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BOVINE MASTITIS

BAYESIAN EVALUATION OF BUDGETS FOR ENDEMIC DISEASE CONTROL; AN EXAMPLE USING A MANAGEMENT CHANGE TO REDUCE MILK SOMATIC CELL COUNT EARLY IN THE FIRST LACTATION OF IRISH DAIRY COWS

S.C. ARCHER[*], F. MC COY, W. WAPENAAR, AND M.J. GREEN

SUMMARY

Reducing the prevalence of heifers with a high milk somatic cell count (SCC) early in the first lactation could be achieved through herd level management changes during the *pre-* and *peri-partum* period, however the cost effectiveness of interventions is unknown. A synthesis of multiple sources of evidence, accounting for variability and uncertainty in the available data is invaluable to inform decision makers around likely economic outcomes of investing in disease control measures. Evidence was synthesised from 2 separate statistical models and previous research, with the outcome for an individual cow or herd assessed in terms of changes in lifetime milk yield, and disposal risk. To extend the analyses of Archer et al. (2014), a single intervention was tested; storage of bedding material inside. Budgets for the intervention were determined for different decision makers based on their minimum expected return on investment, and risk aversion.

INTRODUCTION

For 50% of Irish dairy herds, reducing the prevalence of heifers with high milk somatic cell count (SCC) between 5 and 30 days in milk (DIM) would be associated with savings through increased longevity, and lifetime milk yield (Archer et al., 2013b; c). A reduction in the prevalence of heifers with high SCC early in lactation could be achieved through herd level management interventions targeted at *pre-* and *peri-partum* heifers (Green et al., 2008). Previous studies have identified risk factors for mastitis in heifers (De Vliegher et al., 2012), but the cost and efficacy of particular management changes have yet to be evaluated in the field. Although data on the likely cost effectiveness of management interventions is unavailable, combinations of management interventions have been simulated to investigate the likelihood of particular scenarios (Archer et al., 2014). This information has highlighted the importance of understanding the characteristics of the people making decisions, in order to decide if interventions may be cost effective.

Uncertainty and variability in parameters can be handled with a Bayesian analysis, in which prior knowledge is combined with data obtained from a particular study to generate posterior probability distributions for outcomes that represent the updated state of knowledge, and are inherently useful for decision makers (Spiegelhalter et al., 2004; Bolstad, 2007). As a further aid to decision making, the Bayesian approach can be extended by using micro-

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simulation to generate posterior predictions for particular scenarios. The trajectory of individuals is modelled as if a carefully controlled trial has been conducted, varying only the exposure of interest. This approach is useful when such a trial would be impossible or very expensive (Archer et al., 2013b; c). The aim was to use micro-simulation to determine budgets for a single management intervention to control heifer mastitis in Irish dairy herds, and compare for different decision makers.

MATERIALS AND METHODS

Overview

To extend the analyses of Archer et al. (2014), a micro-simulation was used for a partial budget analysis to estimate the likely economic impact of a single intervention to reduce SCC in Irish dairy heifers between 5 and 30 DIM (SCC1) in terms of change in lifetime milk yield and cow disposal risk (Fig. 1). Potential financial savings associated with applying the intervention were estimated from the mean difference in lifetime milk yield, and disposal risk at herd level with and without the intervention being applied. The probability of cost effectiveness, and maximum rational spend for implementing the change was estimated for different decision makers based on their willingness to pay.

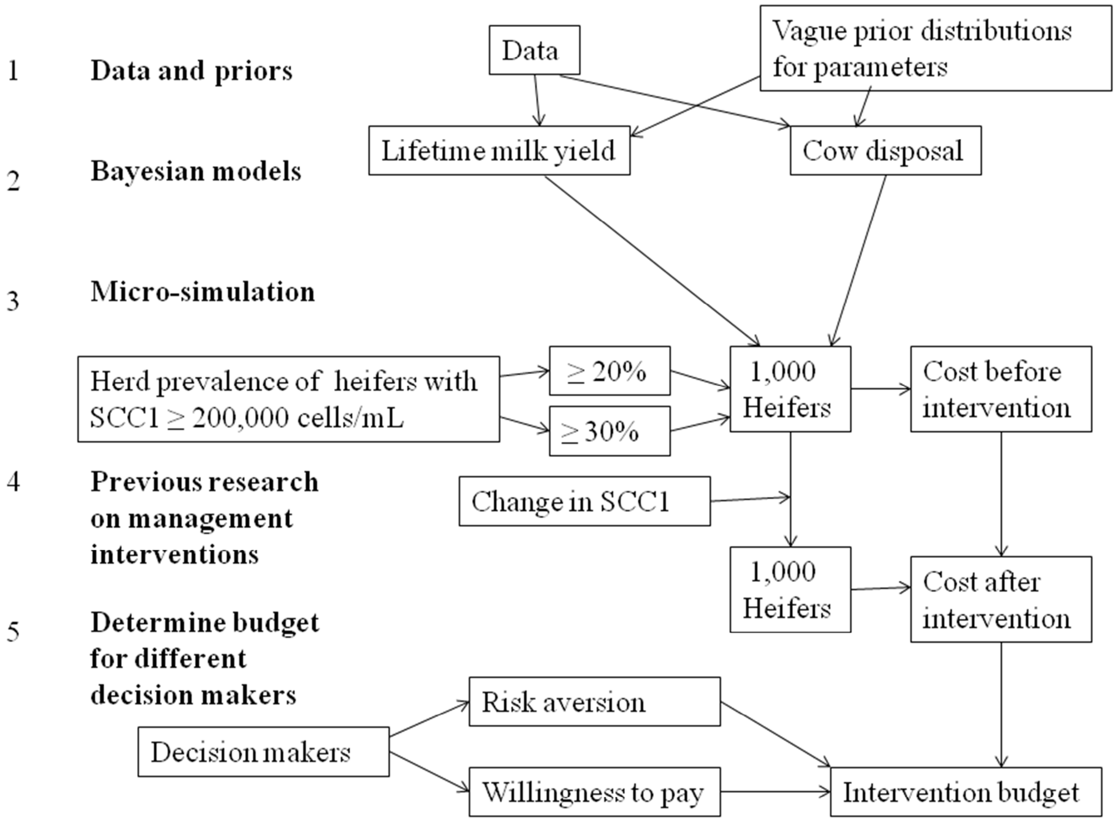

Fig. 1 Overview of the 1-step micro-simulation procedure (Archer et al., 2014)

Lifetime milk yield model (Model 1)

This model assessed the association between the SCC1, and lifetime milk yield for cows in Irish dairy herds (Archer et al., 2013b). The coefficients from this model directly fed into the micro-simulation are summarised in Table 1. Overall, one unit increase in the natural logarithm of (ln) SCC1 was associated with a median decrease in lifetime milk yield of 865 (95% Bayesian credibility interval (BCI) 702 to 1,025) kg.

Table 1. Lifetime milk yield model (in kg; Archer et al., 2013b); 95% Bayesian credible interval for parameters used in the micro-simulation procedure

Exposure (baseline)	Lower 2.5%	Median	Upper 97.5%
Intercept	-4,819	10,950	26,260
Ln[a] SCC1[b] (4.65)	-1,025	-865	-702
First calving February 2007	2,979	4,418	5,832
ln AFC[c] (6.71)	-8,302	-6,906	-5,484

[a] Natural logarithm

[b] First test day somatic cell count record at 5 to 30 days in milk during parity 1

[c] Age at first calving (days)

Cow disposal model (Model 2)

This model assessed the association between SCC1, and survival over a 5 year period from 2005 to 2009, for cows in Irish dairy herds (Archer et al., 2013c). The coefficients from this model directly fed into the micro-simulation are summarised in Table 2. Overall, disposal odds increased by 5% (BCI 2 to 9%) per unit increase in ln SCC1.

Table 2. Cow disposal model (odds ratios; Archer et al., 2013c); 95% Bayesian credible interval for parameters used in the micro-simulation procedure

Exposure (baseline)	Category	Lower 2.5%	Median	Upper 97.5%
Intercept [a]		-6.215	-6.215	-5.809
Ln[b] SCC1[c] (4.64)		1.020	1.052	1.085
TDY1[d] (23 kg)		0.968	0.976	0.983
TDF1[e] (0.04)		0.000	0.001	0.090
ln AFC[f] (6.70)		1.770	2.263	2.930
[ln interval[g]]^1 (2.28)		1.260	1.361	1.473
[ln interval]^2 (2.28)		1.847	1.970	2.100
[ln interval]^3 (2.28)		1.198	1.247	1.298
DIM[h] (< 100)	100 to 199	2.642	2.939	3.264
	200 to 304	5.280	5.883	6.554

[a] Presented on a logodds scale

[b] Natural logarithm

[c] First test day somatic cell count record at 5 to 30 days in milk (DIM) during parity 1

[d] First test day milk yield record (kg) between 5 and 30 DIM in parity 1

[e] First test day fat content record (proportion) between 5 and 30 DIM in parity 1

[f] Age at first calving (days)

[g] 50 day intervals from first calving. Included as polynomials

[h] DIM category in the penultimate interval for each cow. Missing category not shown

One-step micro-simulation model

Implementation: Coefficients from Models 1 and 2 were combined with data from theoretical cows to generate predictions of lifetime milk yield and the occurrence of disposal within 1,750 days from first calving for the ith cow in the jth herd ($y.pred_{ij}$);

$$y.pred_{ij} \sim p(y.pred_{ij} | \boldsymbol{\beta}, X^{sim}), \tag{1}$$

where β is a vector of coefficient distributions (Tables 1 and 2), and Xsim is a matrix of data for simulated heifers. This included an indicator variable to denote a first calving in February 2007 (aged 24 months), and data from a first milk recording (including ln SCC1) at 5 to 30 DIM simulated from observed normal distributions based on ≥ 20%, and ≥ 30% initial herd level prevalence of heifers with SCC1 ≥ 200,000 cells/mL (Table 3). Interval specific indicator variables were used for month of last recording and DIM category in the penultimate interval. To account for variability in parameters this procedure was carried out at each of 10,000 MCMC iterations using WinBUGS 1.4.3 (Lunn et al., 2000).

Economic simulation: At every iteration, the difference in lifetime milk yield for each cow in these scenarios, with or without applying the management interventions, was multiplied by the estimated gross margin (Milk price – variable costs of production) ~ Normal (mean = 0.17, standard deviation = 0.03) €/L (Hennessy et al., 2011), to give the predicted difference in milk revenue. In addition at every iteration, the difference in the number of cows disposed within 1,750 days from first calving for each scenario was multiplied by €1,451 (Kennedy et al., 2011), to estimate replacement costs. Following the assumed management interventions, the cost differences associated with increased lifetime milk yield and decreased cow disposal risk were expressed as a mean financial value per heifer in the herd (Fig. 1). Following 10,000 MCMC model simulations to investigate the full range of scenarios, posterior distributions of total savings per heifer in the herd were plotted as a cumulative frequency distribution to show the probability of different levels of return in an intuitive form.

Table 3. Observed frequency of 7,423 Irish dairy herds, and means (variances) from 233,176 heifers categorised by prevalence of SCC1[a] ≥ 200,000 cells/mL (Archer et al., 2014)

	≥ 20%	≥ 30%
Percentage of herds	59%	26%
Ln[b] SCC1	4.82 (1.47)	5.06 (1.56)
Milk1[c]	23 (30.0)	22 (33.3)
Fat1[d]	0.04 (0.00007)	0.04 (0.00007)

[a] First test day somatic cell count record (cells/mL) between 5 and 30 days in milk (DIM) during parity 1
[b] Natural logarithm
[c] First test day milk yield record (kg) between 5 and 30 DIM in parity 1
[d] First test day fat content record (proportion) between 5 and 30 DIM in parity 1

Simulation of a management intervention: The management intervention tested was storage of bedding material inside such that it is more likely to be dry when used. Dry bedding is less likely to support microbial growth than damp bedding. This intervention has been tested in an intervention study (Green et al., 2007), and can be expected to lead to a normally distributed decrease ln SCC1 with a mean (variance) of 0.15 (0.02; Green et al., 2008). Draws from this distribution were added to the simulated ln SCC1 for each cow (Fig.

1), to determine the impact of the intervention for herds with ≥ 20%, or ≥ 30% initial prevalence of heifers with SCC1 ≥ 200,000 cells/mL.

Willingness to pay

Willingness to pay (k) is defined as the maximum amount a particular decision maker will pay for every €1 of potential saving, and hence the return on investment that would be acceptable (Spiegelhalter et al., 2004). Cost effectiveness is determined by the attitude of the decision maker. The value chosen for k reflects the minimum return on investment the decision maker expects over and above the intervention cost in order that they would consider the intervention to be cost effective. Decision makers typically do not divulge their willingness to pay; therefore a sensitivity analysis is required to evaluate how the incremental net benefit (INB) varies with k where;

$$\text{INB}[k] = k \times \text{difference in savings} - \text{difference in costs, and } k = (0{:}10) \times €0.1. \qquad (2)$$

Appropriate levels of spending for the control of mastitis in heifers during the pre- and peri-partum period are unknown. Therefore, posterior distributions for the maximum intervention cost (when INB[k] = 0) were determined. The maximum intervention cost determines the budget available for implementing the interventions in order that they are considered ‘cost effective’ by a particular decision maker.

RESULTS

Assuming that the management of herds was changed such that bedding material was stored inside and was dry when used, herds with ≥ 30% of heifers with SCC1 ≥ 200,000 cells/mL had 75% certainty of total savings of at least €12 per heifer calved into the herd through increased lifetime milk yield and decreased disposal risk. For herds with ≥ 20% of heifers with SCC1 ≥ 200,000 cells/mL there was only 28% chance that applying the single management change of keeping bedding material dry would be associated with a saving through reduced SCC1. The full range of possibilities is shown in Fig. 2. These savings represent the intervention budget for a decision maker who is content to at least break even on investment. For the higher prevalence herds, Fig. 3 shows that for a given probability of cost effectiveness, the potential budget for this management intervention for the control of heifer mastitis increases with willingness to pay. Even with considerable variation between decision makers, there would be potential to invest up to €4 per heifer in the herd to keep bedding material dry in a worst case scenario where the decision maker must be 80% certain of a return on investment of ≥ 150% (k = 0.4). At the other extreme, the budget would be up to €24 per heifer where the decision maker would be content to have 50% chance of at least breaking even (k = €1; Fig. 3).

DISCUSSION

Budgets for interventions to control mastitis in heifers appear highly dependent on decision makers’ willingness to pay, and hence expected minimum return on investment. In this study, a risk averse farmer with a low willingness to pay may rationally invest up to €4 per heifer calved, which could cover the cost of basic protection to keep bedding material dry. At the other extreme, a farmer who is comfortable with less certainty in the interventions being cost effective and is more willing to pay, would be prepared to invest up to €24 per heifer calved, which over time could make investment in new buildings and facilities feasible (Fig. 3). Importantly, the large range of potential budgets to implement a single specific intervention influences what is practically achievable on particular farms. Therefore understanding the circumstances and characteristics of farmers is critical in order to facilitate changes to improve animal health and welfare.

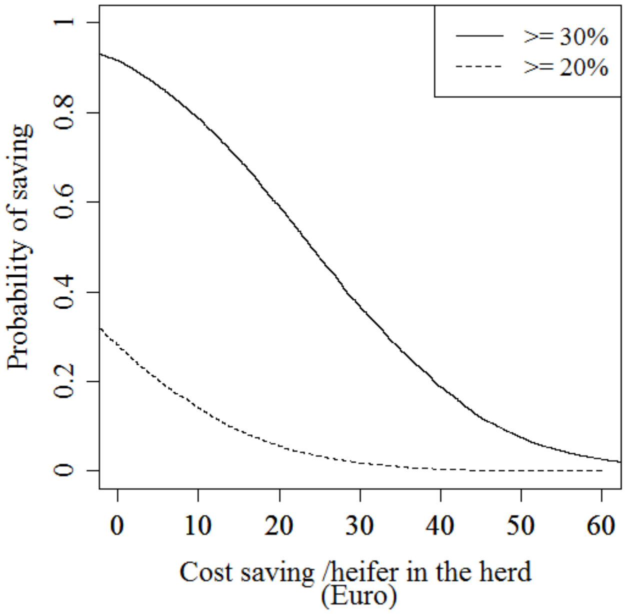

Fig. 2 Posterior predictions of savings through storing bedding material inside

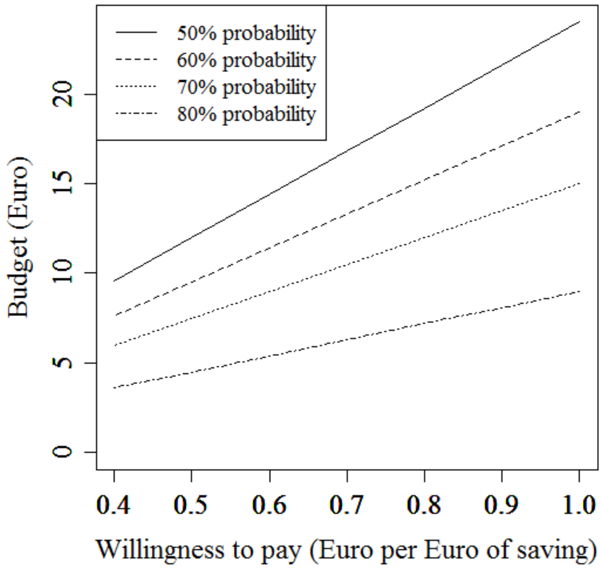

Fig. 3 Impact of willingness to pay and probability of cost effectiveness on budgets to control heifer mastitis through storing bedding material inside for high prevalence herds

Farmers are known to have cost preferences for mastitis interventions which effectively weight costs based on factors such as the practicality of implementing the changes (Huijps et al., 2009). Decision making is therefore complicated by variation in what is deemed 'cost effective' by different individuals. Attitude to risk varies between individuals, and decisions about implementing interventions to control disease must be made based on a level of risk regarding the economic outcome that is deemed acceptable. The expectations of farmers when making decisions around mastitis control are not well understood, and could be affected by their psychological, physiological, and emotional state (Hastie & Dawes, 2001). For instance, pride in keeping cows healthy was an important motivator for mastitis control in Dutch dairy herds (Valeeva et al., 2007). It is hard to put an economic value on emotions such as 'pride' attributable to controlling mastitis, and this could mean 'willingness to pay' exceeds what seems rational based on changes in lifetime milk yield and disposal risk alone. As a result of variation in mastitis risk through time, the efficacy of a management change is initially uncertain, depending on when it is applied relative to the background risk. Background variation in mastitis risk is likely to be related to factors that influence pathogen survival and multiplication in the environment, such as the ambient temperature, humidity, and hygiene of housing. Alternatively, cow factors such as metabolic stress or intercurrent disease may influence susceptibility to mastitis. Therefore, the intervention tested may not always be 'cost effective' on particular farms (Green et al., 2010). Furthermore, effectiveness of the intervention may be influenced by the manner in which changes are applied; if carried out poorly a small or no effect may be observed.

For the intervention to be perceived as 'cost effective', farmers should aim to implement the change for the least possible cost, but within budget. However in practice, the findings from this study only inform rational levels of expenditure for mastitis control in heifers through a single management change, and other interventions may be more appropriate in different circumstances. Furthermore, the single intervention tested would likely only be cost effective for herds that have ≥ 30% of heifers with high SCC1. This differs to the results from a previous simulation in which 3 management interventions to improve environmental hygiene for *pre-* and *peri-partum* heifers were assumed to be simultaneously applied (Archer et al., 2014), as a multi-factorial approach to mastitis control is commonly advised (Green et al., 2007; Anon, 2013). For a holistic approach to the control of heifer mastitis, further research should consider the efficacy, and hence likely budgets to implement management changes based on all known risk factors (De Vliegher et al., 2012). Other costs should be considered, for instance the impact of reducing the prevalence of heifers with high SCC in early lactation on lifetime clinical mastitis costs, and milk quality. Clinical mastitis costs may be partially included in the current analysis through the impact on longevity and hence lifetime milk yield (Heikkilä et al., 2012). In the absence of a record of SCC1, data for heifers with clinical mastitis in early lactation were not available, which suggests the budgets are an underestimate.

The impact of the management intervention in this study was based on research in English and Welsh dairy herds (Green et al., 2007; 2008) and assumed to be applicable under Irish conditions. The magnitude of losses through high SCC1 in English and Welsh herds were similar to those in Irish herds (Archer et al., 2013d), and it is plausible the results are generalisable to these countries. Although the underlying models have been shown to be useful and generalisable to other Irish dairy herds (Archer et al., 2013b; c), further work is needed to validate the cost effectiveness analysis and budgets presented here. Ultimately, this requires observed data on the impact of management interventions on SCC1 in Irish dairy heifers to compare with model predictions. For the cost effectiveness analysis to be useful for decision support in practice, it should be extended to consider other endemic diseases so the relative benefits of control can be compared. A quantitative approach to determining priorities for investment would avoid reliance on subjective opinion (More et al., 2010), and this would be useful for Irish farmers to inform decisions on disease control investments in conjunction with national control plans for several endemic diseases (Anon, 2013). There may be overlapping benefits of certain management changes on multiple endemic diseases which would make them even more economically favourable. A survey of Irish farmers would be useful to further evaluate their 'risk aversion' and 'willingness to pay' for disease control. This information would help refine budgets, and therefore identify achievable farm management changes for validation of efficacy in future studies.

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EFFECTS OF DIFFERENT MASTITIS SUPPORT STRATEGIES ON DAIRY FARMERS' MOTIVATION, PREVENTION BEHAVIOUR, AND THEIR HERDS' UDDER HEALTH STATUS

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SUMMARY

This randomised field trial evaluating support strategies to improve udder health in dairy herds combined epidemiological and psychological data. The results allowed drawing conclusions about the value of targeting farmers' attitudes towards different mastitis prevention behaviours in future mastitis prevention programmes. In addition, the study yielded insights about which support strategies farmers would appreciate most to improve udder health in their herds.

INTRODUCTION

Mastitis is the inflammation of the mammary gland and is the most costly disease in the dairy industry. Mastitis situations differ between dairy herds and a holistic herd-specific approach is needed to improve their udder health sustainably (Lam et al., 2013). The greatest improvement in udder health can be expected when as many management practices as possible are corrected into the desired direction (Green et al., 2007). Moreover, a continued knowledge transfer is assumed to improve farmers' compliance to implement more management changes and to sustainably improve udder health (Lam et al., 2013).

Farmers see their veterinarians as their first source of knowledge in case of udder health problems (Jansen et al., 2009; Lam et al., 2011). Also, veterinary herd-health management is becoming increasingly important and the modern veterinarian needs to be an advice-oriented consultant providing preventive support (LeBlanc et al., 2006; Lam et al., 2011). A trustworthy relationship between a pro-active veterinarian and his/her client is assumed to be an effective animal health support strategy (Derks et al., 2012). Bringing farmers together and letting them share and discuss their udder health experiences in so-called study groups is another support strategy. Farmers seem more willing to accept knowledge from colleagues (Lam et al., 2011) and this may contribute to the optimisation of the mastitis management in their herds (Brightling et al., 2009; Lam et al., 2011). However, the true effectiveness of both support strategies in improving udder health in dairy herds has not been investigated yet.

Farmers' intention to engage in udder health management might be influenced by different factors such as attitudes towards the behaviour, subjective norms or perceived behavioural

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control. The “Theory of Planned Behaviour” (TPB, Ajzen, 1991; Armitage & Conner, 2002) offers a framework to study the psychological factors that influence “intention” and “behaviour.” The importance of specific psychological factors in regard to udder health management and especially in the context of different support strategies has not been investigated yet.

This study was designed as a multi-arm randomised field trial. Its aims were 1) to quantify the effectiveness of 3 mastitis support strategies on the prevalence of high somatic cell count (SCC) cows by comparing them with a negative control group not receiving an intervention (NEGCON), 2) to gain insights in which psychological factors drove farmers to intend and implement behavioural changes in their daily mastitis management, and 3) to determine how the participating farmers experienced the support strategies. Three support strategies were evaluated and included 1) personalised advice (POSCON), 2) personalised advice and veterinary support (VET), and 3) personalised advice and study groups meetings (STUDYGR).

MATERIALS AND METHODS

Based on a sample size calculation, 25 herds were needed for each study arm. Consequently, 100 participating farms were visited by 5 trained veterinarians from September to December 2011 who wrote farm-specific advice reports for the 75 herds belonging to the 3 study arms with intervention. From January to December 2012, the farms were followed up according to their group affiliation. There was a second farm visit after 1 year to re-assess the udder health management on all farms and to evaluate the implementation of the recommended management changes in the 3 study arms receiving a support strategy.

Inclusion and exclusion criteria and enrolment of farms

Dairy herds (*n* = 1,553) with a yield-corrected bulk milk SCC between 200,000 and 300,000 cells/ml in 2010, a herd size of ≥12 lactating dairy cows at each test day, ≥11 test days recorded at the same location (to exclude herds with alpine pasturing) and being located in the French and German speaking parts of Switzerland were eligible for recruitment. One thousand herds, stratified by breeding organisation (Swiss Brown Cattle Breeders’ Federation (*n* = 400), Holstein Breeders’ Federation (*n* = 200) and Swissherdbook (*n* = 400)), were randomly selected and asked to participate in the study. Of herds that responded positively (*n* = 140), 100 herds were selected to participate in the study.

Randomisation and blinding

Assigning herds to the 4 study arms was done in 2 randomisation steps. First, 4 regions with many farms in relatively close proximity were selected to avoid that farmers had to travel far to participate in the study groups. Farms in these 4 regions (*n* = 50) were randomly assigned to the STUDYGR study arm in a ratio of 1:1. The 25 farms not assigned to this study arm were pooled with the 50 farms from the other regions and then randomly assigned to the other 3 study arms in a ratio of 1:1:1. Blinding was not possible in this study because farmers had to actively participate in 1 of the support strategies if they were assigned to one.

Farm visits and advice report

General demographic information (e.g. herd size, herd type and number of cubicles), as well as information about general herd health management, including udder health management, was collected using a standardised questionnaire which was sent to the farmers before the first farm visit. A visit protocol was separately developed to collect information about the housing system, the milking system and the milking hygiene during milking time. Milk samples were additionally collected from all cows with a composite SCC ≥150,000 cells/ml to determine the causative mastitis pathogens. These samples were used to classify herds into herds with contagious mastitis problems, environmental mastitis problems, or both. Based on this field work, every farmer received a standardised, herd-specific report with recommendations to improve the udder health management. The recommendations were prioritised as the 3 most important ones, short term strategies, or long term strategies. The advice reports were sent within a median number of 40 days (range: 19-77 days; $m = 0$) after the farm visit. As the NEGCON study arm did not receive any report in autumn 2011, the median number of days between the first farm visit and the receipt of the report in the other 3 study arms was added to the date of the first farm visit to define $m = 0$ for the NEGCON study arm.

Study arms

The first study arm (NEGCON) served as a negative control group and did not receive any support during the project. The farms were visited and their udder health management was observed but they neither received an advice report nor any actively organised follow-up.

The second study arm (POSCON) served as a positive control group and only received the advice report in autumn 2011. No follow-up was actively organised in 2012 but the farmers were allowed to contact the research team or their veterinarian at their own initiative.

The third study arm (VET) received an advice report at the start of the study and monthly visits of their own private veterinarian during the follow-up period. During these monthly visits, veterinarians were requested to identify newly infected cows in the herds by performing a Californian mastitis test and, subsequently, taking milk samples for bacteriological examination on all cows with a newly composite SCC ≥150,000 cells/ml. If quarters were infected with a major pathogen, the veterinarians were requested to treat them with any registered antimicrobial product commercially available for treatment of mastitis.

The fourth study arm (STUDYGR) received a follow-up that was conducted by organizing study group meetings in 2012. Approximately every 2 months, the research team, in collaboration with an experienced study group moderator, organised study group meetings on udder health for the participants. During those on-farm meetings, farmers continuously discussed the advice that was given. Moreover, they were educated about different topics of mastitis, such as milking technique and milking hygiene, diagnostics, feeding, therapy, etc.

Collected data and definition of outcomes for the epidemiological evaluation of the field trial

Prevalence of high SCC cows: Monthly composite SCC data from January 2010 to December 2012 from all cows of all participating herds were obtained from the 3 Swiss breeding organisations. These data were used to evaluate the monthly herd level proportion of cows with a composite SCC ≥200,000 cells/ml, which was defined as the primary outcome of this study.

Implementation of recommendations: At the second visit to the herds, the implementation of the recommended management changes was assessed as a secondary outcome in the 3 study arms receiving a support strategy. It was determined whether recommendations to optimise udder health were fully, partially, or not implemented, based on on-farm observations by the project team or discussions with the farmers.

Collected data for the psychological evaluation of the field trial

All participants were asked to fill out two psychological questionnaires; one at each farm visit separately. Both questionnaires assessed the psychological variables that are used in the "Theory of Planned Behaviour." Previous research showed the importance of choosing specific behaviour. Therefore, the questionnaire focused on three behaviours that are important for preserving a good udder health: maintaining a high level of health status of the herd, preventing the transmission of bacteria from cow to cow by applying hygienic working procedures, and keeping the environment of animals as hygienic as possible. The items for the scales about attitude, perceived behavioural control, intention, and behaviour were formulated accordingly. Three scales were used as predicting variables for "intention" (3 items), "attitude" (6 items), "subjective norm" (3 items) and "perceived behavioural control" (4 items). The depending variables were "intention" and "mastitis prevention behaviour" (2 items). In the second questionnaire, an additional behaviour scale ("behaviour in the past year," 4 items) was added asking specifically about the behaviour during the intervention study. Finally, participants were asked to evaluate the project at the end of the second questionnaire (Table 6). All items were measured on a 7-point Likert scale. Table 1 shows the final items and scales and gives information about the reliability (Cronbach's Alpha) of the scales which was acceptable or good, except for the scale "mastitis prevention behaviour".

Statistical analysis

Herds needed to have a yield-corrected bulk milk SCC between 200,000 and 300,000 cells/ml in 2010 to be eligible to participate in this study. However, many herds improved their udder health status between 2010 and the time of the first visit in autumn 2011. Therefore, the average yield-corrected bulk milk SCC in the 6 months before receiving the advice report ($m = -6$ to $m = 0$) was used to define herds with a high (≥200,000 cells/ml) or a low (<200,000 cells/ml) bulk milk SCC status at the start of the study ($m = 0$).

The effect of the 3 support strategies on the monthly herd level prevalence of high SCC cows was estimated using population-averaged negative binomial regression models with an autoregressive correlation structure to correct for repeated measures within herds. The study period was defined to range from the 6 months before the receipt of the advice report ($m = -6$) to 12 months after the receipt of this report ($m = 12$). The number of cows with a composite SCC ≥200,000 cells/ml at a certain test day was used as a dependent variable in the model while the natural logarithm of the total number of cows at a certain test day was used as an offset. Study arm (NEGCON, POSCON, VET, STUDYGR), period ($-6 \leq m \leq 0$, $0 < m \leq 4$, $4 < m \leq 12$), bulk milk SCC status (high SCC, low SCC) and all their interactions were evaluated. The 3 study arms with a support strategy were combined into 1 intervention study arm (INTERVEN) if no statistical differences were identified between them.

The implementation of recommendations was evaluated using population-averaged multinomial logistic regression models that corrected for clustering of recommendations within dairy herds. The degree of implementation (not, partially, fully) was used as the dependent variable in these models. Study arm, category of recommendation (milking machine, milking hygiene, environment/housing, dry period, or other), prioritisation of recommendation (3 most important ones, short term, long term), and bulk milk status at $m = 0$ were evaluated as covariates in the models.

Table 1. Final items and scales for the TPB-model, scale reliability (α), mean (*M*), and standard deviation (*SD*) before and after the intervention

Scales and items		Before	After
Attitude[a] To me, keeping my animals' health status as high as possible, is… To me, working hygienically in order to reduce the transmission of germs and bacteria from cow to cow, is … To me, keeping the animals' environment as hygienically flawless as possible, is…	α: *M:* *SD:* range:	.806 6.42 0.58 3.7-7	.902 6.58 0.58 4.3-7
Subjective norms Most work colleagues, who are important to me, believe that it is important… … to keep the animals' health status as high as possible. … to stop the transmission of germs and bacteria from cow to cow. … to keep the animals' environment as hygienically as possible.	α: *M:* *SD:* range:	.846 5.88 0.88 4-7	.904 6.02 0.93 3.7-7
Perceived behavioural control If I pay more attention to my animals' health status, I can reduce the bulk milk somatic cell count in the long term. If I stop the transmission of germs and bacteria from cow to cow, I can reduce the bulk milk somatic cell count in the long term. If I keep the animals' environment hygienically flawless, I can reduce the bulk milk somatic cell count in the long term. If I do more for udder health, I can reduce the bulk milk somatic cell count in the long term.	α: *M:* *SD*: range:	.724 5.28 1.02 2.75-7	.569 5.26 0.86 3.2-7
Intention I want to keep my cows' health status as high as possible. I want to stop the transmission of germs and bacteria from cow to cow as much as possible. I want to keep the animals' environment as hygienically as possible.	α: *M:* *SD:* range:	.745 6.20 1.22 1.5-7	.863 6.35 0.78 2.33-7
Mastitis prevention behaviour I think, I react adequately to mastitis cases in my herd. Recently, I consequently have implemented measures against mastitis on my farm on a daily basis.	α: *M:* *SD*: range:	.416 4.79 1.22 1.5-7	.574 3.95 1.27 1-7
Actual behaviour in the past year In the past year, I have … … supervised my cows' health status much more than before the project. … actively promoted my cows' health status much more than before the project. … actively prevented the transmission of germs and bacteria from cow to cow much more than before the project. … kept the animals' environment much more hygienic than before the project.	α: *M:* *SD:* range:	- - - -	.838 5.04 1.13 2-7

[a] The 3 questions were asked twice with two sets of scale endpoints (1 = unimportant, 7 = important / 1 = useless, 7 = gainful), resulting in 6 items

Moreover, farmers were asked which study arm they preferred to be assigned to during the enrolment phase of the study. The outcome of this random process (assigned to the preferred study arm or not) was additionally evaluated as a covariate. A backwards analysis was used to identify all covariates with a significant contribution ($P < 0.05$) to the final model.

The different scales intended for the "Theory of Planned Behaviour" and the study evaluation questions were tested by factor analysis (varimax rotation) for their dimensionality and with reliability analysis for the quality of the scale (Table 1). Only factors with Eigenvalues > 1 were used to build scales. After reliability analysis (Cronbach's Alpha), the scales were built by calculating the means over the belonging items. Variance analysis for repeated measures (ANOVA PR) and univariate variance analysis (ANOVA) were used to assess the effects of the intervention and the between group differences. Post hoc comparisons (Tukey HSD) were carried out to identify which groups differed significantly from each other. In addition, Pearson correlations and linear regression analyses were conducted to test the TPB-model.

RESULTS

One farmer belonging to the VET study arm was lost to follow up, leaving 99 herds available for statistical analysis. The median herd size was 33 (range: 16 - 125). At the start of the study, 58 herds had a high bulk milk SCC status; the other 41 herds had a low bulk milk SCC status.

Epidemiological evaluation of the field trial

Implementation of recommendations: A total of 875 recommendations, belonging to 77 different recommendations, were given. Of those, 44.3 % were completely implemented, whereas 23.1% were partially and 32.6% were not implemented. No differences between the 3 study arms with a support strategy were observed. Instead, farmers assigned to the study arm of their preference at the start of the study implemented more recommendations than farmers assigned to another study arm (Table 2). Recommendations were significantly more often implemented if they were in the topic of 'milking hygiene', 'environment/housing' and 'other' compared with the topic 'milking machine.'

Table 2. Implementation of recommendations for improving mastitis management according to the final population-averaged multinomial logistic regression model

		Partially implemented	Fully implemented
Variable	Category	OR (95%-CI)	OR (95%-CI)
Topic	Milking hygiene	2.7 (1.7-4.4)	1.8 (1.3-2.6)
	Environment	3.2 (1.6-6.1)	2.1 (1.2-3.5)
	Dry off period	1.8 (0.8-4.4)	0.9 (0.4-1.9)
	Others	11.3 (4.8-26.5)	4.6 (2.1-10.2)
	Milking machine	Ref.	Ref.
Assigned to the preferred study arm	Yes	1.5 (1.0-2.4)	1.6 (1.1-2.3)
	No	Ref.	Ref.

Prevalence of high SCC cows: No statistical differences in the prevalence of high SCC cows were identified between the 3 support strategies (POSCON, VET, STUDYGR). They were therefore evaluated as 1 study arm (INTERVEN). The final statistical model (data not shown) identified a significant 3-way interaction term between bulk milk SCC status at *m* = 0, period, and study arm (INTERVEN vs NEGCON). The observed prevalence of high SCC cows is therefore presented in Fig. 1 to facilitate interpretation.

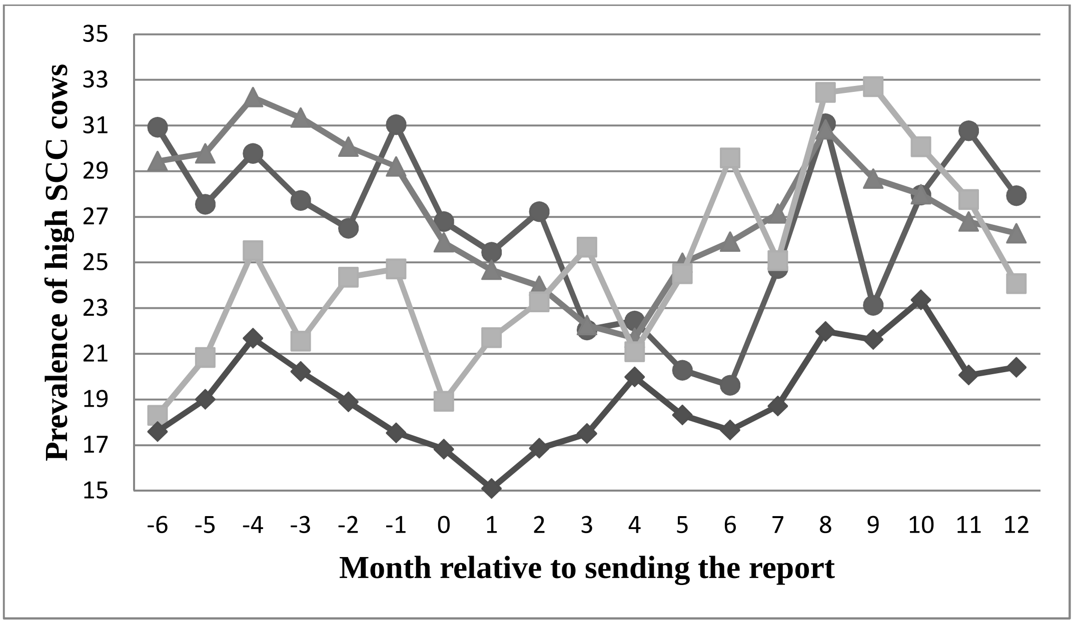

Fig. 1 Prevalence of high SCC cows in herds with a high BMSCC status at *m* = 0 in the NEGCON (○) and INTERVEN (∆) study arms and in herds with a low BMSCC status at *m* = 0 in the NEGCON (□) and INTERVEN (◊) study arms

No significant difference in the prevalence of high SCC cows between the INTERVEN study arm and the NEGCON study arm in the herds with a high bulk milk SCC status at *m* = 0 was identified. However, an intervention effect was observed in the herds with a low bulk milk SCC status at *m* = 0. The prevalence of high SCC cows in the INTERVEN study arm remained at a constant level in these herds, whereas it increased after *m* = 0 in the NEGCON study arm. This became significant in the 4 to 12 months after *m* = 0.

Psychological evaluation of the field trial

Table 3 reports means (*M*) and standard deviation (*SD*), univariate variance analysis (ANOVA), and variances analysis for repeated measures (ANOVA RP) for the scales used in the TPB-model before and after the intervention.

The univariate ANOVAs for the first questionnaire (before) revealed that there were no group differences except for the attitude scale. Post hoc tests revealed that the POSCON study arm had a significant lower positive attitude than the STUDYGR study arm. After the intervention the study arms differed again in their attitude, additionally in their “mastitis prevention behaviour” and their reported “behaviour in the past year.” The VET and STUDYGR study arms reported significantly higher engagement in mastitis prevention behaviour than the NEGCON study arm. The POSCON study arm differed neither from the

NEGCON, nor from the other two study arms in this respect. In regard to the behaviour in the past year all study arms reported a significantly higher engagement in mastitis management than the NEGCON study arm.

The variance analysis for repeated measures comprised three parts (see last column in Table 3). The two factors time (T), study arm (SA) and the interaction term (IA) between those factors were analysed. The factor "time" showed significantly increased "attitudes", "intention", and "mastitis prevention behaviour." The factor "study arm" showed significant differences only for "attitude" and "mastitis prevention behaviour." These results are in line with the univariate analysis. The interaction term showed no significant results.

Table 3. Means (*M*), standard deviations (*SD*), ANOVAs and ANOVAS RP for all scales before and after the intervention

	NEG	POS	VET	STUDY	Total	ANOVA	ANOVA RP
	CON	CON		GR			Time (T):
							Interaction (IA):
	M/SD	*M/SD*	*M/SD*	*M/SD*	*M/SD*	*F/p*	Study arm (SA):
Attitude							
Before	6.44^{ab}	6.21^{a}	6.34^{ab}	6.69^{b}	6.42	3.33	T: *F*(1,95) = 8.10, *p* = 0.01
	.72	.60	.53	.30	.58	.023	IA: *F*(3,95) = 0.11, ns
After	6.63^{ab}	6.36^{a}	6.51^{ab}	6.81^{b}	6.58	2.93	SA: *F*(3,95) = 3.93, *p* = 0.01
	.59	.67	.57	.35	.58	.037	
Subjective norm							
Before	5.96	5.85	5.68	6.00	5.88	.638	T: *F*(1,94) = 2.53, ns
	.69	.94	.97	.90	.88	ns	IA: *F*(3,94) = 0.45, ns
After	6.03	5.88	5.97	6.19	6.02	.495	SA: *F*(3,94) = 0.44, ns
	.89	1.08	.97	.78	.93	ns	
Perceived behavioural control							
Before	5.46	5.27	4.94	5.44	5.28	1.38	T: *F*(1,94) = 0.07, ns
	.98	.75	1.20	1.09	1.02	ns	IA: *F*(3,94) = 0.52, ns
After	5.19	5.37	5.00	5.45	5.26	1.35	SA: *F*(3,94) = 1.54, ns
	1.02	.75	.71	.88	.86	ns	
Intention							
Before	6.21	6.11	6.14	6.33	6.20	.435	T: *F*(1,) = 4.77, *p* = 0.03
	.92	.71	.72	.67	.75	ns	IA: *F*(3,) = 0.63, ns
After	6.28	6.20	6.26	6.67	6.35	1.89	SA: *F*(3,95) = 1.28, ns
	1.05	.75	.72	.43	.78	ns	
Mastitis prevention behaviour							
Before	4.38	4.88	4.77	5.12	4.79	1.62	T: *F*(1,94) = 4.07, *p* = 0.05
	1.48	1.10	.92	1.26	1.22	ns	IA: *F*(3,94) = 0.82, ns
After	4.44^{a}	4.98^{ab}	5.31^{b}	5.44^{b}	5.04	4.28	SA: *F*(3,94) = 3.98, *p* = 0.01
	1.175	.973	.895	1.219	1.129	.007	
Behaviour in the past year							
After	3.66^{a}	4.73^{b}	4.71^{b}	4.80^{b}	4.47	4.36	-
	1.52	1.18	.91	1.50	1.37	.006	-

^{a,b,c}Superscript letters indicate the significant differences identified by the post hoc tests. Differing letters mark significant differences between the means. Scale: 1 = "do not agree at all" / 7 = "agree completely"

Predictor for intention and behaviour

In a next step, linear regression analyses were conducted to identify which psychological factors explained the “intention” to engage in mastitis management and “mastitis management behaviour.” Table 4 shows the Pearson correlations for all included scales at both measurement points. “Intention” correlated significantly with the three predictors “attitude”, “subjective norm” and “perceived behavioural control.“ “Mastitis prevention behaviour” correlated only significantly with “intention” and “attitude.”

Table 4. Pearson correlations, before (*N* = 98) and after (*N* = 99) the intervention

		1.	2.	3.	4.	5.[a]
1. Attitude	before	-	.258*	.178	.714**	.311**
	after	-	. 501**	.302**	.717**	.201*
2. Subjective norm	before		-	.296**	.463**	.124
	after		-	.161	.461**	.184
3. Perceived behavioural control	before			-	.347**	.041
	after			-	.367**	.070
4. Intention	before				-	.270**
	after				-	.343**
5. Mastitis prevention behaviour	before					-
	after					-

[a] **P* < .05; ***P* < .01; ****P* < .001

Table 5 shows the final linear regression models. The study arms were excluded as a fixed effect from the statistical models since no significant effects were observed between them. The regression models for both measure points showed that “attitude” has the highest predicting value for “intention,” followed by “subjective norm” and “perceived behavioural control”. The models explained 60% and 54% of the observed variance of “intention.”

Table 5. Regression analysis for “intention,” before and after the intervention

	Before intervention (N = 98)[a]			After intervention (N = 99)[b]		
	β	*t*-value	*p*	*β*	*t*-value	*p*
Constant		-1.845	.068		-0.75	.453
Attitude	.619	9.308	.000	.600	7.32	.000
Subjective norm	.255	3.725	.000	.133	1.69	.095
Perceived behavioural control	.161	2.394	.019	.164	2.28	.025

[a] Corrected R^2 = .60, *F*(3/94) = 50.30, *p* = .000; [b] Corrected R^2 = .54, *F*(3/95) = 39.15, *p* = .000

Participants’ evaluation of the intervention study

Table 6 summarises the results of the evaluation in regard to the single items and the overall evaluation scale (α = .862). The STUDYGR study arm evaluated the project significantly better than the NEGCON study arm for all items and scales. In addition, the STUDYGR study arm evaluated the project significantly better than the other study arms for some individual items and scales. For example, participants of the STUDYGR study arm perceived that they significantly had learned more than all other study arms.

Table 6. Evaluation after the intervention: Univariate variance analysis (ANOVA)

The participation in this project, …	Total *M/ SD* *N* = 99	NEGCON *M/ SD* *n* = 25	POSCON *M/ SD* *n* = 25	VET *M/ SD* *n* = 24	STUDYGR *M/ SD* *n* = 25	ANOVA *M/ SD* *F/p*
… has called my attention to my “blind spots.”	4.99	4.08^{a}	5.04^{ab}	5.08^{ab}	5.76^{b}	6.55
	1.46	1.44	1.27	1.41	1.27	.000
… has helped me to improve my BMSCC distinctively.	3.92	3.28^{a}	4.08^{ab}	3.75^{ab}	4.56^{b}	2.66
	1.70	1.31	1.85	1.75	1.66	.052
... has taught me new things.	5.08	3.80^{a}	5.08^{b}	5.17^{b}	6.28^{c}	12.45
	1.67	1.87	1.41	1.47	.79	.000
... has given me the opportunity to exchange experiences with mastitis more often with other like-minded people.	3.79	2.60^{a}	2.88^{a}	3.25^{a}	6.40^{b}	30.40
	2.21	1.87	1.90	1.47	.71	.000
... has satisfied my expectations completely.	4.49	3.83^{ac}	4.64^{bc}	3.79^{ac}	5.64^{b}	7.07
	1.77	1.61	1.80	1.89	1.08	.000
... was very profitable for me.	4.77	4.21^{ac}	4.8^{bc}	4.13^{ac}	5.88^{b}	7.11
	1.64	1.59	1.61	1.75	.971	.000
… was of no use for the udder health problems on my farm.	2.83	3.58^{a}	2.64^{ab}	2.92^{ab}	2.20^{b}	3.31
	1.63	1.70	1.41	1.80	1.38	.024
Overall evaluation	4.58	3.69^{a}	4.55^{b}	4.32^{ab}	5.76^{c}	15.55
	1.32	1.20	1.25	1.11	0.76	.000

^{a,b,c} Superscript letters indicate the significant differences identified by the post hoc tests. Differing letters mark significant differences between the means. Scale: 1 = “do not agree at all” / 7 = “agree completely”

Table 7. Study evaluation by the participants of the VET and STUDYGR study arms

	Missings	Yes	No	Don’t know
VET: Monthly vet visits improved udder health in my herd.	0	29%	46%	25%
VET: I will continue the monthly vet visits after the study.	1	9%	44%	45%
STUDYGR: The study groups improved udder health.	1	67%	8%	25%
STUDYGR: I will continue to attend study group meetings, if they are cost-free.	1	79%	0%	21%
STUDYGR: I will continue to attend study group meetings, even if they do cost.	1	21%	8%	71%

The VET and STUDYGR study arms were confronted with some specific questions about their support strategy (Table 7). The participants of both study arms were asked whether their intervention strategy improved the udder health on their farm; 29% of the VET study arm and 67% of the STUDYGR study arm perceived a positive change. Participants were also asked whether they would continue with the experienced support strategy; 9% of the VET study arm and 21% of the STUDYGR study arm agreed, even when the strategy was aligned with additional costs. Since veterinarian visits always involved costs, only the STUDYGR study arm was asked whether they would continue with the study group meetings if they were cost-free. 79% of the participants answered “yes” to this question. The analysis of the “no” and “don’t know” answers showed that 44% of the VET and 8% of the STUDYGR study arm would not further engage in the provided support strategy. Both evaluation questions that involved costs had a high percentage of “don't know” answers (VET: 45%, STUDYGR: 71%).

DISCUSSION

The aim of this field trial was to determine whether 3 support strategies for improving udder health in Swiss dairy herds were able to decrease the prevalence of high SCC cows. However, in contrast to what was anticipated, the support strategies rather prevented an increase in the prevalence of high SCC cows in herds with a low bulk milk SCC than that they improved the udder health situation in herds with a high bulk milk SCC. A variety of reasons may explain the lack of improvement in herds with a high bulk milk SCC. First, it may be that the ineffective recommendations were given or that they were not communicated in the correct way (e.g., too many at once). However, recommendations were based on current scientific knowledge and practical experience. They were proven to be effective previously. In contrast, an incorrect communication could not be excluded as a possible explanation for failure of improvement. Second, the fact that on average only 44% of the recommendations were fully implemented in the current study may explain the lack of improvement in herds with a high bulk milk SCC status at m = 0. A higher compliance in implementing mastitis management recommendations improved the udder health situation in a similar study in the UK (Green et al., 2007) but such an effect could not be observed in the current study (data not shown). The relatively low compliance does not explain the prevented decreasing udder health situation in herds with a low bulk milk SCC status though. Finally, and most likely, farmers may not perceive that they have an udder health problem in their herd, making it unlikely that they will adapt their mastitis management to improve their udder health situation (Janz & Becker, 1984). However, all possible reasons for not observing an intervention effect in the herds with a high bulk milk SCC status remain speculation and further research is needed to explain the effects seen in this field trial.

Swiss dairy farmers receive a financial bonus when their bulk milk SCC is <100,000 cells/ml. This may explain why farmers with a low bulk milk SCC status at m = 0 were able to prevent an increase in the prevalence of high SCC cows using 1 of the 3 support strategies. The advice report, in combination with a possible continued support by their own veterinarian or with their peers, may have given them the necessary support to maintain a good udder health situation in their herds to continue receiving a bonus (McKenzie-Mohr, 2012). Farmers in the NEGCON study arm not receiving any support may have lacked the necessary support to maintain a good udder health in their herds.

Recommendations for improvement of mastitis management were more often implemented when they were in the topic of ‘milking hygiene’, ‘environment/housing’ and ‘other’ compared with the topic ‘milking machine’. These differences can be explained by the fact that adaptations on the milking machine are often expensive and therefore less likely to be implemented (Huijps et al., 2009). The observation that farmers implemented more recommendations for optimising their mastitis management if they were assigned to the study arm of their preference can be used in future national mastitis control programmes. Support strategies should be tailored to farmers’ preference for knowledge transfer (Jansen et al., 2010) in such control programmes.

The present study did not intend to deliberately target psychological changes in the participants. In fact, the study observed what kind of psychological effects were triggered in farmers participating in the different support strategies. In addition, the study assessed which factors predicted mastitis prevention behaviour best and how participants evaluated the different strategies. The participation in the intervention study increased participants’ positive “attitude,” “intentions,” and “mastitis prevention behaviour.” Subjective norms and perceived behavioural control were not significantly affected by the tested support strategies. Group differences resulting from the one-year process could only be observed in the two behaviour scales. As suggested by the “Theory of Planned Behaviour” the intention to engage in udder health management was predicted by “attitude” “subjective norms,” and “perceived behavioural control.” The strongest predictor was the “attitude” towards the behaviours in question. Mastitis prevention behaviour was predicted by “intention,” but as in other studies using the TPB-framework, an intention-behaviour-gap was observed (Sheeran, 2002) suggesting that context factors (workload, bonus system, etc.) may play an important role too. However, targeting positive attitude changes, strengthening the awareness of high peer norms and increasing perceived behavioural control seem to be promising strategies to foster mastitis prevention behaviour. This could be incorporated in addition to different support strategies in future studies.

The participating farmers evaluated the STUDYGR study arm as most positive. They perceived more benefit and experienced more udder health improvement than the other study arms. This may be due to the fact that the farmers were put in an active role by this support strategy that allowed them to experience self-efficacy. This approach would also allow influencing farmers’ awareness of high quality norms (subjective norms), help to reflect the efficacy of different strategies (perceived behavioural control), and increase farmers’ awareness that mastitis management is a long-term investment.

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DRUG RESISTANCE

A SYSTEMATIC REVIEW AND META-ANALYSIS OF FACTORS ASSOCIATED WITH ANTHELMINTIC RESISTANCE IN SHEEP

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SUMMARY

Anthelmintic resistance (AR) in sheep gastro-intestinal nematodes has been reported worldwide. In this study, frequency of treatment, drench-and-shift pasture management, and use of long-acting formulations were positively associated with AR, while there was conflicting evidence on mixed-species grazing. Scant evidence was found on the association between AR and other commonly recommended practices, such as rotation of drug classes or use of combination anthelmintics, suggesting the need for further research. The findings also highlight the need to improve study designs and reporting of research in the field of veterinary parasitology, so results can be used to support evidence-based decisions regarding AR management.

INTRODUCTION

Disease due to gastro-intestinal nematodes (GINs) is a major economic constraint to grazing sheep production worldwide (Knox et al., 2012). Traditionally, GINs have been controlled with broad-spectrum anthelmintic drugs, which are inexpensive and easy to use (Sargison, 2011). However, the routine use of anthelmintic drugs has led to the development of anthelmintic resistance (AR) whereby anthelmintic drugs have reduced or no effectiveness against GINs present within animals (Morgan, 2013).

Anthelmintic resistance is now considered the *status quo* in most sheep-rearing countries (Kaplan and Vidyashankar, 2012), and repeated cross-sectional studies in Europe and South America have shown a worsening situation, with both multi-drug and multi-species resistance being increasingly more common (Papadopoulos et al., 2012; Torres-Acosta et al., 2012). A recent study by Scott et al. (2013) documented the first field case of monepantel resistance, a novel anthelmintic drug which has only been commercially available since 2009. All this highlights the urgent need to identify risk factors associated with AR development, to inform future recommendations on sustainable parasite control (Morgan, 2013).

Current recommendations to delay further development of AR are based either on evidence regarding key molecular processes involved in the selection for resistance (Dobson et al., 2001), or simulation models (Leathwick et al., 1995). More recently, several observational studies and clinical trials have been described (Suter et al., 2004; Leathwick et al., 2008; Waghorn et al., 2009; Calvete et al., 2012). However, these studies are often based on single populations, and sometimes provide conflicting evidence. By way of example,

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mixed-species grazing has been described as both a protective and risk factor for AR in different studies (Eddi et al., 1996; Lawrence et al., 2006). Thus, there is a need for better evidence regarding the impact of these practices.

Systematic Reviews (SRs) and Meta-Analyses (MAs) are considered the best studies for providing high-quality evidence across published literature. Systematic reviews are guided by a pre-defined research question and search strategy, thus ensuring the repeatability and transparency of the process, while the quantitative synthesis of results through the MA improves the precision and external validity of the pooled estimate (Sargeant et al., 2006).

The objective of this study was to perform a SR-MA on factors associated with AR in sheep. Specifically, the objectives were: (i) to review; (ii) critically appraise; and (iii) provide a qualitative and quantitative synthesis of all currently available literature on the topic.

MATERIALS AND METHODS

Research question, search terms and literature search

A protocol was developed, and the research question and search terms were defined following consultation with experts in the field. The population of interest was sheep; the outcome was AR in GINs of economic interest (noted below); while the intervention of interest was factors associated with AR previously described in the literature. The full PubMed search string was: ["small ruminants" OR ovine OR sheep OR ewe* OR ram OR lamb OR wether] and [anthelmintic OR drench OR "macrocyclic lactone*" OR benzimidazole* OR levamisole OR ivermectin OR thiabendazole] AND [[[gastrointestinal OR internal] AND [parasite*]] OR nematode* OR roundworm* OR haemonchus OR trichostrongylus OR teladorsagia OR ostertagia OR oesophagostomum OR chabertia OR nematodirus OR cooperia] AND [resistance OR resistant] AND [breed OR age OR gender OR refugia OR “risk factor” OR association OR exposure OR prevalence OR incidence OR survey OR management OR “anthelmintic dosage” OR drench OR bolus OR “under dosing” OR "route of administration" OR “oral formulation” OR “persistent” OR "long-acting" OR “timing of treatment” OR “pre-lambing treatment” OR “treatment of adult ewes” OR “quarantine” OR “mixed species grazing” OR “rotational grazing” OR "strategic treatment" OR “targeted selective treatment” OR “pasture management” OR “flock size” OR rotation].

A systematic literature search for all publications published between 1st January 1975 to 8th February 2013 was conducted in four electronic databases (PubMed [Medline], Agricola [EBSCO Host], CAB Direct, and Web of Science). Additionally, recent literature reviews and conference proceedings on the topic were hand-searched for relevant studies. Finally, search verification was performed by consulting several experts in the field, and through solicitation of information on two list-serves dedicated to the discussion of small ruminant clinical problems (American Association of Small Ruminant Practitioners®, United States and SheepVets®, United Kingdom). All citations were imported into Refworks© (ProQuest, LLC, Cambridge Information Group; Betheseda, MD, USA) and de-duplicated.

Abstract and full-text screening

References were screened at the abstract level, and, if they met the inclusion criteria defined below, at the full-text level. Screening was conducted by two independent investigators (LCF and AM) using pre-defined screening tools in an electronic SRS nexus review format (Möbius Analytics, Ottawa, Ontario, Canada). Cohen's Kappa (Cohen, 1960) agreement between reviewers was computed, and a $\kappa \geq 0.8$ was considered acceptable.

References were considered eligible if they: (i) described primary research on risk or protective factors of interest associated with AR in the GINs of interest in sheep; and (ii) reported observational, randomized controlled or challenge trial designs; there were no language restrictions. All other references were excluded, and the reasons for exclusion were noted. Any discrepancy regarding a study's eligibility was resolved by discussion between the two investigators; if consensus was not reached, further adjudication by a third investigator (TJO) was conducted.

Risk of systematic bias and data extraction

Two independent investigators (LCF and TJO) assessed all relevant publications for risk of systematic bias using two instruments for experimental (Higgins et al., 2011) and observational studies (Kim et al., 2013). Domains considered for potential bias included: selection, performance, detection, attrition and reporting bias. Publications were then classified as having a high, low or unclear (not reported or unable to assess) risk of bias in each domain based on pre-set definitions.

Qualitative data extraction, also performed by two independent investigators (LCF and TJO), was carried out for information on: (i) study characteristics (language and year of publication, study design and geographical location); (ii) animal characteristics (number of animals in each treatment and control group, and number of animals per farm); (iii) type of protective or risk factor; and (iv) type of outcome measurement (anthelmintic drug(s) investigated, diagnostic test used to define AR, and GIN species investigated). Publications reporting more than one independent study were duplicated and extracted as separate studies, while multiple publications reporting the same study were extracted as a single study.

Quantitative data extraction and meta-analysis

Full-text publications were included in the quantitative synthesis if they: (i) measured AR using a methodology defined *a priori* in the protocol; (ii) investigated a factor of interest; and (iii) reported quantitative results in sufficient detail to estimate the odds of AR in sheep compared to a control group.

If controlling for confounding was performed, adjusted odds ratios (ORs) and covariates were extracted as reported in the study. Otherwise, unadjusted estimates were extracted. If the OR was not reported it was estimated using raw data. Authors were not contacted to add any additional data or offer clarification due to time limitations of the present study.

When factors were reported in more than one independent study, a random effects MA to account for heterogeneity was used to estimate a pooled effect size (ES) for each factor. Statistical heterogeneity was assessed using the I^2 statistic, and a χ^2 test was used to assess statistical significance of heterogeneity within each analysis ($p \leq 0.05$) (Borenstein et al., 2009). Sensitivity analyses through step-wise removal of the publications were performed to determine whether certain publications had substantial impact on the ES estimated in each analysis. Funnel plots were not assessed for determination of publication bias as there were fewer than 10 studies for each included factor (Higgins and Green, 2011). Analyses were conducted in STATA version 12.0 (StataCorp, College Station, Texas, USA) and RevMan (Version 5.2; The Cochrane Collaboration, Oxford, UK).

RESULTS

Literature search, abstract and full-text screening

The search provided a total of 2,914 citations, while another 5 citations were identified through the search verification process. After de-duplication, 1,712 titles and abstracts were screened, of which 131 full-text publications were further evaluated for relevance. Thirty publications were included in the risk of bias assessment and qualitative data extraction, while the remaining 101 full-text publications were excluded because they: (i) only described factors putatively associated with AR and not AR prevalence (n=30); (ii) only described AR prevalence and not factors of interest (n=23); (iii) measured a non-relevant factor or outcome (n=23); or (iv) reported a non-relevant study design (n=16). Three additional publications were excluded because there were no resources for translation (one Arabic and two Slovak publications), while another six publications could not be retrieved.

Risk of systematic bias and data extraction

Thirty publications describing 25 individual studies were included in the methodological assessment for risk of bias; of these, 15 were observational (14 cross-sectional and 1 cohort study), while 10 were experimental (3 controlled and 7 challenge trials). Unclear (not reported, or unable to assess) or high risk of selection bias based on selection of participants was found in 20% (3/15) and 73% (11/15) of the observational studies, respectively, while 60% (9/15) had a high risk of selection bias based on assessment of confounding variables. Unclear and high risk of performance bias was identified in 40% (6/15) and 13% (2/15) of the observational studies, respectively. All observational studies were found to have a low risk of detection bias, while an unclear or high risk of attrition bias was identified in 7% (1/15) and 13% (2/15) of the studies, respectively. Unclear risk of reporting bias was identified in one observational study. All trials were found to have unclear risk of selection bias and a low risk of detection, attrition and reporting bias.

The descriptive characteristics of the observational and experimental studies included in the qualitative synthesis in this systematic review are presented in Table 1.

Meta-analysis

Publications reporting observational study designs (n=13) described 10 individual studies that were included in the quantitative synthesis, of which only one (Lawrence et al., 2006) provided adjusted estimates, controlling for the season in which AR was measured and the use of purposive sampling. All 10 studies reported observations at the aggregated (farm) level.

The 10 studies included described 8 factors; however, 3 of these factors were described in only one study, and could therefore not be synthesized. These included: calibration of the drench gun (OR=3.11; 95% Confidence Intervals [CI]=0.95, 10.15) (Hughes et al., 2007); use of quarantine strategies (OR=0.26; 95% CI=0.09, 0.66) (Suter et al., 2004 & 2005); and annual rotation of anthelmintic drug classes (OR=1.48; 95% CI=0.88, 4.26) (Suter et al., 2004 & 2005).

Table 1. Descriptive characteristics of the 15 observational studies and 10 trials included in a systematic review of factors associated with anthelmintic resistance in sheep

Study (Author and year)	Study design	Country	Protective and/or risk factors reported	Anthelmintic drug	Outcome measurement	Type of GIN[a] recovered
OBSERVATIONAL STUDIES:						
Bartley et al. (2003)	Cross-sectional	Scotland	Farm type	Benzimidazole	EHA[b]	Not specified
Calvete et al. (2012)	Cross-sectional	Spain	Frequency of treatment; mixed-species grazing; rotation of anthelmintic drug classes; under-dosing	Benzimidazole	EHA[b]	Not specified
Chartier et al. (1998)	Cross-sectional	France	Frequency of treatment	Fenbendazole	FECRT[c]	Not specified
Echevarria et al. (1996)	Cross-sectional	Brazil	Flock size; frequency of treatment	Ivermectin, Benzimidazole, Levamisole, Closantel, Combination (Albendazole+ Levamisole)	FECRT[c]	Not specified
Eddi et al. (1996)	Cross-sectional	Argentina	Flock size; frequency of treatment; mixed-species grazing	Benzimidazole, Ivermectin, Levamisole, Combination (Albendazole+ Levamisole)	FECRT[c]	Not specified
Hughes et al. (2007)	Cross-sectional	New Zealand	Quarantine; drench gun calibration; drench-and-shift; mixed-species grazing; long-acting formulations; frequency of treatment	Ivermectin	FECRT[c]	Not specified
Kettle et al. (1981 & 1982)	Cross-sectional	New Zealand	Frequency of treatment	Thiabendazole, Levamisole	FECRT[c]	Not specified

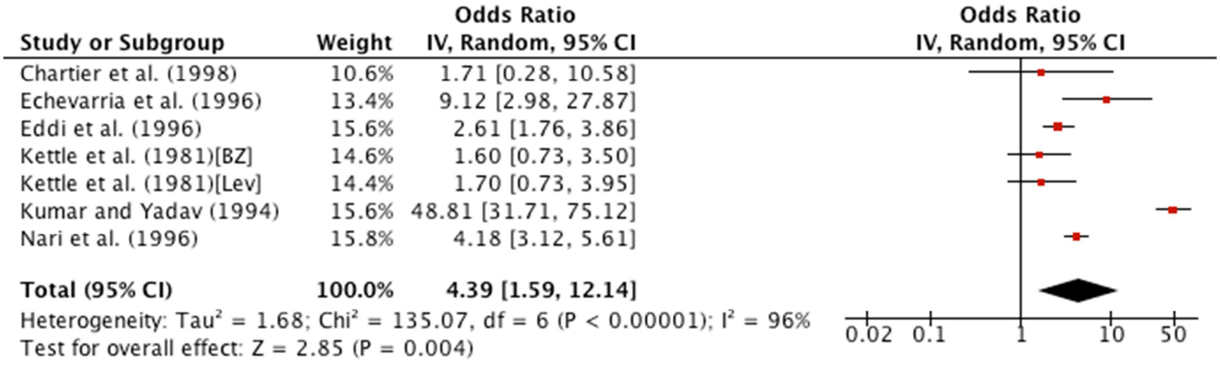
Table 1. Continued

Study (Author and year)	Study design	Country	Protective and/or risk factors reported	Anthelmintic drug	Outcome measurement	Type of GIN[a] recovered
OBSERVATIONAL STUDIES:						
Kumar and Yadav (1994)	Cross-sectional	India	Frequency of treatment	Benzimidazole	FECRT[c]	Not specified
Lawrence et al. (2006) & Waghorn et al. (2006)	Cross-sectional	New Zealand	Long-acting formulations; mixed-species grazing; quarantine	Ivermectin	FECRT[c]	Not specified
Mitchell et al. (2010)	Cross-sectional	Wales	Farm type	Benzimidazole, Levamisole	LDA[d]	Not specified
Nari et al. (1996)	Cross-sectional	Uruguay	Frequency of treatment; flock size	Albendazole, Levamisole, Ivermectin	FECRT[c]	Not specified
Niciura et al. (2012) & Verissimo et al. (2012)	Cross-sectional	Brazil	Quarantine; rotational grazing; Farm type; drench-and-shift; mixed-species grazing; targeted treatment; rotation of anthelmintic drug classes; combination drug formulations; under-dosing	Benzimidazole	Genotyping of F200Y polymorphism	*Haemonchus* sp.
Rendell et al. (2006)	Retrospective cohort	Australia	Long-acting formulations	Ivermectin	FECRT[c]	*Ostertagia* sp.
Suter et al. (2004 & 2005)	Cross-sectional	Australia	Mixed-species grazing; quarantine; frequency of treatment; rotation of anthelmintic drug classes	Ivermectin	FECRT[c]	Not specified
Swarnkar & Singh (2010)	Cross-sectional	India	Flock size; frequency of treatment	Benzimidazole, Tetramisole	FECRT[c]	Not specified

Table 1. Continued

Study (Author and year)	Study design	Country	Protective and/or risk factors reported	Anthelmintic drug	Outcome measurement	Type of GIN[a] recovered
TRIALS:						
Leathwick et al. (2006)	Controlled trial	New Zealand	Timing of treatment; targeted selective treatment; long-acting formulations	Albendazole	FECRT[c]; LDA[d]	*Teladorsagia circumcincta; Trichostrongylus colubriformis*
Leathwick et al. (2012)	Controlled trial	New Zealand	Targeted selective treatment; Combination drug formulations	Ivermectin; Levamisole	FECRT[c]; LDA[d]	*T. circumcincta;* *T. colubriformis*
Martin et al. (1982 & 1984)	Controlled trial	Australia	Drench-and-shift	Thiabendazole	EHA[b]; FECRT[c]	Not specified
Leignel et al. (2010)	Challenge trial	France	Rotation of anthelmintic drug classes	Levamisole; Benzimidazole	FECRT[c]	*T. circumcincta*
Le Jambre et al. (1999)	Challenge trial	Australia	Long-acting formulations	Ivermectin; Moxidectin	Counting of resistant parasite strains	Not specified
Martin (1989)	Challenge trial	Australia	Drench-and-shift	Thiabendazole	EHA[b]; FEC[e]	*Teladorsagia* sp.
Sutherland et al. (2000)	Challenge trial	New Zealand	Long-acting formulations	Ivermectin; Albendazole	EHA[b]; FEC[e]	*T. circumcincta;* *T. colubriformis*
Waghorn et al. (2008)	Challenge trial	New Zealand	Drench-and-shift	Albendazole	EHA[b]; LDA[d]; FEC[e]	*T. circumcincta;* *T. colubriformis*
Waghorn et al. (2009)	Challenge trial	New Zealand	Targeted selective treatment; combination drug formulations	Ivermectin; Levamisole	FECRT[c]; LDA[d]	*T. circumcincta;* *T. colubriformis*
Waller et al. (1989)	Challenge trial	Australia	Frequency of treatment; drench-and-shift; rotation of anthelmintic drug classes	Thiabendazole; Albendazole; Levamisole; Ivermectin	EHA[b]; LDA[d]; TBA[f]	*H. contortus;* *T. colubriformis*

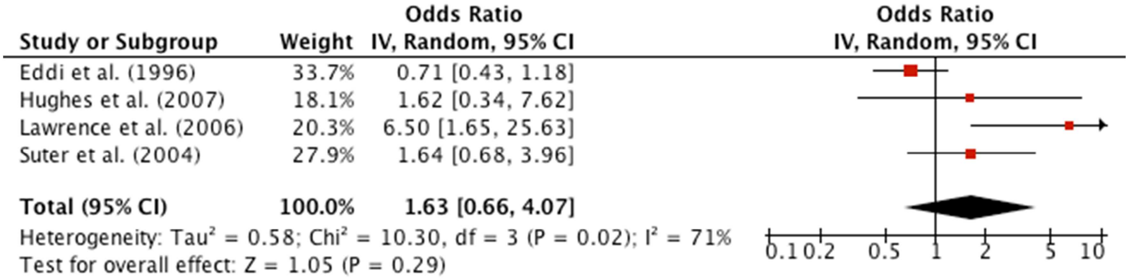
GIN[a]=Gastro-Intestinal Nematode; EHA[b]=Egg Hatch Assay; FECRT[c]=Fecal Egg Count Reduction Test; LDA[d]=Larval Development Assay; FEC[e]=Fecal Egg Counts; TBA[f]=Tubulin Binding Assay



Study or Subgroup	Weight	Odds Ratio IV, Random, 95% CI
Chartier et al. (1998)	10.6%	1.71 [0.28, 10.58]
Echevarria et al. (1996)	13.4%	9.12 [2.98, 27.87]
Eddi et al. (1996)	15.6%	2.61 [1.76, 3.86]
Kettle et al. (1981)[BZ]	14.6%	1.60 [0.73, 3.50]
Kettle et al. (1981)[Lev]	14.4%	1.70 [0.73, 3.95]
Kumar and Yadav (1994)	15.6%	48.81 [31.71, 75.12]
Nari et al. (1996)	15.8%	4.18 [3.12, 5.61]
Total (95% CI)	**100.0%**	**4.39 [1.59, 12.14]**

Heterogeneity: $Tau^2 = 1.68$; $Chi^2 = 135.07$, df = 6 ($P < 0.00001$); $I^2 = 96\%$
Test for overall effect: Z = 2.85 (P = 0.004)

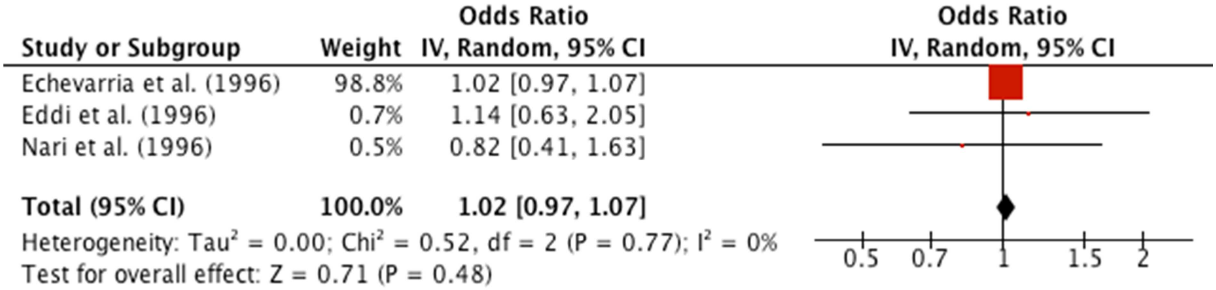
Fig. 1a Forest plot for studies included in the meta-analysis evaluating the association between anthelmintic resistance in sheep and frequency of anthelmintic treatment (*Note:* BZ=benzimidazoles; Lev=levamisole)



Study or Subgroup	Weight	Odds Ratio IV, Random, 95% CI
Eddi et al. (1996)	33.7%	0.71 [0.43, 1.18]
Hughes et al. (2007)	18.1%	1.62 [0.34, 7.62]
Lawrence et al. (2006)	20.3%	6.50 [1.65, 25.63]
Suter et al. (2004)	27.9%	1.64 [0.68, 3.96]
Total (95% CI)	**100.0%**	**1.63 [0.66, 4.07]**

Heterogeneity: $Tau^2 = 0.58$; $Chi^2 = 10.30$, df = 3 ($P = 0.02$); $I^2 = 71\%$
Test for overall effect: Z = 1.05 (P = 0.29)

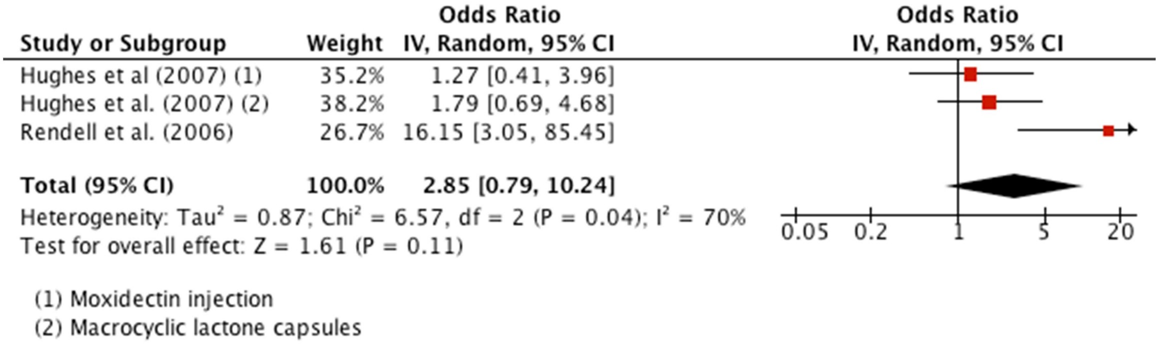
Fig. 1b Forest plot for four studies included in the meta-analysis evaluating the association between anthelmintic resistance in sheep and mixed-species grazing



Study or Subgroup	Weight	Odds Ratio IV, Random, 95% CI
Echevarria et al. (1996)	98.8%	1.02 [0.97, 1.07]
Eddi et al. (1996)	0.7%	1.14 [0.63, 2.05]
Nari et al. (1996)	0.5%	0.82 [0.41, 1.63]
Total (95% CI)	**100.0%**	**1.02 [0.97, 1.07]**

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.52$, df = 2 ($P = 0.77$); $I^2 = 0\%$
Test for overall effect: Z = 0.71 (P = 0.48)

Fig. 1c Forest plot for three studies included in the meta-analysis evaluating the association between anthelmintic resistance in sheep and flock size

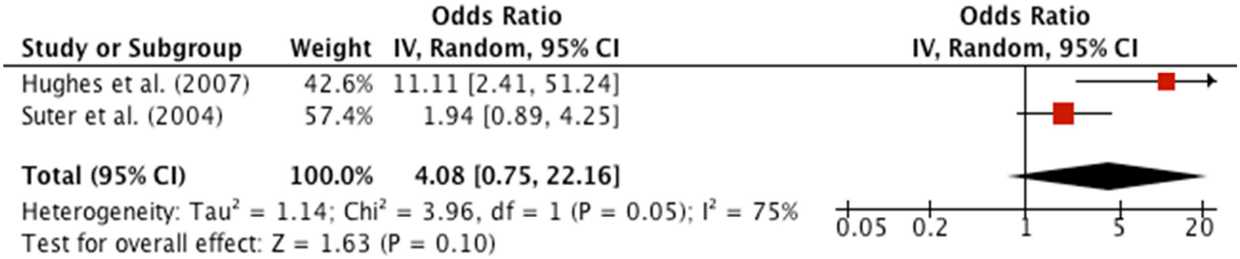


Study or Subgroup	Weight	Odds Ratio IV, Random, 95% CI
Hughes et al (2007) (1)	35.2%	1.27 [0.41, 3.96]
Hughes et al. (2007) (2)	38.2%	1.79 [0.69, 4.68]
Rendell et al. (2006)	26.7%	16.15 [3.05, 85.45]
Total (95% CI)	**100.0%**	**2.85 [0.79, 10.24]**

Heterogeneity: Tau² = 0.87; Chi² = 6.57, df = 2 (P = 0.04); I² = 70%
Test for overall effect: Z = 1.61 (P = 0.11)

(1) Moxidectin injection
(2) Macrocyclic lactone capsules

Fig. 1d Forest plot for studies included in the meta-analysis evaluating the association between anthelmintic resistance in sheep and long-acting formulations



Study or Subgroup	Weight	Odds Ratio IV, Random, 95% CI
Hughes et al. (2007)	42.6%	11.11 [2.41, 51.24]
Suter et al. (2004)	57.4%	1.94 [0.89, 4.25]
Total (95% CI)	**100.0%**	**4.08 [0.75, 22.16]**

Heterogeneity: Tau² = 1.14; Chi² = 3.96, df = 1 (P = 0.05); I² = 75%
Test for overall effect: Z = 1.63 (P = 0.10)

Fig. 1e Forest plot for studies included in the meta-analysis evaluating the association between anthelmintic resistance in sheep and the practice of drench-and-shift

Random-effects MA were performed for the other five factors: frequency of anthelmintic treatment; mixed-species grazing; flock size; use of long-acting formulations; and drench-and-shift pasture management. Figure 1(a-e) below shows the individual and pooled estimates of the studies included (OR and 95% CI), and the heterogeneity of the estimate (I^2 and associated p-value), for each of these factors. In each forest plot, the center of the square represents the individual estimate for that study and the area of the square is proportional to the weight assigned to that study (based on (i) the number of observation units in the study and (ii) confidence intervals). The solid vertical line marks the value at which the factor has no effect on AR levels, while the diamond (♦) at the bottom shows the 95% CI for the pooled estimate.

<u>Sensitivity Analysis</u>

The sensitivity analysis using stepwise removal of publications for frequency of treatment showed that removal of estimates by Kumar and Yadav (1994) significantly reduced the pooled estimate (OR=3.67; 95% CI=2.33, 5.78) and the observed heterogeneity (I^2=56%; p=0.08). Similarly, the removal of Rendell et al. (2006) from the MA for use of long-acting formulations significantly reduced the overall pooled estimate (OR=1.55; 95% CI=0.75, 3.23) and the observed heterogeneity (I^2=0%). No other significant changes were observed when the analyses for the other three factors were repeated with step-wise omission of study estimates.

DISCUSSION

This SR-MA provides further evidence on the association between certain management practices and AR in sheep, while highlighting future research needs. Despite the current relevance of the topic for the sheep industry worldwide, there were surprisingly few studies that investigated the association between putative risk factors and AR in sheep, and fewer still provided sufficient data for a quantitative synthesis.

A high level of heterogeneity in the results was observed in most of the MA results. This was to be expected, as all the studies included were observational studies. While some authors argue that observational studies should not be included in a MA (Lean et al., 2009), others justify their inclusion since certain risk factors can only be investigated this way (Stroup et al., 2000). Given the limited number of studies included in this MA, sub-group analysis or meta-regression to further investigate the heterogeneity between the studies was not possible. Apart from heterogeneous study populations and independent research groups, other possible explanations for the observed heterogeneity are the use of different definitions for putative risk factors by the different studies, and the use of multiple diagnostic tools for AR. The latter is of particular concern, as there is still no consensus on the preferred method for AR diagnosis, greatly impeding the comparison of study results (Knox et al., 2012).

Frequency of treatment was the risk factor for AR most commonly described in the included publications, which is not surprising as for a long time this has often been incriminated as one of the most important contributors to AR development (Sutherland and Scott, 2010). In this study, it was found that flocks that were treated more frequently had higher odds of having resistance, compared to those farms that treated the sheep less often. Higher treatment frequencies increase the selective advantage for resistant parasites, allowing for an increase in the proportion of resistant parasites over time (Sargison, 2011).

Two other management practices that were investigated for their association with AR in the included publications were mixed-species grazing and drench-and-shift. Both practices are based on the concept of *refugia*, which refers to the proportion of susceptible parasites that are not exposed to anthelmintic treatment, either because they are found in the environment or because they are in untreated animals (van Wyk, 2001). Such parasites constitute a reservoir of susceptible genes. Mixed-species grazing with cattle has long been hypothesized as a protective factor for AR, as cattle are generally not infected by sheep GINs and can therefore be used to clean up contaminated pastures (Eddi et al., 1996). However, recent evidence indicates that co-grazing sheep with cattle may actually increase AR levels, as it results in a reduction in the number of parasites in *refugia* on pasture (Lawrence et al., 2006). Results from our study also showed higher odds of AR on farms that practiced mixed-species grazing, though this estimate was not statistically significant, likely due to the limited number of studies. Further research is therefore required to establish the true association between mixed-species grazing and AR.

Drench-and-shift was commonly practiced in the 1980s and 1990s, whereby producers were encouraged to treat their animals with anthelmintic drugs immediately before moving them to clean pastures to reduce GIN re-infection. However, this practice has been found to be positively associated with AR, as resistant parasites that survive the anthelmintic treatment have a selective advantage when infected sheep are put on clean pastures with no susceptible parasites in *refugia* (Abbott et al., 2009). Results from the present study show that the odds of having AR were four times higher on farms that practiced drench-and-shift, compared to those farms that did not. While the pooled estimate was not statistically significant, it does suggest that this practice is associated with AR and should therefore be discouraged.

Another factor frequently investigated in the included publications was the use of long-acting anthelmintic drugs, which delay GIN re-infection. However, as the anthelmintic concentration subsides, both homozygous- and heterozygous-resistant parasites have a selective advantage over homozygous-susceptible parasites, thus allowing for an increase in the number of resistant parasites (Sutherland and Scott, 2010). Results from the present study showed a marginally significant positive association between the use of long-acting formulations and AR, which provides further evidence that use of these formulations could be positively associated with AR. They should therefore only be used when the *refugia* status of the farm is known to be high through grazing management history and repeated fecal egg counts (Sargison et al., 2010).

Three studies reported an association between flock size and AR; higher flock sizes are usually associated with higher stocking rates and, hence, more frequent treatments (Eddi et al., 1996). However, the MA pooled estimate showed no association between flock size and AR levels.

Several other factors could not be investigated in the MA, either because they were only reported in one study, or because they were not reported in sufficient detail to allow for data extraction and synthesis. This highlights the need for further research and better reporting on factors such as the use of combination anthelmintics or annual rotation of anthelmintic drug classes.

There was a variable risk of systematic bias in many studies, which is similar to findings reported by other recent SR-MAs in the veterinary parasitology field (Mederos et al., 2012; Belo et al., 2013). Most of the observational studies included in this review had a high or unclear risk of selection bias as there was no justification for the choice of the study population, and confounding was rarely taken into account. An unclear risk of selection bias was also identified in all of the trials, as none adequately described how blinded randomization was achieved. The STROBE and REFLECT reporting guidelines for observational studies and clinical trials, respectively, have been published recently to help offset this deficiency in reporting. However, Cobo et al. (2011) noted that few authors currently implement these guidelines, and suggested that either authors are unaware of their existence, or the guidelines are too hard to implement in the writing stages if they have not been adequately addressed during the study design and implementation. It is thus important that researchers are familiar with these guidelines early in the research process.

Publication bias could not be investigated in this study due to an insufficient number of studies, which may have influenced the estimates reported in this study. Moreover, although there were no exclusion criteria based on language, three full-text articles that passed abstract screening were excluded since translation was not possible, which might have introduced language bias. Since most relevant studies had an unclear to high risk of bias in many of the domains investigated, it was decided not to restrict the MA to studies with low bias; however, the high risk of bias identified in these studies may have influenced the pooled estimates. Lastly, the high levels of heterogeneity observed in the MA suggest that the pooled estimates should be interpreted with caution.

In conclusion, this SR-MA provides an important overview of the evidence currently available on factors associated with AR in sheep, while highlighting the need for further research, particularly on the impact of mixed-species grazing on AR levels as this management practice is often recommended for sustainable parasite control on both conventional and organic sheep farms. Findings from this study also underscore the need to improve methodological reporting in the field of veterinary parasitology and to standardize methods used for detection of AR, thus allowing for a better comparison and synthesis of results from different studies.

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ASSESSMENT OF THE IMPACT OF FEEDING WASTE MILK CONTAINING ANTIBIOTIC RESIDUES ON THE PREVALENCE OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING E. COLI IN CALVES

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SUMMARY

A longitudinal field study was carried out on a dairy farm known to harbour cefotaximase (CTX-M)-positive *E. coli*, in order to assess the impact of feeding waste milk containing antibiotic residues (WM+AR) on the prevalence of these bacteria in the faeces of calves. Regression analyses were performed on bacterial count data using a population-averaged approach based on generalised estimating equations (GEE). Feeding WM+AR increased the prevalence of calves infected with CTX-M-positive *E. coli*, although this was not statistically significant. It also increased the total amount of *E. coli* and of CTX-M-positive *E. coli* shed in faeces. However, there was no difference between calves fed WM+AR or calves fed milk replacer in the proportion of *E. coli* isolates that were CTX-M-positive. Shedding of CTX-M-positive *E. coli* persisted for longer after weaning in calves fed WM+AR.

INTRODUCTION

Escherichia coli that produce the Extended-Spectrum Beta-Lactamase (ESBL) cefotaximase (CTX-M) are resistant to most third and fourth generation cephalosporins, which are important antibiotics in human medicine. Previous studies have indicated that dairy calves could be a reservoir for CTX-M-positive *E. coli* in the UK (Teale et al., 2005; Liebana et al., 2006 and Watson et al., 2012). One study found 82.8 % of calves on a single dairy farm positive for these bacteria at one day of age (Watson et al., 2012). In addition, farms that had used third and fourth generation cephalosporins in the previous 12 months were almost four times more likely to have CTX-M-positive *E. coli* present (Snow et al., 2012).

It has been suggested that waste milk could be a source of antibiotics for calves which might exert a selective effect on the occurrence of resistance in gut commensal bacteria (Goodger et al., 1993; Aust et al., 2012; Brunton et al., 2012; Duse et al., 2013). Waste milk is milk that is unfit for human consumption and can contain colostrum, milk from mastitic cows or milk from cows treated with antibiotics or other medicines. A recent survey of dairy farms in England and Wales found that waste milk was fed to calves on 83% of farms responding to the survey (Brunton et al., 2012). Comparable findings have been reported in Sweden (Duse et al., 2013). The survey in England and Wales found that cefquinome, a fourth generation cephalosporin, was the cephalosporin most likely to be present in waste

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milk fed to calves. Since the therapeutic administration of third and fourth generation cephalosporins to calves appears to be uncommon, it is possible that exposure of calves to these antibiotics is largely through the consumption of waste milk.

The aim of this study was to assess the impact of feeding waste milk with antibiotic residues on the prevalence of ESBL-producing *E. coli* in the intestinal flora of calves in order to test the following hypotheses: that the feeding of waste milk with antibiotic residues (WM+AR) affects the proportion of CTX-M-positive *E. coli* in the gut of calves and that this will vary with calf age; and that the feeding of WM+AR affects the prevalence of calves infected with CTX-M-positive *E. coli*.

MATERIALS AND METHODS

Animal husbandry

A known ESBL *E. coli* positive dairy farm, also known to use cefquinome, was identified for the study. Fifty Holstein/Angus cross-bred calves were recruited to the study from birth on a rolling basis and signed onto the project licence by a named animal care and welfare officer at the farm. All animal studies were performed in accordance with the Animals (Scientific Procedures) Act (1986) and were approved by the local Ethical Review Committee. A statistical power analysis based on previous prevalence estimates indicated that 25 animals per group would be an appropriate sample size to detect significant differences with 95% confidence and 90% power, assuming a mean CTX-M-positive *E. coli* group-level prevalence of 50% in the control group and 90% in the treatment group.

Calves were left to suckle with their own dam for 24 48 hours from birth prior to recruitment. At recruitment, calves were alternatively allocated to a treatment or control group and housed in two separate groups for the duration of the study. Within each study group, calves were kept in railed pens sub-grouped by age, but with nose to nose contact still possible through railings. Mains water and hay were provided ad libitum. Maize silage was provided ad libitum from approximately four weeks of age. The treatment group of calves was fed WM+AR from cows on the same farm; the control group was fed a powdered milk replacer free from antibiotics according to the manufacturer’s description (Volac Enerlac Instant, Volac). Calves were fed as per the usual feeding routine on the farm. The amount of milk supplied to and consumed by each group was recorded daily. After weaning calves were fed calf rearer nuts mixed into maize silage, restricted to approximately 4 kg per head. Calves were weighed on entry to the study and at 4, 7 and 13 weeks of age. Any treatments given to the calves, or any health events or changes to management practices were recorded. Weighing and faecal sample collections were carried out by the same two fully trained animal handlers throughout the study.

Sampling

Rectal faecal samples were taken from all calves daily for the first week after enrolment (24-48 hours after birth). After seven days following enrolment (calves aged 8-9 days) faecal sampling was reduced to twice weekly until calves were weaned at approximately seven weeks old. After weaning, faecal sampling was reduced to weekly for a further six weeks from the date of weaning. Once calves reached 13 weeks of age (six weeks post weaning) they were signed off the project licence by the named veterinary surgeon and left the study. In total, 25 samples were collected from each calf.

A single sample of waste milk was collected at each feeding time using a 180 ml sterile dipper. A single sample of the powdered milk substitute was collected weekly in the same manner. A 0.75 ml volume of the samples was added to a pre-prepared 0.75 ml volume of HIBB glycerol in a 1.5 ml cryo tube and frozen at -80°C prior to bacteriological analysis. Two further aliquots of 20 ml each were transferred in to two 30 ml polypropylene universal tubes and frozen at -80°C prior to antibiotic residue analysis. Environmental samples from the calf housing were also collected weekly to estimate the burden of infection of ESBL *E. coli* in the environment. A moistened sterile swab was wiped over the area of interest and then placed into a container with 225 ml buffered peptone water (BPW).

Bacteriological and antibiotic residue testing

Isolation and enumeration (colony forming units (cfu) per gram of faeces) of total *E. coli* and CTX-M-positive *E. coli* were performed using CHROMagar ECC and CHROMagar CTX supplement (CHROMagar, France) respectively. For faecal samples, 1 g of the sample was added to 9 ml of BPW, and then 100 µl of ten-fold serial dilutions in sterile phosphate buffered saline were plated onto the two different agars. Plates were incubated overnight at 37°C with the exception of CHROMagar CTX plates which were incubated for ~48 hours at 37°C. Samples yielding less than 10 cfu/g *E. coli* were plated to the same agar after enrichment at 37 °C for 18-24 hours in BPW. In this case, numbers of total and presumptive CTX-M-positive *E. coli* were calculated for the sample after enrichment. If enrichment confirmed the presence of CTX-M-positive *E. coli* a nominal count value of 50 cfu/g was used. Where enrichment yielded a negative result the results were treated as 0. For milk samples, 1 ml of the sample was added to 9 ml BPW. For both environmental and milk samples, a 10 μl loop from each sample was streaked onto CHROMagar ECC or CHROMagar CTX. The samples were then enriched overnight and then any samples which did not produce growth on agar were re-cultured after enrichment. A subset of presumptive CTX-M-positive *E. coli* colonies from CHROMagar CTX (25 per group) were tested for the presence of the CTX-M ESBL genes by PCR with sequencing primers as described previously (Sabate et al., 2002; Carattoli et al., 2008). Forty-five WM+AR samples and three powdered milk samples were screened for antibiotic residues using the method described by Randall et al. (2013). Based on the expected prevalence of CTX-M-positive *E. coli* in WM+AR (4 %; Randall et al., 2013) a sample size of 45 was sufficient to estimate the prevalence of CTX-M-positive *E. coli* in WM+AR with 95% confidence and 5% precision. A single 2 g aliquot of each sample was diluted 100 fold with HPLC-water and analysed externally with a solvent standard calibration curve (range 400 to 50,000 µg kg-1) for the following cephalosporins: cefalexin, cefalonium, cefapirin, cefazolin, cefoperazone and cefquinome.

Statistical analysis

The antibiotic treatment history of the cows contributing to the waste milk and the amount of milk fed at each feeding was recorded. Individual calf tracking forms were completed to record any treatments. Data from these forms, as well as the results from the bacteriological testing and antibiotic residue testing were entered into a MS Access database. Data checking and cleaning were performed in MS Excel and STATA 12 (STATA Corporation, College Station, TX, USA). All analyses were carried out in STATA 12 and p≤0.05 was used as the significance level in all analyses. Data were explored initially at group level using ANOVA or Pearson's X2 tests to examine differences in the proportion of total *E. coli* that were CTX-M-positive, the number of infected calves over time, and the mean number of CTX M bacteria shed over time in each group. These variables were subjected to regression analysis if significant differences were observed between groups in the initial analysis. The rate of colonisation of calves with CTX-M-positive *E. coli* in the two groups was explored using survival analysis.

Three sets of regression analyses were carried out using the CTX-M-positive *E. coli* count data and the total *E. coli* data. A population-averaged approach based on generalised estimating equations (GEE) was used to account for repeated measurements on individual calves over time. For all models, the QIC command in STATA was used to confirm which correlation structure was most appropriate. Three correlation structures were examined: independent, exchangeable and autoregressive. The structure that yielded the lowest QIC value was used. An interaction term between the calf treatment group and sample number (1-25) was included in the models to allow for changes in shedding over time to be examined and compared between the groups. Differences in the proportion of total *E. coli* that were resistant between groups over time were investigated using a model which used a binomial family with logit link function and exchangeable correlation structure. A robust variance estimator was included to provide valid standard errors in case of misspecification of the correlation structure. Two regression analyses were performed using the CTX-M-positive *E. coli* count data. The first explored the proportion of calves that were shedding at any one time in each of the groups using the binomial model approach described above. The second looked at the individual counts of colony forming units (CFU)/g faeces in the calves over time and between groups. CFU data were log10 transformed prior to analysis. This linear model used an exchangeable correlation matrix with a Gaussian family and identity link function with robust standard errors.

RESULTS

Calf Health

The average rate of weight gain was estimated at 4.0 kg per week for the control group and 5.9 kg per week for the treatment group ($p<0.001$). Ten animals were medicated on one or more occasions throughout the study: four calves were treated for diarrhoea, four for umbilical infections, one for both diarrhoea and an umbilical infection and one for a swollen jaw. All of these animals were in the control group which meant that there was significantly higher levels of diarrhoea ($p=0.018$) and umbilical infections ($p=0.018$) in the control group compared with the treatment group. The antibiotics administered to the affected calves were sulfadiazine-trimethoprim, oxytetracyline hydrochloride and amoxicillin-clavulanic acid. Removing medicated animals from the analysis of the animal weights did not affect the overall differences between the two groups. The average volume of WM+AR consumed daily was 116.9 litres, while the average volume of powdered milk consumed daily was 113.7 litres ($p=0.658$).

Contents of waste milk

Cefquinome, a fourth generation cephalosporin, was the most commonly used intramammary and injectable antibiotic in the cows that contributed to each of the daily WM+AR feeds. The subset of samples selected for analysis were found to be highly correlated with all WM+AR samples collected in terms of the antibiotics used in the contributing cows ($r=0.994$; $p<0.001$). Five of the 6 antibiotic compounds screened for were not detected with a detection limit of 400 µg kg-1. In line with the treatment history data, cefquinome residues were detected in 39/45 WM+AR samples, with a concentration range of

390 to 1,700 µg kg-1 and a mean concentration of 746 µg kg-1. Three samples of the milk replacer fed to the control group were also examined and found to be free of any detectable cephalosporin residues (concentration < 400 µg kg-1). Bacteriological testing failed to isolate CTX-M-positive *E. coli* from any of the milk samples. However, 25 of the WM+AR samples yielded purple colonies on CHROMagar CTX media, presumptively identified as Klebsiella spp. based on their colony morphology on the selective medium.

Effect of feeding WM+AR on the proportion of CTX-M-positive *E. coli* in the calf gut

The numbers of total *E. coli* isolated from calves were similar across the two groups, although tended to range higher in calves fed WM+AR. The numbers of CTX-M-positive *E. coli* isolated from calves were consistently higher in the treatment group but tended to follow a similar pattern of decline in number over time compared with the control group. Representative isolates from each group were confirmed as CTX-M-positive by PCR. In the treatment group, 18 isolates were CTX-M group 1 and seven were group 9. In the control group 20 isolates were CTX-M group 1 and five were group 9.

Initial examination of the proportion of total *E. coli* that were CTX-M-positive indicated there was an overall difference between the two groups (p<0.001). Examination of the mean counts at six time points indicated that there was a higher proportion of resistant isolates in the treatment group compared with the control group at four weeks of age (9.02% vs. 0.36%) and eight weeks of age (14.49% vs. 0.002%). The outputs of the binomial GEE model comparing the proportion of *E. coli* that were resistant over time between the groups are presented in Table 1 (model A). The interaction term variable SamGrpXSamNo gives the difference between the slope of the treatment group and the baseline (SampleNo). No significant difference was observed between the two groups in the proportion of isolates that were resistant, or in the changes in the proportion of isolates that were resistant over time.

Effect of feeding WM+AR on the prevalence of CTX-M-positive *E. coli* infected calves

The number of positive samples per calf ranged from seven to 16 out of 25 within the control group, and from 14 to 22 out of 25 within the treatment group. All calves in the control group had shed CTX-M-positive *E. coli* by around eight days of age, compared with six days of age in the treatment group. The survival analysis indicated that there was no difference in the incidence rate of colonisation of calves with CTX-M-positive *E. coli* between the two groups (p=0.736). The proportion of samples that contained CTX-M-positive *E. coli*, averaged across all calves, was 50% for the control group (range: 32% - 64%) and 77% for the treatment group (range: 60% - 96%). In preliminary analysis, a Pearson’s X2 test indicated that there was no difference in the proportion of calves shedding CTX-M-positive *E. coli* at the start of the study, but that a significant difference emerged at around three weeks of age. The outputs of the binomial GEE model comparing the proportion of calves shedding CTX-M-positive *E. coli* over time between the groups are presented in Table 1 (model B). The model indicates there was no significant difference overall between the proportions of animals shedding in the control and treatment groups. However, when accounting for sampling over time, the slope of the proportion shedding in the treatment group declined significantly slower than the control group (p<0.001) indicating that more animals continued to shed in the treatment group over time.

Table 1. Outputs from the three models: A) binomial model comparing the proportion of resistant isolates over time, B) binomial model comparing the proportion of calves shedding over time, C) linear model comparing the rate of shedding over time. Italics denote baseline variables and bold type denotes significant values.

Model	Variable	Coefficient	95 % CI	P value
A	Treatment	0.899	-0.218 – 2.016	0.115
	Control	*-3.871*	*-4.738 – -3.005*	
	SamGrpXSamNo	-0.018	-0.125 – 0.088	0.737
	SampleNo	*-0.009*	*-0.094 – 0.077*	
B	Treatment	-0.277	-1.019 – 0.466	0.466
	Control	*2.012*	*1.482 – 2.543*	
	SamGrpXSamNo	0.116	0.067 – 0.164	**<0.001**
	SampleNo	*-0.154*	*-0.189 – -0.118*	
C	Treatment	1.861	0.543 – 3.179	**0.006**
	Control	*13.604*	*12.492 – 14.717*	
	SamGrpXSamNo	0.046	-0.051 – 0.143	0.355
	SampleNo	*-0.418*	*-0.500 – -0.336*	

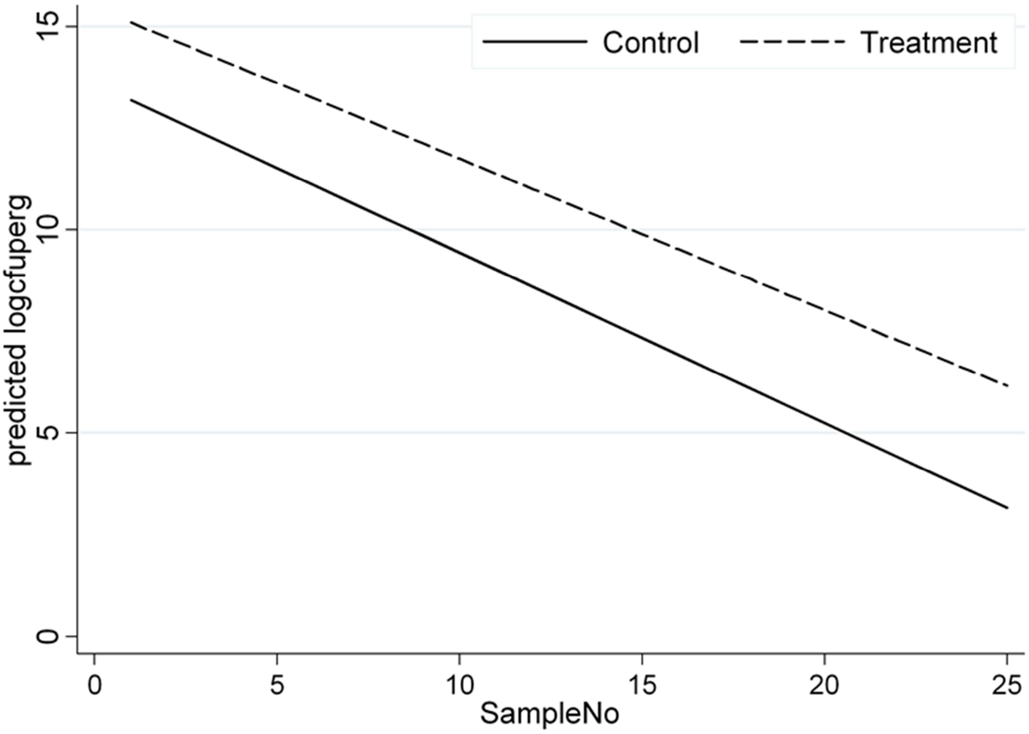

Fig. 1 Fitted line from linear GEE model comparing the degree of shedding of CTX M positive *E. coli* over time by group

The counts of cfu/g faeces ranged from <100 to 2.2 x 107 in the control group (mean: 1.1 x 106) and from <100 to 6.0 x 107 in the treatment group (mean: 2.2 x 106). There was a significant difference in the counts of CTX-M-positive *E. coli* between the two groups at around three weeks of age (ANOVA: p=0.027), but not at other times in the study. A Box-Cox regression indicated that a log10 transformation of the count data was appropriate prior to linear regression. The outputs of the linear GEE model comparing the degree of shedding in the two groups are presented in Table 1 (model C). The model indicated there was a significant difference between the control and treatment groups overall (p=0.006) but no significant difference was observed in the slope of the counts over time (Fig. 1).

Environmental data

One hundred and thirty environmental samples per group were collected throughout the study from eight locations within the pens: bird faeces, dust, feed trough, feeder teats, pen floor, railings, walls and water trough. Equal numbers of samples were taken from each location per group. The location that yielded the largest number of samples containing CTX-M-positive *E. coli* was the water trough (21/34 across both groups). Considering all environmental samples, no significant difference was observed in the number of samples that were positive for *E. coli* or CTX-M-positive *E. coli* between the groups: 60% of samples from the control group yielded *E. coli* compared with 58% in the treatment group (p=0.705); 49% of samples from the control group yielded CTX-M-positive *E. coli* compared with 54 % in the treatment group (p=0.457). Considering the individual sampling locations separately, a significant difference between groups was only found for samples taken from the pen floor, collected using boot swabs (p=0.016).

DISCUSSION

This study was designed to be representative of the standard calf husbandry practices on a typical commercial dairy farm. The sample size enabled the study to be conducted in a field situation, whilst still providing the study with enough power to detect significant associations. The time variable used in the regression analyses was sample number (1-25). As samples were collected at different time points throughout the study (i.e. daily, then twice weekly, then weekly) the amount of time between two adjacent sampling occasions could vary. GEE models account for unequal intervals between observations and this was therefore considered in the analysis. In addition, the results of the survival analysis indicated that there was no difference in the rate of colonisation with CTX-M-positive *E. coli* between treatment groups. This suggests that the selected time variable was unlikely to have had an effect on the associations observed between waste milk feeding, the prevalence of calves with CTX-M-positive *E. coli* and levels of shedding.

The effect of feeding untreated and pasteurised waste milk or bulk milk on calf performance and health was examined by Aust et al. (2012). They observed no significant difference in average daily weight gain between groups. In this study, calves were weighed on four occasions and a significant difference observed between groups over the course of the study. There was no evidence that the calves fed WM+AR consumed a greater volume of milk than those fed powdered milk. The milk composition was not investigated other than for antibiotic residues, so a comparison of the intakes between the groups in terms of energy and protein could not be made. One of the reasons why farmers in England and Wales choose to feed waste milk to calves is because they believe it improves calf growth (Brunton et al., 2012); the findings of this study would appear to support this. Cefquinome was detected in 87% of the WM+AR samples at a mean concentration of 746 μg kg-1 (0.746 mg/l). Orden et al. (1999) calculated the MIC90 of cefquinome against 195 isolates of *E. coli* from calves to be 0.125 mg/l, while previous work at the Animal Health and Veterinary Laboratories

Agency has found the MIC90 of cefquinome against CTX-M-positive *E. coli* from calves to be at least 2 mg/l (R. Horton - unpublished data). This would suggest that the concentrations detected in this study should be high enough to kill susceptible isolates, but low enough to provide a selective pressure in favour of *E. coli* with acquired ESBL resistance. However, it is not known from this study what concentration of cefquinome would reach the *E. coli* population in the intestine. There is likely to be degradation of cefquinome in the abomasum, plus a dilution effect with the consumption of other feeds, water and saliva.

The first hypothesis of this study was that feeding WM+AR to calves would affect the proportion of CTX-M-positive *E. coli* in the gut of calves and that this would vary with calf age. Aust et al. (2012) found the proportion of resistant *E. coli* to be higher in calves fed waste milk compared with those fed bulk milk with no clear temporal pattern observed. The results of this study have shown that there was no difference between calves fed WM+AR or calves fed powdered milk in the proportion of *E. coli* isolates that were CTX-M-positive. This was also observed when considering the effect of sampling over time. However, although no difference was observed in the proportion of *E. coli* that were resistant between the groups, calves in the WM+AR group shed greater absolute numbers of CTX-M-positive *E. coli* than calves in the control group throughout the study, but shedding decreased at an equal rate in both groups. The second hypothesis was that feeding WM+AR would affect the prevalence of calves infected with CTX-M-positive *E. coli*. The GEE model outputs showed no significant difference between the proportions of animals shedding in the control and treatment groups, but a significant difference was observed in the rate at which the prevalence of shedding calves declined. This suggests that infection with CTX-M-positive *E. coli* persisted in a larger number of calves fed WM+AR compared with calves fed a powdered milk substitute. Edrington et al. (2012) reported a slight difference in the prevalence of multi-drug resistant *E. coli* between calves fed waste milk or pasteurised waste milk where the prevalence in calves fed waste milk decreased at a younger age. The results of this study show that although the prevalence of calves infected with CTX-M-positive *E. coli* does decline post-weaning (i.e. once the selective pressure of antibiotics in waste milk has been removed), there was a difference in persistence between the control and treatment groups.

CTX-M-positive *E. coli* were isolated from around half of the environmental samples collected in this study and all sampling locations yielded resistant isolates. Although no overall difference was observed in the proportion of positive samples between the two groups, significantly more pen floor samples were positive in the treatment group. This is likely to be as a result of the greater number of CTX-M-positive *E. coli* shed by calves in the treatment group, which could have contributed to maintaining infection within that group. Although differences between the two groups of calves were observed, the widespread occurrence of CTX-M-positive *E. coli* in the environment of both groups of calves is also likely to have influenced the overall results. The study investigated the influence of WM+AR on the occurrence of CTX-M-positive *E. coli* in calves housed on a contaminated farm. It is possible that removing the effect of the contaminated environment might yield different results, but the study was designed to reflect the field situation and demonstrate the differences likely to be observed when calves were fed WM+AR under such circumstances.

The findings of this study indicate that feeding WM+AR on this farm increases the amount of CTX-M positive bacteria shed in the faeces. Shedding of CTX-M-positive *E. coli* persists for longer after weaning in calves fed WM+AR. This suggests that feeding WM+AR to calves can increase the number of CTX-M-positive *E. coli* in the farm environment due to increased shedding. These findings are applicable to the situation observed on this farm, but may differ on other farms where the contents of the waste milk or other factors such as the degree of contamination of the animal environment may vary. However, similarity was observed between the most commonly used antimicrobials on this farm, and those commonly used on farms surveyed in 2010 (Brunton et al., 2012), indicating that this study is likely to represent the situation on a number of other dairy farms in England and Wales. The feeding of WM+AR did not have a negative effect on calf health, and in fact had a positive effect on weight gain. The benefits to farmers of feeding WM+AR and the degree of the effects observed in the different groups of calves, need to be carefully weighed against the risks of increased dissemination of resistance through feeding waste milk containing third and fourth generation cephalosporins.

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EMERGING INFECTIONS

SCHMALLENBERG VIRUS EPIDEMIC IN THE NETHERLANDS: IMPACT ON MILK PRODUCTION AND REPRODUCTIVE PERFORMANCE IN DAIRY CATTLE

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SUMMARY

This study aimed to quantify the impact of Schmallenberg virus (SBV) on the productivity of dairy cattle in the Netherlands, using census data. Several multilevel multivariable models were applied to capture the different frequency of events and hierarchical structures of the data. There were no SBV-free control herds available. It was therefore decided to let herds act as their own controls by comparing productivity in periods influenced by SBV to historic reference periods.

All fertility parameters were slightly, but significantly reduced between August and November 2011 compared to the reference period in 2009-2010. A reduction in milk yield associated with SBV appeared to be most evident between the end of August and early September 2011.

This study indicates that the direct effect of SBV on the productivity of dairy cattle was limited. The indirect effect on the ruminant industry as a result of international trade restrictions is expected to be much higher.

INTRODUCTION

From the third week of August 2011 onwards, an unexpected number of reports were made by veterinarians regarding acute diarrhoea, drop in milk production and occasionally fever in dairy cattle herds in the Netherlands (Muskens et al., 2012). The cause of the symptoms that were reported was unknown. In November 2011, a new virus ‘Schmallenberg virus’ (SBV), belonging to the Simbu serogroup of the genus Orthobunyavirus of the family Orthobunyaviridae was identified in cattle with similar symptoms in Germany (Hoffmann et al., 2012). Not long after, SBV was found to have caused outbreaks of congenital malformations in lambs and goat kids (Van den Brom et al., 2012) and newborn calves (Hoffmann et al., 2012). The first malformed calf in the Netherlands was notified on December 13th, 2011. By then, the archived serum samples of cows with clinical signs in late August 2011 had been tested using an RT-qPCR test, kindly provided by the Friedrich Loeffler Institute (Germany); SBV had been identified. On December 20th 2011, the Netherlands was the first country to enforce mandatory notification of SBV infection, i.e.

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malformations in newborn ruminants had to be reported to the authorities. In 2012, a serosurvey estimating the ‘final’ seroprevalence after the 2011 epidemic revealed that SBV had spread throughout the Netherlands in cattle, sheep and goats (Veldhuis et al., 2013).

In the Netherlands, a national monitoring system, partly based on routinely collected herd data, is in place to follow trends in cattle health. In the last quarter of 2011, some signs of reduced fertility were observed (unpublished data), indicating a possible detrimental effect of the SBV epidemic. Akabane virus, an Orthobunyavirus like SBV of the Simbu serogroup within the family Bunyaviridae, is associated with reproductive disorders such as abortion, stillbirth, and congenital malformations in foetuses and newborns (Inaba et al., 1975). The effect of SBV infection on general reproductive performance and gestation in cattle, apart from congenital malformations in infected offspring, remains however, to be clarified.

The impact of the SBV epidemic seemed limited in north-western Europe, in terms of the number of affected herds with malformed offspring (European Food Safety Authority, 2012b). Conraths et al. (2013) suggested that the direct economic impact of SBV in the sheep and cattle industry is limited, yet major losses are caused by international trade restrictions. Nevertheless, little is known with regard to the overall within-herd impact of SBV infection (European Food Safety Authority, 2012a). Therefore, the objective of the current study was to quantify the impact of the 2011 SBV epidemic on the productivity of dairy cattle in the Netherlands, using routinely collected census data. As SBV spread throughout the Netherlands within one vector-active season, a group of control herds completely free from SBV was not available in this study. It was therefore decided to let herds act as their own controls by comparing productivity in periods influenced by SBV to reference periods in two previous years.

MATERIALS AND METHODS

Study population

In 2011, the dairy cattle population in the Netherlands comprised 18,589 herds. Most dairy herds can be found in the northern and eastern region of the Netherlands. In addition to the national population of dairy herds (referred to as ‘all dairy herds’), this study assessed the impact of SBV on a subgroup of herds. This subgroup reported malformations in newborn calves during the period in which notification of such symptoms was mandatory in the Netherlands (n=1,301). From these herds, 1,082 herds were classified as ‘probably SBV infected’ based on the pathological findings found in malformed calves; these were included in our study (referred to as ‘notifying herds’).

Data collection

Based on expert opinion, five productivity parameters describing milk production and reproductive performance that were available from routinely collected data were hypothesised to have a possible association with SBV (Table 1). Data regarding fertility were supplied by the Royal Dutch Cattle Syndicate (CRV) for the period January 1st, 2009 to September 1st, 2012. Bulk milk collection data was available from January 1st, 2009 to December 31st, 2011 (2-3 records per week per herd, for 95.8% of the Dutch dairy herds). Using test-day level milk production data, correcting for the number of lactating cows present in the herds, the average milk production per cow per herd per day could be calculated for 77% of dairy herds in the Netherlands. Average milk production per cow was aggregated per week for each herd prior to analysis. Herd sizes and information regarding herd location (province) were obtained from the national Identification and Registration (I&R) database.

Table 1. Overview of parameters that were used to assess the impact of SBV on dairy cattle in the Netherlands in 2011, including their definition and level of analysis

Parameter	Level	Definition
Non-return at day 56 (NR56)	Parity[a]	The absence of a new artificial insemination up to day 56 after initial insemination.
Abortion	Parity	(i) An interval of 100-260 days between two subsequent inseminations, or (ii) an interval of 100-260 days between the last insemination date and calving date.
Short gestation	Parity	A gestation length of 260-274 days, based on the interval between the last insemination date and calving date. The boundaries of the interval represent the 10% shortest gestations in cattle in 2009-2010 (reference period).
Number of inseminations	Parity	The number of inseminations per cow per parity, irrespective of whether the last insemination ultimately led to gestation.
Average milk production per cow per day (kg)	Herd	Calculated by dividing the total amount of collected bulk milk on a given day by the number of animals that joined the test-scheme that day[b].

[a] Parity is defined as the number of gestational cycles of a cow

[b] As the number of animals that joined the test-day was available on a 4-6 weekly interval per herd, this number was interpolated between test-days.

Study period

Given the pathogenic course of SBV infection, several analytical scenarios were carried out to quantify the impact of SBV infection on dairy cattle. Two different study periods in 2011 were defined in which the impact of SBV on productivity was assessed: (i) SBV period (the default), and (ii) specific milk production SBV period. Equivalent periods in 2009-2010 were used as reference periods. By doing so, herds acted as their own controls. Below, the SBV periods are described in more detail.

SBV period – standard definition: It was assumed that the SBV epidemic in the Netherlands started mid-August 2011, based on retrospective detection of SBV-specific antibodies in sheep sera during that period (Veldhuis et al., 2013). This is in agreement with the sudden increase in notifications of diarrhoea and drops in milk production from veterinarians, from the third week of August 2011 onwards. It appeared that the majority of ruminants seroconverted between August and the end of October 2011. It was therefore assumed that a possible effect of SBV on productivity parameters could be expected between August 1st and November 1st 2011 (referred to as ‘SBV period’). The same period in 2009 and 2010 was chosen as the reference period.

Specific milk production SBV period: As the duration of the observed clinical signs during the acute phase of infection, in particular drop in milk production, was short-lived (Muskens et al., 2012) and most seroconversions occurred in September 2011 (Veldhuis et al., 2013), a secondary (smaller) period of interest was defined as August 15th to September 19th 2011 to assess impact on milk production more specifically. Again, the same period for the years 2009 and 2010 was chosen as the reference period.

Data analysis

Different models were applied to capture the different frequency of events and hierarchical structures of the data, which are described in more detail below. For each parameter of interest it was determined when the parameter could have been influenced by SBV infection. Statistical analyses were performed using STATA/SE version 12.1 software. Normality of linear model residuals was checked using normal probability plots. The goodness of fit of logistic models was assessed using Pearson's goodness of fit tests. Overdispersion of Poisson regression models was checked and did not occur.

Milk production: The impact on the average milk production per cow per day was analysed using two different methods. In the first method, a multilevel linear regression model (xtmixed) (Eq.1) was used, classifying milk production records as possibly influenced by SBV infection if the bulk milk collection day was within the SBV period. In the second method, a multilevel linear regression model (xtgee) (Eq.2) was used to predict the herd-level milk production per lactating cow per day in 2011, based on milk production levels in 2009 and 2010. Then, differences between observed and predicted milk yields were analysed for both SBV periods in 2011. A random herd effect was included in both models to account for dependence between milk production records within herds. Also, factors that could influence the level of milk production in a herd, such as herd size (i.e. the number of lactating cows), seasons and region (province) were included. Model 1 (Eq.1) was carried out for both SBV periods, as indicated in Table 2.

$$E(y_{ji}) = \mu_j + \beta_1 SBV_{jiz} + \beta_2 prov_j + \beta_3 nmilk_{ji} + h_j + \varepsilon_{ji} \tag{1}$$

$$E(y_{ji}) = \mu_j + \beta_1 week_j + \beta_2 prov_j + \beta_3 nmilk_{ji} + \beta_4 season_{ji} + h_j + \varepsilon_{ji} \tag{2}$$

Where $E(y_{ji})$ is the average kg milk production per lactating cow per day in herd j in week i, μ_j is the intercept for herd j, $\beta_1 SBV_{jiz}$ is the variable classifying whether observation of herd j in week i was at risk of being influenced by SBV (using time frame of interest z (definition 1 or 2)), $\beta_1 week_j$ the week-number, $\beta_2 prov_j$ the province where herd j is located, $\beta_3 nmilk_{ji}$ is the number of lactating cows in herd j in week i, $\beta_4 season_{ji}$ is the season of the milk production record of herd j in week i, h_j is the random herd effect for herd j and ε_{ji} is the random error for herd j in week i.

Reproductive performance: The impact of SBV infection on the incidence of abortions, short gestations and NR56 were each analysed using a multilevel logistic model (xtmelogit) (Eq.3), given the binomial distribution of the data. An NR56 was classified as possibly influenced by SBV infection if at least one of the 56 days after first insemination was within the SBV period. An abortion or short gestation was classified as possibly influenced by SBV infection if at least one day of gestation was within the SBV period. The number of inseminations per parity per animal was Poisson distributed; therefore a multilevel Poisson regression model (gllamm) was used to analyse the impact of SBV on this parameter (Eq.4). The number of inseminations was classified as possibly influenced by SBV infection if the first insemination occurred within (or just before) the SBV period, and a second insemination had not yet been performed within (or just before) the SBV period. Cows that received multiple inseminations before the SBV period were excluded. A random herd effect was included to account for dependence between fertility records within herds. A random cow effect was included to account for dependence between parity-level records within cows. Also, factors that might influence a cow's reproductive performance in a herd, i.e. age of the cow, herd size, and region (province) were included in all models.

$$logit\left(y_{fert_{ijk}}\right) = \mu_{ij} + \beta_1 SBV_{ijk} + \beta_2 prov_j + \beta_3 hsize_j + \beta_4 age_{ijk} + c_i + h_j + \varepsilon_{ijk} \quad (3)$$

$$ln\left(y_{ijk}\right) = \mu_{ij} + \beta_1 SBV_{ijk} + \beta_2 prov_j + \beta_3 hsize_j + \beta_4 age_{ijk} + c_i + h_j + \varepsilon_{ijk} \quad (4)$$

Where $logit\left(y_{fert_{ijk}}\right)$ (Eq.3) is the dependent variable indicating reproductive event *fert* (non-return (yes/no), abortion (yes/no), or short gestation (yes/no) of cow *i* in parity *k* in herd *j*) and $ln\left(y_{ijk}\right)$ (Eq.4) is the number of inseminations of cow *i* in parity *k* in herd *j*. μ_{ij} is the intercept for cow *i* in herd *j*, $\beta_1 SBV_{ijk}$ is the variable classifying whether observation of cow *i* in herd *j* in parity *k* was at risk of being influenced by SBV (using time frame of interest (definition 1)), $\beta_2 prov_{ij}$ is the province where herd *j* is located, $\beta_3 hsize_j$ is the herd size of herd *j*, $\beta_4 age_{ijk}$ is the age category (nulliparous / multiparous) of cow *i* in parity *k* in herd *j*, c_i is the random cow effect for cow *i*, h_j is the random herd effect for herd *j* and ε_{ijk} is the random error for cow *i* in parity *k* in herd *j*.

RESULTS

An overview of the number of herds per group and their herd size characteristics is provided in Table 2. Data from 2,592,474 dairy cows, out of which 167,332 were from notifying herds, was used. The overall median herd size based on adult cows was 77 cows. Notifying herds were larger with a median herd size of 87 cows.

Table 2. Number of herds, number of cows per herd and total number of cows per group of herds

Group	Number of herds	Number of adult cows per herd[a]		Grand total number of cows
		mean	median	
All dairy herds	18,589	85	77	2,592,474
Notifying herds	1,082	97	87	167,332

[a] > 2 years of age

Reproductive performance

Results of the four fertility parameters in the SBV period(s) and corresponding reference period(s) is shown in Table 3. Observed means are shown at herd level. All fertility parameters were significantly affected in all dairy herds and notifying herds during the SBV period in 2011 in relation to the reference period in 2009-2010. The odds ratio for a non-return at day 56 after insemination between the SBV period and corresponding reference period was 0.77 (95% CI: 0.76-;0.78) and 0.75 (95% CI: 0.72;0.79) in all dairy herds and notifying herds, respectively. This indicates that a return within 56 days after insemination was more likely in the SBV period compared to the reference period. The odds of an abortion increased 1.05-fold (95% CI: 1.03;1.06) and 1.07-fold (95% CI: 1.03;1.11) during the SBV period compared to the reference period in 2009-2010 in all dairy herds and notifying herds, respectively. The odds of a short gestation increased 1.11-fold (95% CI: 1.10;1.12) and 1.14-fold (95% CI: 1.10;1.19) during the SBV period in all dairy herds and notifying herds, respectively. The incidence of insemination increased 1.03-fold (95% CI: 1.03;1.04) and 1.04-fold (95% CI: 1.03;1.05) during the SBV period in all dairy herds and notifying herds, respectively.

Table 3. Results of multivariable logistic and Poisson regression analyses on reproductive performance parameters during the SBV period in Dutch dairy herds and notifying herds specifically, with observed mean, odds ratios (OR), 95% confidence intervals (CI) and *p*-values. Estimates for variables not describing the SBV period are not presented, for details see Eq.(3-4).

Parameter	Group	Category[a]	Mean	OR	95% CI	*p*-value
NR56	All dairy herds	Ref. period	62.5%	-		
		SBV period	56.4%	0.77	0.76; 0.78	<0.001
	Notifying herds	Ref. period	61.5%	-		
		SBV period	55.7%	0.75	0.72; 0.79	<0.001
Abortions	All dairy herds	Ref. period	10.0%	-		
		SBV period	10.3%	1.05	1.03;1.06	<0.001
	Notifying herds	Ref. period	9.4%	-		
		SBV period	10.0%	1.07	1.03;1.11	<0.001
Short gestations	All dairy herds	Ref. period	7.6%	-		
		SBV period	8.4%	1.11	1.10;1.12	<0.001
	Notifying herds	Ref. period	7.2%	-		
		SBV period	8.2%	1.14	1.10;1.19	<0.001
Number of inseminations per cow	All dairy herds	Ref. period	1.95	-		
		SBV period	2.01	1.03[b]	1.03;1.04	<0.001
	Notifying herds	Ref. period	1.96	-		
		SBV period	2.04	1.04[b]	1.03;1.05	<0.001

[a] SBV period: 1-Aug-2011 to 1-Nov-2011

[b] Incidence rate ratio

<u>Milk production</u>

In total, 9,359,980 milk production records were used to calculate weekly averaged daily milk production level per cow per herd. In 2011, a clear absolute minimum in milk production was detected in week 35 (August 29th to September 4th) (Fig. 1).

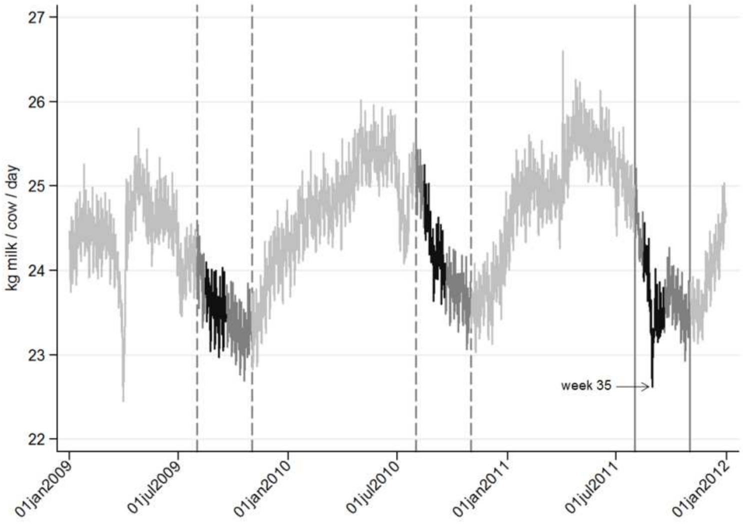

Fig. 1 Average daily milk production (kg) per lactating cow in Dutch dairy herds between January 1st 2009 and January 1st 2012, with SBV period (solid vertical lines) and the corresponding reference periods (dashed vertical lines). The two periods that were assumed to be influenced by SBV are displayed between the bars in dark grey (definition 1) and black (definition 2)

Table 4. Results of multivariable linear regression analyses on weekly averaged daily milk production (kg) per lactating cow per herd during the SBV period (defined using definitions 1 and 2) in Dutch dairy herds and notifying herds specifically, with observed mean (kg), model estimates (coefficient β), 95% confidence intervals (CI) and p-values. Estimates for variables not describing the SBV period are not presented, for details see Eq.(1).

Parameter	Group	Category	Mean	β	95% CI	*p*-value
Milk production by definition[a] [1]	All dairy herds	Ref. period	23.71	-		
		SBV period	23.64	-0.07	-0.10;-0.05	<0.001
	Notifying herds	Ref. period	24.35	-		
		SBV period	24.09	-0.25	-0.35;-0.15	<0.001
Milk production by definition[a] [2]	All dairy herds	Ref. period	24.34	-		
		SBV period	23.55	-0.26	-0.30;-0.22	<0.001
	Notifying herds	Ref. period	24.40	-		
		SBV period	23.96	-0.43	-0.59;-0.28	<0.001

[a] Definition of SBV periods:
[1] 1-Aug-2011 to 1-Nov-2011
[2] 15-Aug-2011 to 19-Sep-2011

Results of the milk production analysis using the difference between observed and expected milk yields (based on model predictions; method 2) were fairly equal to results of the analysis of milk production in assumed SBV periods versus corresponding reference periods (method 1). Therefore, results of method 2 are not shown. During the SBV-infection period, irrespective of definition, daily milk production per lactating cow was significantly reduced (Table 4). Between August 1st and November 1st 2011 (definition 1), the average loss in milk production per cow was -0.07 kg per day ($p<0.001$). This means that the total loss per cow associated with SBV infection was estimated at 6.44 kg. Between August 15th and September 19th 2011 (definition 2), the average loss in milk production per cow was -0.26 kg per day ($p<0.001$). During these 35 days, the total loss per cow was estimated at 9.1 kg.

DISCUSSION

This study aimed to assess the possible impact of the 2011 SBV epidemic on the productivity of dairy cattle in the Netherlands, using routinely collected data. Results indicated an association between SBV and milk production and a number of fertility parameters.

A clear drop in milk production was detected in the Netherlands between the end of August 2011 and the first week of September, in agreement with the first clinical signs reported in adult cattle (Muskens et al., 2012) and our previous findings with regard to the onset of the epidemic (Veldhuis et al., 2013). Nevertheless, the impact of the SBV epidemic in terms of reduced milk yield per cow appeared to be limited at national level. For example, a comparable drop in milk production between the end of March and mid-April 2009 was observed. At that time, no epidemics were recorded that could have influenced the national milk production levels. It is more likely that the drop in milk production was due to the climate during that period, as there had been exceptionally high temperatures and no rain (The Royal Netherlands Meteorological Institute, 2009), and thus, poor pasture quality. Given the quota system in the Netherlands, the economic impact of SBV at national level as a result of reduced milk yield is expected to be low. Nevertheless, the impact per individual farmer could vary and potentially be large, in particular if affected cows do not return to their previous production levels. The largest economic impact of SBV on the ruminant industry as a whole has, however, probably been international trade restrictions.

All fertility parameters analysed in this study (non-return in heat at 56 days after first artificial insemination - NR56), abortions, short gestations and number of inseminations) were significantly reduced between August 1st and November 1st 2011 compared to the equivalent period in 2009-2010. An effect of SBV infection on reproductive performance and gestation in cattle has not been previously quantified. Given the phylogenetic similarities between SBV and related viruses such as Akabane virus and Aino virus, which are known to affect reproductive performance (Inaba et al., 1975; Uchinuno et al., 1998), abortions in cattle as a result of SBV infection have been suggested (Garigliany et al., 2012; Steukers et al., 2012; Varela et al., 2013). For Akabane virus, abortions and stillbirths indicate infection late in gestation (Charles, 1994). There is no documented evidence of early embryonic death, or failure of pregnancy, as a result of Akabane virus infection (Charles, 1994; Kirkland, 2002). However, from all fertility parameters analysed in this study, the number of cows that returned in heat after 56 days after first artificial insemination (NR56) appeared to be the parameter most affected by SBV infection.

Between August 1st and November 1st 2011, a significant increase in short gestations was observed compared to that period in 2009-2010. According to the Identification and Registration data that was used, the short gestations resulted in the birth of viable (ear-tagged) calves. A short gestation in cattle, or more specifically, a pre-term induction of parturition, might be associated with occurrence of a health disorder (e.g. stress or fever) in the pregnant cow at the end of gestation. The exact pathogenic processes leading to reduced gestational length in relation to SBV infection needs clarification. Nevertheless, the direct impact of an increase in the number of short gestations seems limited, as it was not associated with an increase in mortality of newborn (non-ear tagged) calves between August 1st and November 1st 2011.

The impact of SBV infection on milk production and reproductive performance was highest in the subgroup of dairy herds in which at least one malformed calf was notified during the mandatory notification period. It was expected that SBV prevalence in this subgroup was higher than in the overall dairy herd population. The subgroup of notifying herds were most likely infected with SBV, given the pathological findings in the calves submitted for autopsy. RT-qPCR test results of calves' brain tissue were not taken into account when selecting these herds because of possible false-negative results due to the short duration of viraemia of SBV (Hoffmann et al., 2012; Van Maanen et al., 2012).

Given the high seroprevalence in the Netherlands after the 2011 epidemic, dairy herds completely free of SBV were scarce at the time of this study. In August 2012, a voluntary bulk milk survey was carried out to estimate antibody prevalence in bulk milk in the Netherlands (ProMED-mail, 2012). From these herds, 8% tested low positive (N=62) or negative (N=23), meaning that they might be used as a control group for our study. However, this small group of herds was not comparable to the average Dutch dairy herd in terms of herd size, milk production and reproductive performance in 2011 and preceding years. To assure a reliable estimate of the impact of the SBV epidemic without a representative control group, several analytical strategies were applied. First, it was decided to let herds act as their own controls by comparing productivity parameters in periods influenced by SBV to reference periods describing past productivity. Second, these periods were varied and adapted to the pathogenic course of SBV infection. In addition, different statistical models were applied to milk production data which gave similar results. Finally, preliminary results from Germany seem to confirm the Dutch findings (unpublished data). Although all applied analytical analyses suggest similar results, the effect of SBV infection on productivity parameters might be biased. Other factors such as feeding disorders and the relatively wet and cold weather conditions in the Netherlands in the summer of 2011 could have also affected productivity in dairy cattle, in particular milk production. It is however less likely that such factors reduced reproductive performance. Therefore, we are confident that a valid estimation of the impact of SBV on productivity in dairy cattle was obtained in this study.

To conclude, results showed that SBV had a small but noticeable impact on milk production and reproductive performance of dairy cattle. This is in contrast to mortality rates, on which SBV had no or limited impact, as expected given the relatively mild expression of SBV in adult cows and low incidence of malformations in newborn calves (unpublished data). The indirect economic effect on the ruminant industry as a result of international trade restrictions was, however, probably much higher.

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UNDERSTANDING SPATIAL VARIATION IN RISK OF BLUETONGUE OUTBREAKS ACROSS SOUTH INDIA USING A BAYESIAN POISSON REGRESSION MODEL

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SUMMARY

Bluetongue virus (BTV), is an economically important, midge-borne Orbivirus (family: *Reoviridae*) that causes disease in sheep and cattle. In south India, bluetongue disease (BT) occurs annually, whilst sporadic outbreaks occur elsewhere in the country. Within this study, the mean annual number of outbreaks in each district (1992-2009) was examined in relation to land-cover, host availability and climate predictors using a Bayesian generalised linear mixed model with Poisson errors and Conditional AutoRegressive error structure. A rigorous, hierarchical variable selection process was utilised based on information criteria. Land-cover predictors and host predictors outperformed climate predictors as explanatory variables describing patterns in outbreaks. More outbreaks occurred on average in districts with higher coverage of irrigated croplands or standing water following flooding. Number of outbreaks also increased with densities of exotic and local livestock breeds, which may be more susceptible to BTV, and with buffalo density. The value of the resulting cross-India prediction maps of BT risk, to state and district veterinary officers and epidemiologists engaged in surveillance, is discussed.

INTRODUCTION

Bluetongue virus (BTV) is an economically important, midge-borne Orbivirus (family: *Reoviridae*) that causes disease in sheep, deer and cattle (Machlachlan et al., 2009). In south India, the disease is highly enzootic, occuringly annually, with sporadic outbreaks in other parts of the country. The highest impacts are felt by small-holder farmers who suffer direct and indirect losses from bluetongue (BT), but the disease also affects national GDP (~5% of which is made up of animal husbandry and dairy development) and food availability (Ahuja et al., 2008). In many areas affected by BT worldwide, disease impacts have been linked to rainfall patterns (Baylis et al., 1999; Walker et al., 1977). Though monsoons are thought to govern the size and timing of epidemics in India (Prasad et al., 2009), the severity of disease varies substantially between districts, even within areas subject to similar monsoon conditions, suggesting that other landscape and host predictors affect transmission. Midge-borne disease systems have complex ecologies, with transmission often involving several ruminant hosts and biting midge (Culicoides, Diptera:Ceratopognidae) vector species within a single region.

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The epidemiology of BTV in India is complex due to high diversity in viral strains, host populations, geography and environmental conditions. More than 22 BTV serotypes are circulating in the country, from distinct geographic origins (Sreenivasulu, 2004; Maan et al., 2012). A diversity of *Culicoides* species, including both dung-breeding (e.g. *C. oxystoma*, which breeds in buffalo dung) and moist soil breeding species (e.g. *C. imicola, C. schultzei, C. peregrinus*), have been found to be abundant in BT-affected districts in different states (Subba Reddy, 2004; Dasgupta, 1995), although there is a paucity of systematic studies of vectorial capacity and distribution (Ilango, 2005). Past outbreaks of BT have been detected mainly in crossbred and exotic breeds of sheep, whilst local breeds are considered to be relatively resistant, though evidence from detailed breed susceptibility or epidemiological studies is lacking. Worldwide, certain breeds of sheep and wild ruminant species (e.g. white-tailed deer) are highly susceptible to BT disease (Machlachlan et al., 2009). Sheep that are native to tropical and subtropical regions of the world where BTV is enzootic are usually resistant to BT, whereas fine-wool European breeds such as the Merino are highly susceptible. Correspondingly, exotic breeds like Rambouillet and Merino have been reported to be more susceptible than native breeds in India (Lonkar et al., 1983).

Understanding how geographical variability in hosts, vectors and climate interact to produce variation in disease severity across districts requires quantitative methods that can deal with collinearity and spatial dependency in errors, which may arise due to intrinsic processes (such as disease spread between districts) and extrinsic processes (such as autocorrelation in an environmental covariate). In generalized linear models, all parameters are modelled as fixed effects and estimated by Maximum Likelihood (ML) methods. In ecological studies, when there is spatial dependence and key covariates may be missing, the ML approach often leads to unsatisfactory estimates of the district level risk due to extra-Poisson variation. Bayesian generalized linear mixed models (Breslow & Clayton, 1993) overcome these problems by separately modelling the missing covariates and spatial dependence as random effects, through a prior distribution (Clayton, 1995). The Besag-York-Mollie (BYM) model (Besag et al., 1991) can account for spatially structured and spatially unstructured random error variation as well as fixed effects of environmental predictors. Bayesian disease mapping has been developed for many diseases (Besag et al., 1991; Banerjee et al., 2004) using MCMC (Markov Chain Monte Carlo) algorithms for parameter estimation. Recently, an approximate method for parameter estimation in Bayesian frameworks has been proposed by Rue et al. (2009). This uses integrated nested Laplace approximation (INLAs) to estimate the posterior marginals of interest and can be computed easily with vastly reduced computation time (compared to MCMC) in R (R Development Core Team, 2005) using the INLA library (Martino & Rue, 2009). Using this BYM model approach fitted in INLA (INLA-R version by Blangiardo et al., 2012) this paper investigates the role of climate, land-cover and availability of livestock hosts in driving geographical variation in severity of BT outbreaks across districts in south India. The work examines the following hypotheses:

1. severity of outbreaks will be greater in areas with greater availability of land-cover-types containing water bodies or irrigated areas that provide breeding habitat for *Culicoides* (Diptera: Ceratopogonidae) midges;

2. outbreaks will be less severe or absent in areas with closed forest but may occur in open forest types that are used by farmers for grazing;

3. severity of outbreaks will increase as sheep numbers increase, particularly as the number of certain local breeds and exotic breeds increases;

4. severity of outbreaks will increase as cattle and buffalo numbers increase, since disease may circulate silently in these reservoir hosts.

MATERIALS AND METHODS

Data variables

Bluetongue outbreak data: District level (admin-2) monthly BT outbreak data (1992-2009) was provided by PD_ADMAS (Project Directorate on Animal Disease Monitoring and Surveillance) which maintains the livestock diseases database for India and collates outbreak data every month from different sources. The analysis was restricted to data from three states of south India namely Karnataka, Andhra Pradesh and Karnataka, in which outbreaks are regularly reported, with 61 out of 80 constituent districts having ever reported outbreaks of BT over the 18 year study period. Despite the fact that there is high sero-prevalence of BT antibodies in most of the states in India (Bandyopadhyaya & Mullick, 1983; Kakker et al., 2002), there are infrequent clinical outbreaks outside these three states. The mean annual number of outbreaks per district was calculated as the dependent variable.

Land-cover predictors: The proportions of each district covered by ten land-cover classes were extracted from the Globcover land-cover map (Defourny et al., 2006) using Zonal Statistics in ArcMap 10.1 (ESRI, Inc., Redlands, CA,U.S.A.), and they were logit-transformed and each considered individually as predictors in the analysis. The ten classes were selected *a priori* from the original 24 available in Globcover, due to their importance for BT epidemiology either because they were likely to contain favourable semi-aquatic breeding habitat for *Culicoides* vectors (post-flooded/ irrigated cropland, rain-fed and mosaic croplands, water bodies, classes 1 to 3) or because they were likely to be favourable (closed to open forest, classes 4, 5 and 7) or unfavourable for grazing of livestock (closed forest, including different percentages of cover, classes 6 and 8). Urban areas (class 9) and water bodies (class 10) are areas with low livestock density.

Host species and sheep breed data: Densities of host species and individual sheep breeds, namely numbers of indigenous sheep, non-descript sheep, crossbred sheep, goats, crossbred cattle and buffaloes, were extracted from the database of National Livestock census data 2007 (http://www.dahd.nic.in/) and log-transformed. Non-descript sheep breeds are defined as indigenous breeds which cannot be identified or do not have more than 50% similarity to any recognised breed. Out of over 40 breeds of sheep present in India (Patnayak, 1988), only 14 breeds were present in south India.

Rainfall and temperature predictors: Monthly Rainfall Estimates (RFE) were obtained from the NOAA/Climate Prediction centre RFE 2.0 (Xie et al., 2010). Seven year averaged values (2004-2010) of different monsoon rainfall (south-west and north-east) and annual rainfall for districts were extracted using Zonal Statistics ArcMap 10.1 (ESRI, Inc., Redlands, CA, U.S.A.). The annual mean temperature layer was downloaded from Worldclim (Hijmans et al., 2005).

Modelling approach

Relationships between mean number of outbreaks and environmental predictors were quantified using generalised linear mixed models with Poisson errors, implemented in a Bayesian framework. Semi-variogram analysis was conducted on mean number of outbreaks and also on the residuals of a fitted model without spatial error terms to check for residual autocorrelation (Cressie, 1993). In the event of finding significant spatial autocorrelation, an iCAR (Intrinsic Conditional AutoRegressive) error structure was implemented. The probability function for Y is demonstrated in Eq. (1).

$$\begin{aligned}
&\Pr\,(\mathrm{Y} = \mathrm{y}/\mu) = Poisson(\mu_i)\\
&\log(\mu_i) = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_n x_{ni} + \upsilon_i + \nu_i\\
&\upsilon_i \mid \upsilon_{j \neq 1} \sim Normal(m_i, s_{2i})\\
&m_j = \frac{\sum_{j \in N(i)} \upsilon_j}{\neq N_{(i)}}\\
&s_{i2} = \frac{\sigma_{\upsilon 2}}{\neq N_{(i)}}
\end{aligned} \tag{1}$$

Where β_0 is the intercept β_1 to β_n are the coefficients for the fixed effects of predictors (x_{1i} to x_{ni}) in each district i. υ_i is a structured spatial component assuming Besag-York-Mollie (BYM) specification (Besag et al 1991), modelled using an intrinsic autoregressive structure (iCAR). $\neq N_{(i)}$ is the number of districts that share boundaries with the i-th district, and ν_i is the unstructured spatial effect in each district, modelled using an exchangeable prior $\nu_i \sim$ Normal (0, σ_{ν}^{2}).

Model building and selection of predictors: Variable selection is critical to understand the importance of the impact of individual environmental predictors upon the distribution of BT outbreaks, and it can be undertaken using a wide range of frequentist and Bayesian approaches (Efron, et al., 2004; George, 2000 and Miller, 2002). Given the computational efficiency that INLA offers over MCMC methods, we were able to implement a modified All Subsets approach to variable selection, where all possible combinations of the total *p* explanatory predictors, from size 1 to *p*, are fitted (making 2^p-1 combinations in all) and the most parsimonious model is selected using information criteria. Pairwise Pearson correlation analyses were performed on all 32 predictors to identify pairs of predictors that were highly correlated ($r > 0.7$, $p < 0.001$) (leading to the removal of 8 breed and host predictors, and one land-cover class). Since it was impossible, even in INLA, to fit all 33554431 possible combinations of the remaining 25 predictors (9 land-cover, 4 climate, and 12 host predictors), all possible combinations were first fitted within the land-cover, climate and host categories, to identify the best land-cover, climate and host models, respectively. All subsets selection was performed using all the predictors contained in these best category models and the model with the lowest Deviance Information Criteria (DIC, Speigelhalter et al., 2002) was identified as the best model. DIC is a generalisation of the Akaike Information Criterion (AIC), and is derived as the mean deviance adjusted for the estimated number of parameters in the model, compromising between model fit and complexity, and providing a measure of out-of-sample predictive error (Gelman and Hill, 2007). To evaluate predictive performance, district specific predicted posterior mean values were compared with the corresponding observed mean number of outbreaks using pair-wise Pearson's correlation statistics. To test the out-of-fit predictive performance of the model, leave-one-out cross validation statistics, namely Conditional Predictive Ordinates (CPOs) were calculated (Gelfand, 1996). The CPO expresses the posterior probability of observing the value (or set of values) of y_i when the model is fitted to all data except y_i, with a larger value implying a better fit of the model to y_i, and very low CPO values suggesting that y_i is an outlier and an influential observation. When many CPO values cluster near zero, the model demonstrates poor out-of-fit performance. When many CPO values cluster near one, the model demonstrates good out-of-fit performance (Lawson, 2009).

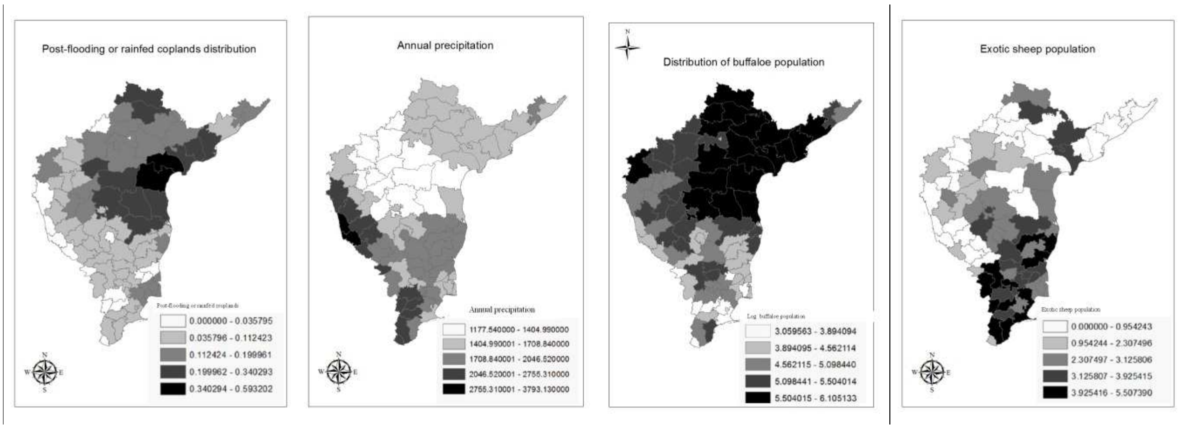


Fig. 1 Maps of potential explanatory predictors affecting average annual number of bluetongue outbreaks at the district level in south India describing: (a) post-flooding or rain-fed cropland landscape (obtained from Defourny et al., 2006); (b) Annual monsoon precipitation (obtained from Xie et al., 2010); (c) buffalo population; and (d) population of exotic sheep breed

RESULTS

The mean annual number of outbreaks per district ranged from 0 to 33.83 (3.74 ± 7.13). The maximum number of outbreaks which occurred across south India in any given year was 1594 in 1998, whilst the district with the most annual outbreaks was Prakasham (Andhra Pradesh); affected by 370 outbreaks in 1998.

Overall, three main groupings of sheep breeds with similar distribution patterns were identified in correlation analyses. Group 1 contained Bellari, Hassan, Kengur and Mandya. Group 2 contained Nellore and Deccani. Group 3 contained Kilakarsal, Madras Red, Mercheri, Nilgiri, Ramnad White, Tiruchi Black and Vembur. Overall, eight breeds of sheep were dropped from further consideration due to their high degree of correlation with other breeds, landscape or climate predictors. The density of buffalo was positively correlated with the land-cover predictors post-flooded or irrigated croplands ($r = 0.673$, $p < 0.005$).

Considering best models within categories (Table 1), the classes post-flooding or irrigated and rain-fed croplands were identified amongst land-cover predictors and were both positively associated with mean annual number of BT outbreaks. Amongst host predictors, densities of sheep of particular breeds (namely Deccani, Bellary, exotic and non-descript sheep) and densities of buffaloes and indigenous cattle were selected. All predictors were positively associated with BT outbreaks except for density of Bellary sheep and indigenous cattle; these were negatively associated with BT outbreaks. Considering the three predictors in the best climate model, annual mean temperature was positively associated with BT outbreaks whilst the north-east monsoon and annual monsoon precipitation amounts were negatively associated (Table 2). Models containing only host or land-cover predictors outperformed those containing climate predictors alone (Table 1).

Table 1. Deviance information criterion (DIC) and effective number of parameters (pD) for the best models describing Bluetongue outbreaks in India. All models are fitted using the Besag-York-Mollie (BYM) specification

Model	Predictors in model	DIC	pD	Difference in DIC from the best model
Host	Indigenous cattle, Buffalo, Deccani sheep, Exotic sheep, Non-descript sheep	173.26	39.08	2.1
Land-cover	Post-flooding, Rain-fed croplands	175.52	37.02	4.36
Climate	Mean temperature, North-east rainfall, Annual rainfall	187.20	45.64	16.04
Combined model	Rain-fed croplands, Indigenous cattle, Buffalo, Deccani sheep, Exotic sheep, Non-descript sheep	171.16	37.17	0

The final combined model, selected from constituent predictors of the best category models, contained all host predictors from the best host model as well as the land-cover predictor rain-fed croplands (Table 1). This combined model had slightly better performance (DIC lower by 2.1 units) than the final host model.

Broad scale spatial autocorrelation in the raw average numbers of BT outbreaks per district was detected (with a range of around 600 km), but negligible spatial autocorrelation could be detected in the residuals of the final fitted BYM model. More-over, the latter mixed model had a lower DIC (DIC=173.26) than models containing either predictors alone (DIC=301.92, pD = 6.976) or spatial random effects alone (DIC= 208.23, pD=57.85), and had less outliers in model predictions (detected from CPO cross-validation statistics). A large proportion of the variance described by spatial random effects was explained by unstructured heterogeneity (>99%) versus structured heterogeneity.

The predicted mean number of outbreaks using the best model (Fig. 2a) shows excellent correspondence with the observed mean number of outbreaks (Fig. 2b), with a correlation coefficient of $r = 0.996$ ($p < 0.005$). The model is able to discriminate between districts with and without outbreaks and also delineates severely affected districts successfully. Areas with a reported absence of BT for the past 18 years include regions with forest cover, at high altitude which have a low sheep population.

DISCUSSION

This study is the first to explain spatial patterns in BT severity in relation to a full range of important environmental drivers. The results indicate that host and landscape heterogeneity was much more important in driving spatial patterns in BT severity amongst districts in south India than heterogeneity in climate conditions. Thus, although monsoon conditions undoubtedly contribute to the disparity in severity of BT between north India (little affected by monsoons) versus south India (heavily affected by monsoons, see Prasad et al., 2009), the study indicates that host and landscape predictors come into play at finer spatial and temporal scales. Together with the finding that models combining different suites of environmental predictors (namely host and landscape predictors) out performed those based on a single suite of environmental predictors, this illustrates the value of considering all potential environmental drivers in the same model framework (*cf.* Purse et al., 2012; Acevedo et al., 2010) and of examining risk predictors at a range of spatial scales.

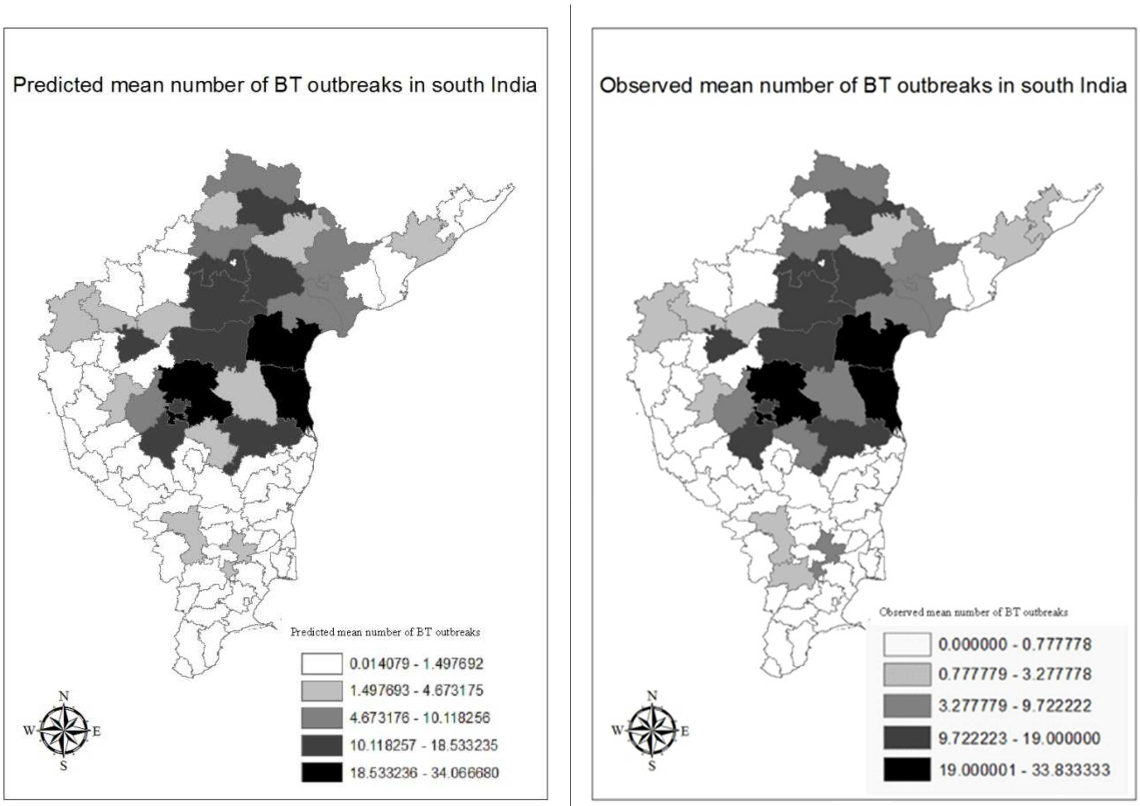
Table 2. Mean coefficient values and credible intervals for fixed effects environmental predictors which describe average annual number of bluetongue cases per district for the best subset models within the land-cover, climate and host categories

Variable	Mean (standard deviation)	Credible interval
	Land-cover	
Intercept	8.4577 (0.9809)	6.6178, 10.4899
Post-flooding or irrigated croplands	1.7907 (0.4278)	0.9736, 2.6616
Rain-fed croplands	1.9566 (0.3859)	1.2369, 2.7595
	Climate predictors	
Intercept	-6.9560 (6.3329)	-19.2905, 5.6826
North-east monsoon	-0.0240 (0.0083)	-0.0408, -0.0082
Annual monsoon	-0.0030 (0.0010)	-0.0050, -0.0010
Annual mean temperature	0.0456 (0.0215)	0.0036, 0.0885
	Host	
Intercept	-9.8765 (2.9653)	-16.209, -4.5230
Indigenous cattle	-1.1987 (0.6339)	-2.4713, 0.0303
Buffaloes	1.6926 (0.5151)	0.7258, 2.7588
Exotic breeds of sheep	0.3764 (0.1327)	0.1215, 0.6453
Bellary sheep	-0.0787 (2.9653)	-0.3145, 0.1530
Deccani sheep	0.4168 (0.1300)	0.1646, 0.6775
Non-descript sheep	1.1371 (0.4558)	0.2803, 2.0794

As hypothesised, districts with a higher land-cover of post-flooding and irrigated croplands suffered significantly more BT outbreaks on average, probably because such landscapes are more likely to contain suitable breeding habitat and *Culicoides* biting midge vectors.

Although the vectorial capacity of candidate vector species has not been well studied in India, three key species, *C. imicola*, *C. peregrinus,* and *C. oxystoma* seem to be abundant in BT-affected areas (Subba Reddy, 2004; Dasgupta, 1995). Populations of *C. imicola* and *C. peregrinus*, which both breed in moist soil, have been significantly associated with irrigated areas or high soil moisture availability elsewhere (*C. imicola* – Acevedo et al. 2010; Conte et al., 2007; *C. peregrinus* – Narladkar et al., 2006). *C. oxystoma*, which breeds in buffalo dung, and its larvae, have been found in both active and abandoned rice paddy fields (encompassed by the irrigated cropland class) elsewhere in Asia (Yanase et al., 2013). It is probable that the extensive rice belt found in Andhra Pradesh (the state most severely affected by BT), that supports high buffalo populations (Fig. 1c) and likely high populations of *C. oxystoma*, makes a substantial contribution to maintaining BT transmission.

The positive effects of particular breeds on outbreak numbers are consistent with previous findings of high susceptibility of exotic and cross-breeds in India (Lonkar et al., 1983), and restriction of previous disease cases to European sheep breeds in south-east Asia (Daniels et al., 2004). Furthermore, antibodies against BTV are reported in local breeds in Indonesia and Malaysia without any clinical signs of disease (Hassan, 1992; Sendow, 1991).



a **b**

Fig. 2 (a) Predicted average annual number of BT outbreaks in south India from the best model; and (b) observed average annual number of BT outbreaks from PD_ADMAS

The finding that areas with high buffalo densities suffer high numbers of outbreaks is consistent with previous observations of high sero-prevalance against BTV without clinical disease in this species (Kakker et al., 2002), and indicates the potential importance of buffaloes as a reservoir and amplifying host. There are range of different hosts and breeds associated with BT outbreaks in this study and this may reflect variation between states in the importance of particular hosts in maintaining BTV transmission. For example, the high populations of exotic sheep breeds may be most important in producing disease cases in Tamil Nadu (Fig. 1d), whilst buffaloes may be playing an important reservoir role, increasing cases of disease in sheep in Karnataka and Andhra Pradesh.

Surprisingly, areas with relatively high annual monsoon rainfall in south India had low numbers of outbreaks on average. This may be because higher rainfall regions without BT cases, for example the western Ghat forests, support very low densities of sheep and other livestock, and therefore probably support low populations of livestock-associated *Culicoides* species.

The finding that a large proportion of the variance described by the spatial random effects was explained by unstructured heterogeneity (>99%, versus the structured heterogeneity), is suggestive of low importance of intrinsic processes such as disease spread between districts, in explaining district-level disease patterns and the potential importance of unmeasured environmental predictors. These could include soil (soil type and water retention capacity) or animal husbandry factors (dung management and use as fertiliser, local drainage and flooding) that may influence breeding site availability and abundance of potential vectors.

The out-of-fit validation statistics and high degree of correspondence between observed and predicted outbreak numbers indicates that the final combined model may be statistically adequate for prediction of disease patterns in the rest of India. Although there are substantial proportions of post-flooding and irrigated cropland across India, the host and breed diversity and overall densities of sheep and buffalo are lower in the north compared to south India and thus the combinations of hosts and vectors, associated landscape and climate factors involved in transmission may differ between north and south. In India, BT risk is currently predicted and mapped at district level for use by the Department of Animal Husbandry using logistic regression models (PD_ADMAS, annual report 2010). The present work complements the existing early warning system by considering an extended suite of potential risk factors, by predicting the mean number of outbreaks, rather than presence-absence of outbreaks, and by accounting for spatial dependence. The resulting risk maps will not only be useful for stakeholders for planning control measures, but also scientists working on BT for planning sero-surveys, vector surveillance and vaccine uptake studies. Future work will aim to predict when a particular district or village within a district will be at risk of severe BT, using novel spatio-temporal modelling techniques and village-level models which consider other mechanisms under-pinning risk, for example herd immunity, and annual and inter-annual variability in monsoon conditions. The disease risk maps will be combined with the surveillance of vectors currently underway in the IBVNet project (http://www.ibvnet.com/) to understand geographical and seasonal variation in the abundance of key candidate vectors.

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DIGGING INTO THE MYTH – AFRICAN SWINE FEVER, RUSSIAN WILD BOAR AND THE ENDEMICITY HYPOTHESIS

H.-H. THULKE*, M. LANGE, H. SIEMEN AND S. BLOME

SUMMARY

African swine fever (ASF) is a highly lethal viral disease of domestic pigs and wild boar. ASF was introduced to the southern Russian Federation in 2007 and is now reported to have spread in populations of wild and domestic suids. An endemic situation in the local wild boar population would significantly complicate the management of the disease in livestock. No sound method exists to identify the characteristic pattern of endemic disease, which describes infection persisting from year to year, between generations in the same wildlife population. In support of urgent management decisions at the wildlife-livestock interface, a new algorithm was constructed to test the hypothesis of an endemic disease situation in wildlife. The approach uses spatial and temporal associations between observed diagnostic data to discriminate between endemic and non-endemic patterns of case occurrence. The algorithm was validated with data from an epidemiological simulation model and applied to ASF data from southern Russia. Based on the algorithm, the null hypothesis of an endemic situation of ASF in wild boar in the region was rejected.

INTRODUCTION

Several introduction events of livestock diseases have occurred where wildlife was found to be involved in the spread of the infection (EFSA, 2012; Beltrán-Alcrudo et al., 2008). The well-documented and close linkage between domestic and wildlife populations, either naturally or anthropogenically mediated, has in addition, created concerns about wildlife as reservoirs of these infections (Bengis et al., 2002; Morgan et al., 2004). These concerns about wildlife host species acting as permanent reservoirs for pathogens are usually due to the acute emergency situation and the limited experience with or knowledge gaps about these infections in the novel ecosystem. Infection circulating in wildlife complicates control and requires extended management measures and hence the earliest insight about a reservoir role may support more reasonable decisions during contingency planning. Invasive spread through naïve wildlife host populations is usually well documented during the epidemic phase after pathogen introduction (e.g. EFSA, 2009); unfortunately there is less documentation right when forecasts are in high demand, to describe whether an introduced infection will die out or enter an endemic phase (e.g. EFSA, 2009), meaning the disease persists year on year, between generations in the same population. Notwithstanding the need for urgent decisions, no standardised approach to acutely report diagnostic records exists that facilitates the discrimination of endemic from epidemic situations. Consequently, any judgment claiming endemicity of an infection in a novel wildlife host population is primarily

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based on a longer duration of presence of the pathogen in the host species, rather than on epidemiological evidence.

A recent example is the emergence of African Swine Fever (ASF) in the Transcaucasia (Oganesyan et al., 2013). The African Swine Fever Virus (ASFV) originates from the African continent (Costard et al., 2009). ASF is a viral disease of warthogs, bushpigs, wild boar and domestic pigs. The disease causes up to 100% mortality in domestic pigs and wild boar (Gabriel et al., 2011; Blome et al., 2012), while warthogs do not develop clinical symptoms (Thomson et al., 1980). ASF is considered an endemic disease in most southern and east African countries, where the virus is maintained either in an ancient sylvatic cycle between warthogs and ticks of the *Ornithodoros moubata* complex or in a domestic cycle that involves pigs of local breeds, with or without tick involvement (Penrith et al., 2009; Beltrán-Alcrudo et al., 2009).

Recent emergence events of the disease in the Caucasus region, Russia, Belarus and the Ukraine (OIE, 2013) pose a threat to the European pig farming industry. The competence of arthropod vectors in this region is unknown (Beltrán-Alcrudo et al., 2008). Suitable control methods other than large-scale depopulation do not exist (EFSA, 2010). Wild boar were regularly involved in the reported outbreaks. There has been discussion of ASF turning into an endemic disease in the wild boar population in southern Russia (EFSA, 2010; Mur et al., 2012; Oganesyan et al., 2013).

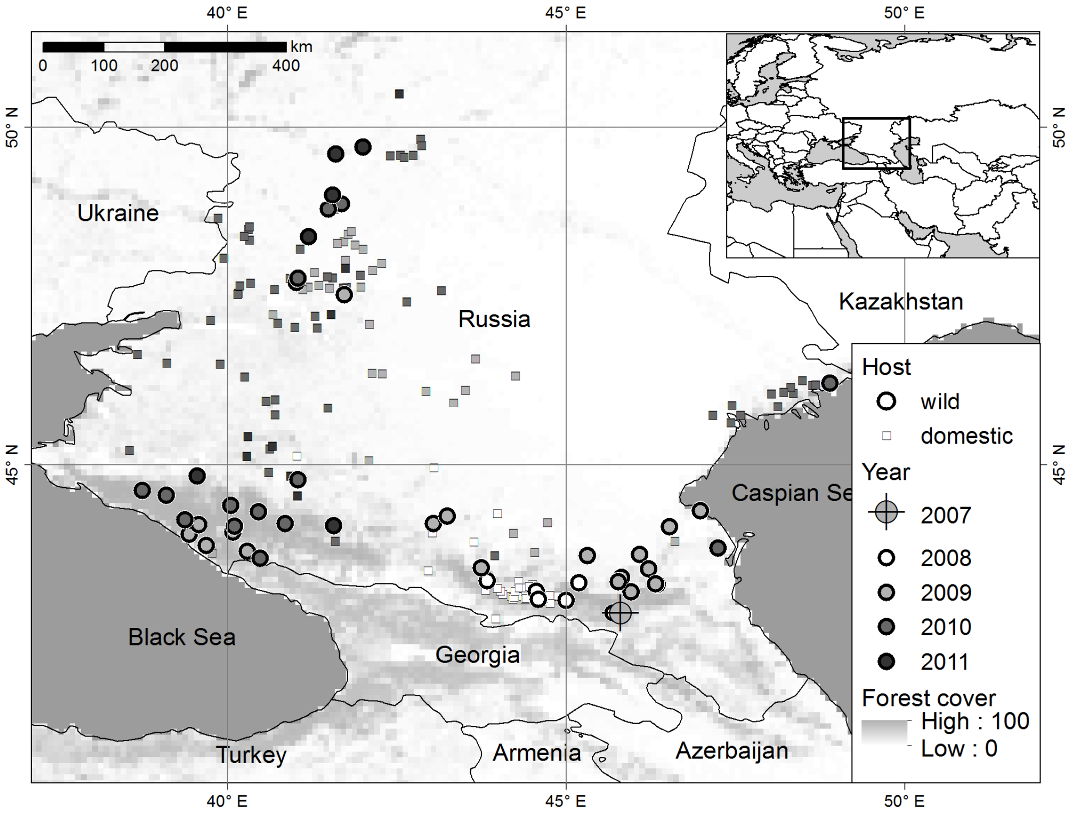

Fig. 1 Map of southern Russia, showing African Swine Fever (ASF) cases in wild boar from 2007 to 2011. Data: ASF reports: OIE, 2013, forest cover: FAO, 2007

The progress of ASFV spread in southern Russia is shown in Fig. 1, highlighting observational reports in wild boar (OIE, 2013). The first case since the 1970s was detected in a wild boar in the Chechen Republic in November 2007 (cross-hair in Fig. 1). In 2008-2010, ASF occurrences spread widely in the southern territories with cases in both wild boar and domestic pigs (white, light and dark grey in Fig. 1). In 2009, the first remote cases were reported in the north of Russia. By June 2011, 189 cases had been reported from the southern territories, of which 52 cases were in free-ranging wild boar. The eastern part of the infected area in southern Russia was particularly dominated by ASFV diagnoses in wild boar. This area would be particularly suitable to use to investigate the potential of ASF to turn into an endemic disease maintained by wildlife hosts. Indeed, having case notifications from at least three consecutive years, the area is considered an ASF endemic area (Khomenko et al., 2013).

A statistical approach is presented to assess the likelihood that endemic disease dynamics led to the observed spatio-temporal structure in the diagnostic records. The approach used temporal permutations of the original data-set to simulate spatio-temporal case detections of a disease persisting from year to year, between generations throughout the same wildlife population. The method is applied to ASF outbreak data from southern Russia between 05/11/2007 and 29/03/2011 (OIE, 2013).

MATERIALS AND METHODS

Test statistic

The test compares the proportion of ‘endemic cases’ between the original data-set and data-sets simulated under the null hypothesis of an endemic situation. An endemic case is a case that occurs within a critical distance d_c around and at least a critical time t_c after any previous case. The proportion of endemic cases is thus (Eq.(1):

$$P_{end}(d_c, t_c) = \frac{1}{n}\sum_{i=1}^{n}\sum_{j=i+1}^{n}\left(d_{ij} < d_c \wedge t_j - t_i > t_c\right); \text{ with } t_i \leq t_j \text{ for all } i<j \qquad (1)$$

where n is the total number of cases, d_{ij} is the Euclidean distance between cases i and j, and t_i and t_j are the time points of cases i and j.

The critical distance d_c reflects the assumed movement range of the host species whilst the critical time t_c represents the time required to solve all infections within an area spanned by the movement range of infected individuals. This period is determined by individual disease courses. Within certain biological limits both parameters may comprise uncertainty and should be varied for different values of P_{end}.

For every combination of t_c and d_c, the proportion of endemic cases P^*_{end} in the original data can be compared to the distribution of P_{end} simulated under the null hypothesis of an endemic situation. The null hypothesis will be rejected at the significance level α% if the original value is less the α^{th} percentile of the distribution.

Concept

The idea of the proposed test is based on a definition of disease endemicity which involves continued circulation and re-appearance in a previously affected area. Contrasting an epidemic situation where a wave of infections is travelling through the host population, infections using the proposed endemicity definition should be detected in sub-populations previously infected (spatially near to prior detection) and late enough after earlier detections to exclude relatedness (with a time-delay relative to prior detection).

If a case occurs in proximity to a previous case, but after a certain minimum time-span, it can be assumed to be unrelated to that previous case in the sense of epidemic spread. The case is then labelled as an ‘endemic case’ by the algorithm. Varying the critical spatial and temporal distance, the proportions of endemic cases can be compared between the original data-set and a number of simulated endemic data-sets. The more the proportion of endemic cases deviates between the original data and the simulated data, the less likely the null hypothesis is to be valid.

Required data

The proposed test is applicable to observational and systematic case reporting data with both period of detection (i.e. virus positivity) and host migration distance being short compared to the space-time horizon of the complete data-set. Each data entry must have time and location information. Moreover, the test application is reasonable only for infections with a definite disease outcome i.e. either dead or immune.

Generation of test data-sets

Hypothetical data-sets are simulated under the null hypothesis of an endemic disease situation. The simulation generates the same number of data points as the observations in the original data, distributed across the same target area and time-period. Even under the null hypothesis, data are not expected to be randomly distributed in time and by location, because observation of case data is usually influenced by spatial structures and temporal processes e.g. restricted habitat or seasonal fluctuations in sampling. To reassemble these characteristics, which are inherent in the investigated observational data, simulated test data-sets were obtained by permuting the spatial component of the original data-set; in other words, the spatial location of case detection is randomly re-associated with the prescribed temporal component of the data. Thus the generated pseudo-random disease report data-sets completely preserve the influence of spatial structure as well as temporal processes that may have systematically biased the original data. The approach can therefore avoid false rejection of the null hypothesis caused by these biases.

Application

The proposed test was applied to data from Russian ASF outbreaks in the southern territories between 05/11/2007 and 29/03/2011.

Study area: The study area comprised the Russian Southern Federal District and the North Caucasian Federal District. The region and the investigated data-set are shown in Fig. 1.

Data: Outbreak report data was obtained from OIE (2013). Each data-point comprises the geographical location, start date and end date. For geographical locations of the entire data-set see Fig. 1. Reports for domestic pigs and wild boar in captivity were excluded from the analysis, resulting in 52 cases of ASF in free-ranging wild boar.

Simulations: The endemicity test was performed for the possible parameter combinations of $d_c \in \{5, 10, ..., 200\,km\}$ and $t_c \in \{5, 10, ..., 200\,days\}$. For each parameter combination

N=499 permutations were generated and the respective proportion of endemic cases was determined (Manly 1997).

Analysis: The test statistic was the proportion of 'endemic cases'. The distribution of the test statistic was achieved using the permutation simulations. The 1st or 5th percentiles of the simulated distribution were used to test whether the null hypothesis may have led to the observed data. Parameter combinations are plotted by the result of this test i.e. likelihood of the null hypothesis being true.

RESULTS

The test statistic of the proportion of 'endemic cases' P_{end} is shown in Fig. 2 for the WAHID data-set for Russia over the range of the critical distance d_{c} and critical time t_{c}. The value of P_{end} increases with increasing d_{c} and decreasing t_{c}, caused by the low probability of very close and/or temporally very distant re-occurrences.

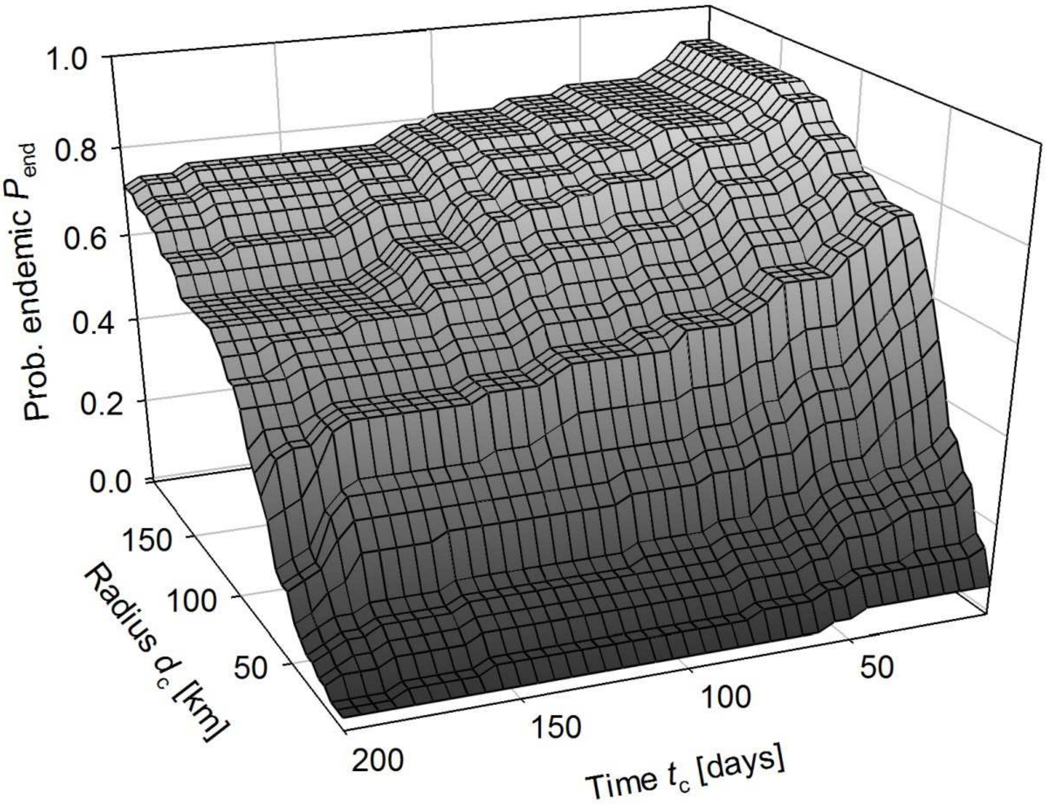

Fig. 2 Test statistic of the original WAHID data-set of ASF in southern Russia

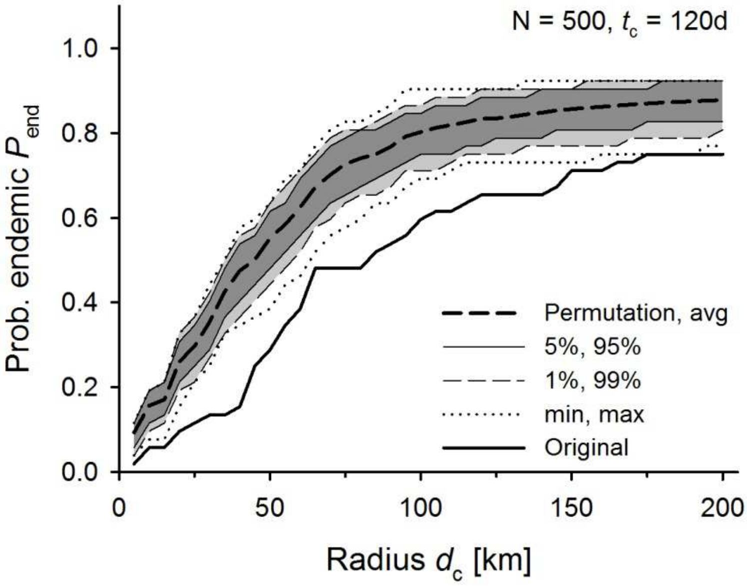

Fig. 3 Cross-section of the values of the test statistic for t_c = 120 *days*, calculated using the WAHID data-set for ASF in Russia and the associated distribution of that value under the null hypothesis.

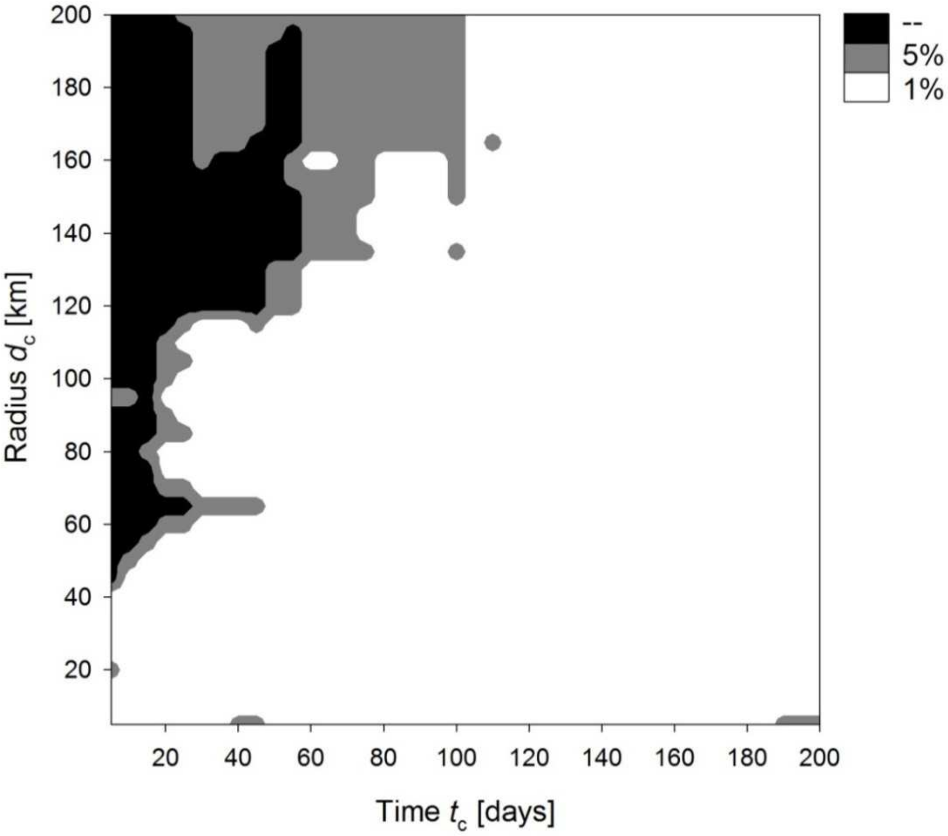

Fig. 4 Results of the endemicity test for the WAHID data-set of ASF in Russia. Contour shapes delineate parameter constellations that result in test statistics outside the 5th (black to grey area) or 1st percentile (grey to white area). Compatibility with the null hypothesis of an endemic situation was only achieved when subsequent cases after a short time but across large distances were eligible to count as ‘endemic cases’

The same information with the critical time arbitrarily fixed at $t_c = 120\,days$ is shown in Fig. 3. Up to a critical distance d_c of $170\,km$, the proportion of endemic cases P_{end} in the original data (bold solid line) lies outside the distribution range of P_{end} of the simulated data-sets (thin dotted line). Compared with N=499 permuted data-sets, the observed value of P_{end} is lower than the 1st percentile of the randomised data (thin dashed line) for any critical distance d_c up to the simulated maximum of $200\,km$. The null hypothesis of an endemic situation can therefore be rejected with 99% confidence for $t_c = 120\,days$ and $d_c \leq 200\,km$.

The results of the endemicity test for all combinations of $d_c \in \{5, 10, ..., 200\,km\}$ and $t_c \in \{5, 10, ..., 200\,days\}$ are shown in Fig. 4 as contours which delineate whether the test statistic fell below two percentile levels of its distribution under the null hypothesis. For a large scope of the explored test parameter space (i.e. t_c and d_c), the null hypothesis of an endemic situation of ASF in southern Russia would have been rejected (grey and white in Fig. 4). The parameter range for which the value of the test statistic was above the 5th percentile, was restricted to a small critical time and a large critical distance (top left in Fig. 4). The transition between the significant and the non-significant regions is located at an average disease spread velocity of around $720\,km/year$, which corresponds to $2\,km/day$. Any lower spread velocity either by smaller critical radius or longer separation time indicates incompatibility of the observed data with the null hypothesis of an endemic situation causing the observed ASF cases in southern Russia.

DISCUSSION

A novel approach to test the compatibility of observational disease data from wildlife with a null hypothesis of an endemic situation was developed in this study. The approach used ASF cases reported from southern Russia between 2007 and 2011. An endemic situation is characterized by disease persisting from year to year, between generations in the same wildlife population. The spatio-temporal description of an endemic situation makes use of a space-time interaction between disease cases, given the repeated clearance of infection from certain local parts of the population e.g. through dead or acquired immunity, and a subsequent re-occurrence after a pool of susceptible hosts has been recovered. Typical field examples are provided by modern moderate strains of CSFV that have circulated for years in European wild boar populations prior to vaccination efforts (Artois et al., 2002; Kern et al., 1999; Kramer-Schadt et al., 2005; Rossi et al., 2005; Leifer et al., 2011). Alternatively, rabies in large rural populations of red foxes in Central Europe exhibits the potential to become endemic; this is illustrated by the historic wave of disease that passed through Europe from the north-east to the south-west in the last century and the subsequent occurrence of thousands of rabies cases all-over the previously infected area (King et al., 2004). Again, the infection circulated until large-scale and long-term vaccination campaigns started (Müller et al., 2005; Pastoret & Brochier, 1998; Jeltsch et al., 1997; Tischendorf et al., 1998).

It was proposed to classify areas where ASFV has been detected into three categories (Khomenko et al., 2013): endemic (ASF reported > 3 years), sporadic (ASF reported for 2 consecutive years) and occasional introductions (single detections of ASF with no secondary spread). The proposed categorisation focuses on the time-horizon of repeated disease reporting. A long period of reporting, particularly in larger regions like southern Russia, does not *per se* provide evidence for an endemic situation. The proposed categories do not describe the spatial dimension of the classified areas. Consequently, these classifiers cannot portray different epidemiological causalities of sequential case detections, either due to an initial undulating forward spread of infection invading a population or the cyclic re-occurrence of the infection afterwards. The proposed endemicity test was constructed to close this gap. For the occurrence of ASF in wild boar in southern Russia, the endemicity hypothesis was rejected with 99% confidence for all biologically reasonably parameter values. Particularly, parameter values assuming any wild boar mediated disease spread velocity (i.e. d_c/t_c) slower than 720 *km/year* were incompatible with the endemicity hypothesis. The spread velocity of 350 *km/year* reported from the study area (Blome et al., 2011), can only be translated into parameter combinations of critical distances and critical time-span that would contradict an endemic status of ASF in wild boar. Finally, the pattern of detected cases differs significantly from the pattern expected in an endemic situation. Dynamic visualisation of the spatio-temporal pattern of ASF cases in the region (see Fig. 1) using a data-movie, can underpin the insights (Thulke et al., 2002). The serial mapping revealed the spatial advance of disease cases towards the north, while areas involved in the first two years were rarely affected in the latter state of the event (Siemen, 2011).

One may argue that the endemicity test was not necessary to reclassify the ASF situation in wild boar in southern Russia, in the light of the huge infectivity of ASF found in experimentally infected wild boars (de Carvalho et al., 2013), the high lethality of the infection (Blome et al., 2012), the likely absence of an arthropod vector (Beltrán-Alcrudo et al., 2008) and because there have been no ASF cases in the area since 2011 (OIE, 2013). Nonetheless, the situation in the southern territories was repeatedly discussed as a possible endemic area because of the presence of the disease over several years (Beltrán-Alcrudo et al., 2008).

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CATTLE HEALTH MANAGEMENT

RISK FACTORS FOR BOVINE RESPIRATORY DISEASE IN AUSTRALIAN FEEDLOT CATTLE: USING A CAUSAL DIAGRAM-INFORMED APPROACH TO ESTIMATE TOTAL EFFECTS OF MIXING AND GROUP SIZE BEFORE FEEDLOT ENTRY

K.E. HAY*, T.S. BARNES, J.M. MORTON, A.C.A. CLEMENTS AND T.J. MAHONY

SUMMARY

A longitudinal study was conducted to investigate risk factors for bovine respiratory disease (BRD) in feedlot cattle. Data from a national cattle movement database were used to derive variables describing animal mixing, grouping and exposure to saleyards. The total and direct effects for each risk factor were estimated using a causal diagram-informed process to determine covariates to include in separate four-level Bayesian logistic regression models. Mixing and group size were important predictors of BRD. Animals not mixed prior to 12 days before feedlot entry and then exposed to a high level of mixing (≥4 groups) had the highest risk of developing BRD (OR 3.6) compared to animals mixed at least 4 weeks before feedlot entry with <4 groups forming the cohort. Animals in groups of ≥100 (OR 0.5) or 50–99 (OR 0.8) formed ≥13 days before feedlot entry were at reduced risk compared to those in groups of <50.

INTRODUCTION

Bovine Respiratory Disease (BRD) describes a complex of diseases involving the respiratory system in cattle. BRD is particularly common where cattle are kept in intensive or confined conditions, such as in feedlots, and is a multifactorial disease; necessary factors include pathogenic organisms, environmental stressors and immunological susceptibility (Edwards, 2010). While early BRD research identified market origin, group size and comingling of cattle close to the time of feedlot entry as risk factors (Martin, 1983; Ribble et al., 1995), these studies lacked sufficient data to disentangle the effects of individual factors and to consider the effect of timing of exposure to these factors relative to feedlot entry. More recent studies have also found that market origin and comingling of cattle from multiple sources are associated with the risk of BRD (Macartney et al., 2003; Sanderson et al., 2008; Step et al., 2008).

Causal diagrams facilitate an informed approach to model building based on *a priori* knowledge and plausible biological pathways. They also allow the separate estimation of both total and direct effects, both of which may be of interest to researchers and industry stakeholders (Dohoo et al., 2009). The direct effect of an exposure is indicated in a causal diagram by a single arrow directly linking the exposure to the outcome. An indirect effect of an exposure is indicated by a pathway through a sequence of arrows passing through one or more intervening variables. The total effect of an exposure variable is the sum of the direct and all indirect effects of that exposure on the outcome.

This paper describes the development of a causal diagram and subsequent analyses to evaluate the total and direct effects of risk factors relating to mixing with other cattle, the number of animals in a group and exposure to saleyards before feedlot entry on the risk of BRD in Australian feedlot cattle.

MATERIALS AND METHODS

Overview of study design

A nationwide prospective longitudinal study was conducted in Australia to evaluate many possible risk factors for BRD in feedlot cattle. Results for the subset of exposures relating to animal mixing, group size and exposure to saleyards are presented in this paper. Managers of feedlots with a minimum capacity of 1,000 head and the necessary resources to keep required records from cattle in study cohorts throughout the study period were invited to participate. A subset of cattle inducted into study feedlots were selected, exposure statuses of animals assessed, and occurrences of BRD identified. Each animal was inducted (identification tagged, weighed, vaccinated etc.) prior to feedlot entry. A cohort was defined as a group of cattle that were assembled together in a feedlot pen following induction. Staff at each feedlot agreed to enrol a particular number of cohorts at regular intervals, but despite attempts to randomise the selection process, the enrolled cohorts were generally selected at the convenience of the feedlot managers.

A total of 35,160 animals were inducted into study cohorts from March 2009 to December 2011. Of these, 35,131 had sufficient data for inclusion in this study, while 29 animals were excluded because they were lost to follow-up before day 50 of the observation period. The study population had a hierarchical structure such that animals were clustered within 1,077 set-13s, which were clustered within 170 cohorts, which were clustered within 14 feedlots. Each animal's set-13 was defined as the group that the animal was part of 13 days prior to induction into the study cohort. The number of animals per set-13 ranged from 1 to 342 with a mean of 33 and a median of 17, and the number of animals per cohort ranged from 17 to 395, with a mean of 207 and a median of 186.

Each animal was monitored from induction until it left the study cohort for any reason, defined by the earliest of the following dates: removal to the hospital pen, moving to a different pen separate from other animals in the study cohort, death or leaving the feedlot. Feedlots were required to record and supply detailed data for all animals enrolled into each study cohort for the duration of the follow-up period. Electronic animal-level files containing induction data stored basic characteristics of each animal (animal identification number, gender, dentition, breed and weight) as well as dates of arrival, induction and the date of the animal's first day on feed (i.e. the first day that it was offered a total mixed ration in a feedlot pen), which may have been prior to or the same day as the induction day. A Microsoft Access® relational database was developed to facilitate data storage and linkage. The Stata® statistical software package (version 12) was used for all data management, validation and preliminary analyses, and MLwiN® (version 2.27) was used to fit final four-level models.

National Livestock Identification System data

The Australian National Livestock Identification System (NLIS) requires that all cattle are individually identified with a unique barcode that may be implanted as an ear tag or a rumen bolus, before they leave the farm of origin. Each farm is identified by a unique Property

Identification Code (PIC). The system relies on registered users scanning the device and inputting data into an online national electronic database every time an animal is moved from one PIC location to another ('transfers'). The source and destination PICs, transfer dates, transfer types and waybill number for all transfers are recorded in the database. Transfer type distinguishes between saleyard transfers and “point to point” transfers between two PIC locations. Information is also stored about the PIC of issue and changes in identification numbers if lost devices are replaced.

NLIS data were obtained for study animals. PICs of issue were obtained for 99.9% of study animals, and 98.8% had recorded movements between PICs. Data validation and correction involved consolidating and simplifying records to create a logical sequence from the PIC of issue to the feedlot for each animal. Where moves are entered into the NLIS database that are not logically correct, the NLIS system imputes a record and date, with the date being one day prior to the next recorded transfer date. Where possible, imputed transfer dates were modified based on common group moves or midpoints between known transfers. Otherwise imputed dates were changed to 180 days prior to a transfer to the feedlot as this was consistent with movement patterns observed among study cattle.

Hypotheses

It was hypothesised *a priori* that the effect of mixing, group size and exposure to a saleyard (a 'saleyard transfer') on the risk of developing BRD might differ depending on the timing of these events in relation to the timing of induction into a feedlot pen. For example, while it was expected that a saleyard transfer close to the time of feedlot entry would increase the risk of BRD, it was hypothesised that the effect of a saleyard transfer at least 4 weeks before feedlot entry may reduce the risk as these cattle would have sufficient time to develop immunity before feedlot entry following exposure to pathogens during the saleyard transfer. Thus, the time points chosen to investigate mixing, group size and saleyard transfers presupposed that these measures were proxies for exposure to pathogens, social and other stressors and a differing response might be expected depending on concurrent exposures and the timing of exposure relative to feedlot entry.

Case definition and exposure variables

The outcome of interest was the development of BRD during the first 50 days following induction. The case definition was based on the clinical signs of disease as recorded by feedlot staff in computerised hospital records that were forwarded to the research team. Clinical signs were recorded after suspected ill animals were removed from their cohort for examination and treatment. Following consultation with feedlot veterinarians, all diagnoses indicating respiratory system involvement were classified as BRD cases by the research team.

Variables were derived from the NLIS data to describe the sequence and timing of transfers and to determine the PIC location and number of animals in a group at particular time points of interest. Because animals were unlikely to be assessed and have records for the occurrence of BRD until after induction, time at risk (TAR) began the day after induction into a study cohort. Hence, the induction date was time at risk zero (day 0). Dates prior to this date were defined as negative TARs. Time points of particular interest were day -28, day-13, day -2 and day 0, and the location, grouping (group-#) and number of animals in the group (e.g. group-13N) at these time points was determined. Variables were derived to describe changes occurring between these time points. For example, mixing prior to day -27 was determined by the number of PICs of issue that contributed animals to form a group-28,, while animal mixing between day -27 and day -13 was determined by the number of group-28s that were combined to form the animal's group-13. These count variables were collapsed into dichotomous variables. As the majority of animals were moved to the feedlot within 12 days prior to induction, the amount of mixing in this period was described by a categorical variable based on the number of group-13s that were combined to form the animal's cohort (1, 2–3, 4–9 and ≥10). These variables were combined to form a single variable (mix history) to describe the animal's lifetime mixing history which summarised mixing during these three time intervals. Three binary saleyard transfer variables were derived to describe whether or not animals had been exposed to saleyards in the intervals pre day -27, day -27 to day -13 -13, and day -12 to day 0.

To avoid potentially invalid assumptions that relationships were linear on the logit scale, all exposure variables were categorised for use in analyses. Definitions of categories were based on prior hypotheses and distributions of exposures across the study population including distributions by feedlots. Despite this, many management-related variables were clustered by feedlot. BRD risk varied markedly by feedlot so it is possible that observed associations may have been confounded by unknown or unmeasured feedlot-level factors. While estimating the total effects of variables of interest was the main focus of these analyses, direct effects were also estimated where these were of interest.

Causal diagram

A causal diagram was constructed to interlink the measured exposure variables with each other as appropriate and with occurrence of BRD in the first 50 days at risk. This resulted in a complex diagram which contained 42 exposure variables. A simplified version of this causal diagram (only including variables relevant to the assessment of the risk factors included in this paper) is shown in Fig. 1. It shows the proposed causal pathways linking the exposure variables describing animal mixing, group size and exposure to saleyards to the occurrence of BRD in the first 50 days at risk, along with potential confounders for either direct or total effects of any of the exposure variables of interest. These additional variables were feedlot move timing, cohort fill duration, total number of animals on feed and induction weight. Feedlot move timing was a 'composite' variable derived from a combination of the interval between arrival at the feedlot vicinity and induction and the estimated duration of transport for those arriving within 12 days before induction. Cohort fill duration was a cohort-level variable indicating whether all of the animals in the cohort were inducted on the same day or the cohort was filled over multiple days. The total number of animals on feed in the feedlot was the estimated monthly average total number of animals in the feedlot during the month that the study animals were inducted. The number of animals in the cohort was the total number of animals that were inducted into the study cohort. Induction weight ('weight') ranged from 196 to 756 kg and was categorised into four categories: 20% of the study population were <400 kg, 31% were 400–439 kg, 34% were 440–479 kg and 15% were ≥480 kg.

Statistical modelling

In estimating total effects, care needs to be taken to adjust appropriately for confounders, including variables that become confounders through conditional associations (Dohoo et al., 2009). Several methods have been proposed for determining covariates to fit when estimating total effects, but they do have similar features (Greenland et al., 1999; Shrier & Platt, 2008; Dohoo et al., 2009; Textor & Liskiewicz, 2011). The DAGitty® software (Textor et al., 2011) was used to identify minimal sufficient adjustment sets to assess total and direct effects of the exposure variable of interest on the occurrence of BRD. A sufficient adjustment set is a set of variables that appropriately controls confounding of the association between the exposure variable of interest and the outcome. When direct effects are required, the sufficient adjustment set also includes all intervening variables between the exposure variable of interest and the outcome. The causal diagram was reproduced within the DAGitty® user interface. Each variable of interest was sequentially identified as the exposure of interest, and the minimal sufficient adjustment sets for both total and direct effects as defined by DAGitty® were noted.

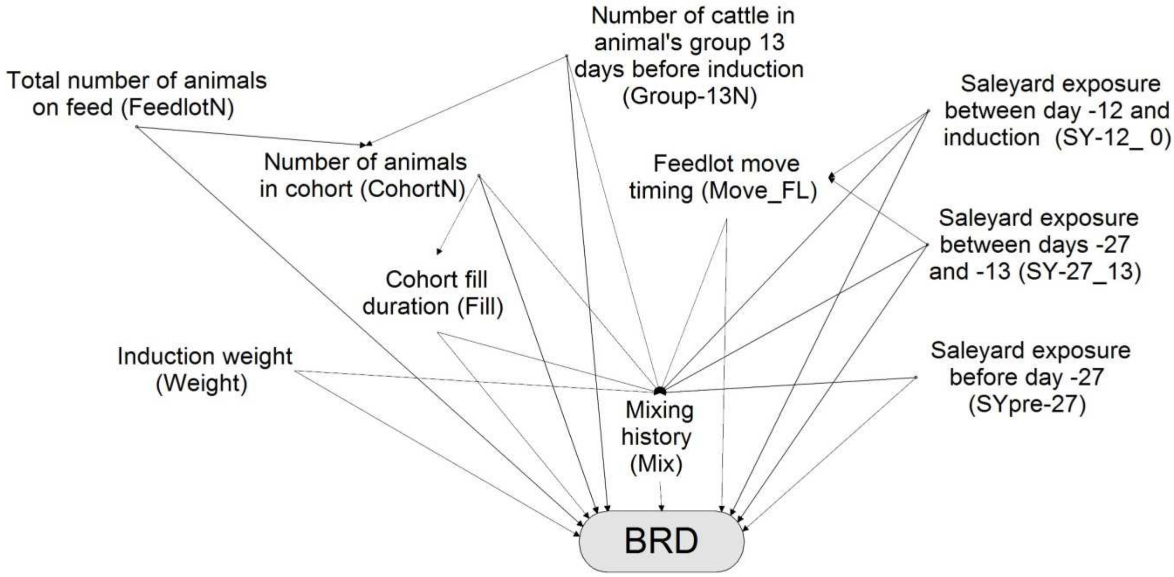

Fig. 1 Causal diagram showing postulated causal paths linking variables related to animal mixing, group size and exposure to saleyards to occurrence of bovine respiratory disease (BRD) in the first 50 days at risk.

The multilevel modelling software package MLwiN® (version 2.27) was used for modelling. It was run from within Stata® using the runmlwin module (Leckie & Charlton, 2013), which facilitates the transfer of data between the two packages. This enabled use of the more flexible multilevel modelling procedures provided in MLwiN as well as the wide range of post estimation commands available in Stata®. For each exposure of interest, covariates for the total effects estimates were first determined by the minimal sufficient adjustment set, and then a model was fitted using second-order penalised quasi-likelihood methods to produce starting values for the second model using Bayesian Markov-chain Monte Carlo (MCMC) methods. The process was then repeated using the adjustment set for direct effects. All models included random effects of feedlot, cohort and group-13 and used Metropolis Hastings sampling methods. Priors were normally distributed with extremely large variances; the default for multilevel logistic models fitted in MLwiN® (Browne, 2012). After a burn-in of 1000 iterations, 10,000 more were run and diagnostic trajectory plots and summary statistics (Browne, 2012) were assessed to estimate the required chain length. Models were then rerun and reassessed. Animal-level variables such as saleyard exposure prior to day -27 displayed good mixing and low autocorrelation, while mix history displayed higher autocorrelation and was slower to converge. Subsequently, MCMC chains were run for between 20,000 and 200,000 iterations to obtain final posterior parameter estimates of mean odds ratios (ORs) and 95% credible intervals.

RESULTS

Descriptive statistics

Of all suspected ill animals removed from their cohort for examination and treatment, 77.3% (6,406/8,285) met the BRD case definition at first examination, comprising 18.2% (6,406/35,131) of the study population. The vast majority of animals that had BRD when first examined were examined during their first 50 days at risk, giving a 50-day BRD incidence risk of 17.6%. This incidence risk does not include BRD occurrences subsequent to diagnosis with another condition at the time of first examination.

Table 1. Distribution of mixing history and estimated odds ratios for the total and direct effects[a] of mixing history on the occurrence of BRD by day 50.

Mix history category[b]	Number of animals (% of animals)	Crude BRD 50-day incidence risk (%)	Odds ratio	95% credible interval
No, no, no[c]	418 (1)	20.6	2.3	(0.4–7.4)
No, no, 2–3	1,489 (4)	19.5	2.3	(1.3–3.8)
No, no, 4–9	3,334 (10)	30.3	3.7	(1.7–7.2)
No, no, ≥10	5,114 (15)	31.4	3.7	(1.7–7.4)
No, yes, yes	627 (2)	17.2	3.4	(1.4–7.2)
No, yes, no[c]	407 (1)	2.5	2.3	(0.5–6.9)
Yes, no, 2–3	3,893 (11)	5.7	Ref. cat.	
Yes, no, 4–9	5,409 (16)	16.4	2.8	(1.3–5.4)
Yes, no, ≥10	7,793690 (22)	20.7	2.2	(1.0–4.5)
Yes, yes, yes[c]	946 (3)	13.7	2.2	(0.9–4.5)
Yes, yes, no[c]	1,958 (6)	3.3	2.5	(0.7–6.6)
Yes, no, no	3,342 (10)	3.4	1.2	(0.5–2.6)

[a] Total and direct effects are the same; model derived from causal diagram (Fig. 1): Cohort fill duration, Weight, SY-12 to 0, SY-27 to -13, SY pre27, CohortN, MoveFL, Set-13N, Mixing history. Number of animals used in model: 34,726

[b] Mix history defined by combination of mixing in three time intervals: pre TAR-27 (yes/no), TAR-27 to TAR-13 (yes/no) and TAR -12 to cohort close (number of set-13s combined: no/2-3/4-9/≥10)

[c] Categories where 7 or more feedlots have no observations

Descriptive statistics for the mixing history variable are shown in Table 1. The most common pattern of mixing involved animals that had been mixed prior to day -27 that joined cohorts that combined ≥10 group-13s ('Yes, no, ≥10, 22% of all animals). Cohorts formed from 4–9 group-13s were also common in previously mixed animals ('Yes, no, 4–9', 16%). A high level of mixing within 12 days of induction was also common in animals not mixed prior to day -27, with 15% of animals in cohorts formed by ≥10 group-13s ('No, no, ≥10') and 10% of animals in cohorts formed by 4–9 group-13s ('No, no, 4-9').

Descriptive statistics for exposure variables relating to the number of animals in group-13 and exposure to saleyards are shown in Table 2. About a third (36%) of the study population had been through a saleyard at least 27 days before induction; while relatively few (6%) had been through a saleyard within 27 days prior to induction.

Table 2. Distribution of group-13 size and saleyard exposure variables and estimated odds ratios for their total effects[a] on the occurrence of BRD by day 50.

Variable, category; number of animals used in model (N)	Number of animals (% of animals)	Crude BRD 50-day incidence risk (%)	Odds ratio	95% credible interval
Animals in set-13; N=35,131				
< 50	13,782 (39)	24.1	Ref. cat.	
50 to 99	9,783 (28)	21.3	0.8	(0.7–0.9)
≥ 100	11,566 (33)	6.9	0.5	(0.4–0.7)
Saleyard transfer prior to day -27; N=34,730				
No	22,223 (64)	18.7	Ref. cat.	
Yes	12,507 (36)	15.7	0.8	(0.7–0.9)
Saleyard transfer between day-27 and day -13; N=35,131				
No	34,162 (97)	17.8	Ref. cat.	
Yes	969 (3)	11.2	1.9	(1.3–2.7)
Saleyard transfer between day -12 and induction; N=35,131				
No	34,200 (97)	17.6	Ref. cat.	
Yes	931 (3)	21.4	2.6	(1.6–4.1)

[a] Model derived from causal diagram (Fig. 1): the minimal sufficient adjustment set for each of these variables was an empty set, so no additional covariates are included in these models

Multilevel logistic regression models

Mixing history had a marked effect on the risk of BRD by day 50. Several categories had increased odds compared to the reference category of animals that had been mixed prior to day -27 from cohorts formed by only 2 or 3 set-13s ('Yes, no, 2-3', Table 1). The risk was substantially higher for animals that were not mixed prior to day -12, but were mixed in the interval between day -12 and cohort close, with the highest risk for those where 4 or more group-13s formed the cohort ('No, no, 4-9' or 'No, no, ≥10'). Animals that had not been mixed prior to day -27 and were then mixed in the interval day -27 to day -13 and again in the interval day -12 to cohort close ('No, yes, yes') were also at markedly higher risk, as were animals that had been mixed prior to day -27 and then subjected to a high level of mixing, where 4 or more group-13s formed the cohort ('Yes, no, 4-9' or 'Yes, no, ≥10'). Compared to the reference category, the odds ratio point estimate for animals mixed pre day -27, but not mixed between day -27 and cohort close ('Yes, no, no'), was close to 1 (OR 1.2), but this estimate was imprecise. Based on the causal diagram, direct and total effects for mixing history were equivalent.

Table 3. Adjusted odds ratios for direct effects group-13 size and saleyard exposure variables on the occurrence of BRD by day 50 based on models derived from the causal diagram shown in Fig. 1.

Variable & category	Odds ratio	95% credible interval
Animals in set-13[a]		
< 50	Ref. cat.	
50 to 99	0.8	(0.7–1.0)
≥ 100	0.6	(0.4–0.8)
Saleyard transfer prior to TAR-27[b]		
No	Ref. cat.	
Yes	1.0	(0.9–1.1)
Saleyard transfer between TAR-27 and TAR-13[b]		
No	Ref. cat.	
Yes	1.3	(0.8–2.0)
Saleyard transfer between TAR-12 and TAR0[b]		
No	Ref. cat.	
Yes	1.6	(0.9–2.6)

[a] Model consisted of CohortN, Cohort fill duration, Weight, SY-12 to 0, SY-27 to -13, SY pre27, FeedlotN, Mixing history, MoveFL and Set-13N. Number of animals used in model: 34,726

[b] Model consisted of CohortN, Cohort fill duration, Weight, SYpre27, SY-27 to -13, SY-12 to 0, Set_13N, Mix history, and MoveFL. Number of animals used in model: 34,726

The estimated total effects of the number of animals in set-13 and exposure to saleyards on the risk of BRD by day 50 are shown in Table 2. Compared to animals from set-13s of fewer than 50 animals, animals from set-13s with 50 to 99 animals were at modestly reduced risk (OR 0.8) and animals from set-13s with 100 or more animals were at markedly reduced risk (OR 0.5) of developing BRD. The direct effects of the number of animals in set-13 (Table 3) were of similar magnitude to the total effects and the 95% credible intervals excluded values greater than one.

The total effect of exposure to saleyards varied with time of the transfer, with markedly increased risk associated with saleyard transfers close to day 0 (SY-12 to 0: OR 2.6; SY -27 to -13: OR 1.9) and modestly decreased risk for saleyard transfers prior to day -27 (OR 0.8) (Table 2). Direct effect estimates are reported in Table 3. There was no evidence for an important direct effect of having been through a saleyard prior to day -27 after accounting for mixing history and several other variables (OR 1.0). The direct effects of saleyard transfer in the periods day -27 to day -13 and day -12 to day 0 were much attenuated after adjusting for mixing, feedlot move timing, cohort fill pattern, induction weight, saleyard transfer in other time intervals and the number of animals in the set-13 and cohort. This indicates that total effects of exposure to saleyards during these period are probably largely due to the effect of mixing. However, an important adverse direct effect of exposure to saleyards from day -12 to indcution was evident (OR 1.6) indicating that exposure in this period has a negative effect over and above the effects of mixing.

DISCUSSION

This study provides powerful evidence that the risk of BRD in feedlot cattle varies markedly with prior mixing history and the number of animals in groups assembled prior to feedlot entry. Management decisions regarding the timing and level of mixing and the number of animals in a group assembled 13 days before induction have the potential to markedly reduce the incidence of BRD in feedlot cattle. If cattle have been mixed prior to a month before entry, fewer than four groups may be combined to form a cohort. If cattle have not been mixed prior to a month before induction, the BRD risk would be expected to be increased, so mixing on a farm for at least a month is recommended before feedlot entry. In addition, animals should be part of a group of at least 50 animals for at least 13 days before entry. Direct purchase of cattle from saleyards for immediate feedlot entry is not recommended; saleyard-sourced animals should be held on a farm for at least a month prior to feedlot entry.

An important finding from this study is that the effects of exposure to saleyards differ depending on the timing in relation to feedlot entry. By separating effects of covariates such as mixing history, feedlot move timing and the number of animals in a group from the saleyard transfer variables, it has been possible to compare total and direct effects. Thus, it was evident that the total effect of saleyard transfer more than 27 days before induction is primarily mediated by factors other than the actual process of unloading, yarding, holding then reloading at saleyards. Similarly, the detrimental total effect of saleyard transfer within 27 days of feedlot entry should be interpreted in combination with the much attenuated direct effect estimates. Saleyard transfers within 27 days of feedlot entry increase risk of BRD but this was at least partially mediated through mixing. However, these results indicate that exposure to saleyards within 12 days prior to feedlot entry further increases risk of BRD over and above effects of mixing.

This study supports prior research that suggests larger group size is protective (Ribble et al., 1995). In defining the group at a consistent time point (day -13) for all study animals, it has been possible to quantify the effect of group size in a manner that enables comparison across study populations.

The use of a causal diagram and the determination and comparison of separate direct and total effects provides informative estimates of effect in often complex situations where particular variables of interest may not remain “significant” in a single multivariable model. The adjusted effects estimated in a multivariable model built using an automated model building process may be direct, partial or total effects and therefore do not necessarily reflect the total causal effect of the exposure variable on the outcome variable (Westreich & Greenland, 2013). This is because with automated model building processes, variable selection is not based on whether variables may be potential confounders or intervening variables for particular exposure-outcome relationships, so some variables that are important confounders may not be included and intervening variables may be included in the model. However, the use of a causal diagram to inform model building can result in uncontrolled confounding if the causal diagram does not accurately capture causal pathways or important confounders are missing from the diagram. It also relies on some assumptions about the directionality of associations and this is not always clear.

Beef cattle management practices in Australia differ from those in North America and Europe in some key aspects. Cattle in Australia enter feedlots at an older average age, often many months after weaning; and it is common for recently weaned cattle to be sold through saleyards or weaner sales and then spend 6 months or more on an intermediate property before being sold to a feedlot (Walker et al., 2007). Accordingly, results of this study may not reflect causal relationships in other countries. In addition, larger capacity feedlots were more likely to participate in the study, so conclusions may not be generalizable to all Australian feedlot populations. However, results should be generalizable to moderate to large feedlot operations and findings relating to saleyard transfers, mixing and group sizes are biologically plausible and consistent with prior research.

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RISK FACTORS ASSOCIATED WITH ON-FARM MORTALITY IN SWEDISH FIRST PARITY DAIRY COWS

M. JANSSON MÖRK*, K. ALVÅSEN AND U. EMANUELSON

SUMMARY

This study included first parity cows (n=76,720) calving between 1 July 2008 and 30 June 2009 in Swedish herds with >40 cows. The effects of potential risk factors on on-farm mortality (death or euthanasia) were analysed using a Weibull proportional hazard model with a frailty effect. The lactations were split in season and lactation stage. The highest hazards were found for trauma and disease, both in the current lactation stage and in the preceding stage. The mortality hazard was higher in early lactation and higher for calving between January and April. Increased age at first calving, dystocia and stillbirth also increased the mortality hazard. Differences were also found between breeds, level of milk yield and genetic merit for milk production as well as farm characteristics. The results in this study show the importance of good management and preventive health actions, especially around calving, to avoid mortality in first parity cows.

INTRODUCTION

High on-farm cow mortality (i.e. euthanasia and unassisted death) is a concern on dairy farms from both an animal well-being and an economic point of view. Increasing mortality has been reported in several countries. Thomsen et al. (2004) showed that the mortality risk among Danish dairy cows increased from 2% in 1990 to 3.5% in 1999. In the USA, mortality increased from 2% in 1995 to 4.6% in 2005 (Miller et al., 2008), and was found to be 5.7% in 2007 (USDA, 2007). Alvåsen et al. (2012) reported that mortality rates in Swedish dairy herds increased from 5.1 to 6.6 deaths/100 cow-years between 2002 and 2010. This is of great concern for the dairy industry and explanations for the increase and possibilities to reduce the mortality rate are sought.

Several risk factors for on-farm cow mortality at herd level have been identified. A recent Swedish study (Alvåsen et al., 2012) found that larger herd size, Holstein breed, and greater herd mean annual milk yield are all factors associated with increased mortality rates. This is in concordance with results from other countries (France: Raboisson et al. 2011; Denmark: Thomsen et al. 2006; the USA: McConnel et al., 2008). Furthermore, no summer grazing (Thomsen et al., 2006; Burow et al., 2011), conventional (compared with organic) herds (Thomsen et al., 2006; Alvåsen et al., 2012) increased proportion of purchased cows (Thomsen et al. 2006; Raboisson et al. 2011) and longer herd average calving interval (Raboisson et al., 2011; Alvåsen et al., 2012) are also factors that have been associated with higher mortality rates.

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There are only few studies on risk factors for mortality at cow level and this has not been studied under Swedish production conditions before. Previous studies have reported greater mortality risk in early lactation (Milian-Suazo et al., 1988; Stevenson & Lean, 1998; Thomsen et al. 2004; Dechow & Goodling, 2008). McConnel et al. (2009) showed that 40% and 50% of deaths occurred by 30 days after calving for younger cows (parities 1 and 2) and for older cows (parities 3 and greater), respectively. These figures show that the risk for mortality increases with higher parity, which is in line with results from Denmark and the USA (Thomsen et al. 2004; Dechow & Goodling, 2008; Miller et al., 2008). Parity and stage of lactation are not modifiable for the dairy farmer, but some other factors may be. It is therefore important to identify other risk factors for mortality at cow level to reverse the negative trend.

The objective of this observational study was to assess associations between cow-level data from the Swedish official milk recording scheme and on-farm mortality in first parity cows.

MATERIALS AND METHODS

Data from herds enrolled in the Swedish official milk recording scheme with a herd size of >40 cows was used. All first parity cows with a calving between 1 July 2008 and 30 June 2009 were included in the study, in total 76,720 cows from 2,084 herds.

Cows were followed from their first calving until they calved again. The event of interest was on-farm death or euthanasia in the on-going lactation. Cows that were culled or sold during the lactation and cows with an on-going lactation after 500 days after calving were right censored. Stated reasons for death or euthanasia and slaughter, respectively, were categorised into ‘Low milk yield’, ‘Fertility disorders’, ‘Udder disorders’, ‘Metabolic disorders’, ‘Claw/leg disorders’, ‘Trauma’, and ‘Other’ (Table 1).

The effects of potential risk factors were analysed using Weibull proportional hazard models with gamma distributed frailty common to cows within herd. For each cow, the observation period was calculated as the time period between the calving starting the study period and the time for the event of interest or time for right censoring. The lactations were split (using the stsplit command in Stata® version 12) at September 1, January 1 and May 1, and at 30 and 100 days in milk. This was done in order to investigate the effect of diseases in different lactation stages and to investigate seasonal effect on on-farm mortality. The corresponding risk factors were included as time-dependent variables in the analysis. Age at first calving was categorised into quartiles. Disease events were categorised into eight disease complexes: ‘Claw/leg disorders’, ‘Infection and parasitic disorders’, ‘Mastitis’, ‘Metabolic disorders’, ‘Other disorders’, ‘Puerperal paresis’, ‘Reproductive disorders’, and ‘Trauma’, and categorised as present/not present in the current lactation stage as well as in the previous lactation stage. Also the total number of diseases was calculated and categorised into ‘No diseases’, ‘One disease event’, and ‘Several disease events’. Data from the first test milking post calving were used to evaluate the effect of production, udder health, and energy balance. The variables were categorised in order to handle missing observations. Only data for the first test milking within 30 days post calving were used, and cows lacking a test milking in that time interval were categorised as missing (i.e. cows that either left the herd before a test milking was performed, had the first test milking more than 30 days post calving or had the first test milking discarded due to e.g. disease). Somatic cell count (SCC) and fat/protein ratio were treated as categorical variables with the cut off at 100,000 cells/ ml and 1.4, respectively. Milk yield, milk fat, milk protein and milk urea were each categorised into ‘First quartile’, ‘Second-third quartile’, ‘Fourth quartile’, and ‘Missing’. Genetic merit for milk production for the dam and sire of the cow, as estimated by the Nordic Cattle evaluation services, was treated as a categorical variable categorised into ‘First quartile’, ’Second-third quartile’, ‘Fourth quartile’ and ‘Missing’.

Potential risk factors were first screened in univariable analysis and variables with a *P*-value <0.2 was considered for multivariable analysis. Correlations between variables considered for multivariable analysis were tested using Spearman's rank correlation coefficients with the intention to excluding one of the variables if the correlation was ≥ |0.7|. Milk yield, milk fat and fat/protein ratio were highly correlated and milk yield was chosen based on higher log likelihood. The full model was reduced by manual backward elimination until all remaining variables had a *P*-value ≤0.05. All variables excluded were then re-entered one by one and kept if the *P*-value was ≤0.05 or if the parameter estimate of another variable changed more than 20% (as indicative of confounding). All statistical analyses were performed using Stata® version 12 (StataCorp LP, College Station, TX).

RESULTS

The probability of survival after calving is presented in Fig. 1. Of the 76,720 first parity cows included, 2,904 (3.8%) died or were euthanized, 12,632 (16.5%) were slaughtered, 439 (0.6%) were sold and 50,845 (66.3%) calved again. The remaining 9,900 (12.9%) lactations were right censored because the observation period ended. Of the dead/euthanized cows and culled cows, 29.5% and 4.8%, respectively, left the herd during the first 30 days of the lactation (median 89 and 252 days for all dead/euthanized and culled cows, respectively). The stated main reasons for death/euthanasia and slaughter are listed in Table 1. Results from the survival analysis are presented in Table 2.

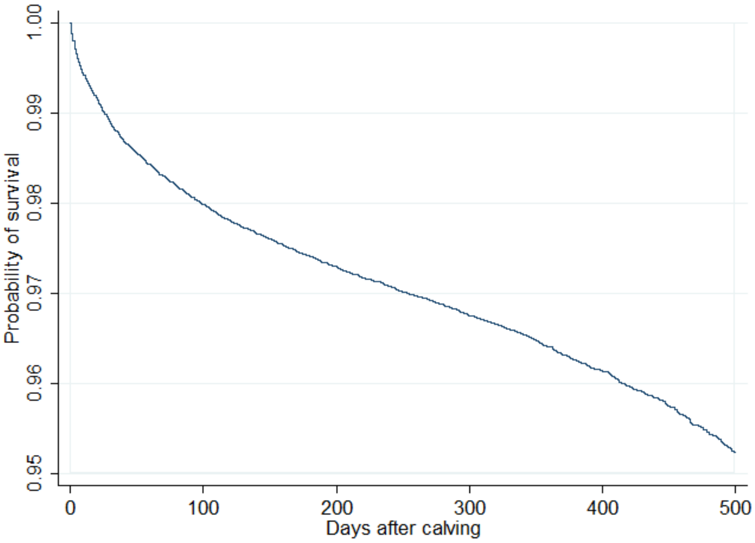

Fig. 1 Probability of survival after calving for Swedish first parity dairy cows

Table 1. Stated reasons for death/euthanasia and culling in a study of cow-level risk factors for mortality in Swedish first parity cows calving between July 2008 and June 2009

Main reason for disposal	Death/euthanasia		Culling	
	n	%	n	%
Fertility disorders	287	9.9	3,413	27.0
Udder disorders	187	6.4	2,839	22.5
Metabolic disorders	93	3.2	30	0.3
Claw/leg disorders	522	18.0	526	4.2
Low milk yield	2	0.1	2,185	17.3
Trauma	372	12.8	164	1.3
Other	1,441	49.6	3,475	27.5
Total	2,904	100.0	12,632	100.0

Table 2. Risk factors for on-farm mortality estimated as proportional hazards by survival analysis of Swedish first parity cows calving between July 2008 and June 2009

Variable	Category	HR[d]	95% CI[e]	*P*-value[f]
Age at first calving	<25.2[a]	1	-	<0.001
	25.2-27.1[ab]	1.08	0.96-1.21	
	27.2-30.0[b]	1.18	1.05-1.32	
	≥30.1[c]	1.45	1.30-1.62	
Breed	Swedish Red[a]	1	-	<0.001
	Holstein[b]	1.27	1.16-1.39	
	Other[c]	0.82	0.68-0.98	
Calving season	Jan to Apr[a]	1	-	<0.001
	May to July[b]	0.78	0.71-0.87	
	Aug to Dec[b]	0.84	0.77-0.92	
Claw/leg disorders	No	1	-	<0.001
	Yes	2.12	1.64-2.73	
Claw/leg disorders previous lactation stage	No	1	-	0.010
	Yes	1.56	1.11-2.18	
Dystocia	No	1	-	<0.001
	Yes	2.13	1.89-2.41	
Genetic merit for milk production, dam	<86[a]	1	-	<0.001
	86-98[a]	0.99	0.90-1.09	
	≥ 98[b]	0.86	0.76-0.96	
	Missing value[c]	1.30	1.12-1.49	
Infection and parasitic disorders	No	1	-	<0.001
	Yes	7.94	6.33-9.97	
Infection and parasitic disorders in previous lactation stage	No	1	-	<0.001
	Yes	3.92	2.69-5.71	

(Table continued on the next page)

Lactation stage				
	0-29 ^{a}	1	-	<0.001
	30-99^{b}	0.63	0.56-0.70	
	100- 500^{c}	0.47	0.43-0.52	
Management type	Conventional	1	-	0.008
	Organic	0.75	0.61-0.93	
Mastitis	No	1	-	<0.001
	Yes	1.67	1.38-2.02	
Metabolic disorders	No	1	-	<0.001
	Yes	7.95	6.46-9.80	
Metabolic disorders previous lactation stage	No	1	-	<0.001
	Yes	3.86	2.75-5.42	
Milking system	Automatic^{a}	1	-	0.002
	Parlour^{b}	1.28	1.08-1.53	
	Carousel^{b}	1.92	1.24-2.98	
	Pipe^{a}	1.12	0.95-1.32	
Milk yield at 1st test milking	<21.8^{a}	1		<0.001
	21.8-30.0^{b}	0.74	0.66-0.83	
	≥30.1^{b}	0.75	0.65-0.86	
	Missing^{c}	2.03	1.59-2.58	
Other disorders	No	1	-	<0.001
	Yes	9.19	7.37-11.45	
Other disorders previous lactation stage	No	1	-	<0.001
	Yes	3.58	2.29-5.61	
Puerperal paresis	No	1	-	<0.001
	Yes	3.38	1.83-6.22	
Reproductive disorders	No	1	-	<0.001
	Yes	1.77	1.37-2.29	
Somatic cell count	<100^{a}	1	-	0.0231
	≥100^{b}	1.15	1.03-1.27	
	Missing value^{ab}	1.18	0.93-1.49	
Stillborn calf	No	1	-	<0.001
	Yes	1.41	1.25-1.58	
Total no. of disease events	0^{a}	1	-	<0.001
	1^{b}	1.64	1.46-1.85	
	≥ 2^{a}	0.97	0.76-1.25	
Trauma	No	1	-	<0.001
	Yes	6.96	5.04-9.62	
Trauma previous lactation stage	No	1	-	<0.001
	Yes	7.26	4.72-11.17	

[a,b,c] Different superscripts within category differ significantly at *P*<0.05

[d] HR = hazard ratio

[e] CI = confidence interval

[f] Significance level (Walds test)

DISCUSSION

The probability of survival for Swedish first parity cows in this study was similar to what has been reported in Denmark and the USA (Thomsen et al., 2004, McConnel et al., 2009). Traumatic events and other disease events constituted the highest hazards for on-farm mortality followed by other disease complexes. The effect of disease was highest in the lactation stage it occurred, as expected, but for several diseases it remained high also in the subsequent lactation stage. Several risk factors related to calving were found of which dystocia increased the mortality hazard the most.

The time before an intervention to reduce the risk of death/euthanasia can have an effect will vary depending on the type of action taken. Improving the housing environment to reduce risks for traumatic events should have a quick impact as well as close monitoring of the cow during and after calving. Better planning of feed rations for heifers, dry cows and newly calved could also have a rather fast effect by preventing negative energy balance and, hence, reducing the risk of disease during the transition period. However, on-farm mortality is a multifactorial problem and changing only single practices may not be enough to get an improved situation. The effect of reducing the age at first calving will probably take longer time and need more effort as this would imply improvements during rearing and better monitoring, to improve for example heat detection efficiency, of the replacement heifers.

The choice of breed in the herd is a more long-term strategy. Although the difference was rather small, the hazard for death/euthanasia was higher in Holstein cows compared to Swedish Red and crossbreed/other breeds. Also, several studies have shown that disease incidences as well as incidence of dystocia and still birth are higher in Holstein compared to Swedish Red (Bendixen et al., 1986, 1987, 1988; Emanuelson et al., 1993; Nyman et al., 2009). Breeding planning is another long-term strategy to reduce on-farm mortality, indicated here by the lower hazard for cows with a dam in the highest quartile of genetic merit for milk production and the higher hazards for cows where the dams' genetic merit for milk production was missing (mostly sired by natural service bulls).

The hazard was higher for cows with low milk yield at the first test-milking compared to cows with higher yield. This is in agreement with studies on herd-level where low producing herds have a greater risk for mortality (Smith et al. 2000; Thomsen et al., 2006; Alvåsen et al. 2013). Miller et al. (2008) did, however, find the opposite effect, with higher death frequencies in high-yielding cows. In this study we used the milk yield at first test-milking whereas the studies mentioned above used herd average milk yield or lactation milk yield and the results are therefore not strictly comparable.

The higher hazard for cows in conventionally (compared with organic) managed herds is in accordance with a study on herd-level risk factors for on-farm mortality in Swedish dairy herds (Alvåsen et al., 2012). The hazard was higher for cows that calved during January to April compared with the rest of the year. Miller et al. (2008) found a higher death frequency in June and the lowest in November and Alvåsen et al. (2012) found a lower herd-level mortality rate in September to December compared to January to April and June to August in smaller herds but no seasonal differences in larger herds. Although the hazard for death and euthanasia was highest early in lactation, calving season and the season when death/euthanasia occurs is not the same which may partly explain the differences found. Season of death/euthanasia was tested in the univariable model but was not statistically significant (Likelihood ratio test, p=0.80).

Other disorders, claw and leg disorders and traumatic events were the most frequent causes of death/euthanasia recorded by the farmers. That ’Other disorder’ was the most frequently recorded reason used indicates an uncertainty in farmer-reported causes of death/euthanasia. McConnel et al. (2009) compared farmers’ perception of cause of death with necropsy examination for 94 cows that died unassisted or were euthanized. They found that the producer was correct in 79% of the cases of euthanized cows and 37% of the cases of unassisted death.

CONCLUSION

This study has identified several risk factors for death/euthanasia. The time around calving seems particularly important as the hazard for death and euthanasia was higher in early lactation and higher for cows with events of metabolic disease, mastitis or claw/leg disorders, all diseases which are more common early in lactation. Several other diseases increased the hazard and there was also a persistent effect of disease and traumatic events. Based on this result, health promotion measures to prevent disease and optimise reproduction seem to be to be the most effective ways to reduce on-farm mortality.

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EVALUATING CALF REARING PRACTICES AND CALF MORTALITY IN DAIRY HERDS: A MULTIDISCIPLINARY APPROACH

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SUMMARY

The aim was to determine why calf mortality between 3 days and 1 year of age in the Dutch dairy industry increased and how to reverse this trend. A multi-disciplinary approach with analyses of census data, a quantitative epidemiological and sociological study, was chosen to study this phenomenon. The results showed that the majority of the calves died in their first month of life. Furthermore, besides IBR-, BVDV-control, and purchase, also statements on farmers' mind-set were associated with calf mortality. The sociological study revealed that, among the farmers, three different phases of awareness concerning calf mortality could be distinguished. Each group of farmers needed to be approached differently by the veterinarian increasing the probability that they would implement provided advice. This approach revealed that advisors should be aware of the attitude and mind-set of farmers and adapt their approach to maximize the probability that advice on improving management is effective.

INTRODUCTION

Calf rearing is an essential part of dairy herd management and is a prerequisite for a productive life time of dairy cows. Calf mortality is one of the parameters that give an indication of the quality of rearing and the welfare of the calves (Ortiz-Pelaez et al., 2008) and is an important cause of economic loss in dairy production (Meyer et al., 2001; Mee, 2008). Many papers have been published that studied mortality in calves of varying ages in other European countries. In those studies, the calf mortality rate between varied between 2% and 6% (Raboisson et al., 2013; Bleul 2011; Ortiz-Pelaez et al., 2008). Although these mortality rates are not completely comparable because reporting systems and definitions of mortality rates in calves differ, multiple studies did report an increase in calf mortality over time (Meyer et al., 2001; Mee, 2008; Bleul, 2011).

In the Netherlands, in 2009 and 2010 a slight increase in ear-tagged calf mortality (3 days-1 year of age) in dairy herds was observed. The exact cause of this increase was unknown. Therefore it was decided to conduct a study with the overall goal to determine which factors are associated with the increase in calf mortality in Dutch dairy herds and how this trend could be reversed. The complete study consisted of three parts that were conducted sequentially. In the first part of the study, routinely available data on calf mortality were analysed and a short questionnaire was send to a panel of 400 dairy farmers. The farmers

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reported that increased calf mortality was caused by factors such as lack of time and priority. Based on the results of this exploratory assessment a field study was designed to determine both management factors and the mind-set and attitude of the farmers towards calf rearing and mortality in the herd. The epidemiological studies mainly focused on knowledge and skills. The identity of farmers however, is more complex. Therefore, in the third part of the project, a sociological study focussing on the farmers' identity was conducted followed by a tailored advice by a veterinarian about possibilities to improve the quality of calf rearing.

This paper describes the methods and results of the different studies that were carried out and discusses the advantages of the complete approach when studying a sensitive subject such as calf mortality.

MATERIALS AND METHODS

Study population and definitions

In this study the definition of a calf is an animal between 3 days, which is the age at which the farmer is obliged to ear-tag the calf, and 1 year of age.

The first inventory was carried out on a group of 400 dairy farmers that voluntarily participated in a marketing panel of GD Animal Health. These herds were geographically representative for the dairy industry but were somewhat smaller compared to the average Dutch dairy herd (83 cattle ≥2 years versus 85 cattle ≥2 years (2010)). For the case-control study on factors that were associated with an increased mortality, 100 case and 100 control herds were randomly selected. A case herd was defined as a farm that belonged to the 10% dairy farms with the highest increase in calf mortality, between 2009 and 2010. In a control herd the yearly calf mortality stayed below the median value of the calf mortality in Dutch dairy herds (<7.7% per year) and remained constant in 2009 and 2010.

For the qualitative, sociological part of the study, 30 farmers with structurally high mortality in their calves were selected. Structurally high calf mortality was defined as the 10% farms with the highest calf mortality in the last quarter of the selection period (1 October-31 December 2012) and in at least five out of ten quarters between July 1st, 2010 and December 31th, 2012. Eventually, 438 dairy herds met all the criteria and were eligible for inclusion in the study. The herds were stratified based on the number of quarters that they belonged to the 10% farms with the highest calf mortality (range 5 to 10 quarters), and a random sample of 17 farms per group were initially selected. The selected farmers were randomly contacted by phone for collaboration until 30 farmers agreed to participate in the study.

Questionnaire inventory and case-control study

The questionnaire for the inventory was short and contained only six questions. The questionnaire focussed on possible factors that could be associated with calf mortality in dairy herds and comprised questions on herd size, calf mortality in 2010, calf mortality in 2010 compared to 2009, the age of death, the main cause of calf mortality in the herd (open question) and in the complete dairy industry (open question). The farmers in the panel were contacted by mail with the request to fill in the on-line questionnaire.

The second questionnaire was a more in-depth questionnaire that contained questions on general management, calving management, rearing and infectious disease management. In addition, nine statements on the farmers' mind-set and attitude towards calf rearing and calf mortality were included. These statements were answered by means of a Likert scale and the answers provided an indication of the mind-set and attitude of the farmers. The Likert scale varied from a score of 1 to 7 where 1: completely disagree, 2: disagree, 3: slightly disagree, 4: impartial, 5: slightly agree, 6: agree, 7: completely agree.

The 200 questionnaires were conducted by phone, by five trained interviewers. Each of the studied farms was randomly assigned to one of the interviewers and the interviewers were not aware whether the farm was a case or a control herd.

Data-analysis

Census data: Census data on calf mortality was available from the identification and registration system (I&R). Dutch farmers are obliged to register all ear tagged calves and the reason for removal (e.g. death, sold, exported) in I&R. The complete I&R dataset comprises information from approximately 612.500 calves present on dairy farms per year and was used to 1) select the herds for this study, 2) to determine at what age calves are at highest risk to die and 3) in which period in the life of a calf the mortality increased most between 2009 and 2010.

Inventory: The results of the short questionnaire were described by frequency and summarizing tables and the open questions were grouped based on the answers that were filled in.

Case-control study: The general characteristics of the herds that were enrolled in the case-control study were presented using descriptive statistics. In addition the mortality rates of calves in both 2009 and 2010 and the differences between both years are presented for case and control herds. Logistic regression models in SAS 9.1 (SAS institute 2006) were used for the analyses. First, all variables derived from the questionnaire were subjected to univariable analyses. Variables with $P \leq 0.25$ were kept for the final multivariable model. The multivariable analysis was done using a backward selection and elimination procedure. Confounding was monitored and the robustness of the final model was checked by carrying out the same procedure with forward selection. In the final model, all biologically plausible 2-way interactions were tested. The residuals of the final model were tested for normality and the Pearson X^2 test statistic was used to determine whether the model used was appropriate. The amount of variance explained by the model was presented by means of the adjusted R^2.

Qualitative sociological study

For the sociological study, semi structured in-depth interviews were conducted amongst 30 farmers that were selected for the study. This means that a list with general discussion topics was proposed and depending on the answer on a question, more specific questions could be asked (Jansen et al., 2010; Vaarst & Sørensen, 2009). The topics were: 1) General questions about the farm and management, 2) importance of calves and calf rearing in the herd management, 3) calf rearing, 4) health of the calves, 5) role of the farmers environment with regard to the calving management. In this part of the study, it was decided to focus on all aspects of calf rearing rather than focussing on the mortality of the calves only. The farmers were not aware of the inclusion criteria for the study (i.e. high mortality) and were asked to participate in a study on calf management/rearing. During the interview, the sociologist started a conversation in which the farmer was stimulated to share his/her experiences with calf rearing and in which calf mortality was one of the topics discussed.

All farmers were visited between March and June 2013 and were interviewed by one sociologist (M. Buddiger). The sociologist had no knowledge on the exact calf mortality in the herd and also did not have a background in livestock science. All interviews were recorded on tape and were transcribed in full afterwards. After this, data was coded and could be separated in different categories based on the “Composition of a mind-set” model developed by Leeuwis (2004). The eight categories of this model were combined into three categories, namely 1) knowledge and skills; 2) trust in social environment; and 3) ambitions and priority. In addition, the main themes that were often mentioned by the farmers with regard to the mortality in the calves in their herds were labelled and could be categorized into three different categories of awareness of their calf rearing problems. After the in-depth interview, the sociologist discussed the results of each specific farmer with a veterinary advisor that was specialized in calf rearing (A. Smolenaars). Thereafter, the advisor visited each of the farms and discussed suitable improvements to increase the quality of calf rearing with the farmer. The aim was to decrease calf mortality in these herds, by levelling with the farmers’ attitude and motives.

RESULTS

Census data and inventory

In 2009 and 2010 the complete dairy industry comprised 18,969 and 18,387 dairy herds in the Netherlands with an average herd size of 82 and 85 milking cows (>2 years) and 33 and 32 ear-tagged calves. The median yearly mortality rate of calves in 2010 was 7.7% and varied largely among herds with a 5% percentile of 0% and a 95% percentile of 36%. In 2009 the median mortality rate in calves was 7.0%. Most calves that died in 2010 in Dutch dairy herds, died in the first month of their lives (Fig. 1).

The highest increase in mortality between 2009 and 2010 was observed in the youngest calves in the first month of their lives. Therefore it was decided to focus the questions in the case-control and qualitative sociological study mainly on the ear-tagged calves between 3 days and 1 month of age.

From the 400 panel members that were send the short questionnaire, 271 farmers (68%) returned the questionnaire and the answers of 236 of these farmers (87%) were complete and could be included in the analyses. These farms had median calf mortality in 2010 of 4% (mean: 7%). In their own herds, most calves appeared to die because of diarrhoea (31%) and other infectious diseases such as BVDV, salmonellosis or Bovine Neonatal Pancytopenia (13%). Twenty-four per cent of the farmers indicated that the cause of death among the calves in their herd was unknown. In addition, 50% of these farmers indicated that this occurred more often in calves originating from primiparous cows compared to calves originating from multiparous cows (Table 1).

Most farmers (40%) indicated that the evolution of the Dutch dairy industry towards a more intensive farming system with increasing herd sizes resulting in less time and priority for the calves, was the most important reason of the increasing mortality rates in Dutch dairy farms (Table 1). In their own herd, health issues such as diarrhoea or (infectious) diseases were most often mentioned as the cause of death in calves.

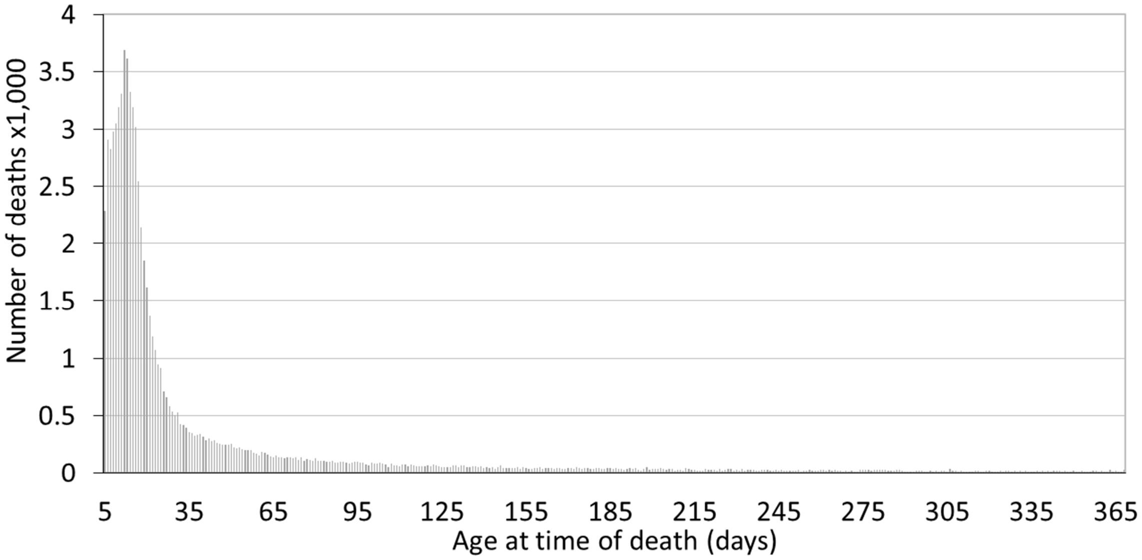

Fig. 1 Distribution of the number of dead calves relative to the age in days at the time of death for ear-tagged calves between 3 days and 1 year of age in Dutch dairy herds

Table 1. Farmers' reported causes of death in their own herd and perceived causes of deaths in general of ear-tagged calves between 3 days and 1 year of age in 2010, according to 236 Dutch dairy farmers

Causes of death	Observed causes of death in own herd[a]	Perceived causes of death in Dutch dairy herds in general[b]
Diarrhoea	31%	-
Diseases like salmonellosis/BVDV/Bovine Neonatal Pancytopenia	13%	3%
Evolution towards a more intensive farming system with less time and priority for the care of the calves	6%	40%
Exact cause unknown, but occurrence more often with calves from heifers compared to calves from multiparous cows	12%	1%
Other (accidents, mineral deficits, respiratory problems, large calves)	26%	15%
Unknown	12%	20%
Genetics/resistance/resilience	-	19%

[a] observed causes of death in their own herd as mentioned by 236 Dutch dairy farmers included in this study

[b] causes of death in Dutch dairy herds in general as perceived by 236 Dutch dairy farmers included in this study

Case control study

After validating the data and removing double and incomplete observations, data of 98 case and 85 control herds remained for the analyses. The case herds were slightly larger than the average Dutch dairy herd (85 cows) with 94 cows >2 years (range 32-332). The 86 control herds were slightly smaller than the average Dutch dairy herds with 80 milking cows >2 years (range 36-154). In the case herds, the mortality rate of calves from 3 days to 1 year of age, showed a median increase of 23% between 2009 and 2010 (Table 2). Whereas the control herds showed a low and stable mortality rate between both years (median difference of -0.5%) (Table 2).

Table 2. Median (mean) number and percentage of dead calves from 3 days to 1 year of age in Dutch dairy farms in 2009 and 2010 and the differences between both years for the cases (98 farms) and controls (85 farms)

	2009		2010		Difference[a]	
	Median number of deaths (mean)	Median mortality (mean)	Median number of deaths (mean)	Median mortality (mean)	Median number of deaths (mean)	Median mortality (mean)
Cases[b]	3	10.0%	10	32.9%	+8	23.3%
	(5)	(13.0%)	(12)	(35.3%)	(+9)	(27.1%)
Controls[c]	1	4.7%	1	4.2%	0	-0.5%
	(2)	(5.6%)	(2)	(4.6%)	(0)	(-1.0%)

[a] Case: belonging to the 10% dairy herds with the largest increase in calf mortality in ear-tagged calves from 3 days to 1 year of age between 2009 and 2010;

[b] Control: stable and below average ear-tagged calf mortality from 3 days to 1 year of age in 2009 and 2010

[c] The difference between 2009 and 2010 were calculated as the average of the difference between the years for each dairy herd in the study

The results of the univariable analyses showed that 19 variables were possibly associated (*P*-value <0.25; Wald test) with the probability of being a case or control herd. From these variables, three factors were related to calving management, six factors involved general management practices, six factors involved measures taken to reduce problems caused by infectious diseases and four out of nine statements remained for inclusion in the multivariable model.

In the final multivariable model, three parameters and one interaction remained. The residuals of the model were normally distributed and there was no indication to assume that the model was not correct, based on the Pearson X^2 statistic (*P*-value>0.05). With this final model, 22% of the probability of being a case or control herd could be explained. The results indicated that farmers that purchased none or up to 2 animals (cows or bulls) had a significant lower odds for being a case herd (OR=0.21 and OR=0.14 respectively) compared to farmers that purchased a larger number of cattle in 2010 (Table 3). Herds that were either IBR free or that vaccinated against BVDV, had a lower odds for being a case herds (OR=0.34 and OR=0.10) compared to herds without an IBR free status and that did not vaccinate against BVDV. Two statements were significantly associated with the probability to be a case herd. The more farmers agreed with the statement “a dead calf is a problem for me, because I have to put it at the roadside for the rendering plant to collect it”, the lower the odds that they were a case herd. In addition, the more farmers agreed with the statement “A still birth from a milking cow is worse than a still birth from a heifer”, the higher the probability that they were classified as case herd (Table 3).

Table 3. Results of the multivariable analyses modelling the probability of being a dairy herd with increased mortality in ear-tagged calves from 3 days to 1 year of age between 2009 and 2010 (case; n=98) compared to being a dairy herd with a below average and stable calf mortality (control; n=85)

Variable	% of occurrence in case herds	% of occurrence in control herds	Odds Ratio	95% Confidence interval	*P*-value (Wald test)
Purchase of cattle in 2010					
Yes, >2 animals	58%	24%	Reference		
Yes, 1-2 animals	7%	14%	0.14	0.04-0.5	0.01
No	35%	62%	0.21	0.1-0.5	<0.01
Interaction between participation in a voluntary IBR program and vaccination status for BVDV					
No IBR free status & no vaccination against BVDV	29%	60%	Reference		
IBR free & no vaccination against BVDV	51%	31%	0.34	0.2-0.7	<0.01
IBR free & vaccination against BVDV	8%	5%	0.30	0.07-1.2	0.10
No IBR free status & vaccination against BVDV	12%	4%	0.10	0.02-0.4	<0.01
A dead calf is a problem for me because I have to lay it beside the road so that the rendering plant is able to collect it					
(1 completely disagree; 7 completely agree)	Mean Case: 3.2	Mean control:4.3	0.79[a]	0.7-0.9	<0.01
A still birth from a milking cow is worse than a still birth from a heifer					
(1 completely disagree; 7 completely agree)	Mean case:2.2	Mean control:1.6	1.24[b]	1.0-1.5	0.05

[a] The higher the agreement with this statement, the higher the probability of being a case herd

[b] The higher the agreement with this statement, the higher the probability of being a control herd

Qualitative, sociological study

The answers of the 30 interviewed farmers were classified into five categories according to the “Composition of a mind-set” model of Leeuwis (2004).

Knowledge and skills: most farmers indicated that they had sufficient knowledge with respect to calf rearing practices (n=28). There were only 2 farmers that stated that they had a lack of knowledge and felt the need to get advice to solve the calf rearing problems they faced. Most farmers said that they knew the critical control points for calf rearing on their farm (n=24). However, solving or preventing problems with respect to calf rearing appeared more difficult because of financial or practical factors.

Trust in social environment: social pressure did not appear to play a major role in the decision making of the interviewed farmers. All farmers stated that they had a good relationship with their advisors such as the veterinarian and the feed supplier. Nevertheless, part of the farmers indicated to have problems with calf rearing and had high calf mortality rates, but did not discuss this with their veterinarian (n=4). The farmers indicated that they only felt social pressure when calves died and had to be placed at the roadside for collection to the rendering plant. Therefore, a few (n=3) of the interviewed farmers had decided to sign a contract with the rendering plant that stated that the dead calves would be collected from the farm property, instead of at the roadside. Twenty-one of the farmers stated to be in good contact with colleagues. However, only two of the farmers were part of study groups in which numbers and figures of herd health indicators were shared and discussed among each other. More than half (n=16) of the interviewed farmers said to have lost faith in diagnostic tests. In addition, even though the farmers stated to have good contact with advisors, they often mentioned not to discuss with their advisors on calf rearing problems.

Ambitions and priority: all farmers seemed to be very passionate with respect to their farms. However, it appeared that half of the interviewed farmers (n=15) found it difficult to find enough time to take good care of the calves. The milking cows often had a higher priority, which for example, had consequences for providing colostrum to new born calves as fast as possible. Part of the farmers experienced this to be a problem, but others state that this was inevitable in their herd management. The farmers were asked to describe the ideal situation for the rearing of their calves and why they did not obtain this ideal situation yet. They indicated reasons such as lack of financial means or were reluctant to change their routine.

Categories of awareness: there were three main themes that could be distinguished among the farmers with regard to their awareness of the mortality among the calves in their herds, where some farmers could be classified in more than one category:

1. Farmers who were only partly, or not at all, aware of high calf mortality and sub optimal rearing (n=17): these farmers stated that they had no, or only limited problems with calf rearing and in their opinion this did not lead to high calf mortality. A part of the farmers did mention to have had problems in the past or had periods with or without problems (n=10). Nevertheless, in all cases they did not experience large difficulties and they were not aware of the fact that the calf mortality in their herds was unusually high.

2. Farmers who felt powerless because of their inability to find a solution for their problems with calf rearing (n=12): these farmers were aware of the fact that they had problems with respect to the rearing of the calves and that these problems led to high mortality rates in their herds. Nevertheless, they experienced powerlessness because they could not find a solution to the problems. Diagnostic tests did not reveal the cause of disease or death and they were often taken by surprise when apparently healthy calves suddenly weakened and died.

3. Farmers who knew they can be inaccurate when it comes to rearing calves, but were reluctant to change this (n=8): These farmers knew that they had problems with their calf rearing practices and that the mortality among the calves was too high. Nevertheless, they stated that they were not able or willing to change their management practices in order to improve the quality of calf rearing.

From the 30 farmers that were interviewed by the sociologist, 27 were visited for the second time by a veterinary expert on young stock rearing. During the second visit the impression of the attitude and motivation of the farmer according to the advisor was not always consistent with the categorisation that was based on the statements of the farmers themselves. From the veterinary advisors point of view, six of the interviewed farmers lacked knowledge about the causes of the rearing problems, while these farmers were convinced that they had sufficient knowledge during the first interview. The veterinary advisor discussed possible improvements in the calf rearing practices in the herds and explained why certain practices could potentially lead to problems. The six farmers eventually became convinced that some problems needed action. Twelve farmers were consistently categorised based on the in-depth interview and the impression of the veterinary specialist. From the remaining farmers the impression of the veterinarian differed from the categorisation based on the farmers' statements.

DISCUSSION

This study described a multifactorial approach with the ultimate goal to improve calf rearing and decrease calf mortality in ear-tagged calves aged 3 days to 1 year in Dutch dairy herds. The results showed that the majority of the calf mortality occurred in the first month of life, which was in accordance with earlier studies on calf mortality (Gulliksen et al 2009; Wells et al., 1996).

The majority of the increase in calf mortality that has been observed in the Netherlands between 2009 and 2010 could be attributed to increased mortality in calves <1 month of age. A first exploration to possible causes for this increase revealed that, besides management, farmers' priorities and attitudes might also have played a role. Furthermore, the case-control study in which dairy farmers with a high increase in calf mortality were compared with dairy farmers with a stable and below average calf mortality, lead to similar conclusions. Not only known management factors such as purchase of replacement cattle or having an unfavourable IBR and BVDV status, which also have been identified in earlier studies (Raboisson et al., 2013; Torsein et al., 2011; Diéguez et al., 2009; Lundborg et al., 2005) were related to the calf mortality but also a few statements on calf rearing and calf mortality. These statements gave again an indication that the attitude of farmers was related to increased calf mortality. This showed that influencing farmers to improve calf rearing quality and decrease calf mortality needs a multi-disciplinary approach as described by Leeuwis et al. (2004) and Kristensen & Enevoldsen (2008). In accordance with our study, Vaarst & Sørensen (2009) also found different attitudes in farmers to be associated with high versus low calf mortality. Thus, in order to return the trend of an increase in calf mortality, the focus should not only be on certain management factors but also on how to influence the attitude of farmers and thus achieve a change in behaviour. Nevertheless, persuading the farmer to implement changes in their daily management is difficult to achieve (Hardemann et al., 2002). Therefore, in the third part of our study we conducted an in-depth qualitative approach into the identity of a group of dairy farmers with structurally high calf mortality. Besides getting more information on the identity of these farmers the aim of this study was also to provide these farmers a tailored advice on calf rearing, taking into account not only the herd management but also the identity of the farmer. The background of this thought was that previous studies showed that a "one-fits-all" communication does not work in stimulating the farmer to implement improvements (Kristensen & Jakobsen 2011; Valeeva et al., 2007) and that tailored advice is needed.

The in-depth interviews showed that the farmers believed to have sufficient knowledge on calf rearing practices, but experienced problems when they had to implement this knowledge. This finding was supported by a study of Lam et al. (2008) who found that even though the farmers believed to have sufficient knowledge on mastitis management this was not always decisive in implementing alterations in herd health management. Nevertheless, in this study, more than half of the farmers did not have an idea about the average calf mortality in Dutch dairy herds and were, therefore, not aware of the fact that they frequently had extremely high mortality rates. In order to make the farmers aware of their problems, the veterinary advisor discussed with them the problems and the possible improvements that could increase the quality of rearing. The farmers stated to be open towards advice, but that the probability that they would follow up on provided advice depended, amongst others, on the way that this advice was communicated to them. In addition, also the practical possibility to implement the given advice in the daily herd management played a role in the probability that the given advice was followed. This finding was supported by the results in the study of Noordhuizen et al. (2008) who found that given advices need to be communicated in a trustworthy and competent way in order to be effective.

Part of the farmers in the study group felt powerless, because they felt unable to find a solution to their calf rearing problems or knew that they were inaccurate when it came to calf rearing. This finding was also experienced by Vaarst & Sørensen (2009) who found that farms with a high mortality in their calves had a basic belief that calf mortality was a permanent crisis that had to be expected on a dairy farm. The difference between the two groups of farmers was that the farmers that experience powerlessness tended to be more open towards advice compared to the farmers who knew that they were inaccurate. The latter group was most difficult to influence, because they were openly reluctant to changing their daily management practices. Possibly, these farmers can only be motivated by obligatory regulations or financial consequences.

Although the 30 farmers included in the qualitative sociological study were not representative for all dairy farmers in the Netherlands, we believe that the three categories of awareness that were identified in this study may also be found when problems with calf mortality arise in any other dairy herd.

In this study a multi-disciplinary approach into calf rearing and mortality of calves between 3 days and 1 year of age was conducted. More understanding was gained in the age categories of calves that were at highest risk for mortality, the factors that were associated with increased calf mortality in dairy herds and the identity of farmers with structurally high calf mortality towards calf rearing. With this information it was possible for veterinary advisors to focus their advice to the rearing of the calves in which most problems occurred (calves <1 month of age). With the information from the in-depth interview with the sociologist, the veterinary advisor was able to give tailored advice to the farmers about risk factors and preventive measures that could be taken in the herd to improve the quality of rearing and decrease the risk of calf mortality.

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NOVEL APPROACHES

SOCIO-PSYCHOLOGICAL VETERINARY EPIDEMIOLOGY. A NEW DISCIPLINE FOR AN OLD PROBLEM

ERWIN WAUTERS[*], CRISTINA ROJO-GIMENO

SUMMARY

Socio-psychological veterinary epidemiology is the study of human behaviour that affects the causes, spread, prevention and control of animal diseases and health problems. This paper reviews the existing literature with two purposes: (1) to draw conclusions and implications for farmers, veterinarians, veterinary and epidemiological researchers, extension agents and policy makers and (2) conduct a methodological review and provide recommendations for future studies. In total, 64 articles were reviewed showing that most of the studies are dealing with health management of dairy cows and have been conducted in Europe. The role of the veterinarian, the effect of knowledge, social influences of the farmer and the effect of epidemic and endemic diseases on farmer's behaviour were often object of study. There was an absence of any clear universally significant factors affecting animal health behaviour but some recommendations and solutions could be identified, such as the positive influence of the veterinarian on the farmer. This influential relationship should be exploited better and targeted communication schemes should be used by veterinarians to deliver information to farmers. Future research should be conducted to inform the farmers about the potential benefits of managing zoonotic diseases. We advocate for a truly interdisciplinary approach where social knowledge will be used by researchers to adapt with the farmers perceptions and behaviour.

INTRODUCTION

This paper reviews past evidence on the social dimension of animal health management. Socio-psychological veterinary epidemiology is the study of human behaviour that affects the causes, spread, prevention and control of animal diseases and health problems. Animal health is becoming an increasingly important topic for several reasons. Firstly, there is increased concern about animal health since many animal diseases are zoonotic, and hence pose a threat to human health (Cleaveland et al., 2001). The associated food safety considerations have induced governments to intervene in the monitoring and control of animal diseases. Secondly, the emergence of new animal diseases has increased due to several factors like increased global transport of both humans and animals and because of climate change (WHO/FAO/OIE) (2004). Thirdly, some animal diseases may induce large economic losses to the agricultural industry (Mc Gowan et al., 2001, Mc Leod et al., 2006). This is partly due to the impact of the disease on production and mortality rates itself, but the economic loss

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also results from trade restrictions imposed on infected countries (Rushton et al., 2012). Fourthly, due to the increased specialization in animal husbandry, the impact of animal diseases and of animal disease control measures has large consequences on the economic viability of the farm. Indeed, specialized livestock farms have in general a smaller operating profit margin compared to mixed farms and arable farms, which means that the leverage effect of revenues and/or operational costs on the gross margin is high. Therefore, small changes in productivity, due to animal diseases or due to animal disease control, might have much bigger consequences on the gross margin and on net income.

There is a large body of literature on veterinary and epidemiological approaches to increase our knowledge about animal health and to design animal health interventions. Yet, managing animal health is to a large extent a farm level issue. Ultimately, veterinary and epidemiological solutions and interventions towards better animal health are to be implemented by farmers. As a consequence, researchers have started to study the behaviour of farmers and other stakeholders relating to the implementation of animal health management practices (AHMP). This is not exclusive to animal health management (Burton, 2004). The behavioural approach has increasingly been applied to several issues in the agricultural domain, such as sustainable land management (e.g. Wauters et al., 2010; 2013), safety behaviour (e.g. Colémont & Van den Broucke, 2008) and conversion to organic agriculture (e.g. McCarthy et al., 2007). Very recently, the veterinary and epidemiological domain has experienced a rapid increase in behavioural studies investigating the human factor in animal health decisions (Fig. 1). This is to the extent that it is almost becoming a new, interdisciplinary subdomain in veterinary epidemiology, just as economics entered the field of veterinary epidemiology several years ago.

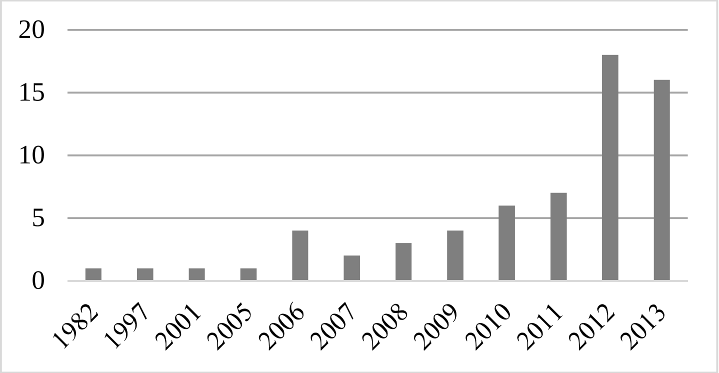

Fig.1 Number of publications related to socio-psychological veterinary epidemiology, per year 1982 - 2013

Inspired by the term social epidemiology, the field that covers research on human attitudes, perceptions and practices influencing human health, we suggest to use the term socio-psychological veterinary epidemiology (SPVE) for this relatively new domain within veterinary epidemiology. As with any developing discipline, it is, from time to time, appropriate to adopt a rear view at both the findings and especially at the assumptions, approaches and methodologies of past studies, in order to provide recommendations for the future development and usefulness of the evolving discipline. This paper provides a literature review of the bulk of past SVPE studies, with two objectives. The first is to summarize and – if possible– draw some universal conclusions on motives, barriers, values and factors that affect how humans manage animal health and welfare in farming systems. The second objective of this literature review is to critically assess the approaches and methods that have been applied and the type of questions that have been studied, in order to provide suggestions for future progress of this discipline, both from a scientific point of view and in terms of the relevance and usefulness of the studies for practical implications.

MATERIALS AND METHODS

For this literature review, the common guidelines for conducting literature reviews and meta-analyses (e.g., Cooper, 1998; Webster & Watson, 2002) were followed. Different search mechanisms were used to identify papers that could be classified as falling within the domain of SPVE. First, keywords such as “animal health behaviour farmers”, “animal disease intervention adoption” and alternatives of this were entered in Scopus, PubMed and Google scholar. A second method of literature search was what is called backward references search, defined as examining the references cited in an article of interest. A third method was backward and forward author search, which involves searching for what the author(s) of an article of interest have published earlier and/or after the article. A fourth method was forward reference search, in which the papers that referred to an article of interest were examined.

The literature search was not restricted to peer-reviewed papers only. Although peer-review is generally considered a good indicator of quality, not every peer-reviewed paper is of the same quality. Further, such restriction would render the literature review prone to publication bias, which refers to the tendency of authors to submit and of editors to accept studies that publish significant results more often compared to studies with insignificant results (e.g., Rosenthal, 1991). Further, as socio-psychological veterinary epidemiology is a relatively new domain, many interesting papers and studies might not have been published in the peer-reviewed literature, but rather have been stuck in the grey literature such as unpublished PhD-theses, research reports or conference proceedings.

After the literature search was completed, all articles were screened for a number of characteristics that were deemed relevant for the purpose of this literature review. Given the purpose of this literature review, a screening framework was developed including year, region, animal species studied, diseases and health problems, scientific approach, methodological approach, survey methodology, data analysis methodology, sample size, investigated variables, sign and significance of all the investigated variables. Universal patterns in the findings were identified, by categorizing investigated variables and results in overarching themes. The results of this are presented in the next section. Next, all studies were compared with respect to the scientific and methodological approaches and the survey and analysis methods. The findings about the scientific and methodological review and suggestions for future progress are presented in the discussion section.

RESULTS

Characteristics of the studies

Most SPVE studies so far are, by large, dealing with the health management of dairy cows, followed by pigs. A small number of papers were dealing with several species. Among the less frequently investigated health behaviour, is the health management of poultry, ruminants (goats and sheep) and beef cattle (Fig. 2).

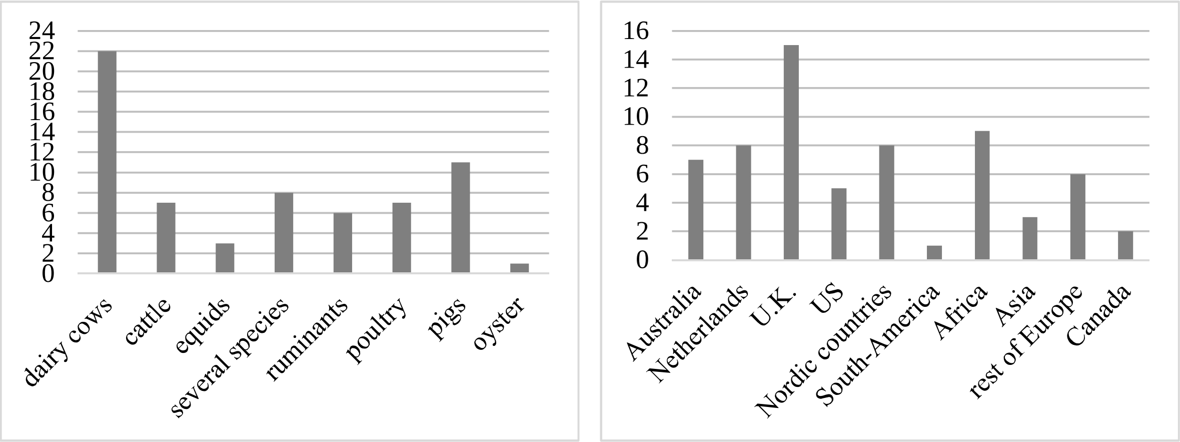

Fig.2 Number of related-SPVE articles per species (left panel) and per region (right panel)

Most studies have been conducted in Europe, most notably in the U.K. and in the Netherlands (Fig. 2). Further, there are a noteworthy number of studies from Australia (Dhand et al., 2011, Elliot et al., 2011, Schemann et al., 2011, Hernandez-Jover et al., 2012a, Hernandez-Jover et al., 2012b, Schemann et al., 2012, Dowd et al., 2013) and from the African continent (Huttner et al., 2001, Bell et al., 2006, Fandamu et al., 2006, Desta et al., 2012, Mather, 2012, Sirdar et al., 2012, Negro-Calduch, et al., 2013) . The low number of studies in the U.S. (Bhattacharyya et al., 1997, Gramig et al., 2010, Delgado et al., 2012, Beam et al., 2013, Bhattarai et al., 2013) is a sign that the SPVE discipline has not permeated the veterinary and agricultural sciences in the U.S. yet.

Figure 3 shows the most popular topics in SPVE-papers so far. The large majority is dealing, either with one specific disease, such as bovine tuberculosis (Enticott, 2012a) or bluetongue virus (e.g. Elbers et al., 2010), or with farmers' implementation of biosecurity measures against a whole range of diseases (e.g. Racicot et al., 2012).

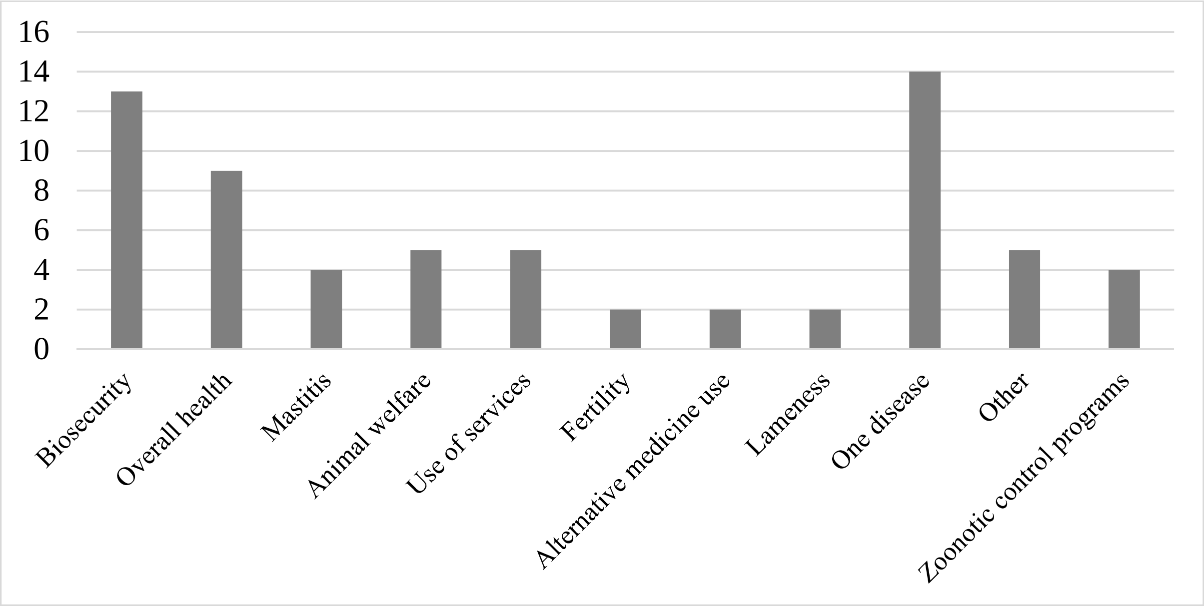

Fig.3 Topics investigated across the reviewed SPVE-related articles (n=64)

Focusing on the type of disease that is being dealt with, about half of all studies are about endemic health problems (Fig. 4). Slightly more than one third of the papers were dealing with both endemic and epidemic diseases, the remainder focusing on epidemic diseases (Cross et al., 2009, Dhand et al., 2011, Schemann et al., 2011, Hernandez-Jover et al., 2012a, Hernandez-Jover et al., 2012b, Mather, 2012, Neupane et al., 2012, Schemann et al, 2012, Zingg & Siegrist, 2012, Carlier et al., 2013, Negro-Calduch et al., 2013, Tornimbene, et al., 2013, Sawford et al., 2013). About 50% of all papers are dealing with non-zoonotic health problems, and less than 15% are dealing with zoonotic diseases only (Fig.4).

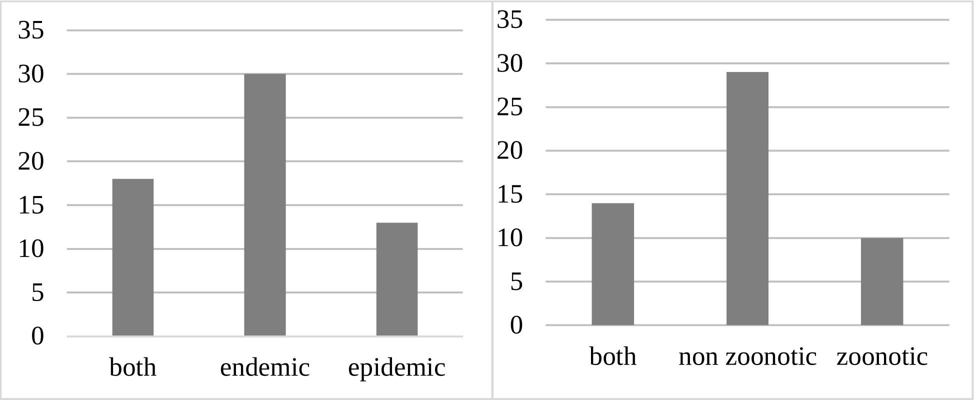

Fig. 4 Division between paper focusing on endemic, epidemic or both type of disease (left panel) and between studies that deal with zoonotic, non-zoonotic or both diseases (right panel)

Main findings in the existing literature

Knowledge: Knowledge is hypothesized to influence behaviour in several ways. First, knowledge about the disease and its potential consequences is thought to raise awareness and induce a need for action. Second, knowledge of potential intervention strategies and their impact is thought to influence farmers to adopt these strategies. Several studies have noticed the lack of knowledge as a constraint to implementing innovations, for instance in the case of pig diseases (Alarcon et al., 2013) on pig farmers, the use of antibiotic registration in France (Dernburg et al., 2007), the lack knowledge of industrial guidelines to control zoonosis by veterinarians in Australia and use of protective equipment (Dowd et al., 2013). These studies recommended establishing study groups and workshops to provide information about the topic.

Farmers who had more fear of getting infected were less acknowledged about the disease. The protection motivation theory (Maddux &Rogers, 1983) suggest that fear is indirectly interrelated with attitude by influencing the appraisal of threat severity. The knowledge of infectious disease lead to an understanding of the disease spread heightening the confidence and reliability of the effectiveness of the protective behaviour. Knowledge has been shown its ability as fundamental motivator to comply with recommended behaviours (Schemann et al., 2012). However, the cross-sectional design of Neupane et al. (2012) did not allow the authors checking for causality on this assumption.

In a qualitative study, Nahar et al., (2012) reported that pig farmers in Bangladesh did not acknowledge that the transmission between humans and pigs was possible, which poses a serious risk for spill-over of diseases from pigs to humans. In Sirdar et al. (2012), the authors reported that the poultry farmers in Sudan were not aware that use of antibiotics on laying hens will affect the eggs and the people consuming them. This misconception that lead to irrational behaviour, can be solved by increasing the level of education of farmers. It is particularly important to identify the means of knowledge transfer in developing countries where literacy may be heterogeneous in the population. It has been shown that the younger the farmers the higher the literacy, therefore the education programs may need to be literacy-targeted for different age groups (Stringer et al., 2011).

Some authors also point to insufficient knowledge of veterinarians. Despite the fact that veterinarians are regarded as trusted sources of information by the farmers, they recognise their lack of knowledge as in Dowd et al. (2013) where less than half of the veterinarians interviewed said that they had a high knowledge about zoonotic infections and 69.3% were not aware of the existence of any industry guidelines or standards relating to zoonotic disease. Postgraduate education in veterinarians has been related with increase of adoption of certain beneficial hygiene practices such as the use protective equipment when performing risk behaviour for contracting zoonotic diseases (Dowd et al., 2013).

<u>Problem recognition and responsibility</u>: Problem recognition refers to the degree to which the farmer sees a problem and thus feels a need for action. It also relates to just how serious he perceives this problem to be. Farmers that perceive major problems with the health and welfare status of their herd are more inclined to adopt certain AHMP (Alarcon et al., 2013, Anneberg et al., 2012, Ellis-Iversen et al., 2011) .

Problem responsibility refers to where farmers put the responsibility to solve a particular problem. There appears to be a difference in this respect between so-called production diseases (endemic diseases with potential negative effect on productivity and profitability, such as mastitis) and zoonotic diseases (diseases that are more a problem in terms of food safety). Hence, different communication strategies and different policy measures will be needed in order to convince farmers to control problems such as salmonellosis compared to problems such as mastitis.

Several studies find that farmers tend not to consider themselves fully responsible for the food safety in the food chain and tend to put the duty on the government, retailers and consumers (e.g. Gunn et al. 2008; Heffernan et al. 2008). Ellis-Iversen and co-workers (2010) reported that all farmers believed to have a social responsibility to produce safe products and control zoonosis on their farms but half of them reported that also the government had responsibility. Zoonotic diseases are a special instance where farmers thought that the government should fund their control (Fraser et al., 2010). However, some studies find that farmers do feel responsible for the food safety for foodborne diseases (i.e., *Salmonella*) (e.g. Fraser et al., 2010, Alarcon et al., 2013). Further, some farmers reported to feel responsible to ensure animal welfare and that inspections were needed to find the illegal farmers (Anneberg et al., 2012). The pride and good reputation of producing safe products for human consumption may prompt farmers to adopt control measures as in the case of Johne's disease (Nielsen, 2011). Different perceptions exist if a disease is epidemic and how it is tackled by the different stakeholders. Several studies have shown that farmers will follow the advice/compulsory rules dictated by the government when there is an outbreak. However, when outbreaks occurred, farmers had not been always happy with the government dictations (Negro-Calduch et al., 2013).

The social influence of the veterinarian: A major result in many studies is that the role of the veterinarian is of paramount importance. Farmers considered the veterinarian as their main advisor (Ellis-Iversen et al., 2010, Hernandez-Jover et al., 2012b, Alarcon et al., 2013, Derks et al., 2013a, Derks et al., 2013b, Espetvedt et al., 2013) and trusted the information provided by them (Garforth et al., 2006). This applies both in the case of an outbreak and in endemic situations. One of the motives of the higher efficiency with which biosecurity measures were applied was the possibility to trust veterinarians and the information received from them (Schemann et al., 2011, Hernandez-Jover et al., 2012b). According to the farmers, there are different kinds of veterinarians: the vet can be seen as a firefighter (Kaler& Green, 2013) or as a source of information that will offer structured solutions to problems. It has been suggested that veterinarians should be more active in showing their role in solving preventable problems (Lievaart et al., 2008).

However, there is evidence that the way veterinarians' deal with farmers and their management of animal health problems leaves scope for improvement. Firstly, several articles suggest that the communication of information to farmers and veterinarians is not sufficient (Hektoen, 2004, Cross et al., 2009, Ellis-Iversen et al., 2010, Leach et al., 2010, Thomsen et al. 2012) and that veterinary communication skills should be improved (Hall & Wapenaar, 2012). To improve communication it may seem useful to provide information to farmers with planned communication strategies, with the target to have a positive effect on the factors that influence behavioural change. Communication should tackle the goals and objectives of the farmers to plan and evaluate herd health management. This is often not the case as it was shown in Derks et al. (2012).

Secondly, there is room for improvement on the relationship between the veterinarian and the farmer (Derks et al., 2013a, Derks et al., 2013b). The level of trust and the relationship the farmer has with his/her veterinarian is an important aspect that influences the degree to which a farmers tends to follow the veterinarian's advice. Establishing a good relationship with the veterinarian often has a positive influence on farmers' animal health behaviour.

A third suggestion from the literature is that veterinarians should be more aware of the goals, objectives and management styles of the farmers. Different farmers have different goals and objectives, influencing how they manage their animals and how perceptive they are to certain messages related to the impact of animal health management (Jansen et al., 2010, Derks et al., 2012). Further, veterinarians and farmers frequently have different perceptions on the importance of animal health management measures. For example, the most important biosecurity measures for farmers and veterinarians are different (Simon-Grife et al., 2009, Cross et al., 2009). Further, farmers tended to give better scores to the overall quality of biosecurity on their farm compared to veterinarians (Simon-Grife et al., 2009, Simon-Grife et al., 2013). In a Danish study (Thomsen et al., 2012), veterinarians and farmers agreed on the most painful diseases for cows, but the scores assigned by the veterinarians were lower than by the farmers. Several papers also suggest that the veterinarian's knowledge about farming systems and farm management economics should be improved. These studies suggest that increased postgraduate education and continuous professional education may help veterinary involvement in proactive dairy cattle disease control on three important diseases (lameness, Johne's disease and mastitis) (Higgins et al., 2013).

Fourth, there is a perceived need for more collaboration between veterinarians and other animal health and technical professionals. Farmers often have to consult other specialists for aspects related to animal health, e.g. fertility management that is being performed by advisors other than veterinarians, nutritionists and trimmers that are consulted for the nutrition and claw health (Derks et al., 2012). This emphasizes the need of veterinarians to work together with other professionals, which will aid to solve more complicated problems or increase their knowledge about more technical aspects of the animal production (Lievaart et al., 2007, Alarcon et al., 2013). Ellingsen et al. (2012) showed that agricultural advisors had more knowledge about organic farming legislation and regulation than veterinarians, According to them the main role of the veterinarians is to treat sick animals while an agricultural advisor's is to guide and strategically help farmers, which requires overall knowledge of legislation. Therefore a collaboration between the two professionals would lead to the success of implementing organic farming correctly. Nevertheless, there are also studies that prove that veterinarians are collaborating effectively with other professionals as in Hall & Wapenaar, (2013).

Social influences other than the veterinarian: Social influences other than the veterinarian relates to the sources of information and their impact. One social influence that has been investigated quite often is other farmers, family and friends. Other producers are often identified as important sources of information (Garforth et al., 2006, Elliot et al., 2011, Hernandez-Jover et al., 2012, Mafimisebi et al., 2012, Alarcon et al., 2013, Espetvedt et al., 2013).

Farmers often regard governments as a negative source of information. The information provided by public research institutes or the state were very poorly considered by farmers (Cross et al., 2009, Ellis-Iversen et al., 2010, Hernandez-Jover et al., 2012a, Hernandez-Jover et al., 2012b, Alarcon et al., 2013). This is particularly worrying in the light of an epidemic disease such as AI which poses a high risk for human health and global economies. In a paper by Negro-Calduch and co-workers (2013) the small-scale poultry farmers did not trust the government-imposed biosecurity measures. The distrust in the government has been further shown in Neupane et al. (2012) were only 1% of the farmers in the study reported that they will report to the authorities in case dead animals appeared on their premises and many farmers considered that the government control and compensation measures were insufficient.

There are papers suggesting that there is a lack of collaborative effort between different stakeholders who are responsible for the implementation of biosecurity measures (Gunn et al., 2008, Alarcon et al., 2013) which could be solved by preparing well-prepared communication, targeting persons that are influential within the farming community (Lievaart et al., 2007, Cross et al., 2009, Elliot et al., 2011). Cross et al. (2009) also highlighted the fact that several stakeholders are not communicating effectively.

Economic impact of the animal health management practices: Regardless of how farmers think about the necessity to act, regardless of their characteristics, they often consider the costs and benefits of specific proposed interventions separately. In this respect, costs and benefits should not solely be thought of as in monetary terms, but also in economic terms. Non-economists generally think of economic impacts in terms of financial, monetary costs and benefits, whereas economists use a broader definition of economic costs and benefits, encompassing issues such as long term benefits, market effects, the value of foregone opportunities, risk, farming-system interactions and other costs and benefits. The economic impact in economic language bears much resemblance to the attitude concept that is used by social psychologists. In social psychology, attitudes are the overarching concept encompassing all subjective –perceived– advantages and disadvantages of a particular action.

Economic impact, or advantages and disadvantages of the proposed practices have been shown to influence the degree of implementation. Non-monetary factors provide motivation and influence the decision of the farmers (Valeeva et al., 2007, Leach et al., 2010). In a paper by Nielsen (2011), 86% of the farmers participating in a voluntary producer-paid scheme to reduce the prevalence of Johne's disease said that they participate because they wanted to reduce the production losses. In a study about the use of protective equipment amongst veterinarians 10.7% of them believed that the cost of protective equipment was too high, which prevented them from using it (Dowd et al., 2013).

Positive attitudes towards biosecurity measures play an important role in the application of biosecurity measures by farmers (Edward-Jones, 2006, Jansen et al., 2009, Elliot et al., 2011, Thomsen et al., 2012).

Farm and farmer characteristics: Farm and farmer characteristics refer to observable characteristics of the farm and the farmers that are thought to influence adoption of practices and behaviour. Ever since Ryan & Gross (1943) showed that adoption is typically uneven from farmer to farmer, researchers have always directed attention to farmer characteristics in an attempt to explain this difference. The most studied factors in this category are age, education and experience (Leach et al., 2010, Toma et al., 2013), herd size, land size and household size (Bell et al., 2006, Bhattarai et al. 2013, Ellingsen et al., 2012, Elliot et al., 2011). In a majority of cases, these variables appear insignificant in explaining the use of certain animal health management strategies, and in those instances where they are significant, their impact is inconclusive: sometimes positive but sometimes negative as well.

Education sometimes has a significant association with the implementation of innovations or new adoptions in a farm. For instance, farmers or animal owners that have a higher education present higher levels of biosecurity on the farm (Bell et al., 2005, Lievaart et al., 2008, Noremark et al., 2010, Schemann et al., 2011, Thomsen et al., 2012). However, schooling did not have a significant relationship with the adoption of preventive measures against avian influenza (AI) (Neupane et al., 2012). Age was usually not significant (i.e. Bigras-Poulin et al., 1982). Still, one study found that young farmers have a riskier biosecurity behaviour than older farmers (Schemann et al., 2011). Gender is mostly not included in the studies. In Zingg & Siegrist (2012) females presented a significantly higher perception of risk than males across three stakeholders groups (farmers, veterinarians and general population).

The type of worker also had a significant association in this study, which may be related to the fact that farm employees tend to work on commercial farms, which have more economic means to buy protective garments, than on small holder farms (Neupane et al., 2012).

Local knowledge and flexibility of practices: Several studies highlight the need to integrate different sources of knowledge and information when implementing a plan to eradicate an emerging disease, something which is in contrast to the strict standards of international organizations (Mather, 2012), who strive to find the best solution and impose this solution to a whole region. In the case of the 2004 outbreak of Highly Pathogenic Avian Influenza (HPAV) in ostriches in South Africa, the authorities put in place a contingency plan according to international standards. However, the outbreak could not be controlled and the disease expanded to the region of Western Cape. There, the decentralized veterinary services created a eradication plan taking into account the local knowledge of the farmers, veterinarians and production systems. They managed to control the disease in the country with less culled animals and in less time than in the other province. Two papers by Enticott

(2012a; 2012b) show that it is the variation, rather than the uniformity of AHMP that are applied, that positively influence the effectiveness of a nation's overall health management.

In a highly pathogenic porcine reproductive and respiratory syndrome (HP-PRSS) context in Cambodia, farmers seemed reluctant to contact veterinarians or local health workers in the case of an outbreak because they can have access to medicines and vaccinations without prescription. Also the fact that they do not know how to contact veterinarians made them reluctant to seek their advice (Tornimbene et al., 2013). In many instances it has been found that the presence of an outbreak will prompt the farmers to call the veterinarian but if the farmers recognise the symptoms they will try to solve the health issue themselves (Kaler & Green, 2013).

DISCUSSION

Implications for policy, research and extension

There is an absence of clear, universally significant factors that influence animal health management behaviour. The OIE, the WHO and other national and international bodies have a task to develop policies to promote global adoption of sound practices to improve animal health and welfare, and prevent foodborne diseases. The sometimes contradictory results observed across analyses make this task particularly challenging . Indeed, the results above tend to suggest a 'targeted policy approach', whereby policy mechanisms are geared to the particulars of a locale or, preferably, to individual farmers and their farm operations. Yet, this may be too pessimistic altogether. Based on the summary of the literature above, some general recommendations can be suggested. Firstly, the role and potential of the veterinarian should be exploited better. This could be done in several non-exclusive ways, such as improving veterinarians' understanding of the farming systems and farm management, improving collaboration between different professionals offering advice to farmers and improving the communication skills of vets. Educational institutes have an important role to play in this respect, by improving the curricula for future vets, including more courses on farming, farm management, farm economics and communication.

Secondly, the economic costs and benefits of the proposed practices should be investigated. The results of such economic studies can be used to inform the decision makers on the most economically profitable interventions. Alternatively, veterinary researchers designing the strategies can try to make adjustments to the proposed intervention, such that the economic impact becomes more beneficial to the ones adopting the practices. In this respect, it is important, primarily, to investigate the economic impact of the proposed strategies, rather than only investigating the economic damage of a particular disease. Subsequently, when investigating the economic impact, researchers should adopt a farming systems approach, in which they recognize possible interactions with other parts and goals of the farm business.

Thirdly, targeted communication schemes can significantly improve animal health management behaviour. Targeted communication schemes should consider farmers' goals and objectives and tackle farmers' key perceptions about the economic costs and benefits. When delivering targeted communication schemes, the potential of social norms and perceptions should be exploited further, using the influential role of the veterinarian and other farmers.

Fourthly, epidemic and zoonotic diseases pose a specific challenge, since farmers do not always feel responsible for the management of such health problems. Hence, the role of government instruments will be particularly important. Further, research could be conducted that can inform the farmers that the potential benefits of managing such diseases are not only reaped by society but that it can have substantial benefits for their farm business as well.

Scientific and methodological review

The adoption of alternative practices by farmers has been a popular topic in the agricultural and rural literature. Depending on what the challenges were, the focus changed from adoption of green revolution innovations (e.g. Ryan & Gross) to more environmentally friendly production practices (e.g. Wauters et al. 2010). An extensive list of papers has been written that review the methodological approaches of such studies. A few methodological issues can have important scientific consequences and therefore, we reviewed the papers based on these few issues. A first aspect is the production of potentially misleading results by quantitative studies without a previous hypothesis or behavioural-ecological framework. Such studies relate a number of characteristics to several measures of adoption. In the empirical literature, almost every measurable farm and farmer characteristics has been at least once found to be statistically related to the adoption of some innovation. However, such studies can suffer from several methodological problems. Poor model specification, due to the lack of predefined framework can lead to omitted variable bias since they use only a subset of the required variables (Lindner, 1987). The most serious problem is a scientific and conceptual one, which arises from not appreciating the importance of the dynamic learning process that is underlying any behavioural change. At any stage of the learning process, differences in observed implementation will be the result of differences in perceptions. Hence, attempts to relate possible explanatory variables to individual behaviour are very likely to produce misleading results (Lindner, 1987), unless the diffusion process is completely over, which is hardly the case in animal health management. Whereas such problems have been very persistent in several adoption areas, we conclude that, in the SPVE domain, these issues are almost non-existent. One reason is probably the fact that the SPVE discipline is relatively young, with the majority of papers published after 2009 and having learned from the past. Most papers that we reviewed use a behavioural framework supporting the quantitative studies, such as the health belief model (e.g. Valeeva et al., 2011) or the theory of planned behaviour (e.g. Elliott et al., 2011).

A second aspect is the benefits of adopting a mixed-method approach to behavioural studies. Mixed-method approaches are methodologies where qualitative research is combined with more traditional quantitative research (Cameron, 2009). Such studies are able to capture much more of the reasoning of the relevant stakeholders compared to purely quantitative studies often relying on closed-question format questionnaires. The qualitative phase of the research can be very beneficial both before and after the quantitative phase. Before the quantitative phase, it can inform the researcher on the broader issues that are related to the specific question, in our case, the implementation of AHMP. As such, it can serve as a basis for the design of the quantitative data collection. Before and after the quantitative phase, the qualitative phase can add new, additional and even conflicting information. After the quantitative phase, it is particularly useful to elicit the more complex reasoning behind the quantitative findings. Most papers in our review use such mixed methods approaches, most of them a sequential mixed methodology in which the qualitative stage precedes the quantitative stage and serves to inform the design of the quantitative data collection. Some studies are purely qualitative, based on in-depth and/or semi-structured interviews with relevant stakeholders. Whereas these studies have the advantage of qualitative research approaches, they have limitations in showing scale, tendencies, representativeness and trends.

Areas of progress

A few potential areas of progress have been identified. The first deals with the place of SPVE in broader veterinary and epidemiological research projects. Up until now, it has too often been the case that the social research about farmers' behaviour is just another work package that needs to be included in order to get project proposals approved, just as the economic work package was a few years ago. Most studies deal with investigating farmers' perceptions, knowledge, willingness to adopt and actual adoption of predesigned animal health interventions. As such, the combination of veterinary and epidemiological sciences on the one hand, and the social sciences on the other, remain multidisciplinary. Each discipline looks at the problem from each respective angle, and the role of the social sciences is often still to investigate why farmers do not use the strategies that are designed by the veterinary and epidemiological sciences. In a truly interdisciplinary or even trans-disciplinary research approach knowledge generated in each discipline would be further used by the other discipline in order to make it more according to farmers' perceptions and suitable for practical application.

A larger number of intervention studies is advocated for. Save for a few exceptions, most studies are adoption studies, that investigate farmers' and/or veterinarians beliefs, perception, intended behaviour and actual behaviour related to a specific or more broad animal health problem. Whereas the results of such studies are very useful to suggest intervention strategies that could actually change animal health management behaviour of the relevant stakeholders, there is a lack of studies that investigate what actually works and what not. A few exceptions are worth mentioning (e.g., Huttner et al., 2001, Stringet et al., 2011).

Lastly, in order for SPVE studies to produce results that can successfully inform policy makers, veterinary researchers and extension agents, we advocate for a continuation of the mixed-method approach as the dominant research paradigm. More specifically, we suggest using three stage mixed-method approaches. In such approaches, the first, qualitative stage, would serve to set the scene and to understand the broader issues, norms and values of the relevant stakeholders. This can also suggest relevant hypotheses to be tested in the second, quantitative phase. This second quantitative phase should be designed according to some behavioural framework in order to avoid omitted variable bias. Further, the measurement and empirical treatment of latent variables such as perceptions, norms and attitudes should be done based on sound scientific and methodological principles. Third, we suggest to use a third quantitative phase, serving two distinct goals. The first goal is to validate the results from the quantitative study and to unravel the more detailed reasoning underlying these results. The second goal is to elicit potential interventions that could be successful in changing the behaviour and to improve the animal health management.

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WHICH CAME FIRST? USING CANONICAL ORDINATION TO EXPLORE INFECTION-INTERACTIONS IN ETHIOPIAN VILLAGE CHICKENS

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SUMMARY

Chickens raised under village production systems are exposed to a wide variety of pathogens, and current or previous infections are an important risk factor for further infections, and can alter the manifestation of each. However, in cross-sectional studies, where the timing of infection is unknown, apparent associations between infections may be observed due to parasites sharing common risk factors. This study examined 8 different infections in 1056 Ethiopian village chickens for potential associations, whilst accounting for common risk factors. Constrained ordination, a technique for analysis of ecological community data, was found to be a useful tool in exploring this complex dataset, and allowing potential relationships to be uncovered and tested. Relationships between *Pasteurella* and *Salmonella*; and between Marek's disease and parasitic diseases were identified, suggesting a number of diseases and their interactions are likely to be important among village chickens, and may be relevant to future development planning.

INTRODUCTION

There is widespread recognition of the importance of village poultry in developing countries. Poultry are easily accessible, even to the poorest households or those with a lack of able-bodied workers, as they require minimal land, labour or financial inputs. They can scavenge for food, and do not compete for food resources with humans. They are normally kept under minimal supervision in close proximity to the household, enabling them to be managed by women and children while placing few additional burdens on these groups. This role in managing a household asset gives women greater control over the income from sale of poultry and eggs, and this can be used to support child education. As such, the improvement programs for family poultry have the potential to contribute to several of the UN's Millennium Development Goals (Alders & Pym, 2009).

Infectious disease is recognised as one of the major constraints to developing backyard poultry production. Control of Newcastle disease virus (NDV) has been identified as the most critical intervention in numerous studies throughout Africa, and other interventions such as improving feed, housing or controlling parasites are only effective if used in conjunction with vaccination (Dwinger & Unger, 2006). Seroprevalence surveys have demonstrated the

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presence of NDV in several areas of Ethiopia (Tadesse et al., 2005; Mazengia et al., 2010; Chaka et al., 2012), although little attention has been paid as yet to control strategies. However, NDV is not the only problem; chickens under a village production system are exposed concurrently or consecutively to a number of different pathogens. In Ethiopia sero-surveys of village chickens have identified the presence of infectious bursal disease, salmonellosis, pasteurellosis and mycoplasma infection (Mazengia et al., 2010; Berhe et al., 2012; Chaka et al., 2012; Jenbreie et al., 2012). Marek's disease has also been identified in village chickens kept under intensive management (Duguma et al., 2005). Parasitic diseases, including coccidiosis, helminths and ectoparasites have also been demonstrated to be highly prevalent in the country (Ashenafi et al., 2004; Tolossa et al., 2009; Luu et al., 2013).

Host survival may be influenced by multiple, coincident infections (Jolles et al., 2008) and there is evidence from studies of wild rodents that existing infections may pose greater risks for further infections than environmental variables (Telfer et al., 2010). Associations between diseases may be the result of direct interactions between the pathogens themselves or indirectly, via the bird's immune system, such that their impact on the host may be altered. Many of these interactions are thought to be mediated through the altered differentiation of T-lymphocytes; whereas protective cell-mediated responses to intracellular microbial pathogens are primarily driven by T helper 1 (Th1) cells, protection to extracellular infections usually requires antibody and primes the immune system towards a Th2-type response. Hosts in a natural environment may have limited ability to effectively mount both types of immune response simultaneously, particularly where they are constrained by limited resources (Jolles et al., 2008). The helminth *Ascaridia galli* has also been shown to reduce the antibody response to vaccination with NDV (Horning, 2003); therefore pathogen interactions may have implications for disease control programmes. Indeed, the health consequences of multiple low-grade infections may become more significant as interventions reduce the impact of single diseases (Pullan & Brooker, 2008).

However, associations between infections may arise because diseases have other risk factors in common, such as similar transmission routes, temporal patterns of exposure, or host factors, such as sex, age, socio-economic status or behaviours (Hellard et al., 2012). The use of generalised linear mixed models, as proposed by Fenton et al. (2010) for the analysis of macroparasite data, can account for such factors, but may be less useful for serological surveys which measure the host's adaptive immune response rather than the pathogen itself. An added difficulty with serology is that it does not necessarily represent current infection; the order of infection is important in determining the immune response to each infection, and it is thought that susceptibility to new co-infections may return to normal soon after the clearance of the first infection (Telfer et al., 2010). Therefore models which assume a directional relationship are unsuited to cross-sectional serological data (Hellard et al., 2012).

Although there have been several laboratory studies of interactions between pairs of pathogens in poultry, in a village situation pathogens exist in communities with the possibility of multiple interactions occurring. This study therefore investigated the epidemiology and ecology of co-infection with a range of pathogens in Ethiopian village chickens, with the main objectives being (i) to assess patterns of co-infection, and (ii) to identify common risk factors for co-infections. The range of pathogens studied necessitated the collection of both serological and parasitological data, and a method of combining these different infection measures was sought. Ordination methods are commonly used in ecology for community analysis: Here they have been applied to this community of pathogens, where each bird may be thought of as a “site”, which has the potential to be exploited by any of the micro- and macroparasite species.

Two main techniques were used to explore this data set. Principal component analysis (PCA) is an ordination technique which seeks to extract the main trends in the data set such that it may be explained by a few linearly uncorrelated principal components, which will be less than or equal to the number of original variables. The main structures in the data may then be displayed in a graph(s) constructed from the reduced set of orthogonal axes. Graphs, known as biplots, can be scaled according either to the distances between sites or the correlations between variables (Zuur et al., 2007). Redundancy analysis (RDA) is a canonical ordination technique which combines regression with principal component analysis, and thus tests the relationships of a set of explanatory variables to the multivariate response data. This study highlights the use of both of these techniques to explore infection-interactions in Ethiopian village chickens.

MATERIALS AND METHODS

This study was approved by the University of Liverpool Committee on Research Ethics (reference RETH000410). The study was carried out between May 2011 and November 2012 in two geographically distinct woredas (administrative districts), Horro and Jarso, within the Oromia region of Ethiopia. Woredas are divided into smaller administrative districts, called kebele, which may comprise several villages. A group of villages which are linked by trading networks is collectively known as a market-shed (Dessie 2003). Within each region two market-sheds and two kebele per market-shed were purposely selected on the basis of their willingness to participate, in consultation with local representatives of the Department of Agriculture and the communities. These kebele were believed, locally, to be representative of other kebele within the woreda. A list of names of all household heads in each kebele, grouped by sub-area, was obtained from the respective kebele agricultural development agents and systematic random sampling was used to select potential participants.

Ethiopian staff fluent in the local languages and in English were recruited and trained to collect data for this study. Each kebele was visited on four occasions; in May/June and October/November in each year of the study. These visits were timed for before and after the main rainy season, which occurs between June and September. Different households were selected on each occasion to take part in a questionnaire survey and for two of their chickens to be sampled. Households which did not own at least two indigenous-breed chickens of over 6 months of age were excluded from the study. Information on management and disease occurrence over the previous 12 months was collected in a questionnaire interview in the local language with an adult member of the household, normally either the household head or the main carer for the chickens. In addition, two chickens of at least 6 months of age were randomly selected from the household flock and each underwent clinical examination, was condition scored using a 0-3 grading scale (Gregory & Robins, 1998), and was scored for lice using a timed count of 3 areas of the body – one side of the keel, the back and the rump - and a total count of lice at the base of the flight feathers of one wing and the tail feathers (Clayton & Drown, 2001). Birds were also scored for the degree of hyperkeratosis of the legs and feet (none, mild or severe), and scrapings were taken to confirm the presence of Cnemidocoptes mutans (scaly leg mite). Data on the chickens’ age, origin and current status of production was collected from the owner. Blood samples were drawn from the brachial vein of the selected birds into sodium citrate and faecal samples were collected from individual birds wherever possible; environmental faecal samples were taken to represent the household, if individual samples were not forthcoming. All samples were kept chilled and were transported to the laboratory for testing.

Serological testing was carried out for NDV antibodies by haemagglutination inhibition (HAI) assays, according to the O.I.E. (2009) protocol, and for other bacterial and viral infections by enzyme-linked immunosorbent assays (ELISA). Infectious bursal disease (IBD) virus antibodies were tested for using a commercial kit (Flockscreen, x-OvO, Dunfermline, UK), while antibody ELISAs were developed in-house for Pasteurella multocida (PM) and Salmonella O9 serotypes, using Salmonella enterica serovar Gallinarum (SG) antigen, according to the protocols by Beal (2004); and to Marek's disease virus (MDV) according to the protocol described by Zelnik et al. (2004). The latter assay required that samples were tested against a lysate of both MDV-infected and uninfected chicken kidney cells to identify false positives. Samples for all ELISA's were tested in triplicate wherever possible, or in duplicate when reagents became limited, and those with high variation between replicates were retested. Positive and negative controls were run on each plate. Optical densities for all ELISA samples were converted into a ratio to the positive control (s:p ratio) to allow comparison of samples tested on different plates, using Eq. (1). Mixed-effects linear regression was used to control for the variation between plates, and identify and exclude any cross-reactors to the MDV assay, and the bird-level residuals were taken as an estimation of the antibody response.

$$\text{s:p ratio} = \frac{\text{Mean sample OD - Negative control OD}}{\text{Positive control OD – Negative control OD}} \quad (1)$$

Faecal samples were examined for *Eimeria spp*. oocysts and nematode eggs using a modified version of the concentration McMaster technique and identified to genus level where possible using published keys (Permin & Hansen, 1998). Only the most common egg types, *which* could be attributed to nematodes of the *Ascaridida* order *Ascaridia galli* or *Heterakis gallinarum*, were counted. These eggs were counted as a single group, as it was not possible to accurately differentiate the two species. Although other types of nematode eggs were seen in low numbers, there were no other frequently occurring species.

Analyses were performed using R software (R Development Core Team, 2008) using the vegan package (Oksanen et al., 2011), and according to the methods described by Borcard (2011). A matrix of response variables was constructed from the ELISA s:p ratios for the viral and bacterial pathogens (IBD, PM, SG and MDV); counts of the three parasites (Ascaridida, Eimeria and lice) and the hyperkeratosis grading. In order to make all response variables dimensionally homogeneous, each variable was scaled by centring values on the mean and dividing by the standard deviation. This ensured additional weights were not given to the parasites with larger ranges of values and fulfils one of the basic requirements for application of PCA. In order to test whether the results were features of the data (different data types, presence of outliers), data were re-analysed following a number of prior transformations, including log transformations of the ELISA s:p ratios, square root transformations of the individual parasite count data, and Hellinger transformations of the parasite count matrix. In addition, data were re-analysed following dichotomisation, using a range of cut-off values for the ELISA data and presence / absence for the parasite counts and hyperkeratosis.

Initially, an unconstrained PCA was carried out on the matrix to identify correlations between parasites; a second matrix of explanatory variables, including household data and bird signalment, was then incorporated using RDA. A category “production status” was used to combine data on sex and the current state of production (in lay, not in lay, brooding eggs, rearing chicks or male). Each centred response variable was regressed on the explanatory variables and the fitted values combined in a matrix. A principal component analysis (PCA) of the fitted values produced canonical eigenvalues and a matrix of canonical eigenvectors, used to calculate the coordinates of the sites (chickens), either in the space of the original response variables, or in the space of the explanatory variables (i.e. on the fitted response variables). The residual values from the multiple regressions were also submitted to a PCA to obtain an unconstrained ordination of the residuals.

The adjusted R2 value was used to test whether the inclusion of explanatory variables was a significantly better fit than the null model, and a forward selection process was used to select those significant variables which explained the greatest proportion of the variance in the response data (Borcard, 2011). Permutation tests were used to test how many RDA axes explained a significant proportion of the variation. The Kaiser-Guttman criterion, which compares each axis to the mean of all eigenvalues, was applied to unconstrained axes, to determine those which explained variation of interest. Biplots and triplots were produced, scaled according either to the distances between observations (scaling 1 in the vegan package) or the correlations between variables (scaling 2).

RESULTS

A total of 1056 birds, 532 from the Horro region and 524 from the Jarso region, were included in the analysis. All birds were found to be in reasonable health at the time of sampling, with only a very few displaying any clinical signs which may have been attributable to the diseases of interest. Only 9 birds over all four seasons of testing were found to be serologically positive for NDV antibodies (HAI titre of 16 or greater); therefore this disease was not included in the ordination analyses. All other infections were present in each village and at each sampling season with the exception of IBD, where no positive birds were found in one Jarso village at any time point, and seropositive birds were detected in only three villages during the second year of sampling.

After constructing the correlation matrix of responses to each infection, PCA was used to explore the variation in the scaled dataset. Regardless of the prior data transformation used, this suggested that three axes represented interesting variation in the data (using the Kaiser-Guttman criterion), and the other axes displayed essentially random variation. However, the first three axes represented less than 50% of the variation, accounting for 17.0%, 14.7% and 13.2 % (in total, 44.9%) respectively on the untransformed data. The majority of the data points were intermediate in all variables, with a relatively small number of birds spread out by the three axes.

The first axis principally represented the variation contributed by the Salmonella and Pasteurella titres, whilst the majority of the second axis consisted of the variation added by scaly leg mite, MDV, Eimeria and, to a lesser extent, lice (Fig. 1a). Lice counts and IBD titre were negatively correlated and formed the principal part of the variation represented by the third axis, and Ascaridida principally contributed to the fourth (non-significant) axis. Analyses following other data transformations showed similar overall groupings, although binarisation emphasised negative correlations between scaly leg and both IBD and Eimeria; whilst Hellinger transformations gave more weight to the distances between non-zero parasite counts and highlighted negative correlations between lice and Eimeria, and between

Ascaridida and scaly leg (data not shown). All PCA's illustrated a strong correlation between Salmonella and Pasteurella which consistently contributed the 1st principal component and therefore the greatest variation in the data.

A forward selection process identified the following explanatory variables for inclusion in the RDA model: bird production status and weight; and at the household level, the use of chemical sprays for parasite control and recent outbreaks of disease in the household's chicks or growing birds. Variables which were tested but not deemed to significantly improve model fit included the season of testing, the village or region, body condition, age of the bird, and where the bird had come from. Although significant, the explanatory variables accounted for only a very small proportion of the total variation in the dataset (adjusted R-squared value 0.03); a permutation test of these axes suggested that 3 of them described significant variation. However, when the household-level variation was partialled out from the model by fitting this as a fixed effect prior to fitting other explanatory variables (the permutation tests used preclude this variable from being fitted as a random effect), this explained around 59% of the variation in disease responses, and left only bird weight and production status as significant bird-level explanatory variables, and only two significant RDA axes (Fig. 1.b).

The residuals from the multiple regressions were also subjected to an unconstrained PCA. The Kaiser-Guttman criterion suggested 4 axes may be required to represent the residual variation of interest in the data, and the groupings of the disease variables remained much as they were before. Salmonella and Pasteurella, although both tending to be have higher values in females, still maintained a positive correlation. Lice, MDV and scaly leg also maintained their previous cluster, and higher values were associated with male birds and those with greater body weights, although Eimeria became more distant from this group.

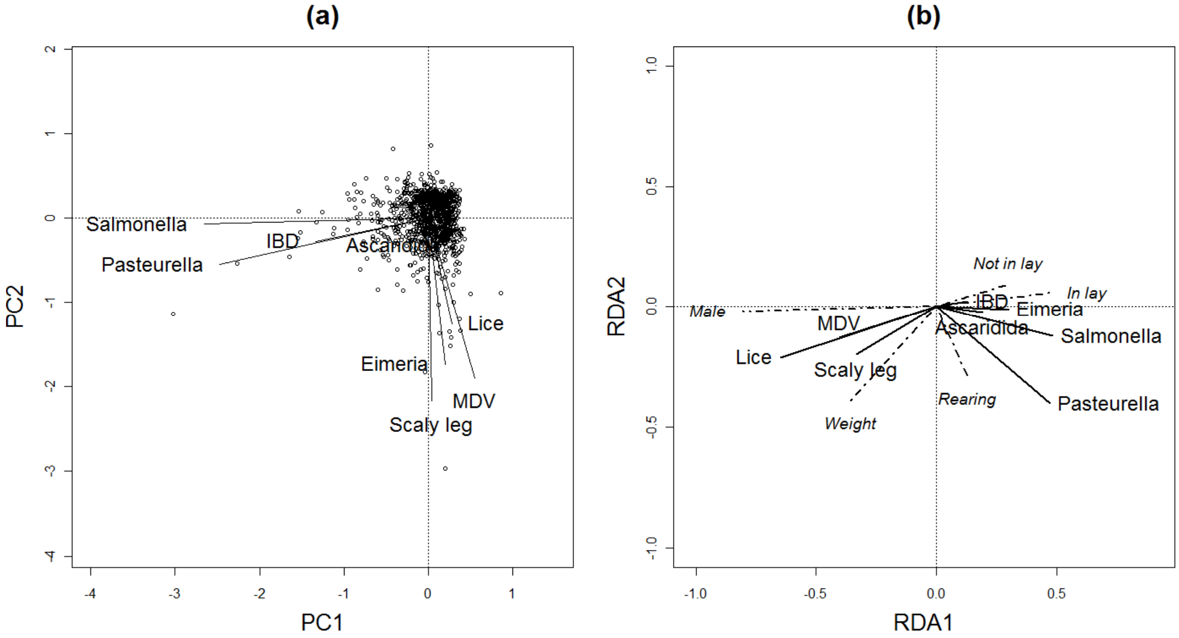

Fig.1. (a) Biplot of the first two principal components, showing correlations between the unconstrained response variables; (b) Triplot showing the relationships between response and explanatory variables between the first two RDA axes. Solid lines represent pathogens (outcomes); dashed lines represent explanatory variables. In both plots, angles between variables represent their correlations.

DISCUSSION

Multiple micro- and macroparasites were found to be circulating within apparently healthy adult village chicken populations in Ethiopia. Traditionally, epidemiology has concentrated on individual diseases, but the effect of other parasites on the infection of primary interest should not be overlooked. However, identifying potential interactions between parasites and testing the strength of these associations, especially when the order of infection is unknown, remains a complex issue. The use of ordination methods was found to be a useful exploratory tool, and appeared to be robust to the combination of different types of response data (ordinal scores, counts and serological titres), perhaps giving it wider applications within epidemiology.

PCA does not require normally distributed variables, but in community ecology count data are frequently transformed (e.g. Hellinger or chord transformations) in order to reduce the effect of large counts and to eliminate the apparent correlation of sites with double zeroes, (i.e. where two species may be absent, but for different reasons; Zuur et al. 2007). Because this transformation gives a Euclidean distance in the space of each site's (i.e. bird's) total parasite abundance, standardising the data gave a large weight to the difference between 0 and 1 parasite, but no difference to the difference between 1 and 60 parasites, if the other parasites were held constant. Since parasite intensity is important for bird health, it was decided that total burden should not be lost from the analysis. In addition, the test methods used for detecting parasite infection are not especially sensitive and the data is likely to contain a number of false negatives.

The serological data, being continuous, did not require prior transformation to avoid the issue of double negatives, and log transformations or dichotomisation to reduce the impact of outlying values resulted in relatively minor differences to the main components. In an analysis of river water quality variables, Cao et al. (1999) suggested transforming data to give weightings to observations which were outside the normal range of each variable, in order to give more biological relevance to the analysis. The untransformed scaled data maintained the distances between those birds whose infection measurements may be considered outliers from the general population. Since these may be of most interest, either in terms of their immune response and infection tolerance or because they are likely to be significant contributors to pathogen spread in this population, analysing and presenting the scaled untransformed data appeared to be of most value in this context.

The results of the ordinations show some groupings of pathogens which may warrant further investigation. The closest correlation was seen between Salmonella and Pasteurella measurements, and this was maintained even after in-common measured risk factors, which explained very little of the variation, were accounted for. As both of these were measured by ELISA, this may be a reflection of individual's ability to mount an adaptive immune response. The second clustering of diseases grouped MDV loosely with Eimeria, and MDV also showed a positive correlation with lice counts and scaly leg scores. MDV would be expected to negatively impact the immune response through lymphocyte depletion, and previous findings have demonstrated that MDV-infected birds are less able to clear Eimeria infections (Biggs et al., 1968). This grouping of diseases was most closely associated with male birds and/or heavier birds in the RDA, whereas the bacterial diseases were associated more strongly with females. Males infected with MDV are less susceptible to the development of tumours (Payne and Venugopal, 2000), and in other avian species larger body sizes have been shown to correlate with greater abundances of lice (Møller & Rózsa, 2005); therefore it may be that males are simply tolerating these infections better. Differences in the male and female immune response to Salmonella enterica serovar Pullorum infection has also been demonstrated (Wigley et al., 2005). These observed groupings may therefore reflect genuine differences in the way that these pathogens interact with the immune system in male and female birds. In addition, unmeasured risk factors, such as different male and female behaviours, may contribute by altering exposure to these different pathogens.

There was a large group of birds which were not spread out by the ordination analyses. Salmonella and Pasteurella antibody distributions were positively-skewed. High antibody titres against Salmonella O9 serotypes may reflect establishment of a carrier state such as that found in some birds following S. Pullorum infection (Chappell et al., 2009). The more moderate levels of antibody titres may represent exposure to S. Enteritidis or S. Gallinarum. Pasteurella multocida is also capable of establishing a carrier state, although the mechanisms and the immune response to this bacterium remain largely unstudied in poultry (Wigley, 2013). This clustering of birds that were intermediate in all antibody measurements may, therefore, suggest most of these birds have been exposed to Salmonella and Pasteurella, but are probably not chronically infected, and are negative for IBD and MDV and all parasites. This group of birds is challenging to interpret; low antibody titres may mean that birds have not been exposed, were infected too long ago to detect the response or simply have a poor immune response, possibly as a result of another immunosuppressive or immune-modifying infection. For the parasite species that were measured more directly, a negative count may again mean a bird has never been exposed, or has been previously infected and cleared the infection.

There were almost no birds which had high responses / counts to more than one pathogen group. This may in part be due to other opposing and mutually exclusive risk factors, such as sex, where males and females tended to be associated with different groups of diseases. Including household as a partial fixed effect in the RDA illustrated the significant contribution of household to many of the disease responses, suggesting that either there are some important, unmeasured, household factors or that exposure to these diseases at any single time point varies significantly even within a village. This may be due to geographical and management factors, but may also be due to the relatedness of birds in the same household giving them similar immune characteristics. The scarcity of co-positive birds may be due to a lack of synchronicity between infections. It is also possible that birds recently infected with two pathogens in a short time period, where we may expect both a high antibody titre and a high parasite burden, frequently do not survive to be measured, hence the lack of these in this dataset. In order to investigate many of these hypotheses, longitudinal studies are required; however, the ordination analyses has proved useful in the generation of these hypotheses, and in illustrating some potential associations, both positive and negative, between different types of pathogens, even with very different types of measurement used.

The impact of these multiple infections in the village chicken population is likely to be of greatest significance within the young stock but any of these infections may potentially impact the adult birds at times of climactic, nutritional or other stress. The results of this study have implications for interventions to improve chicken health and production in Ethiopia. For example, programmes aiming for genetic improvement of indigenous stock by selective breeding (Dana et al., 2011) need to consider the range of infectious diseases to which village chickens are exposed, and be careful not to lose important protective immune traits from the population. Furthermore, interventions such as de-worming could, whilst controlling for one pathogen, have implications for the infection biology and epidemiology of others (Nacher, 2011); therefore any intervention undertaken in this population should carefully monitor any effects on other diseases, especially those with zoonotic implications.

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ACCURATE LIVESTOCK NUMBER ESTIMATES IN MIXED FARMING SYSTEMS THROUGH A MULTI-MODEL APPROACH

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SUMMARY

In many countries with predominantly extensive livestock systems, information on the number of livestock is lacking. However, knowledge on the distribution and abundance of livestock is important e.g. when assessing food security and livelihood in general, making informed decisions on how to plan intervention campaigns or distribute resources such as tick repellants. In those countries decision makers depend on results of expensive livestock surveys. An operational approach to minimize sample size requirements or to improve accuracy of cattle distribution mapping has been developed including internal validation methods and spatial resolution considerations. The approach increases the accuracy of cattle number estimates by applying random forest algorithms. This method increases the amount of available data and therefore lowers the risk of under or overestimation, even when the size of administrative units is small. A resampling procedure allows for accuracy assessment through modelling of error confidence intervals based on variance.

INTRODUCTION

In countries where livestock keepers are not obligated to report birth, decease, intake and off-take of animals, livestock population data is often sparse, inaccurate and incomplete. Therefore important information is lacking to assess e.g. livelihood, food security and transmission risk of diseases among livestock and humans (Kruska et al., 2003). In such situations decision makers generally rely on distribution estimates from survey records or projections of outdated enumeration results (Wanyoike et al., 2005). Given the cost of large-scale surveys, sample size is often restricted and the accuracy of estimates is low. Furthermore, temporal resolution is limited due to the large time span between livestock counts.

In the past efforts have been made to fill this gap and to estimate livestock distribution in areas lacking reliable livestock abundance data. One way to do so is by training statistical models using spatial data such as environmental variables as done in the FAO's Gridded Livestock of the World (Wint & Robinson, 2007). Because remote sensing images with a high spatial resolution are easily accessible (e.g. on a 1x1 km basis), the resulting livestock abundance maps show the same spatial detail. Although this may lead one to assume a high accuracy on a pixel by pixel basis, values cannot be evaluated as is and should be aggregated before being interpreted. The operational scale on which confidence levels become

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acceptable is difficult to determine as validation data is often unavailable in sufficient quantities.

Another method to assess livestock abundance more accurately in data-sparse environments was proposed by the authors in 2012 (Bryssinckx et al., 2012). They used an inverse distance weighted (IDW) interpolation based on observed livestock numbers to make estimates for areas where no samples were taken. Subsequently, pixels on the resulting map were aggregated on different administrative unit levels resulting in significantly lower error values as compared to traditional data processing of livestock survey results. The added value is attributed to data from adjacent administrative units which is included when livestock number estimates are made for the administrative unit of interest. However, only nearby areas contribute to the improved livestock number estimates and the influence of samples outside the administrative unit depends on the course of the borders.

To overcome these issues, an alternative approach was developed using a random forest modelling technique so denominator data from more distant but similar areas are included as well. The method was tested using the existing political borders of local government units within Uganda as well as using artificial areas delineated by a regular hexagon grid. The latter was done to assess operational scales as existing administrative units differ in size even when units from the same administrative level are considered. In addition an internal validation procedure was built upon resampling techniques to eliminate the need for a separate set of samples to allow for error estimation. The aim of this work is to assess if random forest modelling as a data processing technique is able to increase the accuracy of livestock abundance reports based on agricultural survey data.

MATERIALS AND METHODS

Training data

To test different data processing approaches, the Uganda 2008 National Livestock Census was used (MAAIF, 2009). Its size of 964,047 sampled households grouped into 8870 enumeration areas allows for simulating livestock surveys of smaller sample sizes, process these subsets of data and compare the resulting livestock abundance estimates with the reported NLC 2008 figures. During the planning stage of the livestock enumeration in 2008, Uganda consisted of 80 districts which are further subdivided into counties, subcounties and parishes. The most detailed geographical reference of enumeration areas is the parish in which it is located, for which shapefiles are available. Environmental variables which are used to train spatial models were extracted for these parishes and also on subcounty level to assess the importance of spatially aggregating predictor variables which corresponds to not only considering the homestead of herds but also the home range used during daily grazing activities. Predictor variables included estimated human population numbers for rural and all areas (Lapar & Jabbar, 2003), travel time to nearest city (Nelson, 2008), distance to nearest water body (Luke, 1987) and a set of remote sensing imagery such as night-time light (Elvidge et al., 2001), summary statistics of normalized difference vegetation (Huete et al., 2002) and Bioclim (Hijmans et al., 2005) variables representing precipitation and temperature. Another predictor variable was the landcover type as defined by the International Food Policy Research Institute (IFPRI) (Sato & Tateishi, 2002). All predictor variables used are freely available for countries worldwide.

Model development

Random forest was selected as statistical modelling technique because (i) few statistical distribution assumptions are made, (ii) categorical variables are easy to include and (iii) there is no risk for overfitting even when the number of predictor variables is large compared to the number of observations as subsets of training data and predictor variables are taken randomly when training the individual regression trees. This makes this partitioning method very suitable to be used in an automated fashion such as in software packages, also when a wide range of predictor variables is used.

To simulate different sampling strategies sample locations were selected using a (i) regular grid, (ii) systematic sampling, (iii) random sampling, (iv) sampling with differential probability of inclusion, (v) stratified sampling according to landcover classes and (vi) clustered sampling. Because it is not guaranteed that in 2008 households were sampled at the selected locations, for each sample the nearest enumeration area was taken. In case more than one enumeration area share the same location (when more enumeration areas are located in one parish, they all get the centroid of the polygon as georeference), one is selected randomly.

In a first stage, political borders of local government units as of 2007 (the year in which the Uganda 2008 NLC was planned) were used to aggregate model results per administrative unit. Later livestock number estimates were aggregated using artificial administrative units sharing the same area size in order to assess the operational scale. Areas of 1000 to 5000km² with an interval of 1000km² were tested covering average sizes of subcounties, counties and districts in Uganda, and sample sizes included 100, 200, 400, 800 and 1600 enumeration areas per administrative unit.

The model predicted cattle numbers for the same areas for which training data were available, which generalizes livestock abundance data within similar areas and reduces the risk of under- or overestimating livestock numbers. A multimodel denominator estimate was calculated as well. This technique has been applied in a variety of fields of which species distribution modelling is one (Wintle et al., 2003). Two main approaches to combine multiple models are Bayesian inference and multimodel inference (Link & Barker, 2006). Both approaches require weighting of the different models. These weights represent how well each model approximates “truth”. In this study uninformative weights were used (i.e. model results and direct estimates are averaged by calculating an unweighted mean value) as data on livestock populations in countries with predominantly extensive livestock systems is sparse and prior knowledge for assigning weights is inaccurate. For direct estimates, ensemble model and null-model (estimating cattle numbers by averaging direct estimates with the median of sampled cattle numbers), the null-hypothesis that error values were the same was tested using an ANOVA test together with Tukey Honest Significant Difference post-hoc tests in order to rank the different approaches. The R language for statistical computing was used for both data management and statistical analysis.

Error assessment

While in this study error values can be obtained by comparing modeled livestock number estimates with aggregated Uganda 2008 NLC entries, this wealth of data is generally not available in a mixed farming environment. Therefore a resampling procedure was set-up to assess error values based on cattle number estimate variance for a set of 50 subsamples. The resampling procedure was repeated 400 times on different sets of samples and tested for each combination of administrative unit size, sample size and subsample size. The approach was tested for subsets of 30% and 60% of all samples, as well as for one sample being left out of the total set. Linear regression models were used to assess the correlation between variance and error. Because the error-variance ratios are larger for small variance values as compared to large variance values, both variables were transformed in order to estimate error values using a linear model (Equation 1 and Equation 2).

$$\sigma^2{}_t=\sigma^2/(1+\sigma^2) \quad (1)$$

Where $\sigma^2{}_t$ is the transformed variance and σ^2 is the variance among estimates based on the resampling subsets. The transformation ensures that the transformed variance, which is used to estimate error, does not exceed one.

$$\Delta_t=\Delta^x \quad (2)$$

Where Δt is the transformed absolute error and Δ is the error which is raised to the power of x.

RESULTS

Model development

The sum of error values for direct estimates (baseline) and model results were rescaled so they sum up to one. Therefore, Fig. 1 shows that for all sample sizes accuracy was increased when applying the random forest model, except for sample sizes exceeding five enumeration areas per district when only administrative units with high average herd sizes were considered. This was found to be a result of the unbalanced training data as only few administrative units were found with large average herd sizes (>9 animals per household; Fig. 2). The accuracy increase was also found for each landcover class. When considering an ensemble of direct estimates and random forest results, accuracy increases were found in each of the livestock abundance classes (Fig. 3). The post-hoc test indicated that model results outperformed the direct estimates. The null-model performed worst.

When the same test was repeated for the artificial administrative units, similar results were obtained though accuracy levels differed among (i) area sizes for which estimates were made and (ii) administrative unit levels for which predictor variables were extracted. The added value of the ensemble model over direct estimates was most pronounced when predictor variables were extracted on parish level.

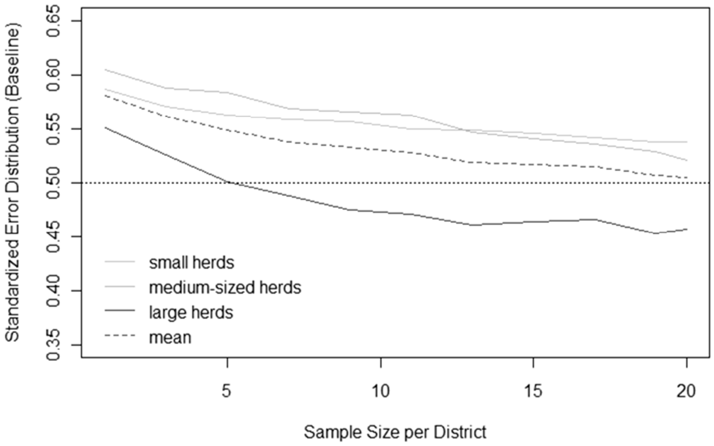

Fig. 1 The standardized error distribution shows the ratio of error values of the direct estimate to those of the model results. Values below .5 denote an accuracy increase when livestock figures were estimated by the random forest instead of by direct estimation

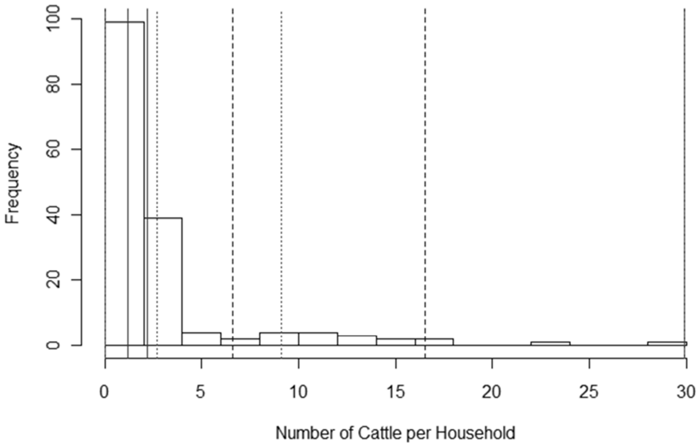

Fig. 2 Distribution of mean number of livestock per household and categorization into three classes using a k-nearest neighbor technique

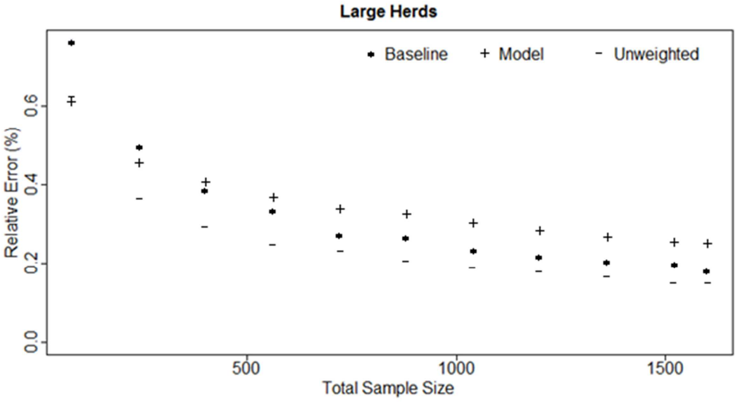

Fig. 3 Ensemble model results (unweighted; -) have lower errors compared to direct estimates (baseline; *) even when model errors (model; +) are higher

Error assessment

When assessing resampling variances and corresponding error values, the absolute error clearly shows a trend of increasing values for higher variances (Fig. 4 – Top left). For relative errors this trend is less pronounced as high relative error values are found for low variances and low relative error values are found for high variances (Fig. 4 – Top right). When evaluating data from all repetitions, a clear upper limit can be observed for 60, 70, 80 and 90% of the lowest absolute error values. Scatter plots show a fast increasing trend of absolute error values which flattens towards higher variances.

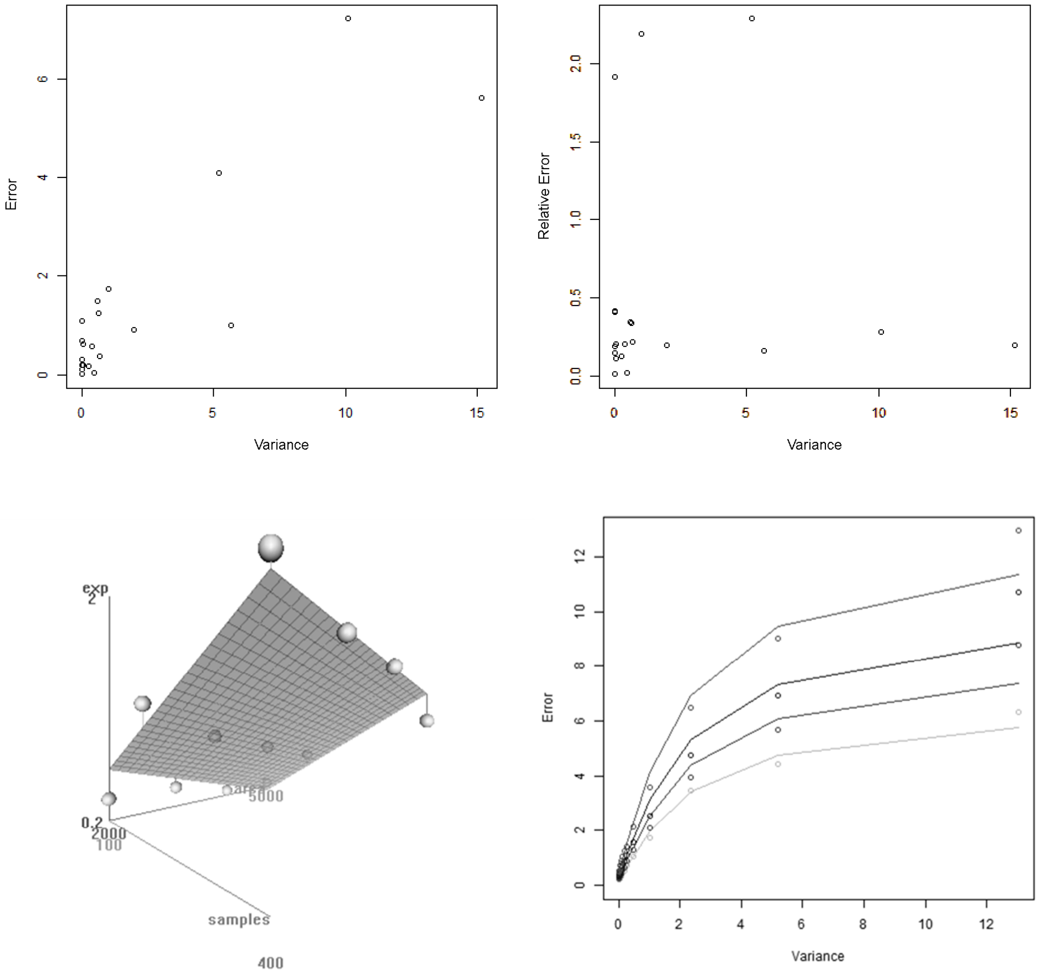

Fig. 4 Top left - Scatter plot of variance and absolute error for one repetition and 20 administrative units. Top right - Scatter plot of variance and relative error for one repetition and 20 administrative units. Bottom left – Exponents used to transform variance in an error prediction model. Bottom right – Modeled (.6, .7, .8 and .9 percentiles; lines) and observed error values (points).

DISCUSSION

Based on the 2008 livestock survey, modelling denominator data showed to be an effective way of improving the accuracy of denominator data estimates as compared to the direct estimate. However, when the results were subdivided by livestock abundance, it became clear that in the few districts with large mean herd sizes, the performance of the predictions was worse as compared to the direct estimate when sample sizes were small.

Observed accuracy differences among the three livestock abundance classes are the result of an unbalanced training dataset with many admin units with small cattle numbers per household and only few admin units where households own many cattle. When using random forest models, accuracy will therefore be highest for admin units in the livestock abundance class which is most common among the training data. As this plays to the advantage of those who will use the livestock distribution maps, there is no need to balance the training dataset by removing some of the administrative units of the largest livestock abundance class. This would only diminish the amount of training data, which is already sparse.

When only a small livestock survey is available and no extensive validation dataset (such as in this test framework) is at hand, there is uncertainty about whether the direct estimates or model outputs are closest to truth. As Wintle et al. (2003) noted, there is considerable risk in ignoring the alternative arguments (i.e. outcomes from other models) when selecting only one of these methods. A more conservative approach is to consider any of the competing models by calculating a mean estimate. When an ensemble estimate was made by computing the unweighted mean of a direct estimate and spatial model prediction, the relative error values were reduced and generally an accuracy improvement was obtained over the entire range of tested sample and herd sizes. This shows the proposed approach to be a robust way of improving denominator data estimates without increasing sample size. This is accomplished through averaging under- and overestimations, and through the introduction of data from outside the administrative unit of interest.

Because a very comprehensive sample size was available for validating the proposed methodology, large sample sizes could be tested. Therefore, the results are considered to be applicable in many countries as sample sizes are usually much smaller in most national livestock surveys covering mixed-farming system. Which additional predictor variables should be added to the model may depend on country-specific situations and might require adaptation of the spatial model.

Although there was no direct linear relationship found between variance within resampling subsets and absolute error, error estimates could be modelled after transforming both variables. Worst case error values for 60, 70, 80 and 90% confidence levels were assessed using this method and found to approach the observed values well. A 30 percent resampling subset has been chosen over a 60 percent subset or one where one enumeration area was left out because of the clear trend of exponent change for the error transformation in both sample as area size dimensions. This benefits the universal application of the model in a broader field of settings.

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ANIMAL CONTACT STRUCTURES

HOW FAR WILL A DOG ROAM? MOVEMENTS OF DOGS IN ABORIGINAL AND TORRES STRAIT ISLANDER COMMUNITIES AND IMPLICATIONS FOR RABIES SPREAD

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B. COOKSON AND M.P. WARD

SUMMARY

Transmission parameters are the core of epidemic models, but they are difficult to estimate, especially when outbreak data are lacking. Investigation of home range (HR) can provide the foundation for such parameter estimation in free-ranging animals. The objectives of this study were to estimate HR and utilization distributions (UD) of 48 domestic dogs in Aboriginal and Torres Strait Islander communities in northern Australia and to compare three different methods of HR estimation. Median core HR (50% isopleth) of the dogs were estimated to be 0.2 to 0.4 ha and for the 95% isopleth 3.4 to 5.5 ha. The HR and UD shapes were found to be principally circular around the owner's house; however, the UD of some dogs covered large parts of their community. HR sizes differed significantly between some of the three methods and isopleth levels used. One of the methods, the Biased Random Bridge approach, provides several advantages for the analysis of this dataset and was therefore found to be most suitable for UD estimation.

INTRODUCTION

Infectious diseases spread with the movement of their hosts. For free-roaming animals, both wildlife or un-restrained domestic animals, investigation of their home range (HR) can provide information on speed and direction of disease spread, e.g. by investigating size, shape or overlapping areas of HR (Pfeiffer & Hugh-Jones, 2002; Laffan et al., 2011). HR of free-ranging animals has been defined as the area an animal commonly uses for normal activities, such as foraging, hunting and breeding (Burt, 1943). The utilization distribution (UD) integrates relative time frequencies with the various areas used by an animal within its HR (Winkle, 1975; Worton, 1989; Millspaugh et al., 2006). It therefore provides information on spatial and temporal use of the area. Several methods based on global positioning system (GPS) data have been described to estimate individual animals' HRs or UDs. These range from simple methods such as the minimum convex polygon (MCP; Blair, 1940; Odum & Kuenzler, 1955), to local nearest neighbour convex-hull construction (Getz & Wilmers, 2004; Getz et al., 2007) to kernel density estimation methods (Silverman, 1986; Worton, 1989; Seaman & Powell, 1996). More recently, the location-based kernel density estimation (LKDE) methods have been extended to use animal movements (instead of locations) as a basis for UD estimation. An advantage of movement-based methods of UD estimation is that

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they take into account the path travelled by the animal between two GPS fixes rather than only the positions of the records. The most recently developed movement based method, the Biased Random Bridge method (BRB; Benhamou & Cornélis, 2010; Benhamou, 2011) is based on a biased random walk model, i.e. the animal movement between two GPS fixes is not purely diffuse, but affected by the drift from one fix to the next (Benhamou, 2011).

Free ranging domestic dogs are numerous in Aboriginal and Torres Strait Islander communities in tropical northern Australia. While several studies have been published on the HR and roaming behaviour of wild dogs – particularly in Australia where a range of studies have been undertaken on dingoes (e.g. Harden, 1985; Thomson et al., 1992a, 1992b; Newsome et al, 2013) – published data on the HR or UD of free-ranging domestic dogs living in human communities globally are scarce. Some reports are available from Brazil (Dias & Guilloux, 2012), Indonesia (Gunata, 2011), the USA (Beck, 2002) and Australia (Meek, 1999). However, roaming behaviour data on dogs in Aboriginal and Torres Strait Islander communities in northern Australia are lacking. These populations pose not only a risk for the spread of existing infectious diseases, including zoonoses (Meloni et al., 1993; Walton et al., 1999; Brown et al., 2006; Barker et al., 2012; Hii et al., 2012), but also potentially for exotic diseases if an incursion in northern Australia occurs.

Rabies is one such exotic disease that poses a threat. Australia is historically free from terrestrial rabies. However, rabies is currently spreading eastwards along the Indonesian archipelago (Tenzin & Ward, 2012) and an incursion into Australia is a genuine threat (Murray et al., 2012). The impact of a rabies incursion on animal and human health, animal welfare and native fauna in Australia is unknown, although hypothesized to be profound considering the emergence of rabies in new regions such as in Bali, Indonesia (Susilawathi et al., 2012; Putra et al., 2013) or Bhutan (Tenzin et al., 2010). The long incubation period complicates epidemiological tracing and selective control measures and once rabies enters the wildlife population, the control will become much more resource intensive. The close relationship between dogs and people in Aboriginal and Torres Strait Islander communities increases the impact of this zoonosis on society.

Theoretical epidemiological models are useful tools to estimate the impact of diseases in non-endemic populations. They allow simulation of the spread of disease and comparison of different control measures and are therefore of critical value for exotic disease preparedness. The accuracy of epidemic models of infectious diseases depends on the quality and suitability of their parameter values. Transmission parameters, depending on the contact rates within the susceptible population and the transmission probability given a contact, are the core of epidemic models but, they are difficult to estimate. When outbreak data are available, transmission parameters can be fitted to these data providing parameter estimation suitable to the outbreak scenarios. A series of epidemic rabies models have been published, including some reporting on transmission parameter values within dog populations (Kitala et al., 2002; Hampson et al., 2009; Zinsstag et al., 2009; Fitzpatrick et al., 2012; Hou et al., 2012; Zhang et al., 2012). However, these studies have been conducted in rabies endemic countries and therefore application of such parameter values for rabies free countries is questionable. When a disease is exotic and outbreak data are therefore lacking, transmission parameter values have to be estimated using a different approach. For free-ranging animals, contact rates can be deduced from estimated intensity of interaction based on the HR and UD (Germain et al., 2008; Cornélis et al., 2011).

The objective of this study was to estimate the HR and UD of free-ranging domestic dogs in remote Aboriginal and Torres Strait Islander communities (we use the term community dogs) in northern Australia. An additional aim was to compare the HR sizes deduced from the UD estimated by three different methods (MCP, LKDE and BRB). The study results provide information usable for transmission parameter estimation that ultimately can be incorporated into models of potential rabies spread in northern Australia.

MATERIALS AND METHODS

Study site and collaboration

The study was conducted in the Northern Peninsula Area (NPA) of Cape York, Queensland, Australia and involved all of the five NPA communities (Bamaga, Injinoo, New Mapoon, Seisia and Umagico), which are located in close proximity (2–4km) to each other. Close cultural ties exists between NPA and the residents on the Torres Strait islands, and movements to and from the islands are frequent. In total, 2272 people live in the five communities (2011 census data, http://www.censusdata.abs.gov.au). The dog population was counted to be 437 during a 2009 census, resulting in a human-dog ratio of 2.7–8.8 per community (Table 1). The study was supported by the NPA Regional Council and was been approved by the Animal Ethics Committee of the University of Sydney (# N00/7-2013/2/6015).

Table 1. Characteristics of the five Northern Peninsula Area communities and number of GPS units involved in a dog roaming study, Australia, 2013

Community	Geographic coordinates (lat/long)	Dog population size	Human population size	Dog-human ratio	No. of units distributed	No. of units for final analysis
Bamaga	-10.883 / 142.383	136	1046	2.7	16	15
Injinoo	-10.933 / 142.333	53	468	3.0	9	6
New Mapoon	-10.861 / 142.382	91	274	8.8	10	9
Seisia	-10.850 / 142.367	51	203	4.0	10	9
Umagico	-10.917 / 142.367	106	281	7.7	11	9
total		437	2272	5.2	56	48

The GPS unit and data collection

Fifty-six CatTraQ™ (www.mr-lee.com) GPS units were fixed on common nylon dog collars. The units were delivered in customized robust plastic cases of about 5x5x2cm size and 56 grams weight. The accuracy of the units was estimated by static tests in which seven units were attached for a period of 24 hours to a bottle of 9% sodium chloride solution to mimic the animal’s body. The mean distance of the recorded locations from their centroid ranged from 14.6m to 22.8m (mean 18.3m). The GPS units were pre-tested on two dogs each located in Camden, New South Wales and the NPA in June and July 2013, respectively, and produced satisfactory results. The time between two GPS fixes was set at 1 minute.

Data collection was conducted during the first week of September 2013, including a total of 56 units on 55 dogs (Table 1). One dog in Umagico lost its unit after less than 24 hours and the unit was replaced. The dogs were identified opportunistically while two teams of researchers and Animal/Environmental Management Workers drove through all parts of the communities searching for households with dogs and owners at home. When identified, the study was described to the dog owner in local language or English and a signed statement of consent was requested of each owner. Two dogs could not be caught to put on the collar and it was left with the owner to attach later. The gender, estimated age and breed of the dogs were recorded as well as the exact time the collar was fixed to the dog. When the collars were retrieved, the exact time when the collars were taken off the dogs and special events (e.g. hunting trips, excursions by car) while the dogs wore the collars were recorded.

Data analysis

GPS data were downloaded using the free software @TRIP PC (http://www.a-trip.com), exported as a csv-file separately for each unit and imported and stored in R (http://cran.r-project.org, version 3.0.1). R was used for further data cleaning and analysis. The coordinates were projected on a GDA94 / Map Grid of Australia zone 54.

Data cleaning: After extracting those GPS fixes from the dataset for the period during which the dogs were wearing the collars, those for which the calculated speed of the dogs was implausible were excluded. Two reasons were identified that can cause such a high estimated speed: the dog was taken by car or errors in the GPS unit. By plotting the GPS fixes, these two reasons could easily be differentiated because the car trips were depicted as a route whereas the non-accurate fixes were shown as single outliers. The speed threshold for the identification of the fixes which should be excluded was set at 20km/h, based on visual inspection of speed histograms and the assumption that a community dog is very unlikely to run at speeds of more than 20km/h during a one minute period. In case of an identified car trip all GPS fixes during the trip were excluded, including those with speeds between two consecutive fixes less than 20km/h.

Home rage and utilization distribution estimations: Three methods were applied to estimate the HR or UD using the R packages adehabitatHR and adehabitatLT (Calenge, 2011a, 2011b): the minimum convex polygon (MCP), the location-based kernel density estimation (LKDE) and the Biased Random Bridge (BRB) method. Two-sample Wilcoxon Rank Sum tests were used for comparison of the HR sizes calculated by the three different methods.

The MCP calculates the area enclosing all GPS fixes, unless it is specified that a given percentage of fixes farthest from their centroid are excluded from the calculation. The reason for excluding outliers (usually the 5% farthest fixes) is that the HR is defined as the area an animal commonly uses for normal activities (Burt, 1943) and that it might be that the animals occasionally roam to locations for reasons other than normal activities. For comparison of MCP with the LKDE and BRB methods, the MCP was calculated for four levels of outliers by excluding 50 (representing the core HR), 25, 15 and 5% of fixes.

The LKDE calculates the UD by placing a bivariate kernel function over each fix (Worton, 1989). The default method of the “kernelUD” function in the adehabitatHR package, which was applied to analyse the current dataset, is the bivariate normal kernel function using a reference bandwidth for the smoothing parameter based on the standard deviation of the coordinates. To derive the one-dimensional HR size from the two-dimensional UD for comparison with the results of the MCP and BRB methods, the "getverticeshr" function was used. This function calculates the minimum area in which the probability of re-locating the animal is equal to a specific percentage value (Calenge, 2011a). The percentage values (or isopleth levels) were set at 50, 75, 85 and 95%.

The BRB method places kernel functions over each step ("track") travelled by the animal between two consecutive GPS fixes and therefore follows the actual animals' movements. This method is described in detail by Benhamou (2011) and Benhamou and Cornélis (2010). The principle is that kernel smoothing is carried out considering not only recorded GPS fixes, but also interpolated locations, whose number depends on the time period between two successive recorded fixes. Additionally, the BRB method allows us to define an upper time limit between two GPS fixes, T_{max}, above which the respective fixes were excluded from the UD calculation. This is particularly useful for datasets, such as the present one, in which the time between two fixes differs greatly. We set T_{max} to 6 minutes, assuming that the dogs' behaviour is approximately homogenous (i.e. the dog moved according to a biased random walk) during this period. The BRB function incorporates tracks covering a distance shorter than a specified limit, L_{min}, by setting the diffusion coefficient D equal to 0 for these tracks (i.e. the animal does not move at all). The value of L_{min} depends on the GPS unit's noise (i.e. mean distance between two consecutive fixes), reflecting that even if the animal stays at the same location, the GPS fixes recorded might be different. We set L_{min} at 45m, which is twice the mean distance between two consecutive GPS fixes calculated from the seven units during the static test. Finally, a parameter defined as the minimum smoothing parameter, h_{min}, has to be specified to incorporate the GPS unit's classical noise (i.e. mean distance of the fixes and their centroid). We set h_{min} to 18 meters based on the results of the static test. Similar to the LKDE method, a one-dimensional HR was deduced from the BRB estimated UD using the "getverticeshr" function for the isopleth levels of 50, 75, 85 and 95%.

RESULTS

Collars retrieval and data cleaning

The collars were collected after they were active for a time period of 23–84 hours (mean 57 hours). Fifty-four of the 56 units could be retrieved, two collars were lost, the two collars left with the owner to attach to their dogs eventually were not attached and four units failed to record GPS fixes. This provided a total of 48 units for further data analysis (Table 1). Both sex were equally represented (25 females and 23 males) and age ranged from 7 months to 12 years (median 3 years).

From ten dogs, 4–93 GPS fixes were excluded due to car trips. The number of fixes excluded because of unreliable outliers involved 16 dogs and ranged from 2–11 per dog. In total, 56,727 GPS fixes were used for further analysis. There was high variability in the number of fixes between individual dogs (42 – 4493, median 1155). The median time difference between two consecutive fixes was 70 seconds (IQR 66–90 seconds, range 55 seconds to 22.9 hours). The proportion of time gaps longer than 10 minutes between two consecutive fixes among all fixes ranged between 0 to 8% (median 2%) per units.

HR and UD shape and sizes

The core HR size derived from the UD (i.e. within 50% isopleth) ranged from 62 m2 to 8.6 ha (median 0.27 ha) for the LKDE method and 0.2–1.1 ha (median 0.40 ha) for the BRB method (Table 2). When applying the MCP method and excluding the 50% of GPS fixes that were farthest away from the centroid, the HR size ranged from 0.02–22.7 ha (median 0.20 ha). The more extended HR (within the 95% isopleth of the UD) was estimated to be in the range of 0.9–86.8 ha (median 3.4 ha) and 2.1–40.5 (median 5.5 ha) by the LKDE and BRB method, respectively (Table 2). Using the MCP method excluding the 5% farthest fixes, the HR was estimated to range from 0.3–104.3 ha (median 4.0 ha).

Table 2. Measures of home range size estimation of 48 dogs based on three different methods: MCP (minimum convex polygon), LKDE (location-based kernel density distribution), BRB (Biased Random Bridge)

	MPC			LKDE			BRB		
	50	85	95	50	85	95	50	85	95
Median	0.20	1.03	3.39	0.27	1.34	3.40	0.40	2.21	5.53
IQR	0.12 – 0.71	0.64 – 3.43	1.87 – 11.04	0.16 – 0.79	0.83 – 3.78	2.04 – 9.34	0.32 – 0.60	1.44 – 3.61	4.23 – 9.47
5 – 95th percentile	0.06 – 5.10	0.31 – 23.60	1.04 – 47.95	0.06 – 5.59	0.49 – 19.86	1.20 – 44.01	0.26 – 0.87	0.94 – 6.13	2.68 – 22.57

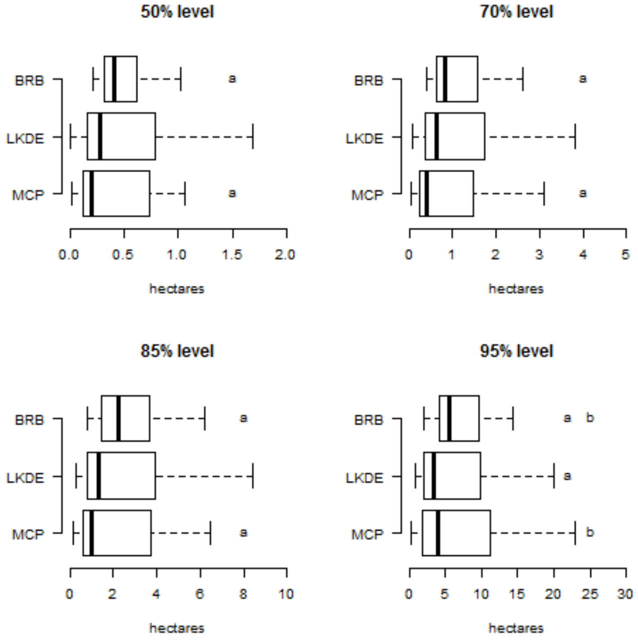

Fig. 1 Boxplots comparing the home range sizes estimated by the three different methods (minimum convex polygon (MCP), location-based kernel density estimation (LKDE) and biased random bridge (BRB)) for four isopleth levels in a dog GPS collaring study conducted in the Northern Peninsula Area of Australia, 2013. a/b = significant differences in HR size

HR sizes differed significantly between some of the methods used (Table 2, Fig. 1). The difference was significant for all of the four HR size levels calculated by MCP and BRB (W ≥ 876, p ≤ 0.043). The difference between the LKDE and BRB methods was only significant for the 95% isopleth level (W=831, p= 0.018), but the p-values were < 0.1 for all the other levels as well. However, the MCP and LKDE methods resulted in HR sizes that never differed significantly (p≥ 0.148). The HR sizes of all four isopleth levels were highly correlated between the different methods (Spearman correlation coefficients ≥ 0.89).

The shapes of the core UDs (50% isopleth) were essentially circular around the dog owner’s house, with the exception of nine dogs that alternated between two or three places. The shape of the 95% isopleth differed more between individuals compared to the shape of the core UDs, although the majority (60%) of the dogs still followed a circular roaming behaviour pattern around their core location. The UD of the six dogs of Injinoo are presented in Fig. 2 as an example of the variability of UD shapes.

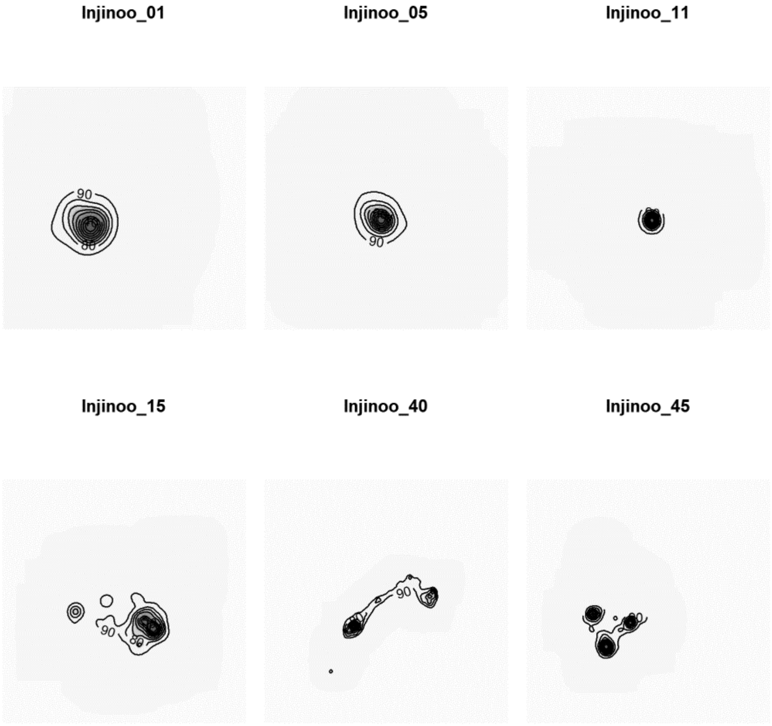

Fig. 2 Utilisation distributions with isopleth lines of the biased random bridge method of the six dogs of Injinoo, in a dog GPS collaring study conducted in the Northern Peninsula Area of Australia, 2013. The darker the area the more frequent used by the dog.

DISCUSSION

Forty-eight free-ranging domestic dogs were tracked in remote Aboriginal and Torres Strait Islander communities in northern Australia over a period of two to three days in order to collect data on their roaming behaviour. Such data can further be used to estimate intensity of interaction and contact rates between the dogs within and between communities, a foundation for estimating transmission parameters for infectious disease models.

This is, to the best of our knowledge, the first study tracking community dogs in tropical Australian regions. Only one study previously reported home ranges of free-ranging domestic dogs in Australia (Meek, 1999). Meek (1999) observed ten dogs in a NSW Aborigine community and the estimated HR showed great variation (1 – 2451 ha) using the MCP method. However, using the 60% isopleth the estimated core activity area was much more homogeneous (<0.02 – 0.93 ha). In Bali, Indonesia, (Gunata, 2011) estimated the average HR sizes of five male dogs (10 ha) to be significantly larger than for five female dogs (5 ha) using kernel density methods. The HR sizes estimated in the current study are comparable with these previous results, with a median core HR using the 50% isopleth ranging from 0.2 to 0.4 ha and a median of the 95% isopleth estimations ranging from 3.4 to 5.5 ha (depending on the methods used). The core HR shape was found to be, with a few exceptions, circular around the owner's house and therefore rarely overlapped with other individuals (except if they were living in the same or close-by household). However, as observed in other studies (Meek, 1999), some individuals roam much further and cover major areas of their communities or occasionally beyond with extended HR sizes up to 40–104 ha. Such dogs are of particular interest for infectious disease transmission and their roaming behaviour can therefore serve as a worst case scenario for the estimation of overall population contact rates and disease transmission parameters.

The external validity of the results with respect to the dog population in the NPA and to other Aboriginal and Torres Strait Islander communities in northern Australia is an issue. As no previous data were available, it is unknown how accurately the roaming behaviour of these dogs represents those of the community dog population. However, a diverse range of UD and HR sizes were observed, sex was equally represented and the age was well distributed. Further research would provide more detailed information on factors possibly influencing the dogs' roaming behaviour, such as season, age, sex or breed; for example by repeating a similar study with the same individuals in other seasons or extending the study site to other communities.

An additional aim of the study was to compare the outcome (HR size and shape) of different methods. The estimated HR size using LKDE and BRB only differed significantly for the 95% isopleth and not for the lower isopleth levels. The MCP method estimated HR sizes were never significantly different from those calculated by the LKDE approach, but were always significantly different from those calculated by the BRB approach, for all of the four examined isopleth levels. This indicates that the HR sizes estimated with the much simpler MCP method provided reasonable results considering all animals overall. However, the MCP method is much more sensitive to extreme values, which always occurred in our dataset: the largest HR size for all four isopleth levels was always found by the MCP method. The recently developed BRB method has several advantages. First, it is based on a more realistic assumption by considering the tracks rather than the GPS fixes to calculate the UD (Benhamou, 2011). Additionally, the method accounts for unequal time gaps between successive fixes by including interpolated fixes and the specification of Tmax. Second, it allows the user to specify several parameters – Tmax, hmin and Lmin – based on the data collected. Therefore, the BRB method better reflects the characteristics and quality of the GPS units by correcting the UD estimation for less precise records. It was observed that this method resulted in the lowest variability of HR sizes for all levels (Fig. 1) which might be explained by the user specific parameter setting.

Although the GPS units used provided useful information for the purposes of this study, they had some limitations compared to more accurate but much more costly wildlife GPS units. First, the accuracy of the units was found to be moderate, which might result in an overestimation of the HR and UD by all three methods. Second, several time gaps were observed when the unit was inactive for a longer period. Fortunately, the BRB method provides two approaches to deal with such gaps, by including interpolated locations between fixes and by defining Tmax above which two fixes are no longer interpreted as consecutive.

Studies on roaming behaviour, home range and utilisation distribution of domestic dogs in general are scarce, and absent – prior to this study – for community dogs in northern

Australia. The information generated by such studies has been identified to fill a knowledge gap in the most recent World Health Organization expert consultation on rabies (World Health Organization, 2013). In the present study, community dogs were found to roam predominantly around their home with a limited home range size. However, a minority of dogs cover large areas of their community, and exceptionally beyond. These far roaming dogs are of particular interest for infectious disease transmission. The recently developed BRB method was found to be most suitable for UD and HR estimation and therefore to provide a foundation to deduce interaction intensity between individuals. Further research investigating factors that influence roaming behaviour, estimation of contact rates and application of the BRB approach would provide more detailed information for ultimately estimating transmission parameter for infectious disease models, such as a rabies spread model in Australia

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WHAT ATTRACTS ELK ONTO BEEF CATTLE PASTURES? A DISTRIBUTION MODELLING APPROACH TO WILDLIFE-LIVESTOCK CONTACT STRUCTURE

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SUMMARY

In Southwest Alberta, beef cattle and free-ranging elk (Cervus elaphus) have similar habitat preferences. Species distribution modelling approaches were used to identify ranch management factors influencing the use of cattle pasture by elk. Data on elk locations were obtained from telemetry monitoring of seven elk herds. Landscape characteristics of pastures were obtained from GIS data, and management practices and elk observations data collected from 15 ranchers during interviews. Pastures used or not used by elk were compared in a logistic regression models and the intensity of use was modelled with a negative binomial regression. Cultivated hay land, mineral supplements, and winter feed-grounds were found to be important attractants of elk on cattle pastures, while pastures with manure fertilization and higher road traffic were less likely to be used by elk. These factors are relevant to assess the potential of inter-species pathogen transmission at this wildlife-livestock interface.

INTRODUCTION

The overlap of cattle pastureland and free-ranging elk (Cervus elaphus) home range in southwestern Alberta provides opportunities for inter-species interactions. Such interfaces between wildlife, livestock and humans are increasingly recognized to be of critical importance in the emergence or re-emergence of pathogens (Woolhouse et al., 2005; Jones, 2011). Eloquent examples of this have been described over the past decade in Michigan, USA (O'Brien et al., 2011) or Manitoba, Canada (Brook et al., 2013) with tuberculosis transmitted between free-ranging cervids and cattle, or in Wyoming, USA, where pockets of brucellosis are maintained at the interface between bison, elk and cattle (Thorne, 1992; Van Campen & Rhyan, 2010). The transmission of pathogens in a multi-host system is influenced by the spatio-temporal contact structure of these host species (Morgan et al., 2004). These spatio-temporal patterns of interaction are influenced by a number of habitat, behavioural, ecological and management factors (Van Campen & Rhyan, 2010). Understanding which of these factors influence interactions between two species, such as cattle and elk, is an essential step in assessing the risk of inter-species pathogen transmission. In Alberta, most beef cattle producers (88%) use a rotational grazing system (Rothwell, 2005). The total herd is often divided in sub-herds that rotate sequentially through the different pastures. Ranchers manage pastures to ensure that the nutritional needs of their livestock are met (energy and protein content of forage, mineral and water) (Alberta Agriculture Food and Rural Development, 2006) and these management practices may have consequences on the distribution of free-ranging elk.

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Species distribution models include a number of statistical techniques describing links between species occurrence or abundance and environmental and geographical characteristics (Elith & Leathwick, 2009). Despite previous evidence that resource availability for wildlife species is influenced by management practices in agricultural landscapes, (Hosten, 2007; Van Campen & Rhyan, 2010; Muhly et al., 2013), species distribution models have only in a few instances included human-managed landscape features (Brook & McLachlan, 2009).

In this paper, elk telemetry data, local ranchers' knowledge of elk pasture use, information on pasture management and cattle rotation practices, and GIS data were used to assess the association between elk use of pastures, and ranch management and environmental factors.

MATERIALS AND METHODS

Study area

The study area was in the foothills of the Canadian Rocky Mountains, in southwestern Alberta, Canada. The landscape is part of the montane ecotype characterized by rolling hills and a mosaic of grass and mixed forest. This ecosystem supports an elk population with 7 main herds ranging in size from 150 to 1300 animals. This area also supports a variety of human uses including logging, oil and gas, recreation and livestock production (Arc Wildlife Services Ltd, 2004). This area covers the municipal districts of Pincher Creek, Crowsnest pass, Willow Creek and Ranchland, while the western and southern sides are bordered by the British Columbia border and Waterton National Park, respectively.

Rancher interviews on pasture and elk observations

Ranches located throughout the study area, overlapping the elk home range and including over 100 adult cows were invited to participate to this study. Fifteen ranches accepted to participate and were interviewed. Orthogonal aerial photographs (obtained from Alberta Sustainable Resource Development 2006) of each ranch were printed on paper. The interview material (available upon request) included 39 questions of which 15 collected geo-referenced information about the ranch management and elk observations. For consistency, the interviewer drew the answer of these questions on the paper maps, following ranchers' descriptions. Information was collected on features known or suspected to attract elk, including: feeders, haystack, cultivated hay land, water sources, winter-feeding areas, and mineral supplements. Ranchers were asked to describe the location and their observations of elk use of these features. Manure fertilization areas were also located on the paper maps. Ranchers indicated the generalized pasture rotation strategy over a typical production cycle by delimitating their pastures on the aerial photographs and providing a number of head (thereafter converted in animal units (AU)) and period of grazing for each pasture. Ranchers were also asked to describe their observations of elk on their land and to identify winter and summer ranges, and calving grounds. Information collected during the interview were then digitalized in ArcGIS 10 (ESRI, Redlands, CA, USA) based on paper maps and interview audio-recordings.

Measure of pasture use by elk

From 2007 to 2012, 182 elk were captured in 7 herds by the Montane Elk Research Program (www.montaneelk.com) and equipped with GPS collars recording elk location every

2 hours. These elk herd are more extensively described in (Muhly et al., 2011; Ciuti et al., 2012a; Ciuti et al., 2012b; Muhly et al., 2013). Annual and seasonal pasture use by elk was assessed using the telemetry data: pastures with at least one intersecting elk location during the specified time period were recorded as “Used” while others were “Not Used” in binary variables. Winter and Summer seasons were defined between May 15th and December 15th based on migration movement patterns (Paton, 2012) and preliminary data seasonality analysis (data not shown). For each pasture, the intensity of elk use was estimated by the total number of intersecting elk locations, annually and seasonally.

Pasture use by elk was also defined from ranchers’ observations of elk on their land in a separate binary variable. If the pasture overlapped with a zone where ranchers reported elk sightings, the pasture was defined as used. In another binary variable, telemetry data and ranchers’ observations were combined and pastures were qualified as used if they intersected at least one telemetry location or rancher observation.

Characterization of pastures

Each pasture was characterized by: the mean ruggedness (derived from 30m digital elevation model, DMTI Spatial®, via University of Calgary), the total length of water access (National Hydrographic Network, obtained from GeoBase©), mean Normalized Difference Vegetation Index (NDVI) obtained from MODIS/Terra Vegetation Indices (NASA Land Processes Distributed Active Archive Center©) averaged over the period of interest, proportion of forest cover (derived from a 16 categories vegetation cover, McDermid et al., 2009), and the average traffic-weighted road density derived from a road network and previously published road traffic model (Northrup et al., 2012). Data collected from the rancher interviews were used to characterized the pasture by: presence of haystack, presence of mineral blocks at fixed sites, proportion of pasture used for winter-feeding or cultivated hay land, presence of feeders, and total number of cattle grazed over time (in AUM: Animal Unit Month). Seasonal data included the cattle use and the mean NDVI. In the year round model, only summer NDVI was used.

Modelling pasture use by elk

To assess the association between pasture characteristics and elk visits, two modelling approaches were used. In a first simplifying approach, the presence/absence of elk on pasture was modelled in logistic regressions. As the count of telemetry data intersecting each pasture is a better indication of the relative intensity of use, this continuous outcome was modelled with a negative binomial regression, appropriate for overdispersed count data. Models with all possible combinations of explanatory variables were systematically computed in the R package MuMIn (R Core Team 2013). Models were ranked by their AICc (corrected Akaike Information Criterion) to select the best performing model. Multi-model averaging was carried out using all models weighted by their Akaike weights. The importance of each variable was obtained by summing the Akaike weights over all models in which the variable appeared (Burnham, 2002). This analysis was repeated with the three outcome variables describing pasture use by elk based on telemetry data, rancher observations and a combination of both. The dataset was limited to private pastures, while grazing cooperatives and public land allotments were excluded from this analysis as management information were not available.

RESULTS

Annual pasture use

Based on the elk telemetry data, both logistic and negative binomial regression models indicated the importance of the positive association between pasture use by elk and ruggedness, summer NDVI values, total pasture area, proportion of cultivated hay and winter-feeding area on pasture, and negative association with the cumulative number of cattle on pasture (in AUM), and the average road density/traffic index (Table 1). Manure spreading only appeared as an important factor negatively associated to pasture use in the logistic regression, while the presence of mineral supplement was positively associated in the negative binomial regression. Similarly, rancher observations indicated the importance of ruggedness, pasture area, manure fertilization, and road density/use with the same direction of association but in contrast, total cattle use had lower relative importance with a positive regression coefficient. Using combined telemetry and rancher observations, among the variable with higher relative importance, the regression of ruggedness, summer NDVI, manure fertilization, hay land and feedstack were comparable. However, although directions of effect were identical, the association was stronger for road density/traffic, winter-feeding, water access, forest cover, and weaker for cattle use and pasture area.

Seasonal pasture use

Multi-model averaging for both winter and summer seasons indicated the positive association and high relative importance of the ruggedness, proportion of pasture used for winter-feeding, proportion of pasture covered by cultivated hay, and presence of mineral blocks (Table 2). In the logistic regression models, regression coefficient for the winter-feeding variable was higher during winter season and manure fertilization had a negative association with elk use in winter, while forest and seasonal NDVI were important and positively associated with elk use in summer. In the negative binomial regression for the summer season, fewer elk locations were observed in pastures that had higher cumulative use by cattle.

Despite a lower relative importance of the road density/traffic index compared to the previous variables, the relative importance was higher during winter season and it was included in the best logistic regression and negative binomial models selected by AICc ranking (Table 3). Road density/traffic was marginally negatively associated with pasture use by elk in both logistic (β=-0.14, p=0.079) and negative binomial (β=-8.742e-02, p=0.095) models. The presence of feedstack on pasture appeared to have a relatively low importance and was not included in any of the best seasonal models.

Table 1. Annual pasture-use models

Use definition	Elk telemetry								Rancher interviews				Telemetry/Interview combined			
Model	Logistic regression				Negative binomial regression				Logistic regression				Logistic regression			
Variables	Rank[a]	w_+[b]	β[c]	S.E.[d]	Rank	w_+	β	S.E.	Rank	w_+	β	S.E.	Rank	w_+	β	S.E.
Ruggedness	1	**1.00**	0.303	6.00e-02	1	**1.00**	9.87e-02	1.85e-02	2	**0.79**	7.74e-02	3.68e-02	1	**1.00**	0.365	7.56e-02
Summer mean NDVI	2	**0.99**	1.75e-03	5.02e-04	5	**0.70**	3.92e-04	1.91e-04	10	0.33	2.76e-04	3.51e-04	5	**0.87**	1.31e-03	5.62e-04
Total cattle (AUM)	3	**0.88**	-3.61e-04	1.52e-04	8	**0.56**	-1.15e-04	6.94e-05	5	0.43	1.49e-04	1.27e-04	10	0.27	-2.95e-05	1.51e-04
Pasture area (m^2)	4	**0.80**	5.03e-07	2.34e-07	2	**0.98**	2.62e-07	7.60e-08	3	**0.59**	2.85e-07	1.88e-07	8	0.37	2.08e-07	2.26e-07
Manure fertilization (%)	4	**0.80**	-1.48	0.711	9	0.44	-0.450	0.368	4	**0.56**	-0.953	0.614	3	**0.89**	-1.77	0.747
Road use/density	5	**0.73**	-0.170	8.95e-02	6	**0.66**	-9.17e-02	4.92e-02	1	**0.98**	-0.284	0.109	2	**0.95**	-0.258	9.89e-02
Hay land (%)	6	**0.66**	1.01	0.576	4	**0.73**	0.545	0.264	11	0.28	-0.136	0.471	7	**0.69**	1.07	0.578
Winter feeding (%)	7	**0.64**	0.933	0.535	7	**0.58**	0.404	0.249	7	0.36	0.443	0.459	4	**0.88**	1.41	0.606
Water access (m)	8	0.43	1.95e-04	1.75e-04	11	0.28	-1.53e-05	6.87e-05	9	0.34	6.99e-05	1.26e-04	9	0.34	1.12e-04	1.51e-04
Forest cover (%)	9	0.34	0.786	1.02	10	0.36	0.234	0.442	12	0.27	-7.62e-02	0.677	6	**0.74**	2.55	1.38
Mineral block (0/1)	10	0.31	0.308	0.423	3	**0.80**	0.380	0.173	8	0.35	0.302	0.330	10	0.27	-9.98e-02	0.476
Feedstack (0/1)	11	0.27	0.159	0.403	12	0.26	-4.31e-02	0.202	6	0.40	0.414	0.363	10	0.27	0.181	0.423

[a] Rank according to AICc (Akaike Information Criterion corrected for small-sample bias)

[b] Cumulative Akaike weights of variables (sum of Akaike weights of models containing the variable); variables with $w_+>0.5$ are bolded

[c] Multi-model averaged regression coefficients (weighted by Akaike weights of each model)

[d] Standard Errors of regression coefficients (adjusted for model selection uncertainty)

Table 2. Seasonal pasture-use models

Use definition	Winter								Summer							
Model	Logistic regression				Negative binomial regression				Logistic regression				Negative binomial regression			
Variables	Rank[a]	w_+[b]	β[c]	S.E.[d]	Rank	w+	β	S.E.	Rank	w+	β	S.E.	Rank	w+	β	S.E.
Ruggedness	1	**1.00**	0.274	5.19e-02	1	**1.00**	0.121	1.94e-02	1	**1.00**	0.261	5.23e-02	1	**1.00**	0.123	2.31e-02
Winter-feeding (%)	2	**0.86**	1.15	0.500	5	**0.62**	0.452	0.264	5	**0.64**	0.892	0.494	9	0.37	0.305	0.307
Hay land (%)	3	**0.84**	1.16	0.524	4	**0.64**	0.503	0.275	4	**0.96**	1.63	0.561	3	**0.91**	0.871	0.340
Mineral block (0/1)	4	**0.83**	0.824	0.365	2	**0.98**	0.532	0.171	3	**0.97**	1.26	0.420	5	**0.58**	0.362	0.221
Manure fertilization (%)	5	**0.68**	-1.30	0.731	8	0.39	-0.430	0.414	11	0.29	-0.408	0.693	11	0.29	-0.237	0.431
Road use/density	6	0.49	-0.117	8.60e-02	6	0.47	-7.30e-02	5.25e-02	9	0.33	-6.97e-02	8.89e-02	8	0.39	-6.64e-02	5.90e-02
Forest cover (%)	7	0.45	0.919	0.725	9	0.33	0.295	0.404	2	**0.99**	3.17	0.963	7	0.42	0.573	0.571
Pasture area (m^2)	8	0.43	2.07e-07	1.85e-07	3	**0.97**	2.59e-07	7.11e-08	6	0.54	2.73e-07	1.89e-07	2	**0.94**	2.91e-07	9.44e-08
Water access (m)	9	0.37	1.10e-04	1.25e-04	10	0.29	-1.57e-05	7.28e-05	10	0.31	4.79e-05	1.50e-04	10	0.30	-7.45e-06	9.30e-05
Seasonal cumulative cattle (AUM)	10	0.33	-1.93e-04	2.38e-04	12	0.26	5.16e-06	1.23e-04	8	0.35	-1.60e-04	1.76e-04	6	**0.56**	-2.06e-04	1.20e-04
Seasonal mean NDVI	11	0.28	-1.96e-04	6.74e-04	7	0.45	-3.45e-04	2.48e-04	7	**0.52**	7.81e-04	5.37e-04	4	**0.81**	6.26e-04	2.45e-04
Feedstack (0/1)	12	0.26	4.55e-02	0.384	11	0.27	-6.66e-02	0.217	12	0.26	0.111	0.406	12	0.26	-3.18e-02	0.253

[a] Rank according to AICc (Akaike Information Criterion corrected for small-sample bias)

[b] Cumulative Akaike weights of variables (sum of Akaike weights of models containing the variable); variables with $w_+ > 0.5$ are bolded

[c] Multi-model averaged regression coefficients (weighted by Akaike weights of each model)

[d] Standard Errors of regression coefficients (adjusted for model selection uncertainty)

Table 3. Best annual and seasonal models selected by corrected Akaike Information Criterion

Period	Model type[a]	Best model[b]	df[c]	logLik[c]	AICc	Adjusted R^2(%)
Annual	LR	[d]HayLand+Rugg+Manure+Road+Wintfeed +Area+Sndvi+TotAUMYr	9	-112.6	244.1	46.2
	NB	HayLand+Rugg+Manure+Road+Wintfeed+Mineral +Area+Sndvi+TotAUMYr	11	-441.1	905.3	28.8
Winter	LR	HayLand+Rugg+Manure+Road+Wintfeed+Mineral	7	-131.7	277.8	33.3
	NB	HayLand+Rugg+Manure+Road+Wintfeed+Mineral +Area	9	-401.6	822.0	28.1
Summer	LR	HayLand+Rugg+Wintfeed+Mineral +Area+Sndvi+Forest	8	-120.3	257.2	42.3
	NB	HayLand+Rugg+Mineral +Area+Sndvi+TotAUMsum	8	-371.5	759.7	24.1

[a] LR= Logistic regression; NB= Negative binomial regression

[b] According to Akaike Information Criterion corrected for small-sample bias (AICc)

[c] Degrees of freedom (df); log-likelihood (logLik)

[d] Area=pasture area; HayLand=cultivated hay land; Manure=manure fertilization; Mineral= mineral supplement; Road= road density/traffic; Rugg=ruggedness; Sndvi=summer NDVI; TotAUM= cumulated annual cattle use in Animal Unit Month; Wintfeed= winter-feeding

Rancher observations

Among pastures reported by ranchers to be used by elk, 79% were in agreement with telemetry data. However, for 29% of all pastures, the classification as ‘used’ or ‘not used’ was in disagreement between ranchers and telemetry data. The Kappa agreement score between pasture-use defined by telemetry or rancher observations was 0.41 (95% CI = 0.30 - 0.53), corresponding to a 'moderate' agreement.

Of 15 ranches, 11 ranchers observed occasional direct contact between cattle and elk (as defined by the presence of both species within 2 or 3 meters of one-another). None of the ranchers intentionally fed elk on their property. Twelve of 15 ranchers expressed concern over elk overgrazing pastures, and consuming cultivated hay and crops. Eight of 15 ranchers observed elk using mineral supplement blocks. Nine of 15 ranchers reported fencing their haystack with elk-proof fences. January and February were identified as the months with higher use of haystacks by elk by 7 and 6 ranches, respectively. Of these ranches reporting elk use of haystack in January and February, 4 and 3 had elk-proof fences, respectively.

DISCUSSION

Elk preference for more rugged, higher biomass (higher NDVI), water access and higher forest cover were consistent with previously reported elk habitat preferences (Kittle et al., 2008; Baasch et al., 2010; Proffitt, 2010). The importance of forest cover was higher in summer, consistently with typical elk summer range preference (Morgantini & Hudson, 1989). The NDVI was positively correlated with elk use in summer, but had a lower importance in winter. In winter, NDVI value of snow-covered areas is close to zero while non-zero values correspond to coniferous forest, providing a poor proxy for available biomass in winter compared to summer (Hird & McDermid, 2009). There was an important negative effect of road traffic on pasture use, as observed in other studies (Rowland, 2004; Proffitt, 2010; Ciuti et al., 2012).

Winter-feeding was found to increase the risk of elk using cattle pasture, particularly during winter. Winter- feeding is usually done until the end of the calving season on pastures with road access and closer to the ranch facilities, increasing the cattle density during this period. Although the higher human activity may deter elk as suggested by the repulsive effect of road traffic, some ranchers have reported elk using winter feed, particularly when the snow pack is deep and resources scarce. One rancher even reported “elk chasing cows off the feed”. Winter is also a period of conglomeration of female and young elk into larger herds. Feed grounds have been shown to increase the risk of brucellosis transmission in elk in the Greater Yellowstone Area (Cross et al., 2007; Scurlock & Edwards, 2010) and bovine tuberculosis in deer in Michigan (Garner, 2001; Rudolph et al., 2006). The combination of higher elk and cattle densities and the presence of common attractants may increase the risk of pathogen transmission by direct or close indirect contact during periods of supplemental feeding.

When mineral blocks had fixed locations year-round (as opposed to rotating with cattle), they were found to increase the risk of pasture use by elk in both winter and summer. This is consistent with rancher observations of elk using mineral supplements. Similarly, local biologists reported the common use of natural mineral licks by both elk and cattle within a few hours of one-another (Mike Jokinen, personal communication). Mineral blocks may therefore be features attracting elk in closer proximity to cattle, and therefore facilitating potential indirect pathogen transmission.

As reported by ranchers and confirmed by this analysis, elk seemed attracted by cultivated hay land, more so during summer (corresponding to period of growth). However, fertilization by manure spreading, typically done on hay land, had a negative association with elk use, as previously documented with other wildlife species (Smith et al., 2008). The extent to which manure fertilization may result in faecal-oral transmission may depend on the level of avoidance of faeces-contaminated pastures and the respective strength of these opposite effects.

There was no association between the presence of haystacks and pasture use by elk. To prevent access of wild cervids to their haystack, most ranchers use elk-proof fencing, which may explain this absence of association. Despite these measures, some ranchers reported damages made by elk on haystacks, particularly during months of January and February, concomitant with the period when this stored hay is used for supplemental winter-feeding. Despite the absence of evidence for haystacks to influence pasture use by elk, occasional intrusions of elk into haystacks may result in pathogen transmission during feeding period through feed contamination.

Pastures with higher cattle use were less likely to be used by elk, as demonstrated in the annual pasture use models and summer negative binomial model, thus possibly reducing the risk of direct pathogen transmission. However, a finer temporal scale analysis is required to identify if this negative association is due to the displacement of elk by cattle (Hosten, 2007), or elk avoidance of over-grazed areas (Coe, 2001). These aspects are important for our understanding of the risk of pathogen transmission, and of the possibility of competition for resources between species.

For both telemetry and ranchers' observations, the year-round models indicated the importance of ruggedness, road traffic, pasture area and fertilization with similar effect directions. However, there was overall a lower agreement between telemetry or rancher reports than previously observed (Brook & McLachlan, 2009). It is important to note that there may be a number of biases in ranchers' observations due to their recall ability, their perception of what a significant event is, and their primary focus on their land or specific parts of their land. However, telemetry data also suffer a number of biases, as it only provides information on collared individuals and may under-represent sub-groups of the elk population where no collar was deployed. As a consequence, it is not possible to be sure that pasture classified as not-used never had elk during the observation period (Keating et al., 2004). Using a combination of telemetry data and reported observations may be an alternative to address the insufficiencies of each data source and reduce classification bias. Using count data in a negative binomial regression may also reduce the effect of this bias by modelling the relative intensity of use instead of the simplistic used/not used dichotomy.

Another source of uncertainty in rancher-reported information is related to the consistency of pasture rotation strategy from year to year. If some ranchers have very consistent strategies and use the same schedule for their pasture every year, it is more flexible for others. Some ranchers adapt their strategy to grass growth and pasture carrying capacity, and their schedule of pasture use and head number may vary between years. This may lead to biases in the estimate of cattle number on each pasture. However, ranchers are fairly consistent in their seasonal use of pastures (winter pasture / summer pasture), and this bias may therefore not have a significant impact at this temporal scale.

Finally, it is important to recognize that this study identified practices influencing the use of cattle pasture by elk, but did not identify interaction *per se*. 'Interaction' can take different definitions depending on the mode of transmission of a pathogen. If this analysis points out to features and management practices that may influence host species interactions, it remains to clearly define different types of interaction relevant to different transmission routes and measure them precisely to obtain a more accurate estimate of the risk of inter-species pathogen transmission.

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A NEW MEASURE FOR PROBABILITY BASED ASSESSMENT OF RISK OF DISEASE INTRODUCTION IN ANIMAL NETWORKS

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SUMMARY

In the design of surveillance of contagious livestock diseases, there is often a desire to target high risk herds. This paper presents a new method to assess herd disease risk in contact networks. By this method a probability of disease ratio (PDR) is calculated. This measure is an improvement to current network measures as it takes direction, temporal order, movement size and probability of disease into account, and can be used for e.g. risk-based surveillance, in the classification of herds in control programmes or in risk factor studies. The usefulness of this method is explored by calculation of different versions of the PDR for Swedish cattle herds in simulated datasets, real animal movement data, and results from a survey of Q-fever in dairy herds.

INTRODUCTION

Direct and indirect contacts between herds may lead to the spread of infectious animal diseases. In particular, movement of live animals can be considered a risk to introduce infection in new herds and regions. Therefore, the number of contacts, or the degree of contact between herds, is of interest in different types of epidemiological investigations. For the purpose of disease surveillance and early detection of disease, there is often a special interest in identifying and targeting herds with an increased probability of infection. In this context, herds that purchase many live animals can be seen as a high risk stratum of the population.

One way to assess the magnitude of between-herd contacts is by applying network analysis, and in recent years this methodology has become established in veterinary epidemiologic research. The different measures that are used have usually been developed within other fields of research and therefore there is a need to evaluate their usefulness within the veterinary field, and also to develop new measures for specific disease control purposes.

Two commonly used measures in network analysis are in-degree and out-degree (Wasserman et al., 1994), which correspond to simple counts of the number of ingoing and outgoing contacts. However, second-order contacts, i.e. one or several steps further back or forward in the chain of contacts, can be expected to play an important part in the process of disease spread, and therefore, these measures may have limited ability to accurately reflect the increased risk of disease within the chain of contacts. A more recent measure, the ingoing infection chain, includes the whole contact chain and also takes into account the temporal

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sequence of contacts (Nöremark et al., 2011). It has been used to analyse contacts between cattle herds as regards live animal trade and potential introduction of disease. However, besides number of herds in the contact chain, the risk of disease spread due to purchase of animals can be expected to vary considerably depending on the expected prevalence of disease and the number of animals purchased. In addition, the expected prevalence may be very different across different strata of the population. Frequent purchases from low risk herds may thus be safer compared to just a few purchases from a high risk herd. In order to better reflect the actual risk from animal movements, these aspects also need to be incorporated in the analysis.

In this study, a new measure that includes the direction and the temporal order of contacts, and the number of animals purchased from each herd was developed. The probability of disease at different levels in the population was also incorporated. The aim of the study was to examine the herd variation of this measure and to investigate its usefulness by applying it on both simulated datasets and real animal movement data from Swedish dairy herds included in a bulk milk survey for *Coxiella burnetii*.

MATERIALS AND METHODS

The method described in this paper expands the network measure ingoing infection chain (Nöremark et al., 2011). One example of a contact chain, and its in-degree and ingoing infection chain, is given in Fig. 1.

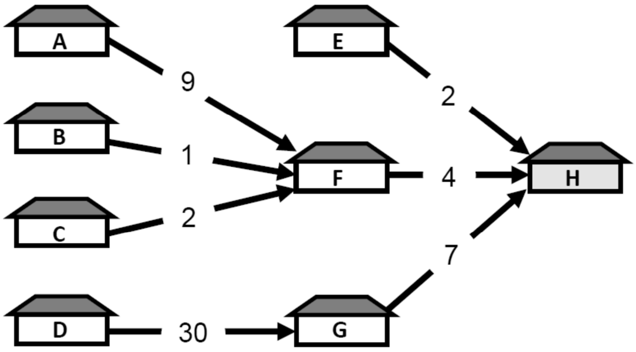

Fig. 1 Contact pattern of a hypothetical herd (H).The herd purchases 13 animals from 3 herds. By this trade there are also indirect trading contacts with 4 other herds. On herd level, the in-degree of herd H in this example is 3 (herd H has direct contact with 3 herds) while the ingoing infection chain measure is 7 (the herd has direct or indirect contact with 7 herds in total)

This type of network analysis is combined with algorithms used to estimate the probability of infection on animal and on herd level, as commonly used in veterinary surveillance (Martin et al., 1992; Martin el al., 2007). The output measure is not a general evaluation of the degree of contacts between units but instead a disease-specific relative ratio of the increased probability of infection due to movement of animals. The input parameters that are applied in the calculations and that need to be set are the relevant time periods and the prevalence of disease on different levels (typically within herd and between herds). In case there is prior information about prevalence discrepancies within the population, (e.g. between regions), this is easily incorporated by applying different input for different strata.

Definition of time periods and sorting of events

Calculations are based on animal movement data with information on source herd, destination herd, date of movement and number of animals moved. The output measures relate to one point in time (t_i) and is based on a defined time period (Δt_{total}) preceding this date. Before estimation, a dataset has to be created by cleaning and sorting animal movement data from the whole extent of Δt_{total}. Similar to the ingoing infection chain measure, the holdings that are connected to the holding of interest through animal movements (i.e. animals entering that holding) are identified. Both direct and indirect movements, and the order in which contacts occur, are taken into account, and the full contact chain of each herd of interest is identified by backward tracing. Here, only movements that occurred within a certain window of time (Δt_{epi}) are allowed in the final dataset. The parameters Δt_{total} and Δt_{epi} are set to reflect the disease of interest.

The time-related parameters t_i, Δt_{total} and Δt_{epi} are illustrated in Fig. 2. The identification of herds in the ingoing contact chain of the hypothetical herd A is made by retrospective backward tracing, starting from t_i. All direct contacts within Δt_{epi} are considered relevant. Indirect contacts of relevance are identified by stepwise backward tracing during Δt_{total}. In each step the time limit of Δt_{epi} is applied. In this example, contacts through movements of animals from herd B to herd A, and from herd C to herd B is included in the analysis. The contact between herd D and herd C is not considered relevant, as it occurs outside Δt_{epi}, counting from the time point of the contact between herd C and B.

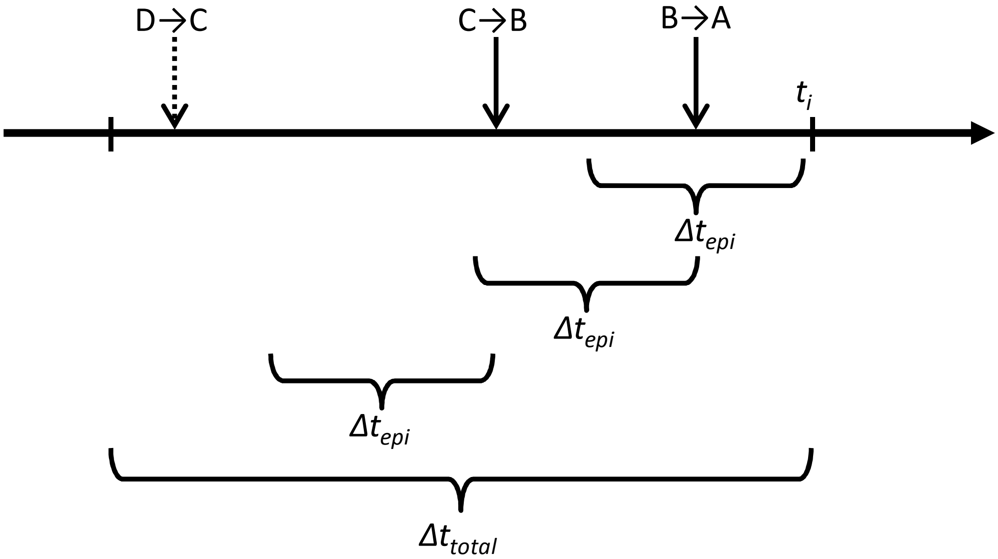

Fig. 2 Timeline illustrating the temporal relationship between t_i, Δt_{total} and Δt_{epi}. Direct contacts that occur during a time interval (Δt_{epi}) before t_i are included in the estimation of the relative increase in probability of disease due to introduction of animals. Vertical arrows indicate time points when movement of animals between herds (A, B, C and D) has occurred

When the relevant contact chains have been identified, the number of animals moved from a source herd to a destination herd can be summarised. The summary is made for each interval in time when the source herd had an unchanged probability of disease – e.g. each period where the source herd did not purchase any new animals. For many diseases there are several different routes of transmission but because this measure is meant to reflect degree of contact through the introduction of animals, only live animal trade is considered.

Calculation of the probability of disease along the contact chain

When the contact chains of the units of interest have been identified, the probability of introducing at least one infected animal $P(intro\ inf)_h$ in the destination herd is calculated for each movement:

$$P(intro\ inf)_h = 1 - (1 - P(D+)_a)^n \tag{1}$$

where n is the number of animals introduced and $P(D+)_a$ the probability of disease within the source herd, i.e. the within-herd prevalence. The destination herd's total probability of introducing disease through the introduction of animals $P(intro\ inf)_{total}$ is then calculated based on this and the general probability of disease at the herd level, $P(D+)_h$, i.e. the between-herd prevalence:

$$P(intro\ inf)_{total} = 1 - \prod\big(1 - (P(intro\ inf)_h \times P(D+)_h)\big) \tag{2}$$

These formulas (1 and 2) can be used to illustrate how the probability to introduce at least one infectious animal varies with the probability of infection within, as well as between, herds. Such illustrations (not shown) clarify how the same contact pattern can constitute very different risks depending on the infection and population of interest.

After each movement, the new expected prevalence $P(D+)_{exp}$ of the destination herd is the $P(D+)_h$ and the probability due to the introduction of animals from other herds:

$$P(D+)_{exp} = 1 - [(1 - P(D+)_h) \times (1 - P(intro\ inf)_{total})] \tag{3}$$

The calculations are performed in a stepwise manner where the probability of disease at each step in the chain of contacts influences the next. One way to do this in practice is to sort the final dataset by time and then perform the calculation for the first movement and then update the probability of disease of the particular destination herd throughout the dataset before continuing the calculation for the next point in time.

Finally, the probability of disease ratio (PDR) reflects the relative increase in probability of disease:

$$PDR = \frac{P(D+)_{exp}}{P(D+)_h} \tag{4}$$

In this last step of the calculations, differences (in the probability of disease) imposed by other risk factors than animal movements can be incorporated. Depending on the purpose of the study and whether information about risk differences is available, the risk factor can be reflected in the numerator and denominator in various ways. In this paper, three combinations are applied; average (A), risk-to-risk (RtR) and risk-to-average (RtA). The corresponding alternative ratios are; $\mathrm{PDR_A}$, where equal herd-specific probabilities of disease are applied throughout the calculations, $\mathrm{PDR_{RtR}}$, where known differences in probability of disease across herds are applied both in the numerator and denominator, and PDR_{RtA}, where herd-specific differences in probability of disease are included in the calculation of $P(D+)_{exp}$ but the average $P(D+)_h$ is used as the denominator instead of a herd-specific $P(D+)_h$. PDR_A and PDR_{RtR} will always be ≥1, while PDR_{RtA} will be ≥0 and <1, or >1, if the herd has a decreased or increased probability of disease due to trade compared to the average.

In real life, the size of the relevant contact network of a herd will be smaller or larger, depending on the disease of interest (i.e. the chosen Δt_{epi} and Δt_{total}). Infections that progress slowly will require a Δt_{epi} of one to five years, or even more, while a shorter Δt_{epi} would be applicable for highly contagious acute diseases. Figure 3 shows the PDR of herd H (from Fig. 1) when applying different combinations of within-herd prevalence and between-herd prevalence in the calculations given the contact pattern described in Fig. 1.

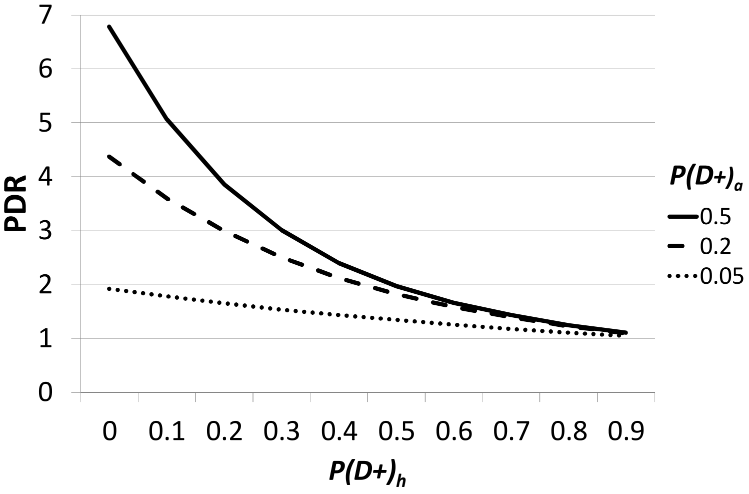

Fig. 3 The probability of disease ratio (PDR) for the hypothetical herd H based on direct and indirect contacts trough movements of animals, at different between-herd prevalence ($P(D+)_h$) and within-herd prevalence($P(D+)_a$).

Herds in Q-fever survey

The method described in this paper was applied on 119 dairy cattle herds in Gotland, Sweden. Bulk milk samples from these herds had been collected and tested for the presence of *Coxiella burnetii* (*C. burnetii*) in June 2011. This bacterium causes Q-fever in livestock which may lead to abortion, stillbirth and metritis, and it is also of interest due to its zoonotic potential (Angelakis & Raoul, 2010, Agerholm, 2013). The samples were analysed using PCR and 64 out of 117 herds (55%) tested positive. Previous studies have shown that the overall herd prevalence of Q-fever in cattle in Sweden is 8% and that Gotland has a significantly higher prevalence of Q-fever compared to other regions (data not yet published).

Information about all movements of cattle between cattle holdings in Sweden from June 2007 till May 2011 (i.e. four years) was retrieved from the national cattle database at the Swedish Board of Agriculture (described in detail by Nöremark 2010 and Nöremark et al. 2011). Based on mandatory reporting made by the farmers, this database contains information on identity of the animal, date of movement, reason for movement, and the identities of the source holding, the destination holding, and the reporting holding. The movements are reported by both the seller and the buyer and in theory there should be duplicate reports of each movement. For this study, only the reports of the buying farmer were used. In total, 1,131,199 movements from 19,134 source holdings to 20,387 destination holdings were included in the final dataset. The total number of active cattle holdings in June 2007 and May 2011 was approximately 28,000 and 26,000, respectively.

Based on the reported movements, the full ingoing contact pattern for each herd was identified, events were sorted and a PDR was calculated as described above. Different combinations of alternative input values for t_i, Δt_{total}, Δt_{epi}, and $P(D+)_a$ were used: t_i was set to May 31st 2011, Δt_{total} was set to 2, 3, or 4 years, Δt_{epi} was set to 90, 180, 365 or 730 days, and $P(D+)_a$ was set to 0.05, 0.10, 0.20, or 0.40. For $P(D+)_h$, prevalence estimates available from a previous national survey was used. The overall between-herd prevalence of 0.08 for all herds, as well as herd-specific prevalence between 0.01 and 0.53, based on region (county), was used.

In addition, the in-degree (Wasserman et al., 1994) and the ingoing infection chain (Nöremark et al., 2011) were calculated, and these measures were based on movements that occurred between June 1st 2009 and May 31st 2011. The association between the contact measures and presence of *C. burnetii* in bulk milk was tested using Wilcoxon rank-sum test.

Software

Data management and statistical analysis was performed using Stata (StataCorp. 2009. Stata Statistical Software: Release 11.2. College Station, TX: StataCorp LP.) and R version 2.15.1 (R Development Core Team 2012 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.). PDR was calculated using Stata while in-degree and ingoing infection chain were calculated using the EpiContactTrace package (Widgren and Nöremark, 2012) in R.

RESULTS

As expected, the PDR for each herd in the Q-fever data varied depending on the input chosen. For all combinations, the difference in PDR between Q-fever negative and Q-fever positive herds was statistically significant. One of the combinations for which this significance was strong, and the one that was considered to make most sense from a biological perspective, was Δt_{total} set to 3 years, Δt_{epi} set to 365 days, and $P(D+)_a$ set to 0.05. In total, 53 (45%) of the herds had not purchased any cattle within the study period. The distribution of the calculated PDR for the remaining 64 herds based on this combination is given in Fig. 4a (PDR_A) and 4b (PDR_{RtR}). Because all herds in Gotland belong to the same risk category (i.e the same county), the distribution of PDR_{RtA} (not shown) is the same as PDR_{RtR}, but with a range from 6.6 to 12.5 instead of 1.0 to 1.9.

The difference between test negative and test positive herds was significant for PDR_A ($p=0.002$), PDR_{RtR} ($p=0.003$), PDR_{RtA} ($p=0.003$) and also for in-degree ($p=0.030$) and ingoing infection chain ($p=0.033$). Box plots of PDR_{RtR} (with the input combination described above), in-degree, and ingoing infection chain for the different herd categories in the Q-fever data, are given in Fig. 5 a-c.

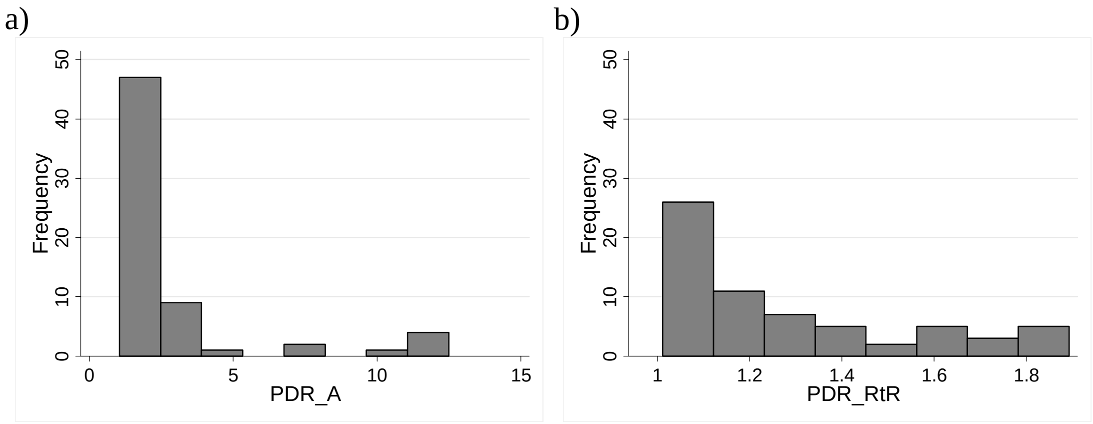

Fig. 4 Distributions of a) PDR$_A$ and b) PDR$_{RtR}$ among herds with at least one relevant purchase within the study period.

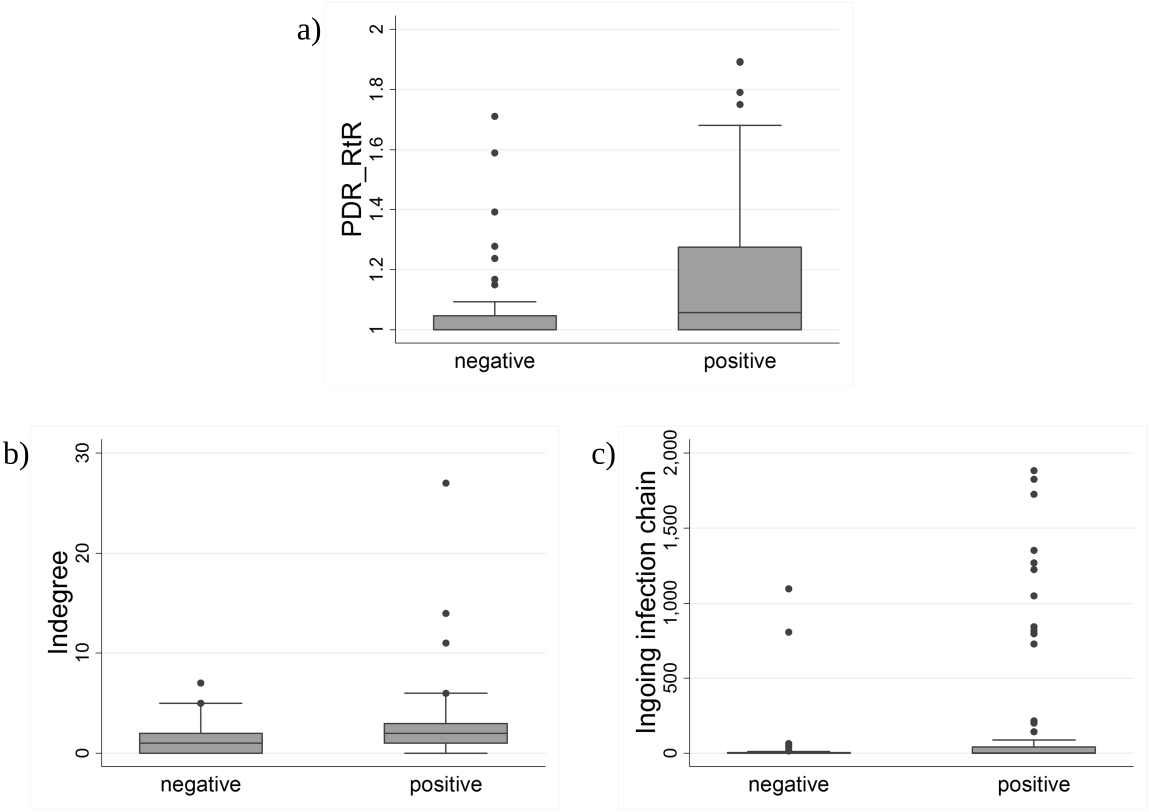

Fig. 5 a) PDRRtR, b) indegree and c) ingoing infection chain by herd category (test negative or test positive as regards C. burnetii in bulk milk).

Out of all Swedish herds that had at least one purchase in the full movement dataset within 365 days before May 31st 2011, 218 herds where above the 95th percentile (n=713) of both PDR$_A$, PDR$_{RtR}$ and PDR$_{RtA}$. For herds that were above the 95th percentile of PDR$_{RtR}$, 326 (45%) out of 716 were below the 95th percentile of PDR$_A$ (Fig. 6a). Moreover, some herds (n=498) that were above the 95th percentile of PDR_{RtA} had values below the 95th percentile of PDR_{RtR} (Fig. 6b).

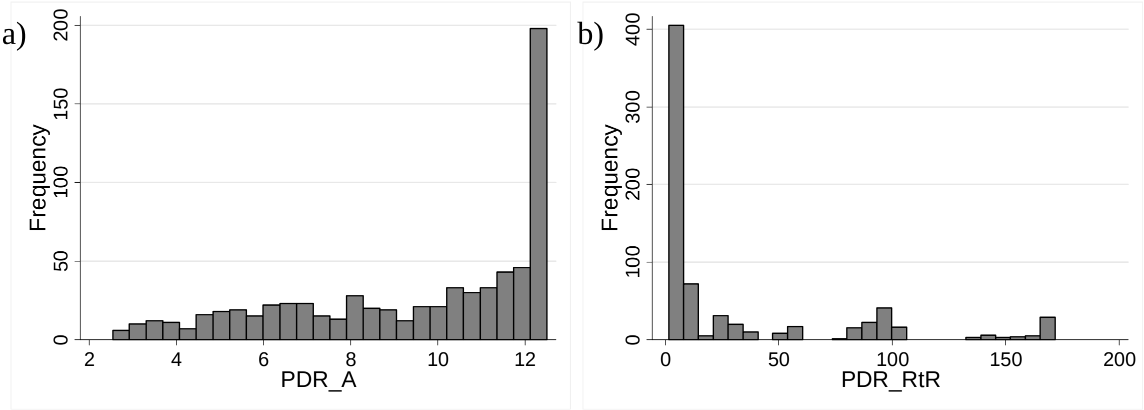

Fig. 6 Distribution of a) PDR_A among herds with a PDR_{RtR} above the 95th percentile, and b) PDR_{RtR} among herds with a PDR_{RtA} above the 95th percentile.

DISCUSSION

The structure and extent of contact patterns between herds through live animal trade come in an infinite number of variations. The risk of introducing infectious disease due to such direct and indirect contacts will depend, not just on the number of contact herds, but also on the number of animals purchased from each herd. In addition, the effective increase in risk will depend on how prevalent the disease in question is. In this paper, we describe and illustrate how this can be incorporated in the estimation of the degree of ingoing contacts in a herd. The method can be used e.g. in the design of animal health surveillance, where there is often a desire to target high risk herds. Such risk-based approaches result in better allocation of resources and improve the performance of surveillance activities. In can also be applied in control programmes where herds need to be classified according to different risk parameters, such as live animal trade, or to represent influential contacts in risk factor studies.

The results show that the PDR is able to capture the different circumstances related to disease prevalence and animal trade contact patterns. In general, the PDR decreases with decreasing within-herd prevalence and increasing between-herd prevalence. For example, if a disease is widely spread within infected herds, the purchase of just a few animals from an infected herd will result in very high probability of introducing disease into the destination herd. Moreover, when disease is widely spread between herds and the probability of disease on herd level is already high, purchase of animals will not have a strong influence on the destination herd’s probability of disease. On the other hand, if the between-herd prevalence is small, the relative increase in probability of disease due to purchase of animals, will be larger.

Common sense suggests that the risk associated with live animal trade will depend on whom you are trading with. Difference in expected disease status of the selling herds is easily incorporated in the calculation of the PDR of a herd. Thereby, PDR can be useful when animal trade contacts need to be assessed for herds under surveillance or within control programmes. For herds that have certified their freedom from infection, e.g. by repeated testing, purchase of animals may have a strong effect on their relative increase in probability of the disease of interest. On the other hand, if they restrict their trade to low risk herds, their PDR will remain low.

If there is a known difference in prevalence, e.g. based on region as in the Q-fever example, this should be included in the calculation of the PDR. It should be noted that for $\mathrm{PDR}_{\mathrm{RtR}}$, the increase in probability of disease of each buying herd is divided by the expected probability of disease of all herds within the same category. Therefore, this PDR only reflects the increase in risk due to trade contacts and does not indirectly represent risk factors that have been used to categorise differences in the prior probability of disease. For $\mathrm{PDR}_{\mathrm{RtA}}$, on the other hand, the increased probability of disease is related to the average probability and herds in high-risk categories will have higher values, not necessary because of trade but because they already had a higher probability of disease than the average herd. We recognise that in some situations, it may be practical to use a ratio that captures both trade and other known risk factors. When it comes to Q-fever, live animal trade is probably not the most important route of transmission. Instead, local spread due to survival of the bacterium in the environment can be expected to play a main part. In other words, in this example $\mathrm{PDR}_{\mathrm{RtA}}$ will reflect not just increased probability due to trade but also due to geographical location. Because of the large regional difference in prevalence of Q-fever in Sweden, the inclusion or exclusion of the risk factor region can result in a considerable discrepancy between $\mathrm{PDR}_{\mathrm{A}}$, $\mathrm{PDR}_{\mathrm{RtR}}$ and $\mathrm{PDR}_{\mathrm{RtA}}$ for some herds (see Fig. 6).

Previous network analysis measures do not account for differences in probability of disease along the contact chain. To just count the number of herds or number of animals (in-degree) has been shown to be useful and it is also a very simple calculation free from prior assumptions. However, sometimes this may not be precise enough. In the present study, the difference in PDR between negative and positive herds was more significant compared to the difference in in-degree or ingoing infection chain. This indicates that a PDR gives a better reflection of the influence of ingoing contacts on infection status. Here, it is important to keep in mind that PDR is a relative and disease-specific measure, and not a general contact measure.

To investigate the influence of changes in input on the resulting PDR, different combinations of prevalence and time periods were used. For some diseases and populations, estimates of prevalence on animal and herd level are limited or absent. In such cases, any biologically reasonable input could be used. In the Q-fever example, detailed information about between-herd prevalence, including regional differences, was available and used. However, less was known about the within-herd prevalence of Q-fever in Swedish herds and a range of inputs between 5 and 40% was used. Although the comparison of results based on combinations with different $P(D+)_a$ is interesting in itself, because it may give information about epidemiological aspects of the disease, we note that the resulting differences between combinations of input are in general quite small. In other words, the usefulness of the PDR does not depend on whether the within-herd prevalence is set to 5% or 10%.

In conclusion, this paper presents a new method to assess herd disease risk in networks. It is an improvement to current network measures as it takes direction, temporal order, movement size and probability of disease into account, and can be used for e.g. risk-based surveillance, in the classification of herds in control programmes or to represent influential contacts in risk factor studies.

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BOVINE TUBERCULOSIS

SURVIVAL AND RISK FACTORS FOR LATERAL SPREAD OF BOVINE TB IN CATTLE HERDS EXPOSED TO INFECTION AT PASTURE IN NORTHERN IRELAND

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SUMMARY

Bovine tuberculosis (bTB), a zoonotic disease caused by *Mycobacterium bovis*, is found in many countries around the world. Northern Ireland (NI) is one region of the United Kingdom (UK) which has a high cattle density and an on-going problem with the infection. This study investigated the risk posed to herds in the proximity of bTB-infected cattle herds in NI during the period 2008-2010. Using survival analysis and a multivariable Cox proportional hazards model, herds exposed to contiguous infected herds were found to have shorter survival times, and exposed herds were 1.53 times more likely to develop a bTB breakdown than unexposed herds. Contiguous dairy herds (Hazard Ratio (HR) =1.55), herds with episodes of bTB in the previous 8 years (HR=1.23), and larger herds (HR=1.002) were at increased risk. The lateral transmission of infection through contact between cattle or wildlife vectors is potentially important in the epidemiology of bTB in NI.

INTRODUCTION

Bovine tuberculosis (bTB), primarily an infectious disease of cattle, is a zoonosis at the interface between farmed animals, wildlife and humans (Michel et al., 2010). The disease is regarded to be one of the most important animal health problems in the world. Within Europe, eradication is required in the Member States of the European Union (EU).

Agricultural census figures record that in NI in 2012 there were 1.63 million cattle in 20,434 herds (DARD, 2012) and NI is one of the most livestock-intensive regions of the UK. The vast majority of cattle are housed over the winter period, typically from November to May, as weather and ground conditions make outdoor production unsuitable. In the summer period cattle are mainly farmed at high animal stocking densities at pasture, and many enterprises are fragmented, with multiple land parcels and the potential for herds to contact multiple contiguous herds (Abernethy et al., 2006a).

A statutory eradication programme for bTB began in NI in 1959 (Abernethy et al., 2006b). All cattle herds in NI have been tested at least annually (Annual Herd Test – AHT) since 1982 using the single intradermal comparative tuberculin test (SICTT), and positive animals are removed and slaughtered, with restrictions being placed on infected herds as per EU legislative requirements (Directive 64/432/EEC). While the vast majority of herds remain officially-bTB-free, herd incidence in 2012 was 7.32% and the achievement of disease

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eradication has remained elusive despite many years of testing and removal of reactor animals.

Following the detection of positive herds ('breakdown herds') by the SICTT or through slaughterhouse surveillance, state veterinary officers conduct epidemiological investigations to try and determine the source of infection. This is often difficult, but based on field investigation and previous experience, lateral spread between neighbouring herds is deemed an important means of disease transmission in NI. Part of the outbreak investigation therefore involves identifying and mapping the contiguous herds. These herds may then be subject to follow-up risk herd testing (Lateral Check Test - LCT) depending on recent herd test history, and these LCTs made up just over one quarter of the annual bTB herd testing conducted in NI between 2008 and 2010.

Previous studies in NI have consistently found an increased risk for contiguous herds neighbouring bTB breakdowns. In a case-control study of risk factors for bTB in NI, Denny and Wilesmith (1999) found that 79% of the fences did not prevent nose to nose contact between adjacent herds with case herds having an average of 13 neighbours. Abernethy (2009) investigated the prevalence of infection in a 1.5 km radius around herds with five or more reactors compared to herds with one or zero reactors, and found that there was significantly more infection around the larger breakdowns, providing evidence of clustering of bTB infection. Developments in strain genotyping have provided further evidence of the fact that bTB outbreaks are often found in localised areas. For example, Skuce et al. (2010) spatially analysed *M. bovis* isolates in NI, and found that there was significant local clustering of genotypes, indicating common sources of infection for local herds. Similar findings on the risks of lateral spread have come from studies in the Republic of Ireland (Griffin et al., 1996; Olea-Popelka et al,. 2006; White et al., 2013) and Great Britain (GB) (Green et al., 2008; Karolemeas et al., 2011; Vial et al., 2011).

The epidemiological literature and field experience therefore provides evidence that contiguous spread is a factor in the spread of bTB infection in both NI and other parts of the British Isles. Based on this evidence, and noting the significance of LCT testing as a proportion of the overall testing programme in NI, this study sought to investigate the importance of lateral spread in the epidemiology of the disease, and therefore to evaluate the usefulness of LCT testing as part of the overall eradication programme. The specific objectives were:

- Determine the relative survival (time without a bTB episode) of herds contiguous to a breakdown herd, in comparison with being contiguous to herds free of bTB infection, between 2008 and 2010.

- Determine risk factors for bTB in herds contiguous to a breakdown herd between 2008 and 2010.

The study used a prospective cohort study (Cox survival analysis) to compare bTB-exposed to unexposed herds, and a generalised linear mixed model (GLMM) to determine whether there was a relationship between the number of reactors in the index herd and the risk of breakdown in the contiguous herd. The main focus of this paper is the prospective cohort study.

MATERIALS AND METHODS

Data sources

A computerised animal health database for cattle has been maintained by the Department of Agriculture and Rural Development (DARD) in NI since 1988 (known as APHIS). This system holds information on every bovine registered in the country with details of its identity, date of birth, movements and test results for bTB at both individual animal and herd level (Houston, 2001). Post-mortem details such as bTB lesions found at routine slaughter (LRS) are also held for each animal.

European subsidies are paid to cattle farmers under the Single Farm Payment (SFP) scheme and are administered by DARD. Information on subsidy claims for the land used to maintain the animals, whether owned or rented, is held on another DARD computer system. These data relate to each field parcel in NI in Geographic Information System (GIS) shape file polygon format with links to the unique business identifier of the subsidy claimant.

Data extraction and manipulation

The cattle herd, bTB test, LRS and movement data were extracted from APHIS and manipulated in Microsoft Access and Microsoft Excel (Microsoft Corporation, Redmond, CA). Statistical analysis was performed using Stata (Release 9.1; StataCorp, College Station, TX) and GenStat (Release 14.1; VSN International Ltd., Hemel Hempstead, UK).

Prospective cohort study – stage 1

Herds not under bTB restriction that had a positive SICTT (1 or more reactor animals) in the first six months of 2008, or which had a bTB-confirmed LRS in the same period were selected as index 1 herds. This produced 786 index 1 herds. These herds were used as markers to identify a bTB-exposed cohort. Index 2 herds were herds which had a negative AHT in the first 6 months of 2008 and had no history of SICTT positives or LRS in the previous 3 years. This provided 6,439 index 2 herds, and these herds were used to identify a non-bTB-exposed cohort.

Models were developed in ArcMap (Release 9.3.1; ESRI, Redlands, CA) to extract the contiguous herd information and link spatial data on land parcels to land usage by individual cattle herds. The objective was to create a dataset of contiguous herds from the index herd dataset. A land parcel was deemed contiguous if its boundary fell within 50 metres of the index herd's land boundary. Two distinct geo-processing models were required to find and map (i) the index herds and (ii) the contiguous herds. A link to the index herd was maintained for each contiguous herd in the output datasets. A bespoke GIS model was run until a contiguous shape file was created for each index herd which were then merged into one dataset.

Prospective cohort study – stage 2

For the contiguous herds related to the index 1 herds, the criteria for eligibility for the study were as follows:

(i) Contiguous herd had not had a SICTT positive or a LRS in the 12 months previous to the date of the bTB breakdown in the index 1 herd.

(ii) Contiguous herd had undergone a SICTT at least once in the previous 12 months.

The eligibility criteria applied for the herds contiguous to index 2 herds were the same as for the index 1 contiguous herds i.e. no SICTT positives or LRS in the 12 months previous to the date of the clear AHT in the index 2 herd. The contiguous herd must also have been tested at least once in the previous 12 months.

This provided a dataset of eligible exposed and unexposed herds for the cohort study. The following types of contiguous herds were removed from the dataset:

- Contiguous herds which were also index herds;
- Contiguous herds which were associated with another herd (e.g. shared management, shared housing, mixing of herds);
- Contiguous herds which had SICTTs with no eligible stock for a bTB test during the period of follow-up.

Cox proportional hazards model

Multivariate analysis of the data was conducted using a Cox proportional hazards model. Variables were selected for both the index and the contiguous herds. For index herds, this included the date of bTB breakdown or clear herd test (marked the start date for follow-up of the contiguous herds), herd size, number of bTB positive animals, bTB history of the herd, cattle movements and number of contiguous herds. Variables for contiguous herds also included the end date event (bTB breakdown or end of the follow-up period), herd location, local bTB herd prevalence and herd type. Exposed and unexposed contiguous herds started accumulating time at risk from the date of the bTB event in the index herd and the date of the clear AHT in the index 2 herds. The survival time was the number of days from the bTB event in the index herd to the outcome (‘failure’) – an SICTT positive or LRS in the contiguous herd. Herds which did not experience a failure in the period ‘survived’ to the end of the follow-up period on 31/12/2010. The bTB event dates were between 01/01/2008 and 30/06/2008. The follow-up periods therefore ranged between 2.5 and 3 years.

The method to select the covariates for the multivariable model was a full stepwise method - forward selection with backward elimination, with a probability to enter (inclusion: p=0.15) and probability to leave (exclusion: p=0.20) at each stage. In each case this probability was calculated by means of a Wald test.

Kaplan-Meier curves for relative survival of exposed and unexposed herds in the study period were created. The hypothesis under test was that exposed contiguous herds would have shorter survival times than unexposed herds i.e. a bTB breakdown would occur more rapidly in herds exposed to a bTB breakdown in a neighbouring herd.

Generalised linear mixed model (GLMM)

A GLMM was created to investigate whether there was a linear relationship between failure in the contiguous herd (contig_failure) and the variables i_pos and i_tot_pos. The variable i_pos was the number of bTB reactors in the index herd on the bTB event date used for the survival study - if the bTB event was a LRS or individual animal test positive, the number of reactors at the next herd test was added to the LRS/individual animal total. The variable i_tot_pos was the total number of bTB reactors or LRS animals from all index herds to which the contiguous herd was exposed for a period of 90 days from the index bTB event date in each index herd.

The relationship between contig_failure and i_pos or i_tot_pos fitted as fixed effects was assessed using a binomial distribution with logit link function, with herd number fitted as a random effect in both models. In each case the fixed effect was assessed by comparing a Wald statistic against an appropriate F-distribution. Results were presented as odds ratios with associated 95% confidence intervals.

RESULTS

Of the 786 index 1 herds, 769 had contiguous data and this provided 13,329 contiguous herd-index 1 herd relationships for 9,693 unique contiguous herds before the eligibility criteria were applied. Seven thousand and sixty-seven of these contiguous herds were contiguous to one index 1 herd only, and 2,626 were contiguous to more than one index 1 herd. For those contiguous herds that neighboured more than one index 1 herd, the date of the earliest bTB breakdown in the related index 1 herds was taken as the bTB event date for the prospective cohort study.

Of the 6,439 index 2 herds, 5,911 herds had contiguous herd data, and this provided 70,842 contiguous herd-index 2 herd relationships representing 23,160 unique contiguous herds. Seven thousand and eight of these contiguous herds were contiguous to only one index 2 herd, and 16,152 were contiguous to more than one index 2 herd - again the earliest bTB event in the index 2 herd was taken if the contiguous was related to more than one index 2 herd.

This provided a dataset (dataset A) with 1,854 exposed herds (herds contiguous to an index 1 herd) and 3,723 unexposed herds (herds contiguous to an index 2 herd). As there was the possibility of clustering in the dataset due to multiple occurrences of some index herds it was decided to select randomly from the multiple occurrence index herds, so that all of the index herds represented in the final dataset were unique. The final dataset for the Cox model (dataset B) had 596 exposed herds and 2,253 unexposed herds, with each contiguous herd related to an index herd. All of the contiguous and index herds were unique, and this was intended to avoid the potential biases that clustering can introduce into the analysis (McDermott et al., 1994).

Univariable results

Ignoring all other factors, exposed herds were 1.58 times more likely to have a bTB breakdown than unexposed herds in the univariable analysis (95% CI, 1.28-1.95).

The Kaplan-Meier curves (Fig. 1) revealed that the exposed herd curve decayed more quickly than the curve for the unexposed herds, demonstrating the higher rate of bTB breakdown in the exposed herds, as per the hypothesis at the beginning of the study.

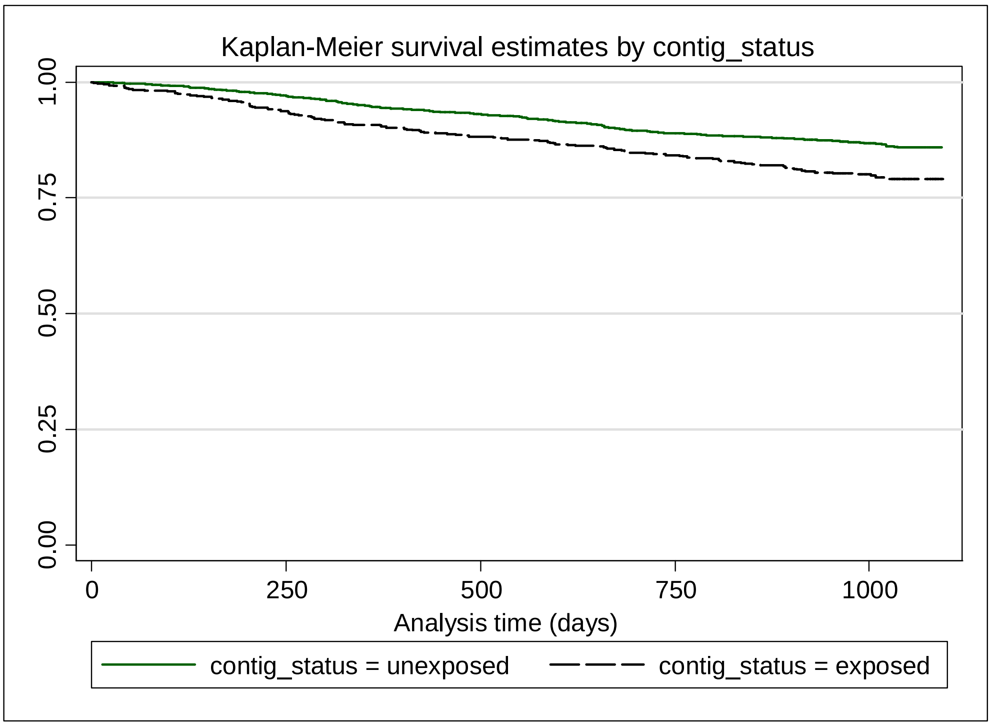

Fig. 1: Kaplan-Meier survival estimates (days) for unexposed versus exposed herds during period of follow-up

Final multivariate analysis

The results of the multivariate Cox model are shown in Table 1.

Table 1: Final multivariate model

Variable	Hazard Ratio	SE	z	p	95% CI	
c_status_expos	1.53	0.19	3.51	<0.001	1.21	1.95
c_tbhistory8yrs	1.23	0.07	3.96	<0.001	1.11	1.37
c_dist_tbprev	1.09	0.03	3.82	<0.001	1.05	1.15
c_movesin	1.001	0.0003	4.33	<0.001	1.0008	1.0020
c_herdsize	1.002	0.0006	2.54	0.011	1.0003	1.0027
c_type_dairy	1.55	0.23	2.94	0.003	1.16	2.07
c_no.herdtests	0.20	0.13	-25.62	<0.001	0.18	0.22
c_tbhistory3yrs	0.83	0.09	-1.63	0.10	0.66	1.04
i_herdsize	1.002	0.0004	4.39	<0.001	1.001	1.003
i_no.contigherd	0.98	0.006	-4.04	<0.001	0.96	0.99

c=contiguous i=index

The following variables significantly increased the likelihood of a breakdown in the contiguous herd:

- Dairy herd type (contiguous herd) (HR=1.55)
- bTB history in contiguous herd in previous 8 years (HR=1.23)
- District bTB prevalence (HR=1.09)
- Contiguous herd size (HR=1.002)
- Number of moves into contiguous herd (HR=1.001)
- Index herd size (HR=1.002)

Generalised linear mixed model

There was no evidence of a linear effect between either the number of index SICTT or LRS positives in the first index herd used for the study (i_pos) or the total number of index SICTT or LRS positives in all of the related index herds (i_tot_pos) as evidenced by the F-values (Table 2).

Table 2: GLMM for i_pos and i_tot_pos

Fixed term	Wald stat.	ndf	F stat	ddf	F pr
i_pos	2.01	1	2.01	228.9	0.158
i_tot_pos	1.60	1	1.60	272.8	0.206

DISCUSSION

As previously described, the majority of studies have found that proximity to a bTB-infected herd is a risk factor in the epidemiology of bTB. The findings of our study confirm that contiguous spread of infection is potentially important in NI, and is in agreement with the earlier studies. Olea-Popelka et al. (2006) recommend that bTB should be investigated at the area level in groups of contiguous herds, and this study corroborates that conclusion, and supports the use of LCT testing.

A few studies have suggested that local spread may be less significant in GB than on the island of Ireland. Wilesmith (1983) reported that just 3.4% of bTB herds were infected from contact with contiguous premises. In another study, 0.6% of breakdowns in South-West England were thought to have been due to contiguous spread (Wilesmith & Williams, 1986). Similarly, Green and Cornell (2005) found that local spread (< 2 km) was not an important feature of spatial spread of bTB in four English counties, postulating that contiguous herd testing may have prevented local spread. They therefore proposed that control policies should focus more on active surveillance and movement controls rather than the possibility of local spread. These differences may be due to local landscapes and the farming practices in different parts of the British Isles. A marked feature of cattle farming in NI is the fragmentation of farm holdings and potential for contact between multiple herds at pasture, as demonstrated by the number of herds which were contiguous to the index herds, with a range of 2 to 135 and mean of 19.48 for the index 1 herds. This contrasts with the number of neighbouring farms in a study of farm contacts in North-West England (Brennan et al., 2008), where the mean was 7.3 (range 1 - 17).

The small effect sizes (Hazard Ratios) found in this study are similar to other studies conducted for risk factors for bTB (e.g. Vial et al., 2011). Exposed contiguous herds were 1.53 times more likely to develop a bTB breakdown than unexposed herds (HR=1.53).

There were also variables which were protective in the contiguous herds such as the number of herd tests conducted in the contiguous herd in follow-up period (HR=0.20) and the total number of contiguous herds which are contiguous to the index herd (HR=0.98).

The mean herd size for the index 1 herds (160) was higher than the mean herd size for the index 2 herds (57), reflective of the fact that breakdown herds tend to be larger herds (Goodchild and Clifton-Hadley, 2001; Vial et al., 2011). The index 1 herds also had substantially more moves into the herd (Mean = 232) compared to index 2 herds (Mean = 30), and the purchase of cattle is a known risk factor for introducing the disease (Johnston et al., 2011).

The Kaplan-Meier curves (Fig. 1) are consistent with the significant difference in survival of the exposed and unexposed herds. What is surprising is that the curve for the exposed herds does not decrease more steeply in the initial follow-up period, and instead declines at a steady rate. This suggests that even though exposed herds are at increased risk of developing a bTB breakdown, it may take some time for infection to develop and then be revealed in the contiguous herd i.e. bTB may not spread very quickly in the proximity of infected herds, and detection may also be delayed, depending on the time taken to conduct a herd test.

Whilst it has been found elsewhere that herds which have more severe breakdowns (with larger numbers of reactor animals in the herd) are at higher risk of developing subsequent breakdowns (Olea-Popelka et al., 2004), this animal-level influence may not be true for the likelihood of contiguous herds developing bTB. This study failed to find a linear relationship between the number of reactors in the index herd and the likelihood of the contiguous herd developing bTB. It may have been expected that more reactors in the index herd would have meant a higher burden of infection with subsequently higher risk of onward herd-herd transmission across boundaries. It had been thought before the study began that features of the index herd breakdown could have been used an investigation setting to predict which contiguous herds are more likely to become breakdowns. The failure to find a linear relationship between number of reactors in the index herd and likelihood of breakdown rules out one obvious route of assessing risk in the contiguous herds. Increasing index herd size does though have an effect on the likelihood of the contiguous herd becoming a breakdown, as can be seen in the final multivariate model (i_herdsize, HR=1.002, p<0.001). The number of herds contiguous to the index herd is also significant, with higher numbers of contiguous herds being protective (i_no.contigherds, HR=0.98, p<0.001). This may be a herd size effect, with more herds indicative of smaller herds, which have less bTB than larger herds.

It was interesting that dairy herds were still highly significant in the final model, even accounting for their larger herd sizes. This may be a result of different grazing patterns and stocking densities between dairy and beef herds. District bTB prevalence as a significant risk factor (c_district_tbprev, HR=1.09, p<0.001) is consistent with the spatial clustering of bTB, and herd size, movements, and previous bTB history in the herd are all risk factors which have been found in other bTB epidemiological studies.

In seeking to apply the findings of this study to cattle farming, the need to reduce or prevent nose-to-nose contact between cattle is one option to seek to reduce spread. This may be achieved through double-fencing, but this is an expensive option for farmers, and they will be particularly unwilling or unable to double-fence land which is rented ‘conacre’ and does not belong to them. A more practical solution may be networks of farmers in localised areas coming together to manage their grazing patterns more effectively to reduce the potential for close contact between neighbouring herds. A strategy used by some farmers is to avoid grazing the perimeter fields of their farm, and instead to use that land for silage production or other crops. Other farmers graze alternative species such as sheep on the boundaries with neighbouring cattle. Scientific knowledge needs to be allied with the practical realities of intensive cattle production to produce workable solutions for farmers which they are more likely to utilize. Having said this, it must be accepted that the potential for reducing badger contact with cattle at pasture is limited given their often wide territorial ranges and ability to negotiate barriers which may keep cattle enclosed or excluded, but not wildlife.

Study limitations

This study uses the best available approximation of land usage by cattle herds. The assumption was made that the data for land parcels was the same as land usage in the three years of the study. Whilst approximately 30% of land in NI is rented on a summer grazing season basis (‘conacre’), and there is the potential for changes to land users between grazing seasons, anecdotal evidence suggests that the majority of land tends to be rented by the same user on consecutive seasons. We are therefore reasonably confident that this was a satisfactory approximation.

The study is based on the detection of bTB in a herd as the event in the survival analysis, and this is determined by the timing of the herd test or detection of bTB at slaughter. While introduction of infection and detection by test or at slaughter are separated in time, we see no way to avoid this disjuncture for a chronic disease such as bTB, where infection often remains unseen and without clinical signs, and the argument may therefore be philosophical rather than practical.

No assumptions can be made in the study about the direction of transfer of infection between neighbouring herds. It is accepted that the index herd may not necessarily have been the source of infection for the contiguous herd. If this provides a bias in the data, it should be a systematic bias across both exposed and unexposed herds.

No data were available on badger presence or absence on farms at a national scale, so unfortunately this variable could not be added to the multivariate model.

Conclusions

The geo-processing models using subsidy data on land parcels, despite the accepted limitations of the data, provided a fairly high degree of accuracy in determining contiguous herds to within 50 metres of the boundary of the index herd. bTB clusters in groups of herds, and herds are at higher risk of breakdown where the local bTB prevalence is higher and where a neighbouring herd is a breakdown. Infection may move across field boundaries by direct nose-to-nose contact between cattle, or through encountering common wildlife sources.

Testing herds contiguous to bTB breakdowns at pasture should remain an integral part of bTB control strategy, and should especially be focused on dairy herds, which are at higher risk of becoming infected when neighbouring herds become breakdowns.

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SPATIO-TEMPORAL TRENDS IN TUBERCULOSIS PREVALENCE IN THE EUROPEAN BADGER (*MELES MELES*) IN THE REPUBLIC OF IRELAND

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SUMMARY

A spatio-temporal analysis of *Mycobacterium bovis* infection in badgers, an important wildlife-reservoir species, was undertaken in the Republic of Ireland. In total, 4,948 badgers with full capture and testing records were analysed using Generalised Estimating Equations (GEE). The overall badger level prevalence was 11.3% (95%CI: 10.5-12.3); sett level prevalence was 15.2% (95%CI: 14.0-16.4). Over the study period (2009-2012), there was a significant decline in the odds of a badger being test-positive. Badgers were more likely to be test-positive if they were male, or a female that had conceived, compared with a female who had never conceived. Badgers were more likely to be test-positive if caught at a sett that was close to other infected setts. The cattle-prevalence of bTB on surrounding farms (500m-1km from capture location) was positively associated with badger test status. There was significant variation in prevalence across counties, with a trend towards greater prevalence in western areas than eastern areas.

INTRODUCTION

It is important to monitor infection levels in wildlife populations in order to assess transmission risk to domestic animals or humans (Morner et al., 2002). Appropriate datasets on spatio-temporal trends in infection prevalence in wildlife populations at large-scales are rare and/or limited (Morner et al., 2002). With diseases such as bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, it is essential to assess the risk posed by wildlife species. Furthermore, as wildlife can act as sentinels for the prevalence of infection in domestic hosts (e.g. badgers and cattle; Murphy et al., 2011), relative infection prevalence within the wildlife population may indicate the local weight of infection within the two-host system.

Badgers are an important wildlife host of *M. bovis*. Badgers infected with *M. bovis* have been reported from a number of countries in Western Europe (Switzerland, France, Spain, Ireland and the United Kingdom; Gortazar et al., 2011), though it is only within Ireland and the United Kingdom that there is unequivocal evidence that badgers are a reservoir species with on-going 'spillback' of infection to local cattle populations (Bourne et al., 2005; Griffin et al., 2005). In the Republic of Ireland, an interim badger culling regime (which reduces badger abundance in geographically distinct 'hot spot' areas; Byrne et al., 2013) is being conducted as part of a range of intervention policies aimed at TB eradication (Sheridan, 2011). As part of this policy, approximately 30% of culled badgers are culture tested for *M.*

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bovis, as a means of surveying the background tuberculosis prevalence within badger sub-populations across Ireland. Here we assess the spatio-temporal trends in TB prevalence in badgers using a large dataset from 2009-2012. We assess the influence of badger attributes and metrics of local- and landscape-scale infection pressure coming from both badger (distance to infected setts) and cattle (cattle prevalence) hosts on the probability of badger testing positive for *M. bovis*.

MATERIALS AND METHODS

The badger and the sampling strategy

Badgers (*Meles meles*) are a medium-sized mustelid species, widely distributed in Ireland but with considerable variation in local density depending on habitat composition, quality and population management history (Byrne et al., 2012a). The species is predominantly nocturnal and resides in setts, which are complex subterranean burrow systems. Badgers were captured as part of a national strategy of targeted culling across the Republic of Ireland, concentrated in areas with chronic cattle herd TB problems (Sheridan, 2011). Each badger survey was instigated as a result of a cattle herd breakdown, where a local epidemiological investigation suggested that badger presence may be a contributing factor. Badgers were captured at active setts over an 11-night capture effort called a ‘capture event’ (Byrne et al., 2013). Badgers were trapped with wire stopped-restraints and dispatched by 0.22 calibre rifle. All badgers removed were sent by courier to a centralized laboratory for necropsy (badgers captured from counties Donegal and Mayo were not processed within this laboratory, due to their distance from this laboratory, and are therefore excluded from this analysis). Couriers transport badgers three times per week, to reduce the period between capture in the field and arrival at the laboratory (usually in 72hrs or less). The majority of badgers were processed (post-mortem and tissue harvesting) on the day of arrival, otherwise the cadavers were kept in cold storage (0º – 1ºC).

Necropsy

Necropsy procedures were standardized and implemented by one experienced veterinary pathologist in one laboratory, allowing for consistent recording and processing over the duration of the study. All badgers were sexed, age-classed (young or adult), and checked for bite wounds or other injuries (e.g. trap related injuries; Byrne et al., in review). Females were also checked to see if they had been pregnant prior to capture by assessing the thickness of the uterine wall, the presence of placental scars and teat morphology.

During necropsy, tissue samples were taken from a systematic sample of approximately one-third of all badgers received from the field. From each of these animals tissue was collected from 16 lymph nodes. Samples were also taken where suspect gross lesions were found at bite wounds and in other organs (kidney, spleen and liver). These tissues were cultured as a pooled sample from each badger. All samples were stored at −20ºC prior to culture.

To reduce cross-contamination, cold detergent was used to sterilise instruments. Implementing more enhanced procedures may increase the sensitivity of badger TB tests (Murphy et al.,2010). Therefore, the prevalence levels reported in this study are probably an underestimate of the true infection prevalence.

Laboratory culturing of samples

Pooled samples from each badger were tested for *M. bovis* via culturing on selective mycobacterial media (similar to Murphy et al.,(2011)). For each sampled badger, test-status was positive if *M. bovis* was isolated by bacterial culture from a sample, and negative otherwise.

Data

Badger level data resided in a number of databases that were joined through data matching processing. The databases included: test-status, post-mortem (PM), capturing history (wildlife) and Geographical Information System (GIS) geodatabases, the latter held the locations of all badger setts at which capturing took place. After employing data-cleaning and data-matching processes, the final spatio-temporal dataset contained full records (i.e. with test-status, PM results, sett histories and location of capture) for 4,948 badgers from culture samples taken during October-2009 to June-2012.

Modelling approach

We created a multi-level model using a Generalized Estimating Equation (GEE) framework, with badger sett being the clustering variable. GEE was chosen as we were interested in Population Averaged (PA – i.e. across cluster (sett)) parameter estimates (Dohoo et al., 2009). The model was specified with a log link function, binomial family, an exchangeable working correlation (matrix) structure with two-level hierarchy with badgers clustering within setts. We employed the ‘robust’ option to avoid issues with misspecification. The GEE-logistic model was evaluated using the goodness-of-fit test (Horton-test; see Byrne et al., 2013).

We used a multi-step model building approach, where variables (see table 1) were screened against the outcome variable using univariable models. Variables were suitably transformed where appropriate (e.g. by including second order polynomials), after assessing the linearity between the outcome and predictor variables using LOWESS smoothing curves (Dohoo et al., 2009). Only variables with associations with the outcome at the liberal p-value of 0.2 were brought forward for further investigations. The predictors were grouped into spatial and non-spatial components. The spatial components included a second order trend surface model using the spatial coordinates (x, y, xy, x^2 y^2 variables) of the badger sett locations to assess if there was underlying spatial variation within the dataset.

We assessed the relationship between the outcome and the distance of the n^{th} sett to its nearest-neighbour sett and nearest-neighbour infected sett. We defined an ‘infected sett’ as a sett with one or more *M. bovis* test-positive badgers removed over the course of the study. We hypothesised that the probability of badger testing positive may be related to the local density of setts (nearest neighbour) and the status of the badgers found within those neighbouring setts (nearest infected neighbour). As badgers tend to use multiple setts within their local environment (within territory), we also calculated the mean distance to the three and five nearest-neighbouring setts, as well as the mean distance to the three and five nearest infected setts. The distance metrics represent local relative density and potential neighbourhood exposure for badgers, respectively. We investigated whether prevalence of TB in cattle herds had an impact on the probability of a badger being test-positive and whether this relationship (if any) was a scale-dependent phenomenon.

Table 1. Spatial and non-spatial potential predictors of the probability of a capture badger testing positive for *Mycobacterium bovis.*

Predictor	**Description**
Spatial	
x-coordinate	Longitudinal geographic coordinate from Irish National Grid (continuous; scaled to 10km)
y-coordinate	Latitudinal geographic coordinate from Irish National Grid (continuous; scaled to 10km)
Nearest-neighbour (1 sett; 3 setts; 5 setts)	Euclidian (straight-line) distance to the nearest-neighbouring sett (Km); Mean Euclidian distance to the three or five nearest-neighbouring setts (Km)
Nearest infected neighbour (1 sett; 3 setts; 5 setts)	Euclidian (straight-line) distance to the nearest-neighbouring infected sett (Km). Mean Euclidian distance to the three or five nearest-neighbouring infected setts (Km). An infected sett is one where a test-positive badger has been found.
Cattle prevalence (Immediate; 250m; 500m; 1000m)	Cattle prevalence (total # standard reactors/total herd population) of the immediate farm (within 15m of sett) or cattle prevalence in all herds within 250m, 500m, and 1000m buffer intersection of each sett respectively.
Weighted cattle prevalence (250m; 500m; 1000m)	Cattle prevalence weighted according to the proportion of land each farm contributed to the buffer intersection (∑(% land * farm prevalence) within 250m, 500m, 1000m of each sett respectively.
Non-spatial	
Age-class	Juvenile, adult, old (categorical)
Pregnancy status	Evidence of previous pregnancies, yes/no (categorical)
Gender	Male, Female (categorical)
Cattle access	Sett accessible directly to cattle, yes/no (categorical)
Weight	Weight of badger (continuous)
Openings	No. of openings to sett (continuous)
Openings used	No. of openings to sett with field signs of activity at time of capture (continuous)
Main	Main sett, yes/no (categorical)
No. badgers	No. badgers removed during current capture attempt (continuous)
Total badgers	Total captures recorded (continuous)
Time	The month of badger capture since the start of the study (month 0 to 32; continuous)

Farm data were derived from the Irish Land Parcel Identification System (LPIS), a high resolution GIS based system of the boundaries of all farms (made up of parcels) within the Republic of Ireland. This dataset is updated annually and contains approximately 95% of all farms nationally. This spatial database was combined with a national animal level TB cattle test dataset (Animal Health Computer System (AHCS), Department of Agriculture, Food and the Marine). We devised two cattle ‘exposure’ metrics, at four different spatial scales (note, we are not assuming a direction with regard to transmission). The immediate herd (within 15m of sett), and herds within 250m, 500m and 1,000m of setts were the four spatial scales investigated. The first metric of cattle prevalence was calculated as the total number of standard reactors (to the single intra-dermal comparative cervical tuberculin test) per 1,000 cattle tested within herds for the year during which the badger capture took place. The second metric devised was a weighted cattle prevalence, where farms that had greater proportion of their land exposed to the badger sett were given a greater weight (∑(% land * cattle prevalence). Finally, we checked to see if there was any evidence of lag effects, by including the cattle prevalence for the year prior to the badger capture event. A backwards selection procedure reduced the number of potential predictor variables to be joined with the non-spatial model building step (see below).

Non-spatial predictors included both badger level and sett-level predictors. Badger level predictors included: gender, weight, age-class and pregnancy status. Sett-level predictors included sett type (main/non-main), access to cattle (binary), openings (number of burrow openings into the sett) and openings used (number of active openings). Additionally, we included the number of badgers captured during the current capture event (No. badgers) and the total cumulative number of badgers removed up to, and including, the capture event (Total badger) as recorded within the database. We assessed the temporal trend in the apparent prevalence over time by including the month of capture as a linear predictor representing the time since study commencement (Time (in months: 0-32)).

Competing models were compared using the Quasi-likelihood Information Criterion (QICμ) values for the GEE models. The model with the lowest QICμ values was considered the model with the best goodness-of-fit to the data amongst competing models (following Byrne et al., 2013). Data manipulation and statistical analyses were completed in Stata® version 11. Spatial analysis was completed using QGIS (QGIS development team) and ArcGIS (ESRI©).

RESULTS

Descriptive analysis

During 2009 to 2012, the overall badger-level TB prevalence was 11.3% (exact 95%CI: 10.5-12.3) and sett-level TB prevalence (% setts that produced at least one test-positive badger) was 15.2% (95%CI: 14.0-16.4). The mean distance between neighbouring infected badger setts was 4.8km (Range: 36m-31km), although the distribution was highly skewed with 95% of infected setts being within 12.6km. Badgers were sampled widely across 24 counties (total counties area: 59,000 ha), with test-positive badgers found in all sampled counties (Fig. 1). There was large variation in the probability of a badger testing positive for *M. bovis* depending on county (Fig. 1). The highest observed prevalence was recorded in counties Clare (20%; 95%CI: 15.9-24.4) and Dublin (20%; 95%CI: 0.5-71.6). However, only five badgers were tested from Dublin, as opposed to 361 badgers tested in Clare. Therefore, the Dublin mean prevalence is an artefact of a small sample size.

Competing models

In comparing metrics of local badger infection risk (distance to nearest neighbouring infected setts; NN_x) and scale dependent cattle exposure (prevalence of cattle TB in local area), a matrix of competing models was produced in which QICμ values were compared (Table 2). For simplicity, findings from the suite of models that used the distance to three nearest infected neighbouring setts (hereafter denoted as *NN3*) were highlighted as they had the lowest mean QICμ values (Table 2). A competing final model using badger infection metric *NN3* and local area cattle TB prevalence at a 250m spatial scale is presented in Table 3. However, the parameter estimates are similar across competing models. There was no evidence of a significant lack of fit to the data across all final competing models (Horton tests: $\chi^2(3df) < 7.76$; $p > 0.05$). The mean within sett cluster working correlation (ρ) was 0.066 across all final competing models.

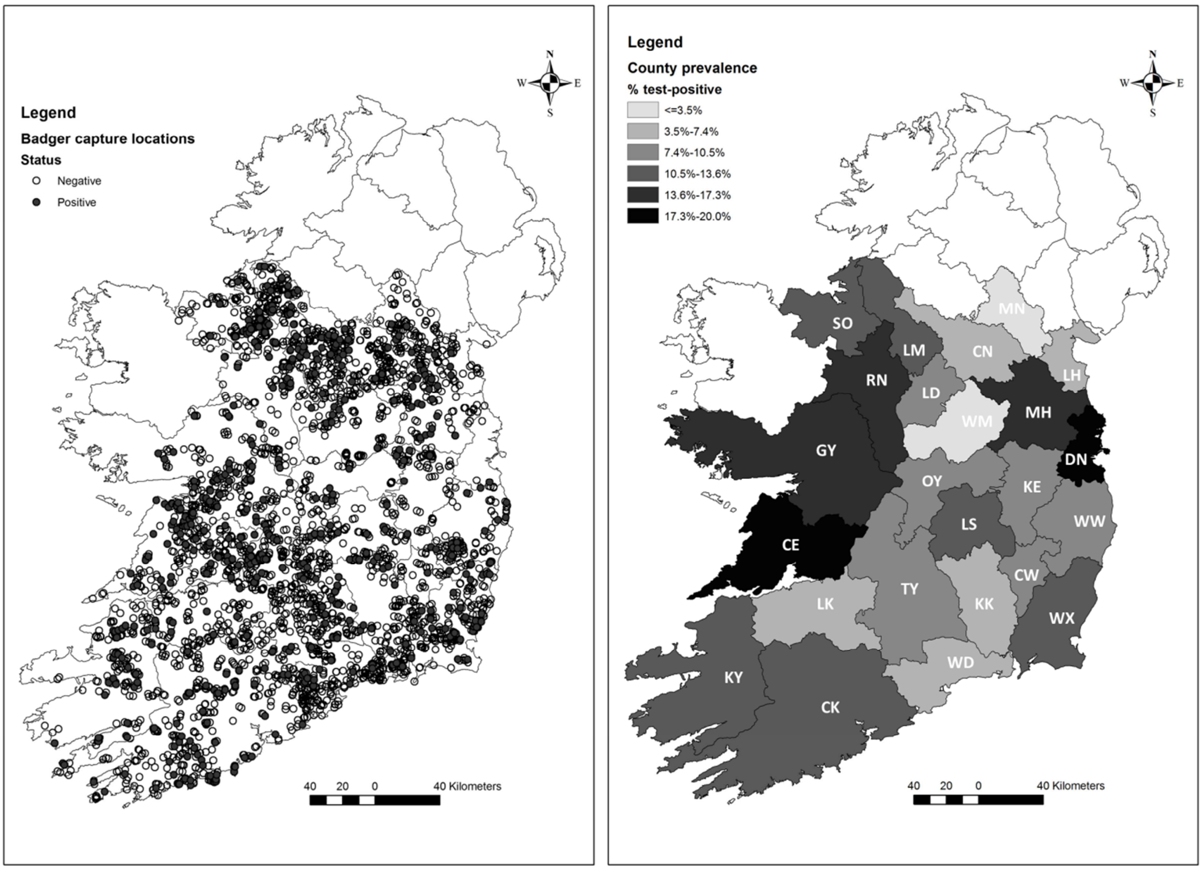

Fig. 1 Location and infection status (left) and observed county prevalence of *Mycobacterium bovis* (right) of captured badgers in Ireland during 2009-2012. SO : Sligo; DN: Dublin; LM: Leitrim; CE: Clare; CN: Cavan; TY: Tipperary; MN: Monaghan; LS: Laois; LH: Louth; WW: Wicklow; RN: Roscommon; LK: Limerick; LD: Longford; KK: Kilkenny; WM: Westmeath; CW: Carlow; MH: Meath; WX: Wexford; GY: Galway; KY: Kerry; OY: Offaly; CK: Cork; KE: Kildare; WD: Waterford.

Spatial effects

An underlying significant spatial trend in the dataset was controlled by including x and x^2 geographic coordinates in the final competing models. This positive association suggested a general east-west gradient in terms of badger TB risk, with the highest probability of being TB positive in the western part of Ireland. There was no significant association between badger test-status and the distance to neighbouring setts (mean distance to one, three or five nearest-neighbours; P>0.05). However, there was a significant association between badger test-status and all metrics of the distance to neighbouring infected setts (P<0.001). Across competing final models, models with the mean distance to three infected nearest-neighbours had the lowest QICµ value (mean QICµ *NN*: 3262; mean QICµ *NN3*: 3164; mean QICµ *NN5*: 3201; Table 3). There were lower odds of a badger testing positive for *M. bovis* with increasing distance to infected setts (Model *NN3* and cattle-prevalence 250m; OR: 0.87; P<0.001 (similar results were found with other *NN* and cattle-prevalence combinations)). Weighted cattle prevalence metrics and prevalence lag effects were not significantly associated with badger test-status at either screening stage of model building (P>0.1) or at the candidate multivariable models stage (P>0.1), and these were therefore dropped from the final competing models. The (unweighted) prevalence of bTB in surrounding cattle herds at short distances from the capture location (immediate farm and within 250m of sett, respectively) had no association with badger test status (p≥0.1); however, there was a significant positive association (Model *NN3*; OR: 1.04-1.06; p=0.031-0.047) between badger test-status and cattle bTB prevalence at greater spatial scales (500m and 1km of sett, respectively). The models with the larger-scale cattle exposure (cattle prevalence at 250m or 500m), had only marginally smaller QICµ values compared with equivalent models without the exposure parameter (ΔQICµ ≈ 1.5). The relation between cattle infection pressure and badger infection pressure (confounders) was assessed by correlating the metrics of local badger infection (*NNx*) with cattle TB prevalence (at the four spatial scales). There was a weak, but significant (mean r = -0.062; P<0.05), negative correlation between the predictors. As cattle prevalence increased, the distance to infected badger setts decreased, highlighting the congruence of infection-intensity between hosts. When nearest infected setts were removed from the competing models, cattle prevalence at smaller spatial scales became borderline significant (cattle-prevalence immediate farm: P=0.040; 250m: P=0.095), while the influence of cattle prevalence at larger scales remained highly significant (500m: P=0.003; 1km: P<0.001).

Table 2. Matrix of Quasi-likelihood Information Criterion (QICµ) values from competing models where badger test-status was associated with local exposures of badger (distance to nearest infected neighbouring setts) and cattle TB risk (cattle prevalence within local environment at immediate, 250m, 500m and 100m scales). Lower QICµ values indicate preferred models.

	Badger infected sett exposure			
Cattle prevalence exposure	***NN****	***NN*3***	***NN*5***	**Mean**
Immediate	3133.58	3041.50	3075.41	3083.49
250m	3050.65	2960.55	2992.57	3001.26
500m	3433.40	3326.80	3368.23	3376.14
1000m	3431.09	3326.38	3367.42	3374.96
Mean	**3262.18**	**3163.81**	**3200.91**	

* NN: Mean distance to 1, 3 or 5 nearest neighbour setts

Non-spatial effects

During 2009-2012, there was a significant decline in the odds of a badger being test-positive (GEE-logit model: per month OR: 0.98; p<0.001). Badgers sampled at the start of the study had a predicted probability of testing positive for *M. bovis* of 0.15 (95%CI: 0.07-0.25), while badgers sampled at the end of the study period had a predicted probability of testing positive of 0.09 (95%CI: 0-0.16). Badgers were more likely to be test positive if they were male (OR: 1.7; p=0.010) or a female that had conceived (OR: 1.9; p=0.002) compared to a female who had never conceived. There was no significant difference between males and female who had conceived (p=0.288), but there was an overall difference between males and females (χ^2 (DF: 2) = 7.00; Prob > χ^2 = 0.030).

Table 3. Logistic model of the probability of a captured badger testing positive for *Mycobacterium bovis* with the lowest Quasi-likelihood Information Criterion (QIC) value, from the matrix of competing models (Table 2).

Test-status	**Odds Ratio**	**Std. Err.**	**z**	**P>z**	**Lower 95% CI**	**Upper 95% CI**
Female * (never conceived)	1					
Male ^	1.550	0.334	2.03	0.042	1.016	2.364
Female ^ (conceived)	1.724	0.364	2.58	0.010	1.140	2.608
Time (in months)	0.981	0.006	-3.41	0.001	0.970	0.992
x (centered; per 10km)	0.972	0.008	-3.4	0.001	0.956	0.988
x^2 (centered; per 10km)	1.003	0.001	2.44	0.015	1.001	1.005
NN3	0.866	0.015	-8.31	<0.001	0.837	0.896
250m	1.001	0.001	1.2	0.230	0.999	1.003

* Referent category.
^ Wald test Male vs. Female (conceived): χ^2 (DF: 1) = 1.13; Prob > χ^2 = 0.288.

DISCUSSION

This study illustrates that TB positive badgers have a wide distribution in Ireland, with infected animals found in all counties sampled. Previous work has also found that badgers can be infected with TB in local areas (>1km away from a culled sett) without recent (within 5-years) TB breakdowns in cattle (Murphy et al., 2011). This is consistent with the view that badgers are a well-adapted host for TB, with intraspecific transmission occurring across subpopulations (Corner et al., 2011). However, given the wide geographic distribution of infected badgers, it is likely that infection was seeded into the badger population on a number of occasions at different locations across the country. The annual testing of all cattle herds occurs throughout Ireland and advanced cases in cattle are now rare (Corner et al., 2011). The risk of cattle-to-badger transmission will be less than where advanced cases in cattle occur, which may suggest that TB was seeded into badger populations in the past when higher prevalence was recorded in cattle.

Spatial effects

There was an east-west pattern with regards the prevalence of TB within badgers, with higher prevalence’s in the west (x and x2 variables significant) of the country. One hypothesis may be that climate may affect TB disease dynamics. However, any biological explanations involving weather is speculative. There may be other variables not controlled for in our model that co-vary with longitude that may affect TB prevalence.

At finer spatial scales, we found TB spatial clustering effects, with badgers being more likely TB positive at closer proximity to other infected setts, and clustering within setts. Such clustering has been reported previously in Ireland (e.g Olea-Popelka et al., 2005); with spatial-clustering occurring at relatively large spatial scales only. This is consistent with the results from our present paper, as we found a steep decline in the probability of being test-positive with increasing isolation. With the metric of three-nearest infected neighbours, we found that most of the increased risk due to spatial clustering occurred at scales of 10km or less. This indicates that the practical scale at which transmission occurs within badger populations is relatively large (Olea-Popelka et al., 2005) and is possibly due to badger populations in Ireland exhibiting frequent trans-territorial movements (scales of ~1-2km) and rare long-distance dispersals (scales of 10-20km; Byrne et al., 2012a; Byrne et al., 2014).

A significant association between metrics of local cattle TB prevalence and the probability of badgers testing positive for TB was found. This finding supports the contention that the two species are epidemiologically linked; however, this study cannot determine the direction of transmission.

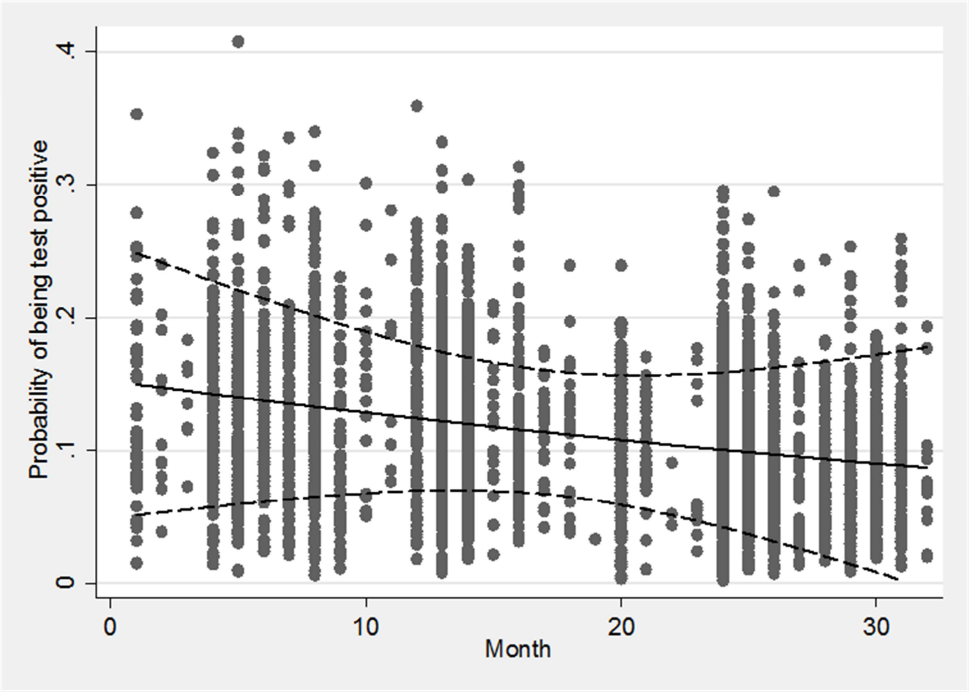

Fig. 2 Predicted prevalence of *Mycobacterium bovis* infection in badgers over time (in months since the start of the study) during 2009 to 2012 in Ireland.

Temporal effects

There was a significant decline in badger TB prevalence from 2009 to 2012, with a decrease from 15% to 9% nationally (P<0.001). A previous study found evidence for a decrease in badger TB prevalence in repeated large-scale proactively culled areas, but equivocal results in local-scale culls in Ireland (Corner et al., 2008). The trends found in this study may represent the removal of higher prevalence individuals during initial captures within TB 'hotspots', with later removals consisting of predominantly immigrant animals i.e. a source-sink dynamic (Byrne et al., 2013). Evidence suggests that badgers from source populations away from TB hotspots in Ireland have lower background TB prevalence levels (Murphy et al., 2011). However, where culling operations fail to reduce population densities to very low levels, surviving badgers may increase their contact-rates with conspecifics, potentially increasing frequency-dependent TB transmission (Woodroffe et al., 2006). A study in England found that repeated proactive culling resulted in increased TB prevalence in badgers within cull areas (Woodroffe et al., 2006). These study populations had high starting badger population densities prior to culling (5.2 badgers/km^2; Smith & Cheeseman 2007; 11.6 ∑trapped badgers/km^2; Woodroffe et al., 2008). With culling efficacies of ~70% (Woodroffe et al., 2008; Smith & Cheeseman, 2007), the post-culled populations may be of moderate density (1.6-3.2 badgers/km2). In comparison, badger populations in unculled areas of Ireland are relatively low (mean 0.8-1.1 badgers/km^2), with culled areas being of lower density again (Byrne et al., 2012b; Byrne et al., 2013). These substantial differences in population density (both pre- and post-cull) in Ireland and England are likely to have significantly affected the transmission dynamics observed between the study populations. Furthermore, in Ireland the alternate host (cattle) exhibited a decreasing trend in TB prevalence over the course of the present study (DAFM statistics 2013), whereas TB prevalence increased over the duration of the English study (Woodroffe et al., 2006). Therefore, if there was cycling of the disease between the two hosts, we might expect a degree of parallel ‘mirroring’ between the trends in both hosts.

Intrinsic effects

The study illustrated that males had a higher likelihood of being TB positive than females. This may be related to behaviour (Corner et al., 2011) and/or intrinsic differences between male and female badger immune systems (Tomlinson et al., 2013). Longitudinal studies of badger populations have found that males have a greater probability of becoming TB positive, and that infected male badgers have shorter life spans than infected females. This has been interpreted as signalling an underlying physiological difference between male and female badgers in mounting immunological responses to infection. Females, when infected, exhibited higher survival and retained reproductive output, irrespective of disease progression (Tomlinson et al., 2013). Males may be more exposed to infection due to behaviour. For example, males have a higher propensity to make interterritorial movements than females (Byrne et al., 2014). Males exhibit significantly more bite-wounds and infected bite-wounds than females. Amongst males (at least in high density populations), breeding males tend to accumulate more bite wounds than non-breeding males and bite wounds can be a risk-factor for TB (Murphy et al., 2010; Tomlinson et al., 2013). Males may also participate more in territorial defensive behaviour, and can aggressively compete with other males for mating opportunities (Byrne et al., 2012a).

Females in our study had lower probabilities of infection than males; however this difference was mainly driven by the significant variation between females who never conceived and those who had. We hypothesise that females may be more important in the maintenance of infection within groups (living longer lives, even when infected, more likely to stay within territory), and males may be more important in terms of spread (shorter lives, more likely become infected, more likely develop progressive disease pathologies, more likely to make inter-territory movements). Females that had never conceived may have been younger animals and would, therefore, have less accumulated time at risk of being infected. Females that had never conceived would have less contact with male badgers through mating, potentially limiting transmission opportunities. A final hypothesis is that pregnancy itself increases the susceptibility to infection through hormonal changes and increased stress reducing immunocompetencies, though there is currently little data to support this speculation in badgers (Pritchard et al., 1987).

Conclusions

We have shown that badger TB-status is affected both by the local-area TB infection levels in badgers and cattle. There has been a decline in prevalence in badgers from culled areas in Ireland, with a decline from 15% to 9% nationally from 2009 to 2012.

Over the same period, herd-level TB prevalence in cattle has declined from 5.0% to 2.3% in Ireland. This is consistent with infection pressure being exerted by both hosts, with interspecific transmission occurring, but the direction of the relative force of infection cannot be ascertained from this analysis. At the individual level, badgers were more likely to be test-positive if they were male, or a female that had conceived compared to a female who had never conceived, and this may be related to differential risk behaviours (movement, aggression, mating) or intrinsic factors (age, stress, immunocompetencies) between the genders. This study highlights the importance of wildlife monitoring of disease during epidemiological interventions to inform the potential effects of wildlife intervention strategies.

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THE RELATIVE EFFECTIVENESS OF TESTERS DURING FIELD SURVEILLANCE FOR BOVINE TUBERCULOSIS IN IRELAND

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SUMMARY

Testing of bovine tuberculosis using the single intradermal comparative tuberculin test (SICTT) is reliant on the skills and experience of the tester. This study applies mixed logistic regression to assess the relative effectiveness of testers. These methods have been used to assess the efficiency of factory surveillance for bTB, however, to the authors' knowledge, they have not been applied to assess the relative performance of testers before.

INTRODUCTION

In Ireland, new bovine tuberculosis (bTB) cases are detected using both field and abattoir surveillance. Field surveillance is conducted through annual testing of all cattle (excluding home bred calves less than 6 weeks of age on the date of the annual herd test) using the single intradermal comparative tuberculin test (SICTT). In this test, bovine and avian tuberculins are used in combination to measure and compare the skin response at 72 [±4] hours following intradermal injection (Monaghan et al., 1994). Individual farmers are required to pay for one full herd test per annum; a direct cost to the Irish farming community of an estimated €23 million in 2006 (DAFM, 2008). In Ireland, this testing is undertaken by private veterinary practitioners (PVPs), under supervision of the Department of Agriculture, Food and Marine (DAFM). PVPs are nominated by, and receive payment directly from, their farmer clients for annual surveillance testing. Abattoir surveillance is also conducted by PVPs, in this case whilst employed as DAFM temporary veterinary inspectors, who palpate, incise and inspect a defined range of body organs and lymph glands in animals at slaughter to determine fitness for human consumption. Abattoir surveillance is generally considered an important surveillance method for detecting herds infected with bTB; between 30 to 35% of herd breakdowns were detected using this method (Abernethy et al., 2013). In Ireland, abattoir surveillance is particularly important in detecting residual herd infection, noting that no further infection is detected during subsequent testing in approximately 80% of these herds (Olea-Popelka et al., 2008). The protocol for the management of M. bovis infected herds is presented in the ‘Handbook for the veterinary management of herds under restriction due to tuberculosis’ (Good et al., 2010).

Variations in the quality of both field and abattoir surveillance have been areas of general concern for some time, noting that aspects of each procedure require subjective interpretation. In previous work, concerns have been highlighted regarding the relative efficiency of factories in detecting lesions among attested cattle (Frankena et al., 2007), although

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discrepancies between factories have lessened over time (Olea-Popelka et al., 2012). A similar study was carried out by Shittu et al. (2013) in Great Britain using a mixed logistic regression model, with similar results to the Irish studies. The differences between abattoirs were due to inherent differences between the abattoirs, and unrelated to the risk profile of cattle slaughtered at each abattoir. Similar concerns are periodically raised about the effectiveness of field surveillance, which is highly reliant on the skill, experience, and conscientiousness of the individual tester (Monaghan et al., 1994; Wahlström, 2004; de la Rua-Domenech et al., 2006). A broad range of factors may adversely affect test accuracy and precision, including the quality of handling facilities, lighting, site selection, hair clipping and the technique of tuberculin administration. This issue has most-frequently been raised in the context of farmed deer (Seifert et al., 1985; Clifton-Hadley & Wilesmith, 1991; Partridge et al., 2008), but is also relevant to cattle. Logically, variation in the conduct and interpretation of the SICTT will adversely affect the effectiveness of the national eradication programme. Wahlström (2004) has identified measurement consistency among veterinarians during tuberculin testing of Swedish farmed deer as one tool for quality control (QC) of tuberculin test performance.

Strategies are in place to maximise the return on money invested, relating to personnel, field surveillance, abattoir surveillance, the laboratory and applied research. There is considerable emphasis on QC within the national programme, and methods are used routinely to facilitate continuous improvement (Duignan et al., 2012). Field inspection of testers has been conducted in Ireland for many years. Since 2008, this has been supplemented with quantitative performance reports, enabling the performance of each tester to be evaluated and ranked using a range of output-based performance indicators (Duignan et al., 2012).

The objective of this study was two-fold, to assess whether there has been any change in the performance of testers in Ireland during 2008 and 2011, and to quantify the relative effectiveness of testers during field surveillance for bTB in Ireland.

MATERIALS AND METHODS

Study population

In Ireland, the national Animal Health Computer System (AHCS) maintains a record of all SICTT testing data, including test date, test type, tester, number of animals tested and number of reactors. For this study, we accessed data on all annual herd screening tests conducted in unrestricted herds (subsequently termed an ‘eligible test’) in Ireland during 2008 and 2011.

In this study, the tester was the unit of interest, and the study population included all testers who carried out at least one eligible test in Ireland during 2008 and 2011. The outcome measure was a herd restriction at the eligible test. At this test, the ‘standard’ interpretation of the SICTT test is applied. An animal with an increase in skin thickness at the bovine site more than 4 mm greater than the increase at the avian site was classed as a ‘standard’ reactor. Additionally, animals may be deemed to be reactors at the discretion of a veterinary inspector (eg. for epidemiological reasons, nature of skin reactions, or based on ancillary blood test(s)). Herds with one or more reactor animals at this test had movement restrictions imposed (a herd restriction) until two clear consecutive tests were achieved.

Statistical analysis

The outcome measure was a herd restriction involving 1 or more reactor(s). A hierarchical logistic regression model, incorporating tester as a random effect, was used to model the probability of a herd restriction. The model was fitted using the GLIMMIX procedure in SAS v 9.3 (SAS Institute Inc., 2003). Testers that tested less than ten herds within the year in question were excluded from the model. The risk factors considered within the model included year of test (YEAR: 2008 or 2011); herd size (HSIZE) at the time of the test; the herd incidence rate (proportion of herds that became restricted) within the county in the previous year (LOCATION); the disease history of the herd (number of days free from bTB prior to the test) (HISTORY); proportion of cows in the herd (PCOWS); month (or season) of the test (MONTH, SEASON) and the number of animals purchased in the previous year (either all animals purchased (BOUGHT) or all animals aged over 12 months purchased (BOUGHTOVER1)). Initially each risk factor was tested within a univariable model. Risk factors that were significant in the univariable analysis (p-value ≤ 0.20) were used to build the multivariable model. Two-way interactions between YEAR and the other risk factors were included in the initial multivariable model. A backward selection procedure was used, to eliminate terms from the model based on a likelihood ratio test ($p > 0.05$). Terms that were excluded at the initial screening stage were tested for inclusion in the final model ($p < 0.05$). An assessment of the goodness-of-fit was obtained by examining plots of studentised residuals. Further, the relationship of continuous variables (HSIZE, LOCATION, HISTORY, PCOWS, BOUGHT and BOUGHTOVER1) with the outcome were examined using a plot of the log odds of the outcome and the continuous variable. Where no obvious linear or polynomial relationship was evident, the variable was treated as categorical. The estimation method used to fit the model was chosen by comparing models using the AIC.

A likelihood ratio test was used to compare the variation amongst testers in each year within the model. The odds of a tester recording a restriction each year were estimated using the random effects from the model along with confidence limits as described by Cohen et al. (2013). These odds ratios were used to identify how many testers had significantly higher or lower odds of recording a restriction compared to an average tester in each year, after accounting for the risk factors included in the model.

Testers were ranked according to the odds ratio (OR) and then grouped (in ten evenly sized groups: OR group) according to this ranking. OR group 0 included the 10% of testers with the lowest odds of finding a restricted herd, and group 9 the 10% of testers with the highest odds.

The average number of herds and animals tested per tester by the OR group were compared using an analysis of variance (ANOVA). Similarly, the average number of reactors per restriction were estimated for the OR groups and differences tested using an ANOVA. In addition, the proportion of herds that had an animal with a lesion at slaughter in the following year was estimated by OR group and tested for differences using a chi-square test.

RESULTS

In total, 1029 testers performed at least one (median 70) eligible herd test(s) in 2008 and 986 testers performed at least one eligible herd test(s) (median 79) during 2011 (Table 1). In 2008, tests were conducted on 89,719 herds, including 2,046 (2.28%) where reactor(s) were detected. During this period, 901 of these 1029 testers conducted 10 or more eligible tests, on

89,257 herds, including 2,031 (2.28%) where reactors were detected. In 2011, tests were conducted on 94,815 herds of which 1,669 (1.76%) were restricted. Of these, 877 testers conducted 10 or more eligible tests on 94,470 herds of which 1,661 (1.76%) were found to have reactors.

Table 1. Distribution of the number of herds and animals tested per tester, the herd restriction rate and animal reactor rate at eligible tests in Ireland in 2008 and 2011

	Lower Quartile	Median	Upper Quartile
2008 (testers = 1029)			
Herds tested per tester	31	70	120
Animals tested per tester	1576	4175	6677
Herd restriction rate per tester (%)	0	1.45	3.75
Animal reactor rate per tester (%)	0	0.05	0.18
2011 (testers = 986)			
Herds tested per tester	35	79	130
Animals tested per tester	1788	4607	7411
Herd restriction rate per tester (%)	0	1.14	2.70
Animal reactor rate per tester (%)	0	0.04	0.12

In the univariable analysis, the proportion of herds restricted was significantly different by each of the risk factors. Based on plots of the logit against each predictor, herd-size was included as a continuous variable using the natural logarithm of herd-size. All other variables were included in the models as categorical variables. Animals purchased over 1 year of age had a lower AIC and were therefore included in the model in preference to all animals purchased. The final model included the risk factors: YEAR, log of HSIZE, the categorical variables: PCOWS, LOCATION, HISTORY, SEASON, BOUGHTOVER1 and the 2-way interactions: YEAR x log of HSIZE and YEAR x BOUGHTOVER1 (Table 2). A model estimated using the laplace method was preferable based on the AIC when compared to the adaptive quadrature method. Models fit using pseudo (or quasi) maximum likelihood (RSPL) did not converge.

There was a significant difference in the random effect of testers in 2008 compared with 2011 (p = 0.039). The variance of the random intercept in 2008 was higher at 0.589 (standard error 0.060) compared to 2011 when the variance was 0.426 (standard error 0.052). Based on the confidence interval of the odds ratio for each tester, the proportion of testers in 2008 with a lower odds of finding a restricted herd compared to the average tester was 0.11% (1 out of 901 testers). In 2011, there were no testers with significantly lower odds compared to the average. The proportion of testers identifying significantly more restrictions than the average was higher in 2008 (8.9%: 80 out of 901 testers) compared to 2011 (4.6%: 40 out of 877 testers).

Table 2. Odds ratios of fixed effects from the multivariable random effects logistic regression model of the probability of a herd being restricted at eligible tests in Ireland in 2008 or 2011, including tester as a random effect.

				95% confidence interval		
Variable	Values		OR	Lower	Upper	P-value
Proportion of cows	0		0.82	0.73	0.92	<0.001
	0.001 - 0.306		0.66	0.59	0.73	<0.001
	0.307 - 0.399		0.68	0.62	0.76	<0.001
	0.400 - 0.486		0.73	0.66	0.81	<0.001
	> 0.486		1.00			
Year	2008		1.67	1.17	2.40	0.005
	2011		1.00			
Log herd size x Year	2008		1.48	1.40	1.56	<0.001
	2011		1.62	1.53	1.71	<0.001
Location (herd incidence rate in the county)	0 - 0.032		0.50	0.42	0.59	<0.001
	0.033 - 0.043		0.63	0.54	0.73	<0.001
	0.044 - 0.053		0.77	0.66	0.89	<0.001
	0.054 - 0.067		0.72	0.64	0.82	<0.001
	> 0.067		1.00			
History (days since previous restriction)	<2365		1.84	1.68	2.01	<0.001
	2366 - 4803		1.38	1.25	1.52	<0.001
	4804 - 8331		1.12	1.00	1.26	0.060
	> 8331		1.00			
Season	Autumn		1.18	1.02	1.37	0.021
	Spring		1.34	1.16	1.54	<0.001
	Summer		1.65	1.44	1.90	<0.001
	Winter		1.00			
Bought in animals aged over 1 (No. of animals purchased) x Year	2008	0	0.82	0.67	1.01	0.064
		1	0.93	0.72	1.20	0.570
		2-7	0.78	0.63	0.98	0.032
		8-18	1.07	0.83	1.40	0.596
		>18	1.00			
	2011	0	0.86	0.74	1.01	0.073
		1	0.82	0.67	0.99	0.042
		2-7	0.97	0.82	1.15	0.717
		8-18	0.76	0.62	0.93	0.008
		>18	1.00			

Table 3. Average number of herds and animals tested per tester by Odds Ratio group in 2008 and 2011

	2008 testers		2011 testers	
OR group	Mean no. of herds tested	Mean no. of animals tested	Mean no. of herds tested	Mean no. of animals tested
0	170	8525	192	8863
1	103	5596	106	5827
2	85	4614	92	5341
3	66	3978	79	4281
4	71	3987	80	4150
5	108	5970	103	5354
6	85	4632	96	5411
7	90	4599	105	4965
8	104	5310	104	5678
9	109	5236	121	5764
p-value	<0.001[a]	<0.001[a]	<0.001[a]	<0.001[a]

[a] p-value based on Anova test

Table 4. Average number of herds and animals tested per tester by Odds Ratio group in 2008 and 2011

	2008 testers		2011 testers	
OR group	Mean no. of reactors per restriction	% of herds with an animal with a lesion at slaughter in the following year	Mean no. of reactors per restriction	% of herds with an animal with a lesion at slaughter in the following year
0	3.1	0.95	6.5	0.70
1	4.7	0.93	5.8	0.83
2	7.2	0.85	4.0	0.80
3	6.6	0.86	3.9	0.88
4	4.7	0.84	5.2	1.03
5	6.7	0.68	3.9	0.82
6	4.5	0.76	4.3	1.05
7	4.5	0.79	6.0	0.77
8	4.1	0.96	4.1	0.93
9	4.0	0.78	3.9	0.80
p-value	<0.001[a]	0.506[b]	0.451[a]	0.158[b]

[a] p-value based on Anova test

[b] p-value based on chi-square test

In 2008, the odds of detecting a restriction ranged from 0.33 to 7.25, while in 2011 the odds ranged from 0.45 to 5.46. There were 725 testers who tested in both years. Testers were ranked from lowest to highest in both years and were grouped into 10 evenly sized groups based on their ranking (group 0 having the lowest odds of recording a herd restriction and group 9 having the highest odds). When comparing the OR groups between the years, there was a significant difference in the OR group of testers in 2008 compared with 2011 (chi-square p <0.001). In addition, the correlation between the rank of testers (from low to high odds) in 2008 compared to 2011 was low (spearman's correlation = 0.314).

The average number of herds and animals tested was higher amongst OR group 0 (the lowest odds group) (Table 3) and there was a significant difference in the average number of herds and animals tested amongst the 10 groups in both years (p <0.001).

There was a significant difference (p <0.001) in the average number of reactors per OR group in 2008 with group 0 having the lowest number of reactors per restriction (Table 4). There was no significant difference in the proportion of herds with an animal identified as having a lesion at slaughter in the following year in either 2008 (p = 0.506) or 2011 (p = 0.158) (Table 4).

DISCUSSION

As highlighted previously, quality control (QC) is an important component of the national bTB programme (Duignan et al., 2012). Field surveillance presents unique QC challenges, given the many factors that can adversely affect the accuracy and precision of the SICTT (Monaghan et al., 1994; Wahlström, 2004; de la Rua-Domenech et al., 2006). These results indicate that good levels of relative testing effectiveness are being achieved by testers in the Irish programme, with very few testers (1 in 2008 and 0 in 2011) identifying significantly fewer restrictions compared to an average tester. Further, variation by tester has decreased over the time period analysed, indicating an increase in consistency of testing, after accounting for other known risk factors. These results can be attributed in large part to the substantial emphasis on QC in the national programme, through field supervision supplemented with quantitative performance reports, benchmarking and corrective action (Duignan et al., 2012). There was a low level of correlation in the rankings of testers in each year, indicating that testers were achieving a different ranking in each year. Therefore, different testers were at the extremes of the rankings in each year suggesting there were no major problems with any one particular group of testers persistently detecting low/high rates of restrictions.

Although there are a number of animal- and herd-level factors that influence the number of herd restrictions, as far as possible, these have been accounted for within the model. This is discussed further below. Therefore, the variation between testers are likely related to tester-level factors, such as testing proficiency, equipment, workload, training, motivation, etc. Some additional herd-related factors may contribute, including the suitability of the testing facilities and standards of cattle handling.

Testers in OR group 0, who had the lowest odds of restricting a herd, on average, tested more animals and more herds than testers in the other OR groups (Table 3). In addition, in 2008 but not 2011, these testers also identified significantly fewer reactors on average compared to the other OR groups (Table 4). On initial consideration, these results suggest an inverse relationship between testing intensity and testing effectiveness, at least in 2008. If this were the case, however, there should be evidence of differences between OR groups in terms of undetected infection in the year following testing. However, this was not the case, in either year, as highlighted in Table 4, given the non-significant association between OR group and lesion detection in animals at abattoir surveillance in the year following the eligible test. This parameter is an important part of ongoing QC efforts through the quantitative performance reports (Duignan et al., 2012), as it provides one measure of herd infection that is independent of tester influence. Lesion detection during abattoir surveillance can be an indication of residual, rather than new infection, particularly if no further infected animals are identified during the herd restriction (Olea-Popelka et al., 2008).

There were a small number of testers in each year with a significantly higher odds ratio than the average. One plausible explanation could be due to an excess of false positive animals in herds tested by these groups, which would result in more herds with just a single reactor animal and, therefore, a lower average number of reactors. Testers in testing groups 8 and 9 tended to identify a similar average number of reactors per restriction as the other groups suggesting that the higher restriction rate is not due to excess false-positive animals.

The modelling results of fixed effects are consistent with those of previous Irish studies, providing confidence in the validity of the final mixed logistic regression model. A number of authors report the results of explanatory modelling, based on either the risk of a herd restriction (based on logistic regression, Griffin et al., 1996; O'Sullivan & O'Keeffe, 1998; Clegg et al., 2008; White et al., 2013) or the hazard of a future restriction (using survival analysis, Olea-Popelka et al., 2004; Wolfe et al., 2010; Clegg et al., 2013; Gallagher et al., 2013). Key risk factors include increasing herd size, a previous history of bTB in the herd and high local herd bTB prevalence. Age or animal class which reflects age has been found to be a significant predictor in other studies (Griffin et al., 1996; Costello et al., 1999; Lenehan et al., 2000; Martin et al., 2002; Martin et al., 2003), which concurs with the finding of an increased risk of a restriction with increasing proportion of cows in the herd. A number of studies have identified purchasing animals as a potential risk factor for restriction (Griffin et al., 1996; Hahesy et al., 1996; O’Keeffe & O’Driscoll, 1997; O’Keeffe & Higgins, 2003; Clegg et al., 2008; Wolfe et al., 2009; White et al., 2013). Consistent with these findings, we found that restriction risk increased as the number of animals purchased increased. We also found a significant seasonal pattern, with higher risk of a restriction in spring and summer months. In addition, the probability of a herd being restricted was significantly higher in 2008 compared to 2011, consistent with the ongoing fall in bTB herd prevalence in Ireland (Abernethy et al., 2013; Gallagher et al., 2013).

In previous studies looking at the performance of abattoirs, Frankena et al. (2007) and Olea-Popelka et al. (2012) used logistic modelling to estimate adjusted risks of submitting lesions and to produce adjusted confirmation rates. Shittu et al. (2013) used mixed logistic modelling to compare slaughterhouse effectiveness. In human medicine, similar models have been used in order to compare the relative effectiveness of care providers such as hospitals. Cohen et al. (2013) compare the two types of modelling (logistic and mixed logistic models) when measuring the relative effectiveness of hospitals. These authors noted that mixed models had the advantage of taking account of the clustered nature of the data and that results for individual tester are shrunk towards the grand mean particularly when based on a small number of tests or when the performance is extreme compared to other hospitals. The odds ratios produced from the hierarchical models are therefore more stable compared to using logistic models.

The current study, focusing specifically on field aspects of the SICTT, is part of a broader national research effort in support of continuous improvement and innovation of QC within the Irish bTB programme. Related research is presented elsewhere (Frankena et al., 2007; Duignan et al., 2012; Olea-Popelka et al., 2012). Lessons from these studies are being used to benchmark performance, to highlight areas where improvements are needed, and to identify opportunities for improved QC implementation. To illustrate, the effectiveness of abattoir surveillance has been substantially improved, once concerns were clearly quantified (Frankena et al., 2007; Olea-Popelka et al., 2012). Tester performance is now assessed routinely in Ireland, as an integral part of the QC programme, based on objective performance measures relating to administrative functions and disease control activities (Duignan et al., 2012). This study provides objective data on the relative performance of testers during field surveillance in Ireland, providing an examination of the variation in tester performance over time. Output from these models will help to monitor and improve the future progress of QC activities.

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BEE HEALTH MANAGEMENT

WHEN EPIDEMIOLOGY MEETS APIDOLOGY: A PAN EUROPEAN SURVEILLANCE PROGRAM ON HONEYBEE COLONY LOSSES (APIS MELLIFERA)

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SUMMARY

For the first time, an active epidemiological surveillance programme on honeybee colony mortality (*Apis mellifera*) based on randomly selected apiaries and colonies has been implemented in Europe. Despite the peculiarities of national protocols, a model of statistical analysis has been developed to analyse and interpret the information in a standardised way. The statistical analysis took into account the dependence of the secondary units (colonies) within apiaries of different sizes. Priority indicators were defined for population, health and performance. In the quantitative illustration (three countries), rates of colony losses during winter ranged from 7.8% to 28.4%. The proportion of apiaries that received a treatment against Varroa was high in two countries (98.5 and 99.5%) and lower in the last country (57.5%). The prevalence of clinical American and European foulbroods was very low at any visit in any countries.

INTRODUCTION

The decrease in honeybee colonies (*Apis mellifera*) in Europe was estimated at 16% between 1985 and 2005, and the reduction of beekeepers at 31% (Potts et al., 2010). Even if the methods used to estimate these numbers were not epidemiologically comparable, the tendency towards reductions in these fields has been hypothesized in more recent and structured studies. From 2008 to 2012, the Coloss project produced some data on honeybee colony losses at European level, and later, worldwide (van der Zee et al., 2012). In France, the rate of colony mortality in the winter of 2011-2012 was lower (17.7%) than the one recorded for the winter of 2007-2008 (29.2% of colony losses) (Holzmann et al., 2011). In Germany, rates of mortality reached outstandingly high rates (37.8%) during the winter 2009-2010 (van der Zee et al., 2012). Outside Europe, in the US, tendencies were similar showing high overwintering mortalities of honeybee colonies (Cox-Foster et al., 2007, Vanengelsdorp et al., 2008).

In this context, the European Commission asked the European Reference Laboratory for honeybee health (EU-RL) for technical assistance in setting up an epidemiological surveillance program on honeybee colony mortality (EPILOBEE). Following the guidelines produced by the EU-RL, the Member states of the European Union answered a call launched by European Commission (European Union reference laboratory for honeybee health 2011).

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Seventeen countries were selected to participate in EPILOBEE (European Commission 2012). For the first time, an active epidemiological surveillance programme on honeybee colony mortality based on randomly selected apiaries and colonies was implemented.

The objective of the program was to quantify the mortality of honeybee colonies in each country on a harmonized basis and to estimate the health of the bee population (Toma et al., 2010).

MATERIALS AND METHODS

Sampling frame

Apiaries were randomly selected in each country from a national list of beekeepers. The sampling frame, based on a two-stage simple random sampling structure, was similar for all the countries participating in the program. Apiaries were the primary units and bee colonies were the secondary units. Subsequently in this paper, i refers to the apiaries and j to the colonies. The apiaries (n) were randomly selected from the list of the total number of apiaries (N) in each country. The number was defined with the expected prevalence and the absolute accuracy. The expected prevalence is the theoretical estimated frequency of a phenomenon (colony mortality for example). This prevalence is usually based on the results from previous studies (Fig. 1). The absolute accuracy is the expected precision associated with the estimated frequency that will define the width of the 95% confidence intervals. Within each selected apiary, the number of selected colonies (mi) from the total number of colonies in the apiary (Mi) was based on a design prevalence. The design prevalence was chosen by each country (Fig. 2). Once the three epidemiological parameters were determined (expected prevalence, absolute accuracy and design prevalence), the number of colonies to be inspected in each apiary was calculated depending on the apiary size according to abacus tables, following a classical method in epidemiology. The size of the colony sub-samples and the probabilities of full inclusion were unequal. This led to mathematical specificities (Ardilly 2006) (see below).

Surveillance protocol

Three visits were performed by bee inspectors before winter 2012, after winter 2012 and during the beekeeping season (spring and summer) in 2013 to estimate the mortality of bee colonies over the winter and during the beekeeping season. Farming practices and clinical manifestations of some infectious and parasitic diseases were recorded by a bee inspector through a detailed questionnaire. The clinical diseases investigated in this program were one parasitic disease (recording of the clinical signs of varroosis and systematic evaluation of the colony infestation with the mite *Varroa destructor* by sampling 300 living honeybees), two bacterial diseases (American foulbrood caused by *Paenibacillus* larvae and the European foulbrood caused by *Mellisococcus plutonius*), three viral diseases (caused by the deformed wing virus [DWV], the acute bee paralysis virus [ABPV] and the chronic bee paralysis virus [CBPV]) and two fungi parasites (*Nosema apis* and/or *Nosema ceranae*). This program was also an opportunity to confirm during the systematic visit of the colonies the absence of two exotic arthropods in Europe affecting honeybee colonies elsewhere in the world: the small hive beetle *Aethina tumida* and *Tropilaelaps* mites. When a colony exhibited clinical signs of a disease at any visit, affected brood or adult bees were sampled for subsequent laboratory analyses to enable disease confirmation.

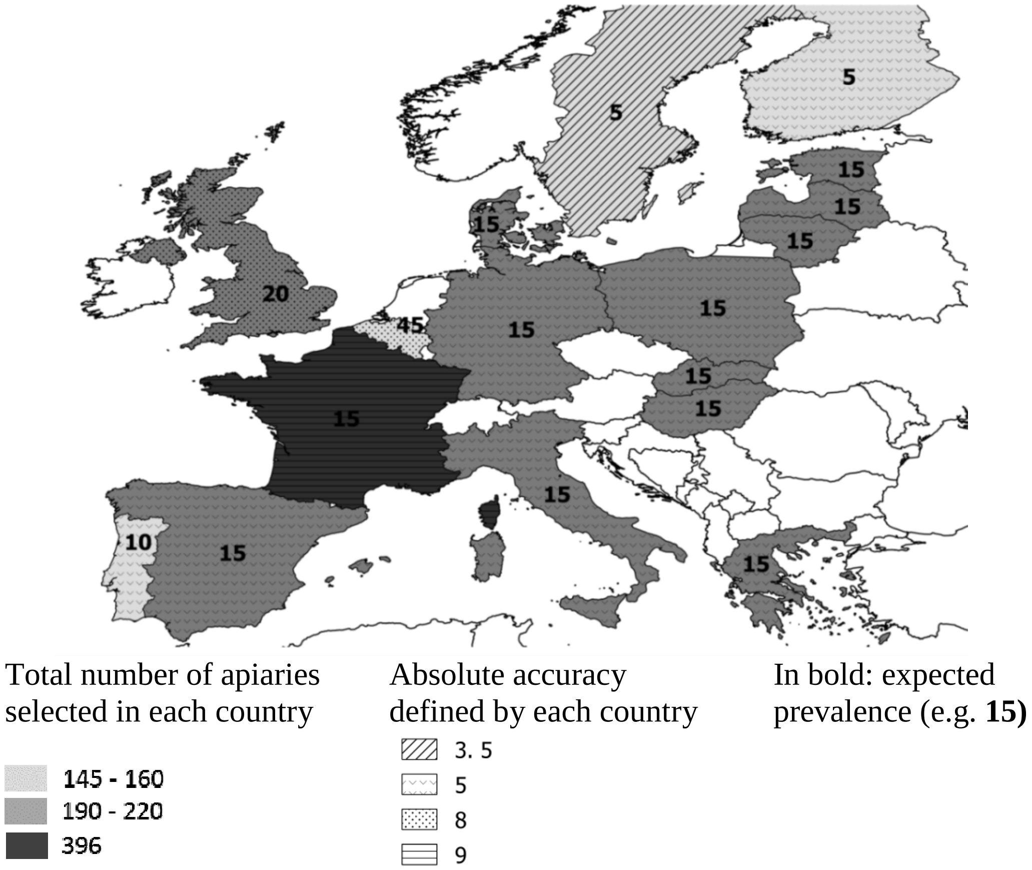

Fig. 1 Sampling plan in EPILOBEE study: total number of apiaries selected, expected prevalence and absolute accuracy

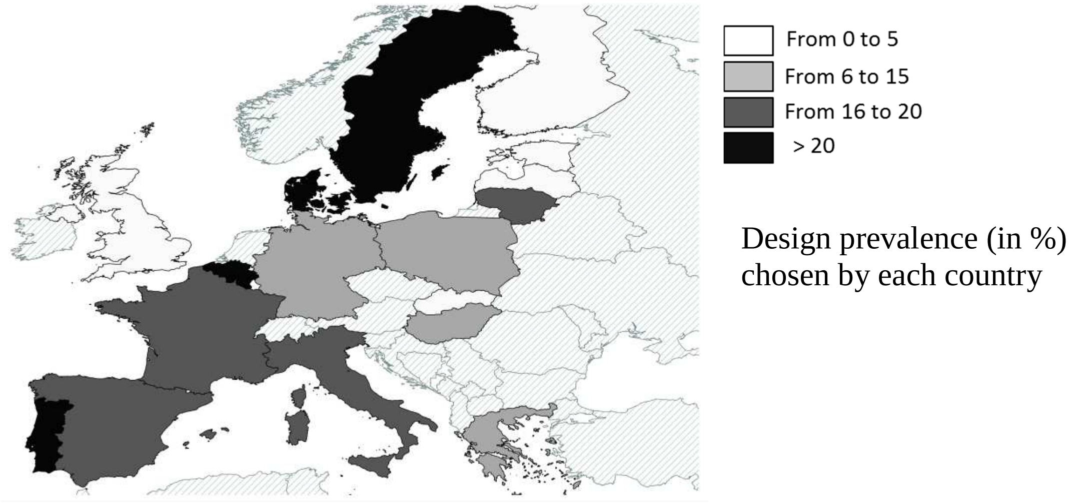

Fig. 2 Design prevalence chosen by each country in EPILOBEE program

Data management

The online database: During each visit, the operator completed a questionnaire and laboratories performed analysis. Data were stored in a standardized way in the European database via a website developed by the EU-RL and the French epidemiological surveillance platform for Animal Health.

Control and cleaning of data: The R program (version 2.15.1) was used to perform the statistical analysis. The control and the cleaning of the data were performed at the EU-RL level using a Deming wheel (Plan-Do-Check-Act) (Tsutsui 1996). R algorithms were used to identify duplicates or nonsense data. The data were consolidated through this automated control. Incorrect and missing data were discarded for calulation.

Calculation of health indicators at apiary level

The indicators were mostly based on proportions, focused on binary qualitative variables, i.e. apiary affected or not by a disease (Table 1). When dealing with proportions and when the numbers were small, the determination of the confidence intervals (CI) was based on the Binomial law (Motulsky 2010). However, an approximation by the Normal distribution could be applied when n was greater than 50 (Ardilly 2006).

Calculation of health indicators at colony level

To calculate the affected rate of bee colonies in a country, unbiased estimators of population have been defined following a statistical technique conventionally used in demography (Ardilly 2006, Tillé 2001). The sample of selected apiaries (S) was defined according to the sampling plan described above, together with the sample of selected colonies (Si) inside the apiary. After the winter, the affected colonies (ai) were recorded amongst the selected colonies. A selected colony (Xi,j) in an apiary (i with j colonies) was qualified as affected (Xi,j = 1) or unaffected (Xi,j = 0). The real figures describing the actual population of a country i.e. the total number of colonies (M) in the population, the total number of affected colonies (X) in the population and the affected honeybee colony rate (ϴ) were unknown. Therefore the parameters were estimated as follow (Ardilly 2006, Sarndal et al., 2003, Tillé 2001). The affected rate (Pi) of honeybee colonies was estimated as per Eq. (1).

$$\widehat{Pi} = \frac{\sum_{j=1}^{mi} Xi,j}{mi} = \frac{ai}{mi} \qquad (1)$$

in a given apiary ($i \in S$).

Given $\boldsymbol{\Theta} = \frac{1}{M}\sum_{i=1}^{N}\sum_{j=1}^{Mi} Xi,j$ and $\mathbf{X} = \sum_{i=1}^{N}\sum_{j=1}^{Mi} Xi,j$ and $\mathbf{M} = \sum_{i=1}^{N}\sum_{j=1}^{Mi} 1$, the unbiased estimators of X, M and θ were $\widehat{\mathbf{X}} = \frac{N}{n}\sum_{i=1}^{n}(Mi\,.\,Pi)$; $\widehat{\mathbf{M}} = \frac{N}{n}\sum_{i=1}^{n} Mi$ and $\widehat{\boldsymbol{\theta}} = \frac{\hat{X}}{\hat{M}}$. Therefore, the estimation of the affected honeybee colony rate can be seen in Eq. (2).

$$\widehat{\Theta} = \frac{\sum_{i=1}^{n}(\mathbf{Mi}\,.\,\widehat{\mathbf{Pi}})}{\sum_{i=1}^{n}\mathbf{Mi}}. \qquad (2)$$

The estimation of the affected honeybee colony rate was a weighted average, by the apiaries size (Mi), of the affected honeybee colony rate of each apiary.

Table 1: Health indicators related to EPILOBEE program

	Apiaries						Colonies					
	Confirmed disease			Suspected cases			Confirmed disease			Clinically disease		
		Incidence			Incidence			Incidence			Incidence	
	Prevalence	Winter	Season	Prevalence	Winter	Season	Prevalence	Winter	Season	Prevalence	Winter	Season
Varroosis (clinical signs)	CI	CI	CI	CI	CI	CI	CI	CI	CI	CI	CI	CI
American foulbrood	CI	CI	CI	CI	CI	CI	CI	CI	CI	CI	CI	CI
European foulbrood	CI	CI	CI	CI	CI	CI	CI	CI	CI	CI	CI	CI
V. destructor (phoretic)	CI	n.a.	n.a.	n.a.	n.a.	n.a.	CI	n.a.	n.a.	n.a.	n.a.	n.a.
DWV	CI	n.a.	n.a.	n.a.	n.a.	n.a.	CI	n.a.	n.a.	n.a.	n.a.	n.a.
ABPV	CI	n.a.	n.a.	n.a.	n.a.	n.a.	CI	n.a.	n.a.	n.a.	n.a.	n.a.
CBPV	CI	CI	CI	CI	CI	CI	CI	CI	CI	CI	CI	CI
Tropilaelaps spp.	CI	n.a.	n.a.	CI	n.a.	n.a.	CI	n.a.	n.a.	CI	n.a.	n.a.
A. tumida	CI	n.a.	n.a.	CI	n.a.	n.a.	CI	n.a.	n.a.	CI	n.a.	n.a.

n.a. : not applicable
C I : the indicator is calculable

To calculate the 95% CI of the affected rate, the dependence of the secondary units was taken into account: $\hat{\theta} \pm 2 \times \hat{V}(\widehat{\theta})$ with $\hat{\mathbf{V}}(\widehat{\theta}) = \frac{\mathbf{1}}{\hat{\mathbf{M}}^{\mathbf{2}}} \hat{\mathbf{V}}(\hat{\mathbf{z}})$ (Ardilly 2006). $\hat{V}(\hat{Z})$ is the estimation of the variance of the total of the Zi,j, the linearised variable of Xi,j (Deville 1999) θ being unknown, the calculation of $\hat{Z}i, j$ was not possible. Subsequently to calculate $\tilde{Z}i, j$, the approximation of $\hat{Z}i, j$ can be seen in Eq. (3).

$$\hat{\mathbf{V}}(\hat{\mathbf{z}}) = \mathbf{N}^{\mathbf{2}} \left(\mathbf{1} - \frac{\mathbf{n}}{\mathbf{N}}\right) \frac{\mathbf{Sa}^{\mathbf{2}}}{\mathbf{n}} + \frac{\mathbf{N}}{\mathbf{n}} \sum_{\mathbf{i=1}}^{\mathbf{n}} \left(\mathbf{Mi}^{\mathbf{2}} \left(\mathbf{1} - \frac{\mathbf{mi}}{\mathbf{Mi}}\right) \frac{\mathbf{Sbi}^{\mathbf{2}}}{\mathbf{mi}} \right) \tag{3}$$

where $Sa^2 = \frac{1}{n-1} \sum_{i=1}^{n} \left(\hat{T}\imath - \frac{\hat{T}}{N}\right)^2$ and $\hat{T}\imath = Mi \sum_{j=1}^{mi} \frac{\tilde{Z}i,j}{mi}$ et $\hat{T} = \frac{N}{n} \sum_{i=1}^{n} \left(\frac{Mi}{mi} \sum_{j=1}^{mi} \tilde{Z}i, j\right)$; and $Sb^2 = \frac{1}{Mi-1} \sum_{j=1}^{mi} \left(\tilde{Z}i, j - \left(\frac{1}{Mi} \sum_{j=1}^{mi} \tilde{Z}i, j\right)\right)^2$; $\tilde{Z}i, j = 1 - \hat{\theta}$, if the colony i,j was affected and $\tilde{Z}i, j = -\hat{\theta}$, if the colony i,j was not affected. To perform this calculation, the package Survey in R was used.

Epidemiological indicators

Three typology indicators are usually used in an epidemiological surveillance system: the population indicators which describe the population under surveillance, the health indicators which illustrate the health status of the population (prevalence and incidence, see Table 1) and the performance indicators which assess the quality of the surveillance (Dufour & Hendrikx 2011, Toma et al., 2010). The inventory, implemented through the guidelines and the standardized questionnaire reported more than one hundred indicators adapted to honeybee health specificity (AFSSA 2009, Chauzat et al., 2009, Dominguez et al., 2013). To assess performance of the surveillance network only, 15 indicators were listed relating to the achievement of samplings, the production of reports to the different stakeholders and the training of bee inspectors and laboratories. Subsequently, a DELPHI method was used to select the most relevant indicators (Observatoire régional de la santé et des affaires sociales 2009), by using seven experts from Denmark, France, Italy and the U.K. The lists of all indicators were individually sent to the experts. Each expert had to score all indicators according to their importance from 1 to 5 (1 not important- 5 really important). Subsequently, scores were averaged, leading to the selection of nine priority indicators (3 indicators for each section: population, health and performance).

Population indicators

The three most relevant indicators were kept for further analysis after DELPHI selection:

1. The size of the apiaries (Mi), i.e. the number of colonies within an apiary

2. The veterinary treatments against *V. destructor*, evaluated by the proportion of apiaries that received a miticide treatment.

3. The risk of introduction of one of the two exotic parasites assessed by the proportion of apiaries located near an airport or a harbor (less than 10 km).

Health indicators

Three indicators were selected for the subsequent analysis, all related to apiary level.

The overwintering mortality rate of bee colonies: The overwintering mortality rate of honeybee colonies was the proportion of dead colonies (aiw) recorded at V2 out of the randomly selected colonies at V1 (miw), weighted by the number of colonies in the apiary V1 (Miw). The mortality rate was calculated as per Eq. (4) with $\mathrm{Pi_w} = \frac{\mathrm{ai_w}}{\mathrm{mi_w}}$.

$$\frac{\sum_{i=1}^{n} \mathrm{Mi_w} \times \mathrm{Pi_w}}{\sum_{i=1}^{n} \mathrm{Mi_w}} \times 100\% \qquad (4)$$

The prevalence of apiaries clinically affected by American foulbrood: Prevalence was calculated for visit n (V1, V2 or V3) as per Eq. (5).

$$\frac{\text{Number of apiaries visited and clinically affected by AFB at Visit n}}{\text{Number of apiaries sampled at Visit n}} \text{ x } 100\% \qquad (5)$$

The prevalence of apiaries clinically affected by European foulbrood at each visit: Prevalence was calculated for visit n (V1, V2 or V3) as per Eq. (6).

$$\frac{\text{Number of apiaries visited and clinically affected by EFB at Visit n}}{\text{Number of apiaries sampled at Visit n}} \text{ x } 100\% \qquad (6)$$

Performance indicators

Five categories of information are needed to monitor the activity of active surveillance programs: data collection, monitoring, reporting, network management and training (Sorbe & Hendrikx 2011). According to the DELPHI selection, three indicators were calculated to estimate EPILOBEE performance.

RESULTS

The results presented in this paper are numerically illustrated with three countries which the authors voluntarily make anonymous (named A, B and C). Data presented below were extracted from the database late December 2013. The three countries had sampling frames giving the same probability of apiary inclusion for the whole country. The absolute accuracy was 8, 5 and 5% and the design prevalence 5, 10 and 30% for the countries A, B, and C respectively. The priority indicators related to population, health and performance are quantitatively illustrated in Table 2. In the sample, the small apiaries (less than 50 colonies) were the most present in all countries representing from 50.7% to 100% of the total apiary population. The median numbers were 3, 20 and 12 colonies per small apiary for the countries A, B and C respectively (Table 2). Apiaries of medium size (more than 50 colonies and less than 150 colonies) had a median number of colonies of 76 and 59 respectively for countries B and C. The countries A and C had no large apiaries (more than 150 colonies).

The risk of introduction of the two targeted exotic parasites was low in country A (less than 12% of the apiaries were located near an airport or a harbour) or very low (less than 1% - countries B and C). Rates of colony losses during winter ranged from 7.8% to 28.4%. Systematic samples were correctly implemented in two countries. All first visits were achieved in all countries. Rates of achievement of second and third visits were lower, but within an acceptable range (from 94.4% to 99.4%). The proportion of apiaries that received a treatment against *Varroa* was high in countries A and B (98.5 and 99.5%) and lower in country C (57.5%). The prevalence of clinical American and European foulbrood was very low at any visit in any countries.

Table 2. Priority indicators for population, health and performance from the EPILOBEE program quantitatively illustrated for three countries

	Countries		
	A	B	C
Population indicators			
Size of apiaries (%)			
<50	100	50.7	96.4
>50 ; <150	0	37.1	3.6
>150	0	12.2	0
Veterinary treatments against *V. destructor* (% apiaries)	98.5	99.5	57.5
Proportion of apiaries near a harbour (%)	10.6	0.51	0
95 % CI	6.2 – 15	0-2.7	0-2.6
Proportion of apiaries near an airport (%)	11.6	0.51	0.7
95 % CI	7.1-16.1	0-2.7	0-4
Health indicator			
Overwintering mortality rate (%)	28.4	7.8	15.0
95 % CI	24.2 - 32.6	5.5 - 10.2	10.3 – 20.2
Prevalence of clinical American foulbrood (%)			
At visit 1	0.00	0.02	0.00
95 % CI	0.00-0.02	0.00-0.04	0.00-0.03
At visit 2	0.00	0.00	0.00
95 % CI	0.00-0.02	0.00-0.02	0.00-0.03
At visit 3	0.00	0.01	0.00
95 % CI	0.00-0.02	0.00-0.03	0.00-0.03
Prevalence of clinical European foulbrood (%)			
At visit 1	0.02	0.01	0.00
95 % CI	0.01-0.04	0.00-0.03	0.00-0.03
At visit 2	0.01	0.00	0.00
95 % CI	0.00-0.04	0.00-0.02	0.00-0.03
At visit 3	0.01	0.00	0.00
95 % CI	0.00-0.03	0.00-0.02	0.00-0.03
Performance indicators			
Rate of systematic samples (%)	96.3	100	13.5
Rate of achievement of the visits			
Visit 1 (%)	100	100	102.1
Visit 2 (%)	98.5	97.9	94.4
Visit 3 (%)	98.4	99.4	94.8

DISCUSSION

Representativeness and accuracy of population indicators and health indicators

The standardization of the 17 national protocols made the use of common indicators possible to assess honeybee populations and colony health. However, some national peculiarities in sampling frames should be taken into account for the calculation of some indicators and in the interpretation of results. The design prevalence varied widely from one country to another, from 0% (i.e. all colonies were visited in a given apiary) to 45%.

The sampling frame: The sampling frame was dependent on the availability of lists of beekeepers and apiaries. In most countries it was difficult to have exhaustive and updated lists. In some countries the registration of beekeepers was voluntary or mandatory for less than two years (see Chauzat et al., 2013 for more details).

Impact of health regulations and medical treatments performed between visits: Some diseases were regulated at European level e.g. American foulbrood (European Commission 1992) or at national level (varroosis for instance in France, Hungary and Italy). This policy required in some cases drug treatments or destruction of affected colonies, leading to some biases in the calculation of prevalence (and incidence) and mortality rates. Similarly, beekeepers could have performed medical or technical treatments after a positive diagnosis following a visit. The improvement of health conditions of apiaries when participating in a surveillance program has been already shown elsewhere (Chauzat et al., 2010).

The technical ability of the bee inspectors and the laboratories: A colony was considered to have a disease if clinical signs were observed and if laboratory analyses confirmed the suspicion. The specificity of the network is considered high because the bee inspectors were experienced and properly trained to be able to recognize the clinical signs. In addition documents to identify the main diseases in the field and on the proceedings of the visits were produced by the EU-RL and allowed harmonized processes. They were provided to all countries in order to make sure that the diseases would be equally detected in any country. Given that all colonies were systematically visited by bee inspectors, it can be ascertained that the sensitivity of the surveillance was high in the case of clinically expressed diseases (Dufour & Hendrikx 2011). Moreover, each Member state had a national reference laboratory involved in EPILOBEE to perform analyses on honeybee diseases or to coordinate a network of laboratories. Some of the laboratories had only recently begun performing analyses on honeybee diseases while others had more experience (Chauzat et al., 2013, Hendrikx et al., 2010).

Health indicators at apiary level

The status of affected apiaries depended on the colony sampling frame within the given apiary, defined by the design prevalence. There were 95% chances to detect at least one affected colony in the randomly selected colonies if the disease prevalence was at least equivalent to the design prevalence. An apiary declared unaffected may actually be affected but the size of the second degree sample (mi colonies) did not allow detection of disease presence. However, in many cases, most of the colonies (sometimes all) were sampled, consequently increasing the probability of disease detection. The comparison of these proportions between countries without the same design prevalence is not straightforward and requires careful interpretation. The same caution should be applied to the calculation of the health indicators at colony level.

Performance indicators

Performance indicators are used to monitor the quality of the surveillance continuously and objectively (Dufour & Hendrikx 2009). The indicators set up at European level to evaluate EPILOBEE indicated good achievement rates for the visits in any of the countries. However the low result of the indicator of achievement for honeybee sampling in country C allowed the detection of a difficulty in the implementation of EPILOBEE sampling program. Consequently, actions should be taken to identify and overcome these problems.

A unique program

So far, EPILOBEE was the only active surveillance program implemented at a European level with such an amount of apiaries and colonies (van der Zee et al., 2012, van der Zee et al., 2013, Vanengelsdorp et al., 2013). The statistical methods used in EPILOBEE have never been implemented in any bee health surveys before (Genersch et al., 2010, Holzmann et al., 2011, Potts et al., 2010, van der Zee et al., 2013, Vanengelsdorp et al., 2011, Vanengelsdorp et al., 2013). The calculation of the indicators for population, health and performance was developed and implemented following the same procedure in all countries. This has been lacking in apiculture descriptive surveys so far (Hendrikx et al., 2010).

CONCLUSION

For the first time a European wide program on honeybee health was implemented with epidemiological standardized methods. Epidemiological surveillance methods developed for other animal diseases were adapted to the specificity of honeybee husbandry. The DELPHI method helped to identify the most relevant indicators at European level. The checking and the cleaning of the data following a Deming wheel ensured the validity of the calculation. The indicators were calculated according to a standardized method in all countries. However, each country should identify all the potential biases due to their sampling frames before interpreting the results. Overall, the national indicators developed through EPILOBEE allowed a better understanding of the honeybee health issues in Europe.

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AN IMPROVED METHOD FOR QUALITATIVE RISK ASSESSMENT - VALIDATION AND APPLICATION TO A BEE PEST

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SUMMARY

A consensus-based import risk assessment approach was developed combining methods applied in the fields of plant health and animal health. It provides (1) an overall risk score per risk pathway and (2) the possibility to rank risk factors between pathways in absence of quantitative data. This method was applied to assess the risk of entry of a bee pest. Expert elicitation sessions were run as is done for the evaluation of the probability of entry performed in pest risk assessments of invasive species in the plant health field and an overall risk score for each pathway was determined by combining risk scores per pathway using a combination matrix used in animal health risk assessment. Validation of this improved method was performed by comparing the determined overall risk scores with overall risk scores that the individual experts assigned at the beginning of the process based on their personal experience and knowledge.

INTRODUCTION

Risk assessment aims to provide an objective evaluation of a risk in which assumptions and uncertainties are clearly considered and presented. Since the World Trade Organisation (WTO) agreement on the application of sanitary and phytosanitary measures (referred to as the SPS agreement) came into force in 1995, a risk assessment is needed to provide the justification for measures in human, animal and plant life or health. At the moment, the application of this concept is still expanding and its importance continuously increases (Jenkins, 2013). The constant evolution of our society and environment creates an everlasting flow of new risk questions that need to be addressed during the decision making process (Finkel, 2011; Gallager et al., 2013; Heinala et al., 2013). Therefore, new risk assessment methodologies need to be developed continuously to provide risk estimates for the risk managers.

The risk assessment methodologies used in different fields are often similar, but the structure and the terminology are often adapted to specific applications. For instance, risk assessment methodologies are available for entry of different types of animal diseases (Wieland et al., 2011; Akoll et al., 2012; Corbellini et al., 2012; Forland et al., 2012). They are designed for pathogens like animal viruses which need a host in order to replicate and spread the disease and hence do not consider the transfer of the pathogen to a suitable host once the pathogen arrives into a new area. However, this is an important step in a pest risk assessment since a pest can move independently from a suitable host to another one. There are several risk assessments performed for plant pests (Jeger et al., 2012) and some for animal pests (e.g. Brown et al., 2002) but they lack an overall risk score per risk pathway.

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The assessment of the risk of entry of the small hive beetle (SHB, *Aethina tumida* [Coleoptera: Nitulidae]) into the European Union (EU) was used as an example for the development of a consensus-based risk assessment approach that provides both an overall risk score per risk pathway as well as the probability to rank risk factors between pathways, even in the absence of quantitative data.

The SHB is a flying coleopteran native to sub-Saharan Africa. Since 1998, the parasite has spread to North America, Latin America and Australia (Hood, 2004; Neumann & Ellis, 2008). The life cycle begins with the adult SHB laying eggs, usually in irregular masses in crevices or into sealed brood cells within beehives. The emerging larvae begin to feed on brood comb, bee eggs, pollen and honey within the beehive. This leads to a significant reduction in brood area and damages to European honey bee colonies whereas a reduction in pollen stores is the main impact of an SHB infestation in African honey bee colonies (Ellis et al., 2003). At maturation, the larvae seeking light, exit the beehive, where they fall to the ground, burrow into the surrounding soil and form a pupal chamber. Emerging adult SHBs are attracted to colonies, beehives, honey storage, extraction facilities and some ripe fruits, where they find food and reproduce (Mayr et al., 2003; Graham et al., 2011). A complete assessment of the risk of entry of different bee pests into the European Union is provided in an opinion of the Panel for Animal Health Welfare of EFSA (EFSA, 2013). In these proceedings, the novel risk assessment methodology is fully described and explained for further use by illustrating how it can be applied to answer the risk questions: ‘What is the risk of SHB entry via import of live bees?’ and ‘What is the risk of SHB entry via import of bee products for use in apiculture, and used beekeeping equipment?’

MATERIALS AND METHODS

A priori rapid risk assessment by individual experts

A group of persons with expertise in bees, bee pests or risk assessment defined qualitative categories of risk and the level of uncertainty associated with certain risk scores (Table 1 and Table 2). Then three experts with scientific background in bee biology and SHB were consulted to estimate the risk of entry of SHB into the EU as well as the corresponding uncertainty. The experts applied the categories during a rapid a priori assessment. The experts provided their risk scorings individually on paper in order to minimize influence among the group during the risk estimation.

Table 1. Definition of risk categories used for scoring during a rapid a priori risk assessment by individual bee experts

Name	Explanation
Negligible	The conditions of the pathway do not allow the pest to enter the risk assessment area.
Low	It is unlikely that the pest will enter the risk assessment area through this pathway.
Moderate	The pest may enter the risk assessment area through this pathway to a low amount.
High	The pest may enter the risk assessment area through this pathway to a relevant amount.
Unknown	The conditions of the pathway are mostly unknown.

Table 2. Definition of uncertainty categories used to score levels of uncertainty

Name	Explanation
Low	No or limited information or data are lacking, incomplete, inconsistent or conflicting. No subjective judgement is introduced. No unpublished data are used.
Medium	Some information or data are lacking, incomplete, inconsistent or conflicting. Subjective judgement is introduced with supporting evidence. Unpublished data are sometimes used.
High	The majority of information or data are lacking, incomplete, inconsistent or conflicting. Subjective judgement may be introduced without supporting evidence. Unpublished data are frequently used.

Consensus-based risk assessment

After the individual scoring had been done, the two risk questions were discussed amongst the bee experts and risk assessors. It became clear that the risk of SHB entry via import of bees is largely dependent on the characteristics of the consignment. Therefore, the design of consensus risk pathways related to SHB entry via bee imports represented these differences in bee consignments. Similarly, the risk question on SHB entry via import of bee products and used beekeeping equipment was subdivided into two alternative risk pathways (Fig. 1).

At the beginning of the assessment different pathways were identified. The design of each risk pathway was based on plant health risk assessment methodology and follows the course of the pest from the origin of infestation to possible hosts into the EU (EFSA, 2010a). The first step of each pathway is the assessment of the association of the pest with the pathway at origin, the second step is the survival of the pest during transport and the third step is the transfer of the pest to a suitable host in the EU (Fig. 1). Per step of each pathway, a detailed discussion took place on the risk factors that could influence the number of pests entering the EU.

Secondly, risk categories were defined and applied for qualitative risk scoring of the three steps per risk pathway: association of the pest with the pathway (Table 3), the survival of the pest during transport (Table 4) and transfer of the pest to a suitable host (Table 5). After the categories were agreed upon, the most appropriate risk and uncertainty scores were selected from these lists (Tables 3-5) and the main rationale for every score was specified. The scoring was done in a horizontal way across the different pathways: the ‘association of the pest with the pathway at origin’ was first scored in the pathway ‘voluntary import *A. mellifera* queens’ (A_1 in Fig. 1), then in the pathway ‘voluntary import of *Bombus* spp. queens’ (A_2) and so on until the last pathway ‘import of beekeeping equipment’ (A_7). The same horizontal scoring was also applied to the steps ‘survival of the pest during transport’ (T_1 to T_7) and the steps ‘transfer of the pest to a suitable host in the EU’ (H_1 to H_7). This approach enabled direct comparisons and ranking of risk scores across the pathways.

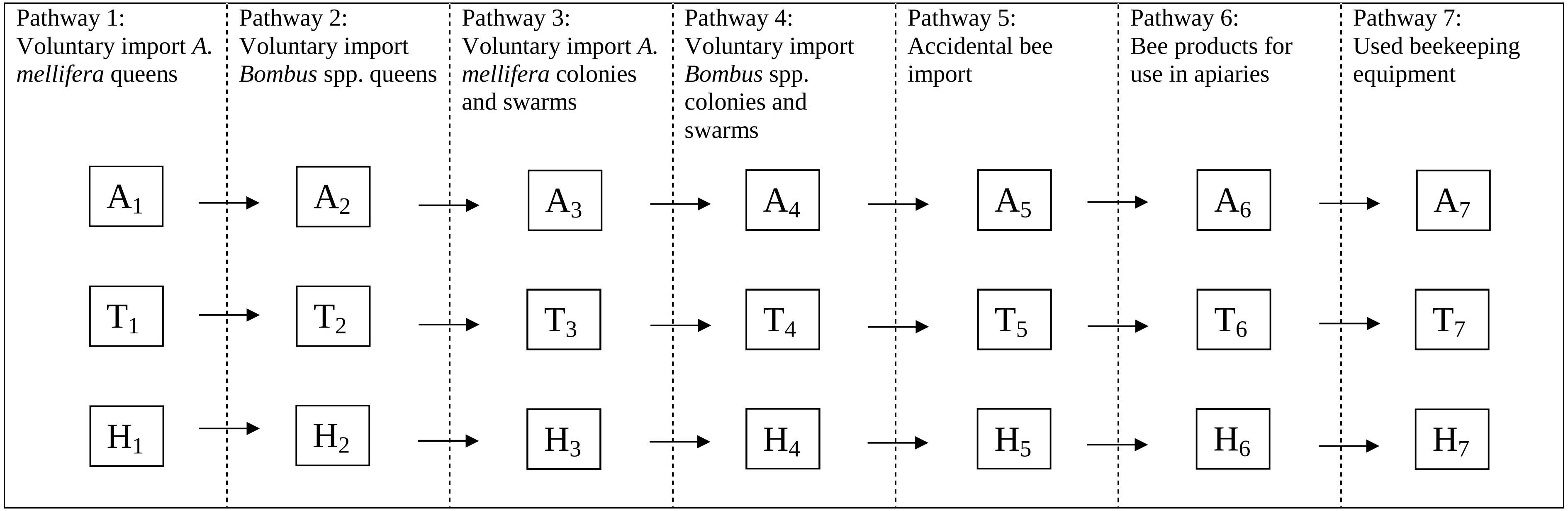

Fig. 1 Pictogram showing how scoring of risk and uncertainty was performed horizontally across the different risk pathways as indicated by the arrows. The risk steps ‘Association of the pest with the pathway at origin’, ‘Survival of the pest during transport’ and ‘Transfer of the pest to a suitable host’ are presented by A, H and T respectively, with the number of the pathways represented as subscripts

Table 3. Definition of risk categories used to score the step ‘association f the pest with the pathway’

Name	Explanation
Negligible	The pest does not or only occasionally associate with the pathway at origin
Low	The pest is rarely associated with the pathway at origin
Moderate	The pest is frequently associated with the pathway at origin
High	The pest is regularly or usually associated with the pathway at origin
Unknown	The association of the pest with the pathway at origin is unknown

Table 4. Definition of risk categories used to score the step ‘survival of the pest during transport’

Name	Explanation
Negligible	The pest will be killed during transport
Low	It is unlikely that the pest will survive transport
Moderate	The pest survives transport to a low extent
High	The pest mostly survives transport
Unknown	The survival of the pest during transport is unknown

Table 5. Definition of risk categories used to score the step ‘transfer of the pest to a suitable host’

Name	Explanation
Negligible	The pest is not able to transfer to a suitable host
Low	It is unlikely that the pest will transfer to a suitable host
Moderate	The pest may transfer to a suitable host to a low extent
High	The pest may transfer to a suitable host to a relevant extent
Unknown	The transfer of the pest to a suitable host is unknown

The next part of the consensus-based risk assessment was the determination of an overall risk score for each pathway by combining the step-wise scores along each pathway. This was achieved using a combination matrix that has been previously used in animal health risk assessment fields (Beckett, 2007; Wieland et al., 2011; EFSA, 2010b). This combination matrix (Table 6) was used to combine two consecutive risk estimates based on the assumption that the subsequent event is conditioned on the occurrence of the previous event and an increased risk would not be meaningful.

The consensus-based risk assessment used the uncertainty scores determined during the *a priori* rapid risk assessment (Table 2). A combination of uncertainty scores was not applied in the risk assessment. In all cases, the highest uncertainty score among the individual steps of a pathway was used.

Table 6. Combination matrix used to define the overall risk score per pathway

	Following event				
Previous Event	0-Negligible	1-Low	2-Moderate	3-High	4-Unknown
0-Negligible	0-Negligible	0-Negligible	0-Negligible	0-Negligible	0-Negligible
1-Low	0-Negligible	1-Low	1-Low	1-Low	1-Low
2-Moderate	1-Low	1-Low	2-Moderate	2-Moderate	2-Moderate
3-High	1-Low	2-Moderate	2-Moderate	3-High	3-High

A two-dimensional scheme was applied to facilitate a rapid comparison of the results within and between the risk pathways and could allow the identification of risk and uncertainty patterns (Table 7). The different risk scores are presented by different shading and the different uncertainty levels are indicated by varying font size.

Table 7. Two-dimensional scheme incorporating both risk and uncertainty;
N, negligible; L, low; M, moderate; H, high; U, unknown; NA, not applicable

		Uncertainty		
		1-Low	2-Medium	3-High
Risk	0-Negligible	N/L	N/M	N/H
	1-Low	L/ L	L/M	L/H
	2-Moderate	M/ L	M/M	M/H
	3-High	H/ L	H/M	H/H
	4-Unknown	NA	NA	U/H

Validation of the consensus-based risk scoring

The risk and uncertainty scores obtained by the consensus-based risk assessment were compared with those obtained by the *a priori* rapid risk estimation given by the individual experts. This comparison allowed the validation of the results (in case of identical risk scores) or evoked further discussion on the identification of the risk factors responsible for a divergent risk score (in case of different risk scores).

RESULTS

A priori risk assessment by individual experts

Three experts with a scientific background in SHB were consulted to estimate the risk of entry of SHB into the EU as well as the corresponding uncertainty. Two experts considered the risk of SHB entry via import of live bees high whereas one expert considered this risk moderate. A low level of uncertainty was nominated by all three experts (Table 8). The experts gave the same risk scores for SHB entry via import of bee products for use in apiaries and used beekeeping equipment as they did for the risk of SHB entry via bees, but the level of uncertainty was higher for entry via bees (one scored low and two scored moderate).

Table 8. Overview of the risk assessment of SHB entry; L, low; M, moderate; H, high. The different risk scores are presented by different shading and the different uncertainty levels are indicated by varying font size.

Risk of SHB entry via import of	Rapid *a priori* risk score given by individual experts			Consensus risk pathway of SHB entry reached through discussion	Consensus risk score given by a group of experts			Overall risk of SHB entry derived from a combination matrix: risk/uncertainty
	Expert I (risk/uncertainty)	Expert II (risk/uncertainty)	Expert III (risk/uncertainty)		Association of the pest with the pathway at origin: risk/uncertainty	Survival of the pest during transport: risk/uncertainty	Transfer of the pest to a suitable host: risk/uncertainty	
Bees	H/L	H/L	M/L	Voluntary import *A. mellifera* queens	H/L	M/M	M/H	M/H
				Voluntary import *Bombus* spp. queens	M/M	M/M	M/M	M/M
				Voluntary import *A. mellifera* colonies and swarms	L/L	H/L	H/L	L/L
				Voluntary import *Bombus* spp. colonies and swarms	L/L	M/L	H/L	L/L
				Accidental bee import	H/L	H/L	H/L	H/L
Bee products and equipment	H/L	H/M	M/M	Bee products for use in apiaries	H/L	H/L	H/L	H/L
				Recycled beekeeping equipment	M/L	H/L	H/L	M/L

Consensus-based risk assessment

Seven risk pathways were identified and agreed amongst the experts (Fig. 1). Each pathway consisted of three steps: association of the pest with the pathway at origin (A), survival of the pest during transport (T) and transfer of the pest to a suitable host (H). The pathway model used follows the course of the pest from the origin of infestation to possible hosts into the EU.

For the individual steps of the pathways the consensus-scores revealed high risk for all segments of the pathways ‘accidental bee import’ and ‘import of products for use in apiaries’.

All pathways that did not involve importation of queens were scored as being associated with low uncertainty. Although the risk scoring (as compared between the two pathways that referred to importation of live queens) were rather similar, the pathway involving *A. mellifera* queens involved a perceived greater risk in step one and a higher uncertainty in step three.

The final step was the determination of an overall risk score for each pathway by combining the risk scores for each step of the pathway using a combination matrix. Voluntary imports of colonies and swarms were perceived to result in a low risk of SHB entry into the EU with a low uncertainty level (Table 8). Voluntary imports of queens and imports of used beekeeping equipment were judged to have a moderate risk, with a moderate to high uncertainty (*Bombus* spp. versus *A. mellifera*) and a low uncertainty, respectively. A high risk of SHB entry with a low uncertainty was judged to be associated with accidental bee imports and imports of bee products for use in apiaries.

Validation of the consensus-based risk scoring

The direct comparison between the rapid *a priori* and the consensus-based risk estimates was not possible since the two original risk questions were subdivided in seven consensus risk pathways. The indirect comparison, however, triggered a discussion amongst the bee experts on their scorings and their rationale. This was facilitated by the application of a two-dimensional scheme for risk and uncertainty (Table 8). For the first risk question on the risk of SHB entry into the EU via import of live bees, the experts agreed that ‘accidental bee import’ was the pathway with the highest risk. Swarms and colonies of *A. mellifera* have been reported in different types of (non-bee) consignments. They can be infested with SHB since the pest is attracted to honey bees and an infested consignment might not be detected. Entry of SHB via import of queen bees was confirmed to have a moderate risk. Import of an SHB-infested *A. mellifera* queen shipment has been reported in the past but fast detection and instant reaction prevented the transfer to a suitable host in the EU. The risk of SHB entry via *Bombus* spp. is also moderate since there are no field survey data on the biological association of SHB with bumble bees at present. Finally, the risk of SHB entry via import of swarms and colonies was considered high by the experts, however, the risk of entry of this pest into the EU is low because import of swarms and colonies is not permitted according to the current legislation. For the risk question on the risk of SHB entry via importation of bee products for use in apiaries and used beekeeping equipment, the experts confirmed that the risk pathway of bee products has the highest risk since they are attractive to SHB. Measures can be taken to reduce the risk of SHB import via bee products for use in apiaries, but they were not considered during the risk assessment. The risk of SHB entry via import of used beekeeping equipment is moderate, as food for SHB is present only as a contaminant of the consignment, and imports of used beekeeping equipment are less frequent.

DISCUSSION

The consensus-based risk assessment approach and its validation have been developed by combining elements from several reported risk assessment methodologies (e.g. EFSA, 2010a) in order to give decision makers a clear answer on the risk of SHB entry into the EU.

The use of a generic pathway that is applicable to all pathways (e.g. import of live bees versus import of bee products) was very important to allow the horizontal scoring. The inclusion of pest transfer to a possible host into the risk assessment is a prerequisite for entry of a pest into a new area, whereas this step is not included in classical import risk assessment procedures in the field of animal diseases since most pathogens can only survive with their host and are transported together. The risk scores were defined specifically for every step of the risk pathway prior to scoring in analogy with the plant pest methodology as it results in a higher clarity and transparency compared to the use of general definitions of risk scores that are applied to all steps of the pathway in the animal health methodology. Scoring of steps of risk pathways is done in both plant pest and animal health risk assessment methodologies, but at a different level of detail. Moreover, all steps were horizontally scored across the pathways to keep the mind setting comparable between the pathways. The details of the approach enabled the ranking of risks across the different risk pathways. The feature in particular could be very useful to risk managers as it would allow them to prioritize actions. It was only the integration of the combination matrix as used in animal health risk assessments (Beckett, 2007; Wieland et al., 2011; EFSA, 2010b) that paved the way to an overall risk score per pathway. This final step is not performed in the plant pest methodology but is useful to the risk managers since it generates one risk estimate per risk pathway.

Experts with a thorough scientific background in biological and epidemiological aspects of SHB infestations and knowledge of the actual legislative situation on trade in live bees, bee products for use in apiaries and used beekeeping equipment were selected. However, complete independency of the experts complying with the selection criteria could not be guaranteed due to the limited size of the scientific community investigating bee diseases. To handle the influence from one expert to another during the consensus-based risk assessment, the bee experts were asked to give an *a priori* rapid risk and uncertainty estimation. The results of the scoring given *a priori* by individual experts can be compared with the outcome of the consensus-based risk assessment. This approach helps to detect possible bias in the results of the consensus-based approach.

The bee experts were not familiar with any risk assessment procedure before being involved in answering the two risk questions on SHB entry into the EU, which could have led to misinterpretation of the risk question or imprecise implementation of the risk and uncertainty scores during their *a priori* risk assessment. Nevertheless, looking back to the individual risk and uncertainty scores, triggered the critical review of the consensus overall scores and the identification as well as formulation of their corresponding rational.

The results intend that the pest was more reliably detected when bees are imported on a voluntary basis (e.g. import of queen bees by a beekeeper) compared to accidental import where bees are unintended present in a non-bee consignment (e.g. bees adhering to a plane). According to the EU legislation, queen bees can be accompanied with maximum twenty attendant bees when they are imported into the EU. On the other hand, hundreds to thousands bees can be present in consignments of swarms and colonies, but their import into the EU is not permitted. Also the imported bee species influences the risk of SHB entry into the EU since *Bombus* spp. are considered a less likely host than *Apis mellifera*. The example shows that elements from established risk assessment methodologies can be combined to generate an improved method when a new risk question has to be answered.

In conclusion, the presented methodology of the consensus-based risk assessment enables scientific experts without experience in risk assessment to provide clear and well-structured answers that decision makers could use as basis for management procedures, even in cases where limited quantitative data are available.

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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 (£40 sterling to cover two years' membership) along with this application form. Overseas members without British bank accounts are requested to pay 2 - 3 years in advance. Cheques should be in £ sterling and drawn from a British bank. British members should pay future dues by standing order (forms are available from the Secretary or Treasurer). Payment can also be made by credit card; the appropriate form is available from the Society's website, http://www.svepm.org.uk/, or from the Secretary or Treasurer.

Please send this form to the Society's Treasurer:

Dr Marnie Brennan
School of Veterinary Medicine and Science
Sutton Bonington Campus
College Road
Sutton Bonington
Leicestershire LE12 5RD
UK

TEL +44 (0) 115 951 6577
FAX +44 (0) 115 951 6415
Email: marnie.brennan@nottingham.ac.uk

Please turn over

INTEREST GROUPS

Please tick appropriate boxes to indicate your interests:

☐ Analytical Epidemiology (Observational Studies)

☐ Quantitative Epidemiology & Statistical Techniques (Incl. Statistical/Mathematical 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.

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.

April, 1982
Revised March, 1985; April, 1988; November 1994, March 2014
Corrected January 1997; April 2002