting up of program management and leadership structure (OIE, 2014), both at the national and state levels.

Farmer education would be an essential component of any control program. Farmers should be educated about the method of transmission of the disease, the use vaccination, and the use of biosecurity practices – especially, the restriction of movement of stray dogs/cattle and visitors onto the farm, the introduction of only test positive animals to the farm, the use of artificial insemination or only test negative bulls, and the use of appropriate biosecurity for movements between farms. However, just education is rarely sufficient (Mankad, 2016; Ritter et al., 2017); behavioural research would be required to understand the barriers to adopting these practices. It is obvious that professionals and experts from several sectors would be required for establishing a successful control program using a One Health approach.

Further research is required to develop a better quality vaccine, to investigate if vaccine guns can be used for vaccine delivery, to identify biosecurity practices suiting the Indian climate and conditions; to investigate the reasons for stray cattle problem and to identify solutions for their management; to develop protocols for abortion investigation; and to investigate the role of other species in brucellosis epidemiology in India. However, this does not mean that the brucellosis control program cannot or should not start until answers to these questions are available. The control should be instituted as soon as possible and then refined as further information is available.

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20:20 IN 2020 REFLECTIONS ON IDEAL: WHAT WE HAVE LEARNT FROM A UNIQUE CALF COHORT STUDY

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SUMMARY

The year 2020 marks a decade since the final visit was made in the ‘Infectious Diseases of East African Livestock’ (IDEAL) project. However, data generation from samples obtained during this ambitious longitudinal study still continues. As the project launches its extensive open-access database and biobank to the scientific community, we reflect on the challenges overcome, the knowledge gained, and the advantages of such a project. We discuss the legacy of the IDEAL project and how it continues to generate evidence since being adopted by the Bill & Melinda Gates Foundation (BMGF) funded Centre for Tropical Livestock Genetics and Health (CTLGH). We also examine the impact of the IDEAL project for each of the stakeholders (the animal, the farmer, the consumer, the policy maker, the funding body, and the researcher and their institution) involved in the project and provide recommendations for future researchers who are interested in running longitudinal field studies.

INTRODUCTION

Constraints on livestock production in Africa are varied and include nutrition, management, access to markets, natural catastrophes and importantly infectious disease (Perry and Grace, 2009). Studies of infectious diseases of livestock in sub-Saharan Africa have most usually focused on single specific infections such as trypanosomiasis or theileriosis / East Coast Fever (ECF) which are known to cause serious constraints to farming systems in the global south. However, livestock in extensive systems are routinely and simultaneously exposed to a wide variety of pathogens whose impacts on animal health are unlikely to be independent of one another (Petney and Andrews, 1998; Cleaveland et al., 2001), with evidence that pathogens interact to influence the outcome of the infection for the host or even the epidemiology of infecting pathogens (Cox, 2001; Lello et al., 2004). For example, in buffalo the presence of worms enhances bovine tuberculosis infection severity (Ezenwa et al., 2019). It is therefore necessary to study the entire burden of infectious diseases rather than single diseases on their own in order to accurately understand their full impact. The Infectious Diseases of East Africa

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Livestock (IDEAL) project was conceived to specifically address this problem (Bronsvoort et al., 2013).

The IDEAL project aimed to use a longitudinal cohort study of cattle to (1) address the underlying lack of baseline epidemiological data about infectious disease in Western Kenya and (2) to investigate the concept of a ‘good calf’ by focusing on what combination of infectious disease exposure, genetic traits, maternal effects, and life history result in a healthy productive calf, or conversely, a poorly grown or dead calf. In other words, why do some individuals get an infection and survive, whilst others experience the same infection but get sick or die?

It is now a decade since the final visit was made to the IDEAL project, and as samples and data from the IDEAL project are adopted by the Bill & Melinda Gates Foundation (BMGF) funded Centre for Tropical Livestock Genetics and Health (CTLGH), it is therefore timely to assess the impact and legacy of this ambitious (in name as well!) IDEAL project. Therefore, the form of this paper is to collect together the findings and impacts of the IDEAL project, from the point of view of the project’s stakeholders.

MATERIALS AND METHODS

The IDEAL project was longitudinal field study of new born calves carried out between October 2007 and October 2010 in Western Kenya (Bronsvoort et al., 2013). It was funded by the Wellcome Trust and conducted collaboratively by the University of Edinburgh, the University of Pretoria, the University of Nottingham, the International Livestock Research Institute (ILRI), and the Kenyan Department of Veterinary Services. The design of the project is described fully in Bronsvoort et al. (2013) but the key points are described for the reader here.

During the study, 5337 visits were made to 548 East African shorthorn zebu newborn calves who were recruited from 20 sublocations (the smallest administrative unit in Kenya). These sublocations were all within a 45 km radius of Busia town (**Fehler! Verweisquelle konnte nicht gefunden werden.**). The study site covered an area extending from Lake Victoria to the foothills of Mount Elgon, thus representing five agroecological zones. Recruitment was achieved through farmers reporting every calving event to their village elder, who informed the assistant chief of their sublocation who in turn contacted the IDEAL project staff. After receiving all the calving reports, one or two cattle were randomly recruited into the study each week. Recruitment was carried out in a five-week cycle with four of the 20 sublocations being visited each week to ensure an even distribution across all sublocations through time, and therefore season, and to enable a logical and efficient work pattern.

Farmers were compensated at a rate agreed with the staff of the District Veterinary office; this comprised the estimated cost of raising the calf for one year as calves were nominally owned by the project for that year. Calves were raised according to the farmer’s practices. However, if the calves became ill then they were treated by and at the discretion of the project vet. Veterinary products were administered to project calves to ensure welfare, but after such treatment calves were dropped from the study and their data censored.

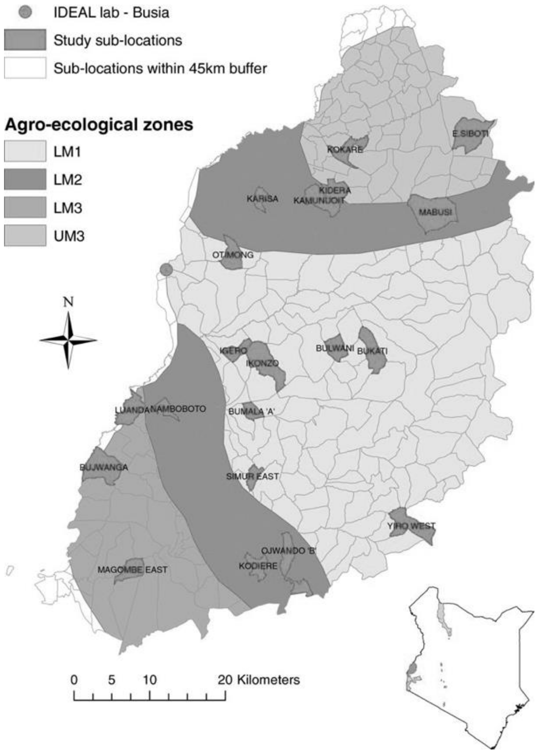

Fig. 2 Map of Kenya showing the study area, agro-ecological zones and sublocations. The sampled sublocations are highlighted. Figure taken from Bronsvoort et al. (2013).

Data was collected about the farm, the farmer, the dam and other animals on the steading. Each calf was visited every five weeks from birth and a clinical examination was conducted at these visits. Interim visits also occurred if clinical episodes were reported by the farmer, and a post-mortem was carried out if the calf died. Blood and tissue samples were collected in association with all visits and screened using a range of diagnostic tools. Weight data, clinical data, haematological values, and post-mortem examination of animals that died while participating in the study formed the outcomes of interest.

During the period of fieldwork, prior to being placed into the biobank, samples collected were tested for a limited number of micro-organisms in a field laboratory. Pathogen identification during the field collection phase concentrated on what was possible, what was probable, but also on what was affordable, and consequently represented mainly those things that could be observed through light microscopy or using commercial ‘penside’ kits. Faecal samples were unable to be stored, but all tissue and blood samples were recorded into the biobank.

The data gathered during the field phase of the study was initially stored in a Microsoft Access database which was designed for easy and secure collection of information. It was subsequently transferred to a relational MariaDB[†] database that could be accessed and updated remotely giving all staff access to the data for analysis. The data was held in 217 tables in a relational database format.

In 2019, the data in the MariaDB database was aggregated into seven tables and a Python web application was developed. This application includes a custom search form which enables users to query the data in a user-friendly way without writing code. Thus, the IDEAL data is

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[†] free and open source fork of MySQL

now accessible through an open access web-hosted database. This links to the biobank samples, providing a resource for researchers who had no original involvement with the project. The website has request options, which provides a method for interested researchers to request access to the biobanked samples. The website, with the data and request options, has recently been launched and is available at http://data.ctlgh.org/ideal/

RESULTS AND DISCUSSION

The IDEAL project allowed us to generate a dataset that is unusually comprehensive, not just for cattle but for any host in any setting. Information gathered during the project was used to provide baseline information on prevalence of pathogen exposure in calves in states of health and disease (clinically silent and clinically observable manifestations of infection such as mortality, clinical syndromes and poor growth). Below we examine the results of the IDEAL project in terms of the impact it had on each of the stakeholders (the animal, the farmer, the consumer, the policy maker, the funding body, and the researcher and their institution) before discussing the logistical lessons we learnt as a result of running such a study.

The Impact on Knowledge and the Research Community

Over 50 different pathogens (or evidence of exposure to pathogens) were initially identified during the project (Bronsvoort et al., 2013). Haemoparasites and gastrointestinal parasites were the predominant finding, with nearly all the cattle infected in their first year of life. The longitudinal data allowed us to more definitely associate these identified pathogens with incidences of disease and production loss, and to demonstrate where infection / exposure had occurred without such impacts. Certain pathogens showed significant impact at the population level, notably *Theileria parva* (East Coast Fever, ECF) and *Haemonchus placei* (haemonchosis) being the main causes of death for 33 (6%) and 10 (1%) of calves respectively (Thumbi et al., 2014). However, that leaves 503 calves that did not succumb to these infections.

This variability in calf performance could be investigated further due to the comprehensive data obtained by the IDEAL project. The study design enabled estimates to be made on the impact of co-infections on survival, health, productivity in the form of growth, and on haematological variables. The impact of the two ‘common killers’ was identified to be influenced by a number of factors; the presence of other infections, the genetic composition of the individual, and life history traits of the calf such as certain husbandry practices (Bronsvoort et al., 2013; Murray et al., 2013; Thumbi et al., 2013a; Thumbi et al., 2014).

As *T. parva* was the most significant cause of mortality, it was of interest to identify differences between those calves that did and did not die from the infection, not least because, as mentioned above, almost every individual in the cohort was exposed. This became the focus of the co-infection and mortality work. It was identified that the presence of *Trypanosoma* spp. in the life of the calf was associated with calves that subsequently died of ECF (Thumbi et al., 2013a; Thumbi et al., 2014). In addition, those calves that had higher faecal worm egg outputs at the visit before death were also more likely to die when infected with *T. parva* (Thumbi et al., 2013a; Thumbi et al., 2014).

A significant and possibly clinically relavent interaction was identified between prior seroconversion to *T. mutans* and a reduced risk of death following a subsequent infection with *T. parva* (Woolhouse et al., 2015). Whereby, in individual calves, evidence of previous exposure to a less pathogenic species of *Theileria* resulted in an 89% reduction in mortality associated with a later *T. parva* infection (Woolhouse et al., 2015). This finding triggered a number of further investigations following the conclusion of the IDEAL project (more below).

In addition to mortality, *T. parva* also impacted on production through a decreased growth rate. However, when cattle were co-infected with *T. parva* and *T. mutans* they experienced a relatively higher growth rate compared to when they were only infected with *T. parva* as a single infection (Thumbi et al., 2013b). In contrast, when cattle where infected with *T. parva* and *Anaplasma marginale* they experienced relatively lower growth rated compared to either infection on its own (Thumbi et al., 2013b).

IDEAL also demonstrated there was a lack of provision of housing for cattle, minimal use of veterinary services and lack of planned breeding (Bronsvoort et al., 2013). These factors in addition to infectious disease pressures were all limiting the potential of livestock production in this region (Bronsvoort et al., 2013; Thumbi et al., 2013a; Thumbi et al., 2013b; Toye et al., 2013; Thumbi et al., 2014).

Infection with gastrointestinal worms, especially increased burden, was unsurprisingly found to be associated with decreased productivity (assumed from increased faecal worm egg count (FWEC)). Losses were estimated to result in a 3.3% decrease in growth rate (Kg/day) for every 1000 increase in Strongyle eggs per gram of faeces (Thumbi et al., 2013b). *H. placei* as the dominant species was correlated with changes in blood parameters (decreased packed cell volume, white blood cell counts, and total serum protein (van Wyk et al., 2014; Callaby et al., 2015)).

IDEAL also investigated the genetic bases of disease. Initially, all the calves were genotyped using a 50K SNP-chip. The IDEAL calves were purposively recruited to be the indigenous East African shorthorn zebu breed and the genetic analysis showed the large majority of these animals were genetically homogenous with an admixed genetic constitution of 84% Asian zebu and 16% African taurine ancestries (Mbole-Kariuki et al., 2014). However, a few individuals revealed evidence of recent introgression with European taurine breeds, which is thought to be the legacy of abandoned breeding programmes which happened in the area (Mbole-Kariuki et al., 2014). Significantly, these European taurine introgressed animals were more likely to experience clinical illness (Murray et al., 2013). In addition, a number of the animals were found to have low levels of heterozygosity (i.e. inbred animals), and these were more likely to die or experience clinical illness (Murray et al., 2013).

The IDEAL biobank (currently held at ILRI in Nairobi) has allowed on-going analysis of samples. A subset of IDEAL cattle were genotyped with the Illumina BovineHD Genotyping BeadChip and these were integrated with other bovine genomes collected from across East Africa, and candidate regions of positive selection were identified for the breed, with a taurine zebu admix hybrid postulated to have been selected for fitness with respect to reproduction and survival. QTLs associated with these traits have been identified as targets for future breeding programmes (Bahbahani et al., 2015; Bahbahani et al., 2017). The samples have also been used in projects which were not envisaged when the project was conceived. For example, genotyping of the DNA from a cohort of the animals, together with the clinical records, has been used to extend the findings from a separate study aimed at elucidating the genomic basis of tolerance to ECF (Toye et al., pers. comm.). The samples will also be key to validating a deep sequencing approach to determining the haemoparasite burden in individual animals, which will prove invaluable in future epidemiological studies.

In addition to the research outputs from the project, the training legacy of the project is substantial and significant. IDEAL has produced six PhD theses, four MScs by research and two members of support staff were supported to complete a taught master's programme. All students have remained in science. We regard this as a significant contribution to training the next generation of tropical animal health researchers.

Lastly, the collaborations developed have been invaluable, and they continue in new projects. For example, two of the IDEAL PIs are the co-leads of the 'Animal Health and Genetics Program' of the CTLGH. The data and knowledge gain by IDEAL have helped a number of other studies to start, based on the information and methods gained from the study. As mentioned above, the finding of heterologous protection to *T. parva* induced by co-infection with *T. mutans* is the subject of a Gates Foundation-funded study to assess the feasibility of using *T. mutans* as a commercial alternative control method for ECF. Furthermore, two major projects, led by Professor Eric Fèvre of the University of Liverpool and ILRI, have also been undertaken in Busia which build on the results and infrastructure from IDEAL. The PAZ project (People, Animals and their Zoonoses, www.zoonotic-diseases.org/project/paz-project/) was targeted at endemic, neglected zoonoses in livestock and humans, and the impact of co-factors on the epidemiology of, and burden imposed by, these diseases. Whilst, the ZooLinK project (Zoonoses in Livestock in Kenya, www.zoonotic-diseases.org/project/zoolink-project/) seeks to enable Kenya develop an effective surveillance programme for zoonotic diseases, integrated across both human and animal health sectors. The activities of both projects were centred around the Busia laboratory.

Since IDEAL, Kenya has moved to a decentralised system of Government, with counties being the governing unit. The County Government of Busia together with ILRI is working towards commissioning Busia as a Government surveillance site, which would extend the laboratory's impact into new areas.

The Impact for Policy Makers

In terms of impact for policy makers, IDEAL showed that it was possible for a project to set up and maintain a functional laboratory and keep it running in rural Kenya. As previously described, this laboratory is still being used by other projects, thus it has become an important legacy and has also enhanced the diagnostic capacity within the region.

IDEAL was funded as a research project not a development project and so training of the famers and extension was not part of the funded grant. Nevertheless, an end of project meeting was held with the Kenyan agricultural department and the veterinary office. This provided the initial findings to policy makers, potentially offering evidence or context to their work.

The Impact on Calves and their Owners

Findings such as the effect of breed improvement programs on the genetics of livestock within the region have a direct impact upon calves and their farmers, since IDEAL showed little evidence of success at the attempts to upgrade local cattle (for improved milk production) with exotic European breeds. However, there seems to be potential for improvements through selection from the local breeds. For instance, there was huge variation (10-fold) in growth rates of the calves and so it is likely other productivity parameters such as milk production are equally variable (Bronsvoort et al., 2013; Lesosky et al., 2013; Thumbi et al., 2013b). Therefore, a greater understanding of why past efforts failed is needed and greater efforts at breed improvements through planned selection should be considered.

Such selection could also occur for parasite burdens. Calves that were born small were found to have higher burdens (if FWEC can be assumed to represent burden) of strongyles, and strongyle egg count was shown to be heritable (h^2= 23.9%, SE = 11.8%, Callaby et al., 2015). Therefore, genomic selection could be used to breed cattle with lower worm burdens, or that have the potential to cause less environmental contamination with eggs so reducing transmission.

In addition, it may be possible to exploit the relationships occurring between co-infecting parasites to aid cattle health. IDEAL showed that a number of calves were found to have multiple infections at the same time. Some of the co-infections appear to have synergistic effects i.e. infection with one pathogen making the effect of another more serious. It could therefore be possible to exploit these co-infections to produce relatively easy and sustainable interventions. For instance, the mortality rate from ECF was higher in animals which had high strongyle worm burdens (Thumbi et al., 2014). In other words, de-worming could have the added benefit of reducing mortality due to ECF.

In terms of disease awareness, IDEAL found that there was a mismatch between farmers' perception of the risk and reality of disease threats (Bronsvoort et al., 2013). However, this could be because the project concentrated on those diseases affecting youngstock and therefore farmer perceptions may represent both the youngstock and the adult breeding stock within the herd. Farmers in the region would therefore benefit greatly from training and education in the actual disease threats facing their herds and how to recognize them, the losses that these diseases may cause, and the treatments and preventative measures available to reduce those losses.

IDEAL was also able to contributed to improved diagnostics and treatment of cattle in this region by developing a live weight-heart girth relationship for accurate dosing of East African shorthorn zebu (Lesosky et al., 2013). Furthermore, IDEAL trained local animal health assistants and employed technicians from local institutions, thus building veterinary capacity in the region.

Finally, it must be noted that although this knowledge has been gained it remains in the research silo unless this knowledge is accessible to and accessed by policy makers and NGOs working in this area. Local meetings in Busia were arranged in the aftermath of the project and major findings were presented to animal health assistants. As calves left the study farmers were provided with a report of the pathogens that had already been identified to have infected their calves. This mainly comprised the gastrointestinal and haemoparasites. It is timely to consider how the new and more integrated knowledge can be presented and used by stakeholders.

Logistical Lessons

The IDEAL project proved to be a tremendously challenging project. Firstly, there were logistical challenges in setting up a field laboratory, and running sophisticated laboratory equipment despite poor electricity and water provision, lack of internet and a long supply chain. In order to achieve this, the project refurbished and equipped a Kenyan Directorate of Veterinary Services (KDVS)-laboratory in Busia. All the microscopy and faecal culture was carried out here, and for more complex diagnostic tests, especially those that required DNA clean rooms, samples were transported to ILRI and the University of Pretoria. These issues were anticipated and planned for. For example, to mitigate losses, samples were taken in duplicate and only one of the duplicates was transported at any one time to Nairobi.

Furthermore, biobanking of the material has allowed more diagnostic tests to be carried out as need has arisen or as new tests have become available.

There were similar challenges in setting up the recruitment and monitoring of calves from such a young age (time critical recruitment) over a wide area with poor accessibility (bad roads, floods, etc.). Data collection commenced at a time when mobile phone ownership was becoming common in the study region. Hand held data recording devices were available and affordable, and towards the end of the data collection period, broadband internet arrived in Kenya. In current times, the use of apps such as Open Data Kit (ODK, Hartung et al., 2010) have helped to ease and simplify these challenges, making data collection easier.

Even with the frequent visits and extensive visit protocol, it quickly became apparent that the 5 weekly sampling strategy was too long to pick up the fine scale dynamics of infection. In the initial stages of project a group of calves was visited weekly, but this was abandoned early on as it was realised that such an approach would be unsustainable without substantial and impossible levels of investment in more field teams (people and vehicles). This has meant that much of the clinical change (other than long term impacts such as growth or permanent outcomes such as death) went largely unobserved. In contrast to the host outcome data, defining the phenotype from an infection status point of view has become both easier and cheaper in recent years and will allow further enrichment of the data. For example, the development of new diagnostic tools, such as the ‘haemobiome’ offers quantititative identification of many haemoparasites from a single ‘test’. Such high resolution phenotypes offer the potential to understand the dynamics between coinfecting pathogens (Hemmink et al., pers. comm.). This can be linked with the phenotype and genetic data already gathered, further enhancing the questions that can be asked.

Looking back at the local legacy, IDEAL used a structured reporting system for calf births where the farmers reported every calving event to the village elder, who informed the assistant chief of the sublocation who in turn contacted the IDEAL project staff. By involving the whole community in the project in this way IDEAL could successfully engage local stakeholders and recruit individuals into the study, and encourage community buy-in.

On reflection, one of the investments made by IDEAL that had most value was a several month long dry run of the field study. This added to the duration and expense of the project, but it paid off many-fold by revealing any barriers and, at the same time, giving the project team the most effective possible training. We would recommend repeating this exercise for similar future projects. Furthermore, a labatory management workflow list was generated from the visit automatically and this ensured that labatory staff were able to efficiently manage their work load and avoid mistakes. Investment was made at the start of the project to ensure that the systems were robust, and once the project was in the main recruitment phase this system was reliable

The project period encompassed a period of unexpected turmoil in Kenya when post-election violence erupted across the country in 2008. IDEAL also had to contend with these unanticipated events. This is one of the challenges of such study designs; the power of the longitudinal study can be affected by external events, with the gaps caused by the unrest evident in the dataset. Not only were a number of routine visits missed, but clinical events passed unnoticed leaving the phenotypes of at least six calves incompletely described as they died unobserved by the project.

Unplanned for logistical failures were also burdensome for the project, both because of the cost to repair things, but also because failure of infrastructure had the potential to lead to loss of samples. For example, frequent power failures over loaded a generator. The repairs were on-going, and the automatic switch failed to reliably work. This meant staff motorbiking into the labatory from home overnight when a power cut or a failure in the generator was detected. Water was a constant issue. Catchment from the roof did not provide adequate supply for the intensive labatory work that was occurring, and water had to be transported in tankers from a river to fill the supply tank. This was costly in terms of both money and time, and was stressful. Weekly teleconferences were an essential part of project management, especially as the for the majority of the project the field site was managed locally by two PhD students.

Post-mortems were carried out under difficult conditions, and around half way through the project a post-mortem facility had to be built to avoid the risk of contaminating the areas around the government building with waste animal material. Gross post-mortems were recorded in a standardised way and analysed locally. However, final diagnosis was delayed until completion of the study to allow sensible work flows. Despite substantial investment in testing and man-hours spent pulling together diagnostic information, in 29% of cases it was not possible to assign a cause of death. This is not unusual. For example, for those cases presented for abortion to the Animal and Plant Health Agency (APHA) and Scotland's Rural College (SRUC) veterinary surveillance centres in 2017, 13% remained undiagnosed (Animal and Plant Health Agency, 2018).

As with all projects, IDEAL was restricted by budget and decisions that were made early on restricted conclusions once analysis began. For example, there was not budget or time to collect immunological markers such as cytokines. However, some of these can be tested using the stored samples. A key lesson learned from the project is understanding and achieving the balance between a large enough population to provide statistical power for, say, genetic analyses, with one that is small enough to obtain detailed clinical information and sampling at a suitable frequency. It is clear that, given the enormous amount of effort and resources expended in collecting the samples and associated metadata in longitudinal studies, every effort should be made to anticipate and accommodate the needs of analyses beyond the current project, but that context and time specific priorities are made and have to be accommodated and accepted.

Finally, database management on this scale is a significant challenge and preparing such a database to even minimal open access standards needs its own budget. It is only due to significant amount of time and funding from the Bill & Melinda Gates Foundation that 10 years later, this database is ready to be opened to the public. Converting the data from a form that allowed it to be efficiently collected (resulting in over 217 interlinking tables) to a form that allows it be efficiently queried has been a major and specialist undertaking. One of the lessons learnt from this project has been the need to plan for that and invest in expert advice in the earliest stages of the process.

The Future of the IDEAL Project

Data generation from samples obtained during this ambitious longitudinal study still continues and is driving further research, enabled by the launch of its extensive open-access database and biobank to the scientific community (http://data.ctlgh.org/ideal/). As shown above, the project has also driven numerous other studies particularly focusing on the control of ECF. In addition, a new haemoparasite typing tool developed from the IDEAL samples, the ‘haemobiome’, now offers the research community a method to quantitatively identify many haemoparasites from a single procedure. The findings from these higher resolution phenotypes for each calf will be published soon (Hemmink et al., pers. comm.). Used in combination with new high density SNP genotyping of the IDEAL cattle will drive deeper understanding of the coinfections occurring within the region and allow us to identify novel genomic markers for growth, disease resistance and survival.

Concluding Remarks

In summary, IDEAL showed that the cattle existed in conditions of exceptionally heavy infectious disease pressure and minimal investment in prevention or treatment. Although most calves experienced multiple infections by potentially fatal pathogens, more than 80% survived, and approximately 50% of animals were not observed to suffer clinical illness. Coinfections with other pathogens partly determined whether or not calves experienced clinical disease. Moreover, a significant fraction of mortality and ill health was attributable to adverse effects of inbreeding and European taurine introgression in affected calves. There was variation in growth rates but, nonetheless, some calves managed impressive weight gains despite multiple infections and living in a harsh environment.

In terms of study design, the real value of IDEAL has come in the effort that was taken at the outset to ensure the long view. Availability of mobile connections, the careful use of sample workflow models, and fastidious attention to detail led to early systems designed to build resilience and longevity. The database and bio-bank has allow the legacy to grow and it has proven to be a worthwhile investment, forming one of the major assets of the CTLGH. It has also allowed us to develop our understanding of the differences identified between these calves, and to begin to better describe that difference leading to the development of interventions such as that for ECF and breeding practices which improve cattle productivity.

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SOCIETY FOR VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

PAST VENUES AND ORGANISERS OF ANNUAL MEETINGS

Year	**Venue**	**Organiser(s)**
1983	Southampton	Davies & Thrusfield
1984	Edinburgh	Thrusfield
1985	Reading	Thrusfield
1986	Edinburgh	Thrusfield
1987	Solihull	Thrusfield
1988	Edinburgh	Thrusfield
1989	Exeter	Howe
1990	Belfast	McIlroy
1991	London	Jones
1992	Edinburgh	Thrusfield
1993	Exeter	Howe
1994	Belfast	Menzies
1995	Reading	Paterson
1996	Glasgow	Reid
1997	Chester	Clarkson
1998	Ennis, Ireland	Collins
1999	Bristol	Green
2000	Edinburgh	Thrusfield & Mellor
2001	Noordwijkerhout	van Klink
2002	Cambridge	Wood & Newton
2003	Warwick	Green
2004	Martigny	Stärk
2005	Nairn	Gunn
2006	Exeter	Peeler
2007	Dipoli	Virtala & Alban
2008	Liverpool	Pinchbeck & Robinson
2009	London	Verheyen & Pfeiffer
2010	Nantes	Fourichon & Hoch
2011	Leipzig	Thulke & Lange
2012	Glasgow	Parkin & Others
2013	Madrid	Martínez-López & Vargas Alvarez
2014	Dublin	More & Collins
2015	Ghent	Mintiens & Dewulf
2016	Elsinore	Nielsen & Halasa
2017	Inverness	Gunn & Reeves
2018	Tallinn	Viltrop
2019	Utrecht	van Schaik

PAST PRESIDENTS

1982-'83	G. Davies	2001-'02	A.D. Paterson
1983-'84	P.R. Ellis	2002-'03	L.E. Green
1984-'85	G. Gettinby	2003-'04	J.L.N. Wood
1985-'86	R.W.J. Plenderleith	2004-'05	E.G.M. van Klink
1986-'87	M.V. Thrusfield	2005-'06	D.J. Mellor
1987-'88	K.S. Howe	2006-'07	E. J. Peeler
1988-'89	A.M. Russell	2007-'08	J. R Newton
1989-'90	S.G. McIlroy	2008-'09	L. Alban
1990-'91	J.E.T. Jones	2009-'10	D.U. Pfeiffer
1991-'92	J.M. Booth	2010-'11	L.A. Kelly
1992-'93	E.A. Goodall	2011-'12	C. Fourichon
1993-'94	R.G. Eddy	2012-'13	T.D.H. Parkin
1994-'95	J.T. Done	2013-'14	K.L.P. Verheyen
1995-'96	G.J. Gunn	2014-'15	K. Mintiens
1996-'97	M.S. Richards	2015-'16	H.H. Thulke
1997-'98	J.D. Collins	2016-'17	A. Lindberg
1998-'99	F.D. Menzies	2017-'18	L.R. Nielsen
1999-'00	K.L. Morgan	2018-'19	M.L. Brennan
2000-'01	S.W.J. Reid	2019-'20	K.M. McIntyre

EXECUTIVE COMMITTEE 2019-2020

K.M. McIntyre (President), M. Brennan (Senior Vice-President), G. van Schaik (Junior Vice-President), P. Robinson (Honorary Secretary), D. Brodbelt (Honorary Treasurer).

Honorary Auditors: Fraser Menzies & Keith Howe

LIFE MEMBERS

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

PLENARY TALKS

Year	Gareth Davies Lecture	Conference Opening Plenary
2020	Jonathan Rushton How GBADs will link to clinical practice and veterinary epidemiology	Matthew Stone The policy perspective and science in an evidence-based policy structure Sam Thevasagayam A funder's perspective of disease studies
2019	Simon More Perspectives from the science-policy interface	Marion Koopmans To jump or not to jump: viruses at the human-animal interface
2018	Klaus Depner African swine fever: Lessons learned about the epidemiology, politics and practical implementation of control measures	Päivi Rajala-Schultz Veterinary epidemiology at the intersection of livestock disease, production and animal welfare
2017	Theresa Bernado TRENDS: Technology, Research, Epidemiology, Networks, Data & Surveillance	Tine Hald Source attribution: Translating science into public health action
2016	Bernhard Url The foundation of science-based risk assessment for decision support on food safety and animal health in EU	Mirjam Nielen Evidence-based veterinary medicine needs clinical epidemiology
2015	Piet Vanthemsche Preventive Veterinary Medicine as an essential part of sustainable animal production	Crawford Revie Hype and Hysteria: Should veterinary epidemiologists really care about Big Data?
2014	Ian Gardner Bridging the gap in infectious disease epidemiology between aquatic and terrestrial food animals: challenges and future opportunities	Nils Toft Confessions of a wannabe Bayesian
2013	Andreas Hensel Dioxins, EHEC and strawberries: Risk assessment and risk communication in practice	José Manuel Sánchez-Vizcaíno The Spanish experience on the control and eradication of infectious diseases: from the old to the current system
2012	Stuart Reid Evidence-based prevention: well done or rare	Didier Boichard Genomic selection: an opportunity for improving health of farm animals
2011	Karin Schwabenbauer From science to policy - the case of classical swine fever (CSF) control	Dominic Mellor The trouble with epidemiology: the tyranny of numbers

2010	David Waltner-Toews Beyond one world, one health and ecohealth…what's out there?	James Wood From pathogen adaption to host ecology: epidemiological and experimental contributions to the understanding of emerging infectious diseases
2009	Jørgen Westergaard The interaction between veterinary science, legislation and management in animal disease control in the European Union	Katharina Stärk Food safety challenges in a global market – are we ready?
2008	Paul Fine Infectious disease eradication – meanings and implications	Kenton Morgan For the benefit of Mr Kite
2007	Yrjö Gröhn Food supply veterinary medicine: Modelling of production, health and food safety	Laura Green Improving Animal Health
2006	David Galligan From partial budgets to real options - concepts in animal health economics	Nigel French Understanding human exposure to zoonoses from food and the environment: The application of molecular tools and modeling
2005	Bill Reilly From TB to VTEC: The changing epidemiology of foodborne zoonoses	Simon More Towards eradication of bovine tuberculosis in Ireland: A critical review of progress
2004	Ulrich Kihm BSE and the stable to table concept	Gary Smith Spatial models of infectious disease in the USA: a crisis of conference and confidentiality
2003	Sir David Cox The current state of statistical science	Ynte Schukken Molecular and mathematical epidemiology of bovine mastitis
2002	George Gettinby Informatics and epidemiology – the first 400 years	Bryan Grenfell Deterministic and stochastic influences on the dynamics and control of infectious diseases
2001	Will Houston Science politics and animal health policy: epidemiology in action	Mart de Jong Design and analysis of transmission experiments
2000	Jim Scudamore Surveillance – past, present and future	Dirk Pfeiffer Spatial analysis – a new challenge for veterinary epidemiologists

1999	Aalt Dijkhuizen The 1997/98 outbreak of classical swine fever in the Netherlands: lessons learned from an economic perspective	Mark Woolhouse Understanding the epidemiology of scrapie
1998	Wayne Martin Art, science and mathematics revisited: the role of epidemiology in promoting animal health	

SOCIETY FOR VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

APPLICATION FOR MEMBERSHIP

Name ....

Address ....

....

....

....

Telephone: ....

Fax: ....

E-mail: ....

Signed .... Date ....

Please enclose the membership fee (£60 sterling to cover two years' membership) along with this application form. Overseas members without British bank accounts are requested to pay 2 - 3 years in advance. Cheques should be in £ sterling and drawn from a British bank. British members should pay future dues by standing order (forms are available from the Secretary or Treasurer). Payment can also be made by credit card; the appropriate form is available from the Society's website, http://www.svepm.org.uk/, or from the Secretary or Treasurer.

Please send this form to the Society's Treasurer:

Dr 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