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THE USE OF SYSTEMIC ANTIBIOTICS FOR DRY COW THERAPY; A BAYESIAN INVESTIGATION OF VETERINARY BELIEFS USING PROBABILISTIC ELICITATION

H.M. HIGGINS·, I.L. DRYDEN AND M.J. GREEN

SUMMARY

The beliefs of practising veterinary surgeons regarding the efficacy of systemic antibiotics as an adjunct to intra-mammary dry cow therapy were quantified as probability density functions using probabilistic elicitation. Cluster sampling selected 24 veterinarians for interview within five practices in England. Major variations in beliefs existed both within and between veterinary practices with implications for decisions currently being made on-farm. The majority of veterinarians holding extra qualifications shared a confidently pessimistic belief in the efficacy of systemic antibiotics. Bayesian models were used to investigate the strength of future evidence required to (rationally) change the beliefs of these practitioners, *given* their current views. This study highlights the importance of determining practitioners' beliefs *before* designing research studies; the current strength and diversity of beliefs amongst practitioners will affect the sample size and study design required to change clinical decision making. Further research into the heterogeneity of veterinarians' beliefs and decision-making is required.

INTRODUCTION

The most common reason for prescribing long-acting antibiotic products to adult UK dairy cattle is for the purpose of dry cow therapy and several licensed intra-mammary dry cow therapy (IDCT) products exist. Systemic antibiotics may be administered as an adjunct to IDCT and this is currently common clinical practice in the UK. However systemic antibiotics are not licensed for dry cow therapy; for this purpose they can only be administered under the prescribing cascade at the discretion of individual veterinarians, who must justify the "off-licence" use.

The focus of this research was to numerically capture the clinical beliefs of practising veterinary surgeons regarding the efficacy of systemic antibiotics when used in combination with IDCT. There is a recognised lack of robust data addressing this question, despite the fact that the responsible use of antibiotics in medicine is crucial, with bacterial resistance an ever-increasing concern. There were two key goals: (i) to evaluate the variation in veterinarians' beliefs and to gain insight into any diversity in the use of systemic antibiotics for dry cow therapy; (ii) to investigate the types and strength of future research evidence that would be required to change the beliefs of practising veterinary surgeons regarding the efficacy of systemic antibiotics, *given* their current clinical opinions.

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A statistical technique called probabilistic elicitation was used to capture the veterinarians' beliefs as probability distributions (often termed "prior beliefs" or simply "priors"). Probabilistic elicitation has been applied in a wide variety of fields and extensive literature exists (O'Hagan et al., 2006). Once numerically quantified as probability distributions, the *diversity* and *strength* of beliefs amongst a population can be studied and also incorporated into Bayesian models. Bayes' Theorem mathematically describes the rational way to update an initial state of knowledge about an unknown variable to a new state of knowledge in the light of new data. For a single continuous unknown variable θ, Bayes' Theorem can be written as

$$\pi(\theta|\boldsymbol{x}) \propto \pi(\boldsymbol{x}|\theta)\pi(\theta) \quad (1)$$

where $\pi(\theta)$ is the prior probability density function (or prior belief) , $\pi(\boldsymbol{x}|\theta)$ is the likelihood (based on new data $\boldsymbol{x}$) and $\pi(\theta|\boldsymbol{x})$ is the posterior probability density function containing all available information about θ after "seeing" the new data. Equation 1 says that the extent of any logical change in belief depends on *both* the prior belief, $\pi(\theta)$, *and* the strength of the new evidence, $\pi(\boldsymbol{x}|\theta)$; it is intuitively evident that veterinary surgeons currently holding very sceptical (or enthusiastic) beliefs will require different strengths of new evidence to change those beliefs, relative to ambivalent colleagues.

The likelihood, $\pi(\boldsymbol{x}|\theta)$, can be modelled based on different simulated data, $\boldsymbol{x}$, to represent varying strengths of new evidence. The actual beliefs of veterinarians, $\pi(\theta)$ (captured by probabilistic elicitation), can be combined with the likelihood using Bayes' Theorem to determine the strength of future evidence required to (rationally) change the beliefs of the veterinarians such that the majority will subsequently share a similar view, $\pi(\theta|\boldsymbol{x})$, given any diversity in their current beliefs, $\pi(\theta)$.

The aim of this research was to conduct a probabilistic elicitation to quantify and interpret the strength and diversity of beliefs of practising veterinary surgeons regarding the efficacy of systemic antibiotics as an adjunct to IDCT.

MATERIALS AND METHODS

The elicitation process can be considered to have three distinct phases each of which are equally important (Garthwaite et al., 2005; O'Hagan et. al., 2006): (i) preparation (including identification and recruitment of experts, definition of variables, task structure), (ii) the elicitation itself, (iii) further analysis dependent on the purpose of the elicitation and including an assessment of phases (i) and (ii). Each phase is described in turn.

Identification and recruitment of veterinarians

The target population comprised all veterinarians regularly involved in treating dairy cattle in England. The study population was the subset of veterinarians working within travelling distance of the first named author's location; an area 100 miles in radius and centred on the University of Nottingham. The on-line database (http://www.rcvs.org.uk/) supplied by the Royal College of Veterinary Surgeons (R.C.V.S) provided a searchable sampling frame of veterinary practices (clusters). Only clusters that treated cattle and contained at least one veterinarian holding the R.C.V.S post-graduate Certificate in Cattle Health and Production ("CertCHP") were selected. Within the study area this yielded 13 practices (labelled 1-13 for anonymity) containing 77 practitioners treating dairy cattle in total.

Practitioners were paid at a rate of £100 per hour (pro-rata) for their time in order to encourage participation and minimise the non-response rate; this was the main sampling cost. It was desirable to use an equal probability selection method ("epsem" sample) with individual veterinarians having the same probability (1/77) of selection (Barnett, 1974). A two-stage cluster design was used whereby clusters were selected with a probability proportional to their size (i.e. the number of veterinarians they contained that treated dairy cattle) and then five veterinarians were selected randomly within clusters. A "with-out replacement" systematic method was used to avoid the same cluster being selected twice, as follows (Kalton, 1983). Each cluster was assigned as many numbers as its size; such that cluster 1 (7 veterinarians) was assigned numbers 1-7, cluster 2 (8 veterinarians) numbers 8-15 and so forth. Dividing total size (77) by 5 (the pre-determined number of clusters to be selected) gave a sampling interval of 15.4. The random number generator function in the software programme "R" version 2.10.1 (R Development Core Team, 2009) was used to select a number between 1 and 15.4, say 9, to choose the first cluster, (here cluster 2). Then 15.4 was added to 9 to give 24.4 (rounded down to 24) to deterministically select the next cluster and so forth. This sampling design produced an "epsem" sample of 24 veterinary surgeons which also met the financial/practical constraints placed on the study. Data were collected on five separate days over a three-week period from 23rd June to 7th July 2010. Two pilot interviews with clinical academics from the University of Nottingham were conducted prior to main data collection to test that the method was tenable.

Definition of variables and task structure

The question of interest was "do systemic antibiotics in combination with IDCT offer a clinically worthwhile benefit over IDCT alone"? Thus it concerned a contrast between two unknown variables; the overall cure rate achievable with treatment 1 (denoted θ_1) and the overall cure rate achievable with treatment 2 (denoted θ_2) where "treatment 1" refers to IDCT alone and "treatment 2" refers to systemic antibiotics in combination with IDCT. The variables, θ_1 and θ_2, are probably regarded as *dependent* by the majority of veterinarians; given knowledge that θ_1 is lower than expected, many veterinary surgeons may believe (and it is biologically possible) that θ_2 will also be lower. To quantify beliefs relating to two variables in full requires their joint probability distribution which makes the task of elicitation considerably more complicated (O'Hagan et al., 2006). To avoid this complexity it is sometimes possible to re-structure the problem so that is it expressed in terms of *independent* variables; the joint distribution is then just the product of the marginal densities and the task simplifies to eliciting beliefs about the marginal distribution for each variable separately. This elicitation task was re-structured by defining an additional variable, θ_3, the overall cure rate achievable with treatment 2 given treatment 1 has failed. With this definition for θ_3, θ_2 is given by

$$\theta_2 = \theta_1 + (1 - \theta_1)\theta_3 \quad (2)$$

Thus θ_1 and θ_3 were elicited separately to obtain the marginal distributions of each; denoted $f(\theta_1)$ and $f(\theta_3)$. Since $0 \leq \theta_1 \leq 1$ and $0 \leq \theta_3 \leq 1$, parametric distributions from the beta family were fitted to the elicited values for θ_1 and θ_3 since this family has flexibility to cover a wide range of beliefs and avoids any issues with impossible events. Hence

$$\theta_1 \sim beta(\alpha, \beta)\,; \quad f(\theta_1) = \frac{1}{B(\alpha,\beta)}\theta_1^{\alpha-1}(1-\theta_1)^{\beta-1} \quad \text{and}$$

$$\theta_3 \sim beta(\alpha', \beta')\ ;\ f(\theta_3) = \frac{1}{B(\alpha',\beta')}\theta_3^{\alpha'-1}(1-\theta_3)^{\beta'-1}$$

where $B(\alpha,\beta) = \int_0^1 \theta^{\alpha-1}(1-\theta)^{\beta-1}\,d\theta$ and $\alpha > 0$, $\beta > 0$ are the hyperparameters for the marginal distribution of θ_1 and similarly $\alpha' > 0$, $\beta' > 0$ for $f(\theta_3)$. The assumption of independence between θ_1 and θ_3 was considered acceptable to veterinary surgeons and hence the joint distribution of θ_1, θ_3 is given by $f(\theta_1,\theta_3) = f(\theta_1)f(\theta_3)$. Since T: $(\theta_1,\theta_3) \quad (\theta_1,\theta_2)$ is a one-to-one transformation of continuous variables, the joint distribution of θ_1 and θ_2 involved a standard calculation using the Jacobian to give

$$f(\theta_1,\theta_2) = \begin{cases} \phi\theta_1^{\alpha-1}(1-\theta_1)^{\beta-2}\left(\frac{\theta_2-\theta_1}{1-\theta_1}\right)^{\alpha'-1}\left(\frac{1-\theta_2}{1-\theta_1}\right)^{\beta'-1} & if\ 0 \le \theta_1 \le \theta_2 \le 1, \\ 0 & otherwise, \end{cases} \qquad (3)$$

where ϕ is a normalising constant; $\phi = \frac{1}{B(\alpha,\beta)}\frac{1}{B(\alpha',\beta')}$. However, structuring the problem according to Eq. (2) also necessitates that θ_2 is greater than or equal to θ_1. It raised the question of whether this would impose an unrealistic constraint on the veterinarians' beliefs. However, whilst it is biologically possible that $\theta_2 < \theta_1$ (i.e. that less cows would be cured by administering systemic antibiotics in addition to IDCT), the authors believed that the majority (if not all) veterinarians would take the view that at worst $\theta_2 = \theta_1$. Nevertheless, the belief that $\theta_2 < \theta_1$ was still elicited separately to capture this opinion, should it exist.

Probabilistic elicitation method

The technique is an iterative process that usually involves eliciting a small number of summaries of the expert's belief, fitting parametric distributions to them and assessing the adequacy of the fit (Garthwaite et al., 2005). The Sheffield Elicitation Framework (SHELF, O'Hagan and Oakley http://www.tonyohagan.co.uk/shelf/) was used in this study; it is a freely available package of guidance documents, templates and software specifically designed for carrying out probabilistic elicitation. Current best practice for probabilistic elicitation was employed whereby an interview between the first author and each practitioner was conducted and feedback carried out using SHELF software, version 1.01. This involved presenting the fitted distribution to the veterinarian graphically and describing some of the implied (but not elicited) probabilities. The clinician then had the opportunity to disagree with any implied assertions which were revised until the fitted distribution was considered a fair reflection of their beliefs. This is essential because it is impossible to directly judge if any elicitation has been "successful"; apart from elicitation itself, there is no other method to establish what the person actually believes (Garthwaite et al., 2005).

For each variable (θ_1 , θ_3) five values were elicited to allow a distribution to be fitted: the plausible range (minimum to maximum), median and two further probability judgements (lower and upper quartiles). Hence the total number of observations per veterinarian was 10. The R code provided in SHELF was used to fit beta distributions to θ_1 and θ_3. This uses numerical optimisation based on the simplex algorithm (Nelder & Mead, 1965) to select the "best" fitting

hyperparameters by minimising the sum of the squared differences between the fitted cumulative distribution and the elicited cumulative distribution.

For the elicitation itself, veterinarians were asked to consider commercial dairy cows at the point of drying-off which had a "chronic" intra-mammary infection (in one or more quarters) with unknown but major pathogens; "chronic" was defined as a somatic cell count greater than 400,000 cells per ml at both of the two monthly milk recordings prior to drying off. Cows received either treatment 1 or treatment 2 and it was assumed that no other treatments were given until calving. Cows were "cured" if they were free from any major intra-mammary pathogens at calving. Note that the actual product choice for both treatment 1 and 2 was left to the discretion of the veterinarian; interest resided in the generic use of antibiotics administered by the systemic route for dry cow therapy. However it was stipulated that the choices must be products the veterinarians would actually prescribe in practice, with the objective of optimising cure rates.

A standard script was used for consistency (available on request). The task was presented as a probabilistic judgement in relative frequency terms for simplicity. Hence the question was based on the number of cows (out of 100) that it was believed could be cured with each treatment and the associated uncertainty around this number. However with this context it was important to avoid any possible confusion between *epistemic* uncertainty, originating from imperfect knowledge, and *aleatory* uncertainty, arising due to randomness (O'Hagan et al., 2006). Of interest was an epistemic variable, the *overall* cure rate achievable with each treatment. Aleatory uncertainty due to the context of considering only a theoretical sample 100 cows was not of interest and it was undesirable for veterinarians to include this extra uncertainty in their assessments; the standard script contained a statement to clarify this.

The elicitation was also designed to avoid *heuristics* which are mental strategies people use to make numerical assessments in the face of uncertainty; they can be effective but may lead to severe systematic bias and error. A large number of heuristics have been identified (Cooke, 1991; Garthwaite et al., 2005; O'Hagan et al., 2006). In particular, "anchoring-adjustment" heuristics were avoided by deliberately not providing initial values or describing a specific clinical scenario. This type of heuristic is also important with respect to the order in which elicited values are requested; experts were asked for their plausible range first so that further judgements were made relative to this. There is also substantial evidence to suggest that people do not assign enough probability to the tails of their distribution; careful phrasing of questions about the plausible range is required. For example, the minimum value was established by asking "tell me the least number of cows you believe will be cured such that you think it is *extremely* unlikely (but not impossible) that less than this number would cure".

Information was gathered during the interview concerning key features of both the individual veterinary surgeons and the veterinary practices they worked in. The raw data were entered into Microsoft Excel (Version 2007, Microsoft Corp). All data analyses were carried out within the R programming environment; version 2.10.1 (R Development Core Team, 2009).

Classical multidimensional scaling

For this analysis the elicited values from the two pilot interviews were included taking the total number of veterinarians from 24 to 26. If the vector of 10 elicited values for each veterinarian is denoted $\boldsymbol{x}_i$ $(i = 1 \ldots 26)$ then the squared Euclidean distance between the vectors for veterinarian i and veterinarian j is given by $(\boldsymbol{x}_i - \boldsymbol{x}_j)^T(\boldsymbol{x}_i - \boldsymbol{x}_j)$ and this was used as a

measure of the dissimilarity in veterinary beliefs. The 26 by 26 "distance" matrix, ***D*,** used to classically scale the data was given by

$$\boldsymbol{D} = [d_{ij}] = \sqrt{(\boldsymbol{x}_i - \boldsymbol{x}_j)^T(\boldsymbol{x}_i - \boldsymbol{x}_j)}$$

where i, j = 1, ..., 26. The standard "goodness of fit" statistic was calculated; it describes the proportion of the total variation accounted for by the first m dimensions and is given by $\sum_{j=1}^{m}\lambda_j / \sum_{i=1}^{10}\lambda_i$ where λ_j are the eigenvalues of the centred inner product matrix, obtained by centering the squared distance matrix, ***D***, times -1/2 (Mardia et al., 1979; Cox & Cox, 2000). This was used to determine the appropriate number of dimensions in conjunction with a scree plot. Interpretation of the principal coordinate axes was facilitated by inspection of the associated eigenvectors.

Bayesian analysis

Two synthetic models for the likelihood were used to illustrate how the beliefs of the veterinarians should (rationally) change in the light of new evidence. The aim was to investigate the strength of evidence (new data) that would be needed to convince the majority of the practitioners in this sample that treatment 2 *does not* offer a clinically worthwhile benefit over treatment 1, given their own current beliefs regarding efficacy. This aim stemmed from the fact that treatment 2 is off-licence, lacks solid evidence supporting its efficacy and its use may contribute to antimicrobial resistance. For this analysis a threshold was used; a "clinically worthwhile benefit" was taken to be a minimum of a 10% improvement in the probability of cure with treatment 2 over treatment 1 (equivalent to an odds ratio ≥ 1.5).

Model 1 - Synthetic data from a single clinical trial following a binomial distribution: This model comprised a Bayesian simulation for each individual veterinarian, to update their prior beliefs with synthetic data from a single clinical trial and thereby derive posterior distributions for their beliefs in additional systemic dry cow therapy. Hence the likelihood was based on a synthetic single clinical trial whereby infected cows are randomly assigned to either treatment 1 or treatment 2. The outcome is binary; an infected cow either cures or does not cure. The data were assumed to follow a binomial distribution

$$X_1 \sim Binomial(n_1, \theta_1),\ X_2 \sim Binomial(n_2, \theta_2),\ X_3 \sim Binomial(n_3, \theta_3)$$

where out of n_1 infected cows given treatment 1, X_1 cows are cured with probability θ_1, out of n_2 infected cows given treatment 2, X_2 cows are cured with probability θ_2 and out of $n_3 = n_1 - X_1$ infected cows *not* cured by treatment 1, $X_3 = X_2 - X_1$ cows are cured using treatment 2 with probability θ_3. Synthetic data for the likelihood were generated for different sized trials; 30, 50, 250, 500, 750 and 1,000 cows in each treatment arm. In each case, θ_1 and θ_2 were set equal so that $\theta_3 = 0$; the synthetic data suggested there was no difference between the two treatments. The Bayesian model was given by

$\theta_1 \sim Beta(\alpha_i, \beta_i)$;	Prior belief for θ_1
$\theta_3 \sim Beta(\alpha'_i, \beta'_i)$;	Prior belief for θ_3
$X_1 \sim Binomial(n_1, \theta_1)$;	Likelihood for θ_1
$X_3 \sim Binomial(n_3, \theta_3)$;	Likelihood for θ_3

where α_i ,β_i and α'_i,β'_i are the fitted hyperparameters for the i^{th} veterinarian (i =1, ..., 24). This model is a standard conjugate analysis and using Eq. (1) the posterior distributions for both θ_1 and θ_3 can be derived explicitly to give

$$\theta_1 \sim Beta(\alpha_i + X_1\ , \beta_i + n_1 - X_1); \quad \text{Posterior distribution for } \theta_1$$
$$\theta_3 \sim Beta(\alpha'_i + X_3\ , \beta'_i + n_3 - X_3); \quad \text{Posterior distribution for } \theta_3$$

However, the posterior distribution of primary interest was the odds ratio (OR) θ_2 to θ_1 for each veterinarian, which was inferred using stochastic simulation from Eq. (2) and by using OR=$(\theta_2/1-\theta_2)/(\theta_1/1-\theta_1)$.

Model 2 - Bayesian random effects meta-analysis of data from five small scale trials: A large single trial (as considered with Model 1) is rare in veterinary medicine. Rather, clinical trials are often small scale and sporadically carried out; typically data will accumulate over many years. However if the trials are all of similar design a Bayesian random effects meta-analysis model can be used to combine the information from different trials (Spiegelhalter & Best, 2003). Model 2 comprised a Bayesian simulation for each individual veterinarian, to update their prior beliefs with synthetic meta-analysis data and thereby derive posterior distributions for their beliefs in additional systemic dry cow therapy.

Synthetic data from five small and equally sized trials (100 infected cows in each treatment group) were generated so that the total number of patients in the five trials combined was 1,000. Similar to Model 1, the data from each trial were assumed to follow a binomial distribution and θ_1 and θ_2 were set equal so that $\theta_3 = 0$; the synthetic data from each trial suggested there was no difference between the two treatments.

This model assumed that the true effect (on a log-odds ratio scale) δ_i for trial i was drawn from a population distribution which allowed the odds ratios in the different trials to vary. A conventional random effects model was used whereby the distribution for δ_i was assumed to be $\delta_i \sim N(\delta, \sigma^2)$ so that the mean was equal to the "true" log odds ratio δ and the standard deviation between trials σ was assigned a reference prior, uniformly distributed on the interval [0,100], i.e. $\sigma \sim U[0,100]$ (Higgins & Spiegelhalter, 2002). The priors for δ were obtained (using stochastic simulation) from the elicited priors for θ_1 and θ_3 by firstly using Eq. (2) to derive the prior distribution for θ_2 and then using the following to derive δ

$$\delta = log_e\left(\frac{\theta_2/1-\theta_2}{\theta_1/1-\theta_1}\right) = logit(\theta_2) - logit(\theta_1)$$

The model (which is not conjugate) consisted of

$\theta_1 \sim Beta(\alpha_i, \beta_i)$;	Prior belief for θ_1 for each veterinary surgeon
$\theta_3 \sim Beta(\alpha'_i, \beta'_i)$;	Prior belief for θ_3 for each veterinary surgeon
$X_1 \sim Binomial(n_1, \theta_1)$;	Likelihood for θ_1 for each synthetic trial
$X_3 \sim Binomial(n_3, \theta_3)$;	Likelihood for θ_3 for each synthetic trial
$\delta_i \sim N(\delta, \sigma^2)$;	Prior distribution for the log odds ratio between trials
$\sigma \sim U[0,100]$;	Prior distribution for the between trial standard deviation

where α_i ,β_i and α'_i,β'_i are the fitted hyperparameters for the i^{th} veterinarian (i =1, ..., 24). The posterior distribution of primary interest was (as for Model 1) the odds ratio θ_2 to θ_1 for each veterinarian and was obtained in this model by taking the exponential function of δ.

Software developed by the "BUGS" project (Bayesian inference Using Gibbs Sampling; http://www.mrc-bsu.cam.ac.uk/bugs/welcome.shtml) was used in a form embedded within R, the library "BRugs" (Thomas et al., 2006) to run both models which uses Markov chain Monte Carlo (MCMC) stochastic simulation.

Sensitivity analysis

Sensitivity analysis is important to investigate any imprecision in the priors introduced by fitting parametric beta distributions to a small number of elicited summary statistics (Garthwaite et al., 2005). It is desirable that the analysis is robust to "realistic" alternative choices of family, in the sense that the veterinarians would be likely to recognise the fitted distributions as a reflection of their own beliefs. Hence alternative choices for the parametric distributions (including the normal and gamma families) were explored and the analysis re-run to investigate the consequences on the size of trial required.

RESULTS

Descriptive statistics

In total 24 veterinarians from five practices were interviewed for up to 40 minutes each; four veterinarians from one practice and five from each of the other four practices. Figure 1 shows the location of the practices in the sample area and those visited.

The non-response rate was zero. In terms of "type of species treated" by the practice, two were "mixed species", the remaining three were "farm and equine only", "farm only" and "dairy only". The veterinarians within the clusters varied widely with respect to several important characteristics likely to influence clinical beliefs. Seven of the veterinarians held extra (cattle-related) qualifications; five held the CertCHP, two held the Diploma in Bovine Reproduction, one held both. The number of years qualified varied from 9 months to 26 years (median 7 years). The "percentage of current time spent working with dairy cattle" ranged from 15-100% (median 80%). The "percentage of that time dedicated to dairy preventive medicine work" varied from 0 to 50% (median 16%). Number of "dairy clients primarily responsible for" ranged from 0 to 35 (median 10.5). Number of "days dedicated to dairy cattle-specific continuing profession development in the last 12 months" varied from 0-20 (median 4). Gender was split 20 males to 4 females. There were 11 assistants and 13 partners; all worked full-time. All six UK veterinary schools were represented at least twice. Liverpool graduates dominated (46% of the sample) possibly reflecting this school's geographic proximity to the study population. Two veterinarians qualified abroad. The sample appeared to be a "fair representation" of the different types of veterinarians and veterinary practices that treat dairy cattle.

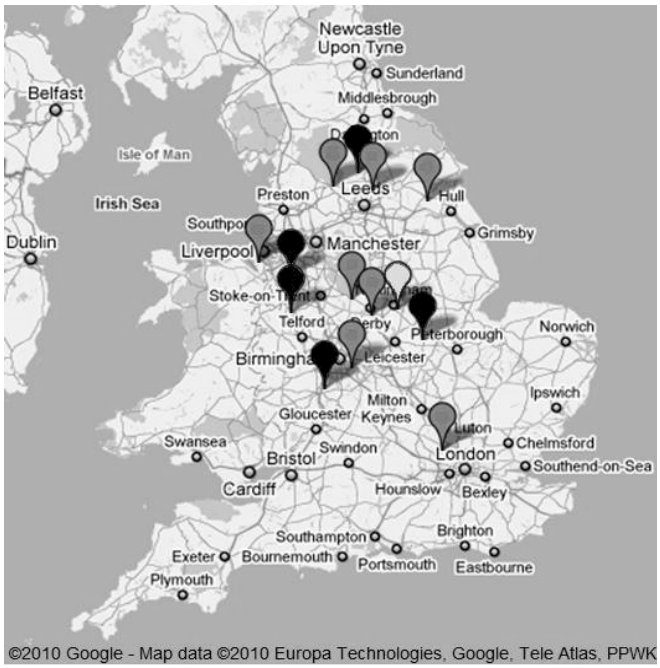

Fig. 1 Map of England showing all 13 veterinary practices (clusters) within the sample area; clusters visited are shaded in black, Nottingham is marked in light grey.

When asked for the probability that $\theta_2 < \theta_1$ (i.e. that the additional use of systemic antibiotics would result in less cows cured) 15 of the 24 veterinarians stated P ($\theta_2 < \theta_1$) = 0 and the remainder gave P$(\theta_2 < \theta_1) \leq 0.05$. Hence structuring the task according to Eq. (2) was considered justifiable and Eq. (3) was used to infer the joint distribution $f(\theta_1, \theta_2)$ for each veterinarian. Figure 2 displays the joint distributions as contour plots and illustrates the diversity in clinical beliefs; there were striking differences both within and between practices with respect to central location and dispersion of beliefs.

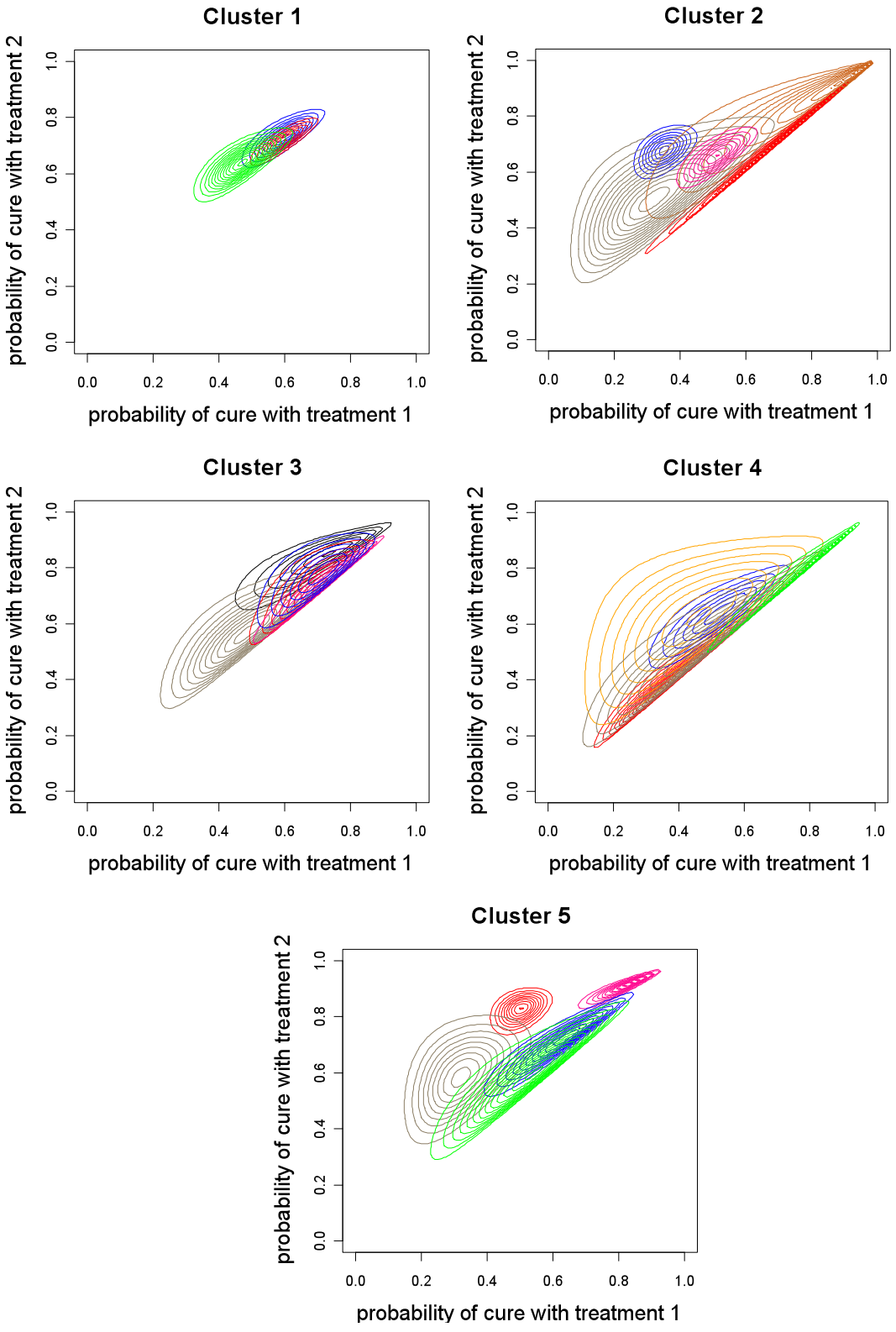

Fig. 2 Joint probability distributions for each individual veterinarian displayed as contour plots and grouped by veterinary practice (cluster). Treatment 1 = intra-mammary dry cow therapy (IDCT) alone. Treatment 2 = systemic antibiotics plus IDCT. The "probability of cure" refers to commercial dairy cows at calving, who at the point of drying-off received either treatment 1 or treatment 2 and had chronic intra-mammary infections.

Classical multidimensional scaling

The first three (of 10 possible) dimensions accounted for 95.3% of the variation and were sufficient to portray the data structure. The first principal coordinate axis was interpreted as an "overall measure" of the veterinarians' belief for θ_3 . Hence it contained information related to all five elicited judgements for this variable and therefore incorporated not just the belief regarding the median, but also the uncertainty (minimum and maximum cure rates achievable) and an impression of the shape of the elicited distribution. Similarly, the second principal coordinate axis was interpreted as an "overall measure" of the veterinarians' belief for θ_1. The third principal coordinate axis was a contrast between the elicited minimum and maximum cure rates for θ_3. It reflects the veterinarians *uncertainty alone* in θ_3.

The two-dimensional map for one of the three planes is presented in Fig. 3. Each number represents an individual veterinarian, with those from the same practice sharing the same first digit (e.g. 11, 12, 13, 14 all worked for the same practice, labelled "1"). A square box highlights veterinarians holding extra-qualifications (denote "expert"). "A" and "B" represent the clinical academics from the University of Nottingham.

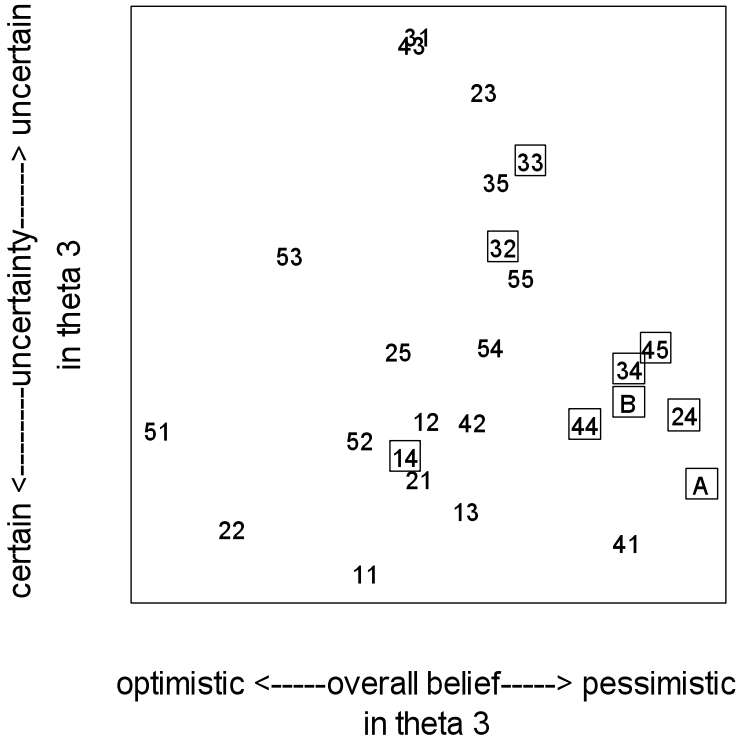

Fig. 3 Classical scaling of the data: Each number or letter represents an individual veterinarian. Theta 3 is the probability of cure with treatment 2, given treatment 1 has failed.

Figure 3 reveals that six of the nine "expert" veterinarians (clustered in the lower right corner of the map) shared the same *confident pessimism* for θ_3 and whilst two experts (32 and 33 from the same practice) were slightly more optimistic, they were also much more uncertain. Only one expert (14) had "confident faith" in θ_3. Inspection of the other two planes (not shown) revealed that seven of the experts shared their optimism for θ_1 (the success of IDCT alone), whilst the other two experts (32, 14) were more pessimistic.

Two notable outliers (in all three planes) were 41 (representing confident pessimism) and 51 (confident optimism) about both θ_1 and θ_3. It can be seen from both Fig. 2 and 3 that the veterinarians in practice 1 are located closest together, suggesting that they held the most similar beliefs (and with a similar level of firm confidence) of any practice. In particular, they all believed systemic antibiotics would cure a minimum of 20% more cows (given IDCT had failed). In comparison, the veterinarians in practice 5 showed a much greater diversity of opinion and strength of belief (Fig. 2 and 3); nevertheless, all the veterinarians in practice 5 did agree that systemic antibiotics would have at least some benefit. In contrast, everyone in practice 3 agreed that using systemic antibiotics may not improve cure rates at all; a view also shared by 4 of the 5 veterinarians in practice 4.

Bayesian analysis

Figure 4 shows the 95% credible intervals for the prior distributions of the odds ratio for the 24 veterinarians (dashed lines). It reveals that only one veterinarian had their entire 95% prior credible odds ratio interval *below* 1.5, whilst six veterinarians had their entire 95% intervals *above* 1.5 (recall that an odds ratio ≥ 1.5 was taken to indicate that systemic antibiotics *do* provide a clinically worthwhile adjunct to intra-mammary dry cow therapy). Odds ratio ≥ 1.5 indicated that treatment 2 offered a benefit when compared to treatment 1.

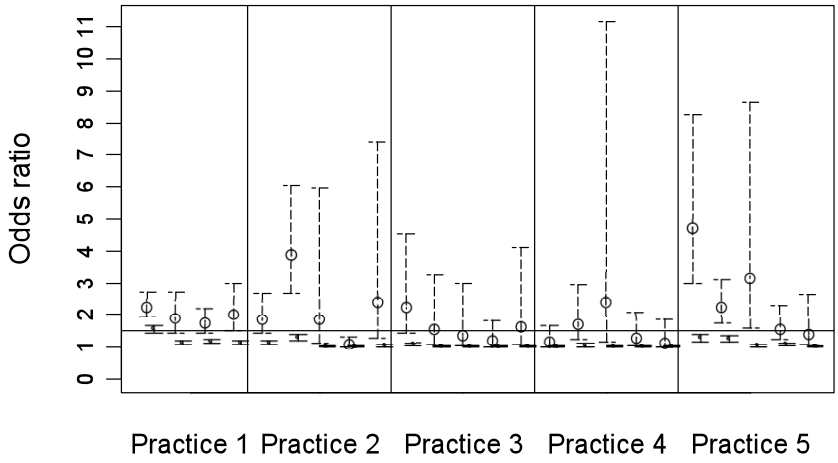

Fig. 4 For the 24 veterinarians, 95% credible intervals for the odds ratio; prior beliefs (dashed lines), posterior beliefs (solid lines). The likelihood was based on synthetic data from a single trial (that showed no difference between treatment 1 and 2) and size = 1,000 cows (Model 1).

Model 1 - Synthetic data from a single clinical trial following a binomial distribution: MCMC simulation was used with three chains, a total sample size of 30,000 and a "burn-in" of 1,000 iterations. The chains visually converged almost immediately; Gelman-Rubin statistic convergence to one. Figure 4 (solid lines) shows that a single trial designed with 500 cows in each treatment group (trial size = 1,000) and showing no difference between treatments, would be required to convince 23 of the 24 veterinarians that treatment 2 was not clinically superior to treatment 1, in the sense that 23 veterinarians would then have their entire 95% posterior credible odds ratio intervals less than 1.5. To convince everyone would require a trial size of 2,000.

Model 2 - Bayesian random effects meta-analysis of data from five small scale trials: MCMC simulation was used with three chains, a burn-in of 5,000 and total sample size 75,000.

The chains were checked visually for equilibrium; the Gelman-Rubin statistic converged to one. With this model, only six vets would have their entire posterior 95% credible odds ratio intervals less than 1.5, contrasting with the 23 vets who were convinced following a single trial with the same total number of patients involved.

Sensitivity analysis

For realistic alternative distributions the results were very similar to the original analysis; the number of clinicians swayed at each trial size altered by at most two. Hence any imprecision arising in the priors associated with fitting parametric distributions was not a primary concern with respect to making a decision on the strength of future evidence required.

DISCUSSION

There were major variations in practitioners' beliefs with respect to the efficacy of systemic antibiotics for dry cow therapy, both between individuals within a practice and between practices. Striking differences in the *strength* of belief were also apparent. Both confident optimism *and* confident pessimism for the combined use of systemic and intra-mammary antibiotics were observed, alongside several veterinarians who had considerable uncertainty. This wide diversity in beliefs is likely to result in very different decisions being taken on farm, with considerable discrepancies in the treatments received by dairy cows at drying-off and the total quantity of systemic antibiotics being administered. The observed variation raises considerable concern over whether or not antibiotics are being prescribed consistently and appropriately; any widespread misuse (or over use) of antibiotics has serious implications.

Explanation of the observed variation is likely to be multi-factorial and reasons for it could include: availability/cost of products and their marketing by pharmaceutical companies, differences in how veterinarians source and critically appraise information, the lack of robust data, under and post-graduate education, the creation and persistence of dogma, the absence of national guidelines, personality traits, farmer perceptions/demand and demographic factors. This research raises important questions about the heterogeneity in beliefs and decision-making by veterinarians in general. Whilst it would be undesirable for veterinarians to be completely unified in their clinical approaches, broad agreement is important for both the credibility of the veterinary profession and to ensure a consistent delivery of healthcare to dairy cattle. Further research is required in this area.

Interestingly the majority of "expert" veterinarians in this sample shared a confidently pessimistic belief in the combined use of systemic and intra-mammary antibiotics, given intra-mammary antibiotics alone has failed, offering some support for the idea that "post-graduate education" is a factor related to the observed variation in beliefs. Moreover, whilst the beliefs of "expert" veterinarians do not constitute fact, they are nevertheless important, especially in the absence of robust data. Overall, when placed in the greater context of an escalating global threat of bacterial resistance to antibiotics, the justification for the off-licence use of systemic antibiotics for dry cow therapy becomes difficult.

This study has demonstrated how probabilistic elicitation can be used to capture the diversity and strength of veterinarians' beliefs for subsequent use as prior information in a Bayesian analysis. Here we have used two synthetic models for a Bayesian analysis and illustrated how the clinical beliefs of these 24 practitioners should (rationally) be updated in the light of new evidence. This may be described as a *true* Bayesian approach in the sense that

genuine prior knowledge has been utilized in a scientific and transparent way; it differs to the majority of published Bayesian analyses that use a variety of theoretical prior beliefs.

A major advantage of adopting a true Bayesian approach is that by eliciting clinicians' beliefs before designing clinical trials, sample size calculations are greatly facilitated by placing the proposed trial in the *context of* current clinical opinion. Despite increasing use of this approach in human medicine over the last decade (Spiegelhalter et al., 2004), the authors are not currently aware of any veterinary research trials that have been designed in this way. Yet research efforts will obviously be targeted more effectively if trials destined never to sway clinical opinion from the very outset are avoided. This is particularly important where there are strongly held and/or diverse pre-existing clinical beliefs amongst veterinarians and it is postulated that this may frequently prove to be the case. An important reason why veterinary research may have failed in the past to evoke important changes on farm could be due to the strength of the research produced relative to the strength and diversity of practitioners' beliefs. We would urge more consideration of this issue.

Possible reasons why probabilistic elicitation has not been used more widely in the veterinary field include the complexity of the task, cost and time considerations (Berry & Stangl, 1996). Indeed, as Spiegelhalter et al. (2004) commented "turning informally expressed opinions into a mathematical prior distribution is perhaps the most difficult aspect of Bayesian analysis". However it is hoped that the development of freely available software (such as SHELF), along with the major benefits to be derived from conducting productive research, will provide motivation. In particular, it is essential that veterinary research which has implications for wider society (e.g. in terms of public health, or informing government animal health policy on a national scale) is designed with due respect for the pre-existing beliefs of *all* relevant stakeholders; particularly when the anticipated outcomes may be highly controversial, costly to implement and/or require co-operation from those stakeholders.

Cluster sampling was used in this research and this method is only efficient when the clusters are internally as heterogeneous as possible with respect to survey variables and between cluster variation is small; the "clustering principle" (Stuart, 1983). Initial concern that the variables of interest (clinical beliefs) may be fairly homogenous within clusters (because veterinarians would discuss and share ideas amongst themselves) proved unfounded; as previously mentioned, striking differences in beliefs were observed both within and between clusters. Hence, for the purposes of eliciting veterinarians' beliefs, cluster sampling may prove to be cost-efficient. The sample size in this study was 24 and one third (24/77) of the study population were interviewed. In a recent systematic review involving 33 published elicitations, Johnson et al. (2010) reported median sample size as 11; therefore the sample size used in this study was considered appropriate for a probabilistic elicitation.

The R.C.V.S database was searched by selecting veterinary practices containing at least one veterinarian holding the "CertCHP". It was believed that post-graduate qualifications may strongly influence clinical beliefs and it was of interest to include a number of "expert" veterinarians in the sample. This necessitated deliberate selection but accordingly, caution should be taken making any inferences to wider veterinary populations. The non-response rate was zero and thus veterinarian-induced selection bias was avoided; the relatively short interview time and payment for veterinary time may have influenced this appreciably.

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HORSE INDUSTRY PARTICIPANTS' PERCEPTIONS OF BIOSECURITY EFFECTIVENESS: VETERINARIANS EMERGING AS IMPORTANT INFORMATION PROVIDERS

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SUMMARY

In 2007, Australia experienced its first ever outbreak of equine influenza (EI), resulting in the issuing of biosecurity guidelines by animal health departments. Biosecurity uptake however is likely to be heavily influenced by perceived effectiveness of the recommended measures. In 2009, interviews were conducted with 200 randomly selected horse owners/managers from highly affected EI regions within the state of New South Wales. Perceived biosecurity effectiveness (low/high), as determined by participants' responses to a 17-item question on the effectiveness of various measures, was used as the outcome in logistic regression analyses. Participants who experienced EI infection in their horses in 2007 were less likely to perceive measures as effective and those who received infection control information from a veterinarian during the outbreak were more likely to perceive these measures as effective. These results should be considered when designing infection control programs, in order to promote favourable perceptions and support increased compliance.

INTRODUCTION

In late August 2007 Australia experienced its first ever outbreak of equine influenza (EI), a highly contagious respiratory disease affecting all members of the Equidae family. EI causes high morbidity but low mortality and is transmitted by direct contact between horses as well as by indirect contact via people, fomites or wind. The EI virus escaped from the Eastern Creek quarantine facility in Sydney, New South Wales (NSW) following the importation of infected horses from Japan (Callinan, 2008). Subsequently the virus spread through major parts of the state of NSW and into south-eastern parts of the state of Queensland. The outbreak lasted for four months from 24th August 2007 to 25th December 2007. In order to control, contain and eradicate the outbreak, federal and state animal health authorities implemented outbreak control measures including movement restrictions, vaccination, quarantining of properties and issuing of biosecurity guidelines to horse owners (NSW DPI, 2007a; DEEDI, 2010). The biosecurity guidelines included measures related to personal hygiene, equipment hygiene and access control. The biosecurity measures were recommended based on expert advice, as at the time of the outbreak, no scientific studies had been conducted to study the effectiveness of on-farm

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biosecurity measures for preventing infection with equine influenza. This study was conducted to investigate the perceived effectiveness of these recommended biosecurity measures.

A large body of literature exists in human health disciplines that supports a range of theories for human behaviour modification, particularly in regard to health-protective behaviours. A recent review by Bish and Michie (2010) examined 26 papers and concluded that a greater belief in the effectiveness of recommended behaviours to protect against a disease is an important predictor of behaviour during pandemics. An initial study conducted by the authors of this paper suggested that Australian horse owners who believed in the effectiveness of their current on-farm hygiene measures were more likely to have high compliance with recommended biosecurity measures (Schemann et al., 2010). The current study was conducted to investigate why some owners consider biosecurity practices to be effective while others do not and what the drivers influencing these perceptions are. This information is important for animal health authorities in order to influence voluntary compliance with biosecurity policies through extension activities. Specifically, knowledge of factors influencing perceptions of biosecurity effectiveness will inform the design of infection control programmes for future exotic disease incursions or for the control of endemic diseases.

MATERIALS AND METHODS

Horse premises located in regions of NSW considered 'at risk' during the 2007 EI outbreak were randomly selected using computer generated pseudo-random numbers from a dataset of premises tested for EI supplied by the NSW Department of Primary Industries (DPI). 'At risk' regions were defined as restricted areas and special restricted areas according to the risk-based zoning system implemented by the NSW DPI in its Equine Influenza Protection Plan (NSW DPI, 2007b). A detailed description of the study design, sample size calculation, inclusion and exclusion criteria and the enrolment process for this study is provided in Firestone et al. (2010). A total of 270 horse owners and managers were asked to participate in the study by a personally addressed letter and up to three follow-up telephone calls to assess eligibility. Of the 270 owners, 38 were deemed ineligible for a concurrently conducted study (Firestone et al., 2010), whilst another 32 premises owners or managers declined to participate (13 premises owners and managers who experienced EI in their horses and 19 who did not). Ultimately 200 owners and managers were enrolled. The University of Sydney's Human Research Ethics Committee approved the study protocol (07-2009/11840).

Two interviewers (KAS and SMF) conducted face-to-face structured interviews with enrolled horse owners and managers using a pilot-tested questionnaire between July and October 2009. The questionnaire contained a total of 61 questions, however only 20 were used in the current study. The remaining 41 questions were exclusively used to investigate factors influencing the spread of equine influenza onto horse premises (Firestone et al., 2010). Of the 20 questions, 16 were closed or semi-closed; the other four questions were open. The questionnaire was designed to obtain information about demographics of participants, the nature of their involvement with horses, their sources of information about infection control during the 2007 EI outbreak, their attitudes towards effectiveness of biosecurity measures, and their attitudes towards a potential future outbreak. The two interviewers piloted the questionnaire on four horse owners/managers to ensure similar method and response recording. The questionnaire was modified as a result of this feedback to reduce ambiguity. The collected data were entered into a purpose-built Microsoft Access 2007 database (Microsoft Corporation, Redmond, WA, USA).

Data cleaning and all statistical analyses were conducted in SAS Version 9.2 (SAS Institute Inc., Cary, NC, USA).

The outcome variable in this study was an index of horse owners'/managers' perception of the effectiveness of biosecurity measures (high or low) in the event of a future EI outbreak. The index was based on a 17-item question asking interviewees whether they considered each of the 17 measures as 'very effective', 'partially effective' or 'not effective' for protecting their horses from EI infection in a hypothetical scenario of an EI outbreak in the area the following day. The 17 biosecurity measures of interest were based on those recommended by the NSW DPI for EI control during the 2007 outbreak (NSW DPI, 2007a) and are presented in Table 1. From the responses to the 17 questions, a median response was calculated for each person interviewed. The outcome variable was then reclassified into a binary outcome measure, in which those who considered more than 50% of the 17 measures as 'very effective' were reclassified as 'high effectiveness' group and the remaining respondents were reclassified as 'low effectiveness' group.

Table 1. Equine influenza biosecurity measures used to create a binary biosecurity effectiveness rating as the outcome variable in a study conducted with 200 horse owners and managers in New South Wales, Australia in 2009.

Biosecurity measure	n[a]	Very effective (%)	Partially effective (%)	Not effective (%)
Not sharing horse gear	189	95	5	0
Controlling who has access to your horses	199	91	8	1
Reducing your own contact with other horses	200	88	11	1
Reducing your horses' contact with other horses	199	86	10	4
Ensuring that feed and bedding comes from a clean source	192	86	10	4
Reducing visits by horse professionals to the property	197	86	10	4
Complying with movement restrictions	200	85	9	6
Using soap when washing your hands	198	78	15	7
Washing your hands before contact with your horses	198	72	20	8
Changing your shoes on arrival at your property	198	69	22	9
Disinfecting horse floats before use	183	66	21	13
Ensuring that any new horses are isolated from your other horses	181	65	14	21
Changing into clean clothes on arrival at the property	196	63	28	9
Ensuring all visitors use a disinfectant footbath	193	63	23	14
Disinfecting vehicles entering your property	192	50	27	23
Showering on arrival at your property	190	44	36	20
Cleaning your horse gear before use	191	39	23	38

[a] 'Not applicable' responses were treated as missing values.

Table 2. Explanatory variables analysed for associations with horse owner/manager perception of high biosecurity effectiveness in a study conducted in New South Wales, Australia in 2009.

Variable group	Variables
Participant demographic and geographic information and horse involvement	Age [a]; Gender [b]; Number of horses [c, d]; Involved in horse racing [d, e]; Involved in equestrian events [d, f]; Involved in horse showing [d]; Involved in rodeo-style horse events [d, g]; Horse breeders [d]; Horses kept for other owners [d]; Horses kept only for recreation [d]; Horses used for farm work [d]; Premises enterprise type [b, d]; Income derived from horses [a, d]; Regional cluster [b, h].
Infection control information sources during the 2007 EI outbreak	Used internet; Used general media [i]; Used other horse owners; Used veterinarian; Used non-veterinarian horse professional [j]; Used Australian Horse Industry Council (AHIC); Used state Department of Primary Industries (NSW DPI); Used association/ society [k]; Used retailers [l].
2007 EI outbreak experience and biosecurity perceptions	Premises infected with EI?; Protection zone during 2007 EI outbreak [b]; Perceived stringency of own biosecurity measures applied during the 2007 equine influenza outbreak [a]; Perceived level of stringency during EI compared to horse owners in the neighbourhood [a]; Perceived level of preparedness for a future equine influenza outbreak [a]; General interest in infection control information [a].

EI = equine influenza; All variables are binary (1=yes, 0=No) unless indicated otherwise. [a] Ordinal variable; [b] Categorical variable; [c] Continuous variable; [d] Variables relate to status at the start of the EI outbreak on 25th August 2007; [e] Horse racing includes thoroughbred and harness racing. [f] Equestrian events include dressage, eventing, showjumping, endurance. [g] Rodeo-style events include camp-drafting, cutting, barrel-racing. [h] Regional clusters as per Cowled et al. (2009); [i] General media include television, newspaper, radio. [j] Non-veterinarian horse professionals include farriers, dentists, chiropractors, trainers, coaches. [k] Associations/ societies include breed and sporting associations and societies. [l] Retailers include feed and horse equipment retailers.

All 29 explanatory variables investigated in this study are presented in Table 2. The variable 'Regional cluster' was derived from research on the 2007 EI outbreak conducted by Cowled et al. (2009), who created it based on an interpolated surface of date of onset of clinical signs, geographic data and location of the infected properties from the NSW DPI data set. The distributions of explanatory variables were assessed by calculating frequencies and relative frequencies. Further, contingency tables of the explanatory variables for the two categories of the biosecurity effectiveness index ('high effectiveness' and 'low effectiveness') were examined. The unconditional association of explanatory variables with the binary outcome variable was assessed in univariable binomial logistic regression analyses facilitated by UniLogistic SAS macro (Dhand, 2010). Variables with a univariable likelihood-ratio chi-square p-value of <0.20 were tested for collinearity in pairs by calculating Spearman's rank correlation coefficient (ρ) and Pearson chi-square test. Additionally, variables were assessed for missing values and variables with >10% missing values were initially excluded from analyses and subsequently retested in the final model. Multivariable binomial logistic regression models were constructed using in-house developed SAS macro MultiLogistic (http://vetsci.usyd.edu.au/biostat/macros/) with a manual forward stepwise approach to evaluate the association of explanatory variables with the outcome variable after adjusting for each other. The variables which achieved statistical significance (p-value<0.05) in multivariable models

were retained in the final model. The gender and age group of the participants were considered to be potential confounders and forced into all models irrespective of their *p*-values. Two-way interactions of the explanatory variables in the final model were tested for significance at $p<0.05$. To assess the effect of clustering of observations from the same region, a regional cluster-level random effect term was added to the final model. Potential interviewer bias was assessed by addition of an interviewer-level random effect term to the final model (Hox, 1994). Intra-class correlation (ICC) was then calculated for each random effect using the latent variable approach (Dohoo, 2009). Outliers and influential observations were evaluated by residual diagnostics. Goodness-of-fit of the final model was assessed using the Hosmer-Lemeshow technique (Hosmer and Lemeshow, 2000).

RESULTS

Age, gender and horse premises type distribution of 200 horse owners and managers interviewed in the study are shown in Table 3. The majority (63.5%) of interviewees experienced EI infection in their horses during the 2007 outbreak. A total of 166 horse owners/managers were included in the 'high effectiveness' group due to their median response to the 17 biosecurity measures being 'very effective' for protecting their horses from EI infection in case of a future outbreak.

A total of 10 variables were unconditionally associated with biosecurity effectiveness perception at the univariable level cut-off *p*-value of <0.20 (Tables 3-4). In brief, horse owners who kept horses only for recreation, were involved in horse racing, or who experienced EI infection in their horses were less likely to perceive measures as effective. In contrast, greater perceived preparedness for a future outbreak and greater interest in general infection control information was associated with greater perceived effectiveness of biosecurity measures. There was no multicollinearity ($\rho>|0.70|$; Pearson chi-square $p<0.05$) among the 10 variables and only one of the variables ('Perceived level of preparedness for a future EI outbreak'), displayed some (2%) missing observations, thus all 10 variables were included in multivariable analyses.

The final model for high perceived biosecurity effectiveness for protection of horses from EI infection is presented in Table 5. There were no differences in perceived biosecurity effectiveness between males and females and between different age-groups. Most significantly, those receiving infection control information from a veterinarian during the 2007 EI outbreak were 5.5 times more likely to believe the measures to be effective. Interviewees who experienced EI infection in their horses during the 2007 outbreak were four times less likely to consider biosecurity measures effective, compared to those who did not experience EI infection in their horses. Analysis also revealed that those associated with equestrian centres or riding schools and those on farms were more than five times less likely than people associated with small acreage horse premises to deem measures effective (Table 5). Two-way interactions among the variables in the final model were not significant at a significance level of $\alpha=0.05$. When added to the final model, the variation due to the interviewer-level random effect term equalled 0 and hence this effect did not account for any proportion of the total variance. Similarly, when the regional cluster-level random term was added to the model, its intra-class correlation accounted for only 0.01% of the total variability and it was consequently not included in the final model. The Hosmer-Lemeshow goodness-of-fit chi-squared *p*-value=0.870, indicating good overall fit of the model to the observed data. A few influential and outlying observations were observed but were not more than expected.

Table 3. Univariable logistic regression results for demographic and horse involvement related variables associated with high biosecurity effectiveness perception ($p<0.20$) in a study conducted with 200 horse owners/managers in New South Wales, Australia in 2009.

Variable	Biosecurity effectiveness			Odds ratio (95% CI)	*p*-value
	High Freq (Row %)	Low Freq (Row %)	Total		
Age					0.89[a]
<35 years old	19 (86%)	3 (14%)	22	1.00	
35-54 years old	122 (82%)	26 (18%)	148	0.74 (0.17, 2.38)	
≥55 years old	25 (83%)	5 (17%)	30	0.79 (0.15, 3.63)	
Gender					0.81[a]
Female	111 (83%)	22 (17%)	133	1.00	
Male	55 (82%)	12 (18%)	67	0.91 (0.43, 2.02)	
Premises enterprise type					0.07
Small acreages/ homes with horses	84 (90%)	9 (10%)	93	1.00	
Equestrian centres or riding schools	26 (72%)	10 (28%)	36	0.28 (0.10, 0.76)	
Farms – cattle, sheep or cropping	30 (81%)	7 (19%)	37	0.46 (0.16, 1.39)	
Commercial studs	15 (83%)	3 (17%)	18	0.54 (0.14, 2.62)	
Agistment- horses kept for other owners	11 (69%)	5 (31%)	16	0.24 (0.07, 0.88)	
Horses kept only for recreation					0.08
No	142 (85%)	26 (15%)	168	1.00	
Yes	24 (75%)	8 (25%)	32	0.55 (0.23, 1.42)	
Involved in horse-racing					0.10
No	153 (85%)	28 (15%)	181	1.00	
Yes	13 (68%)	6 (32%)	19	0.40 (0.14, 1.21)	

[a] Confounding variables were included irrespective of their *p*-values.

Table 4. Univariable logistic regression results for variables related to the 2007 outbreak of equine influenza and associated with high biosecurity effectiveness perception ($p<0.2$) in a study conducted with 200 horse owners/managers in New South Wales, Australia in 2009.

Variable	Biosecurity effectiveness			Odds ratio (95% CI)	*p*-value
	High Freq (Row%)	Low Freq (Row%)	Total		
Protection zone during the 2007 EI outbreak control					0.04
Special restricted area	47 (75%)	16 (25%)	63	1.00	
Restricted area	119 (87%)	18 (13%)	137	2.25 (1.05, 4.79)	
Premises infected with EI during the 2007 outbreak?					0.03
No	66 (90%)	7 (10%)	73	1.00	
Yes	100 (79%)	27 (21%)	127	0.39 (0.15, 0.91)	
Perceived level of preparedness for a future EI outbreak					0.06
Unprepared	17 (71%)	7 (29%)	24	1.00	
Prepared	93 (83%)	19 (17%)	112	2.02 (0.70, 5.40)	
Highly prepared	55 (92%)	5 (8%)	60	4.53 (1.29, 17.13)	
General interest in infection control information					0.09
Not interested	18 (72%)	7 (28%)	25	1.00	
Interested	52 (79%)	14 (21%)	66	1.44 (0.48, 4.08)	
Very interested	96 (88%)	13 (12%)	109	2.87 (0.97, 8.08)	
Used a veterinarian as infection control information source during the 2007 EI outbreak					<0.01
No	33 (67%)	16 (33%)	49	1.00	
Yes	133 (88%)	18 (12%)	151	3.58 (1.65, 7.80)	
Used a retailer as infection control information source during the 2007 EI outbreak					0.04
No	134 (81%)	32 (19%)	166	1.00	
Yes	32 (94%)	2 (6%)	34	3.82 (1.08, 24.33)	
Used a non-veterinarian horse professional as infection control information source during the 2007 EI outbreak					0.17
No	118 (86%)	20 (14%)	138	1.00	
Yes	48 (77%)	14 (23%)	62	0.58 (0.27, 1.26)	

EI = equine influenza

Table 5. Final logistic regression model for high biosecurity effectiveness perceptions of 200 horse owners/managers in a study conducted in New South Wales, Australia in 2009.

Parameters	b	SE(b)	Adjusted odds ratio	(95% CI)	*p*-value[a]
Constant	1.28	0.76	-	-	-
Age					0.884
<35 years	0	-	1.00	-	-
35-54 years	-0.23	0.70	0.80	(0.20, 3.12)	-
≥55 years	-0.42	0.85	0.66	(0.12, 3.47)	-
Gender					0.924
Female	0	-	1.00	-	
Male	0.05	0.47	1.05	(0.42, 2.61)	
Used a veterinarian as infection control information source during the 2007 EI outbreak					<0.001
No	0	-	1.00	-	
Yes	1.70	0.47	5.49	(2.20, 13.70)	
Premises infected with EI during the 2007 outbreak?					0.012
No	0	-	1.00	-	
Yes	-1.43	0.57	0.24	(0.08, 0.73)	
Premises enterprise type					0.016
Small acreages/ homes with horses	0	-	1.00	-	
Equestrian centres or riding schools	0.05	0.47	0.15	(0.05, 0.48)	
Farms – cattle, sheep or cropping	-1.76	0.68	0.17	(0.05, 0.65)	
Commercial studs	-1.26	0.83	0.28	(0.06, 1.45)	
Agistment- horses kept for other owners	-1.36	0.71	0.26	(0.06, 1.04)	

Log likelihood= 29.7; d.f.=9; $P<0.001$; Goodness-of-fit deviance χ^2-test statistic p-value=0.870.

[a] p-values based on Wald χ^2-test of significance

DISCUSSION

This study was conducted to identify factors associated with horse owners'/managers' perception of high biosecurity effectiveness. In this study, three factors were important for the perception of high biosecurity effectiveness: whether infection control information was received from a veterinarian during the 2007 EI outbreak, whether horses became infected during the 2007 outbreak and the premises type.

The most important factor in the final model described whether or not participants received EI infection control information from a veterinarian during the 2007 outbreak. Interestingly, those who did were 5.5 times more likely to judge the recommended EI biosecurity measures as effective. This finding was unexpected as previous research with UK cattle and sheep farmers had concluded that attitudes towards biosecurity did not appear to be influenced by information sources per se (Heffernan et al., 2008). The UK study found that veterinarians were a primary source of information for cattle and sheep farmers, however it hypothesised that the only occasional contact between farmers and veterinarians was unlikely to create behavioural change within an outbreak situation (Heffernan et al., 2008). Our results suggest that contact and information exchange between veterinarians and horse owners/managers during an exotic disease outbreak was sufficient to result in altered perceptions about biosecurity effectiveness. This result is not so atypical when considered in light of the biosecurity engagement guidelines report recently published by Kruger et al. (2010). The report outlines several enablers to effective biosecurity engagement between government personnel and horticulturalists and many of those enablers are present in the relationship between equine veterinarians and horse owners, potentially catalysing the engagement process. Veterinary clinics are generally well established institutions with long-term key staff such as the practice owner/principal veterinarian. Long-standing service together with demonstrated responsiveness and commitment by veterinarians to routine horse health consultations with owners/managers enables a gradual and continual development of trust. This may explain why information delivered by a veterinarian is an important factor for the biosecurity effectiveness perceptions of horse owners and managers. The finding suggests that the network among horse owners and equine veterinarians is an already existing network, which should be tapped into for the effective delivery of biosecurity information, especially in an outbreak situation.

Another important factor, additional to whether or not a veterinarian provided infection control information, was whether or not a premises became infected with equine influenza during the 2007 outbreak. Not surprisingly, owners and managers of infected horses were less likely to believe in the effectiveness of protective measures. One can assume that this association between the perception of poor effectiveness and becoming infected is linked to having performed biosecurity measures during the 2007 outbreak and despite taking this protective action, experiencing EI possibly due to local virus spread facilitated by cough droplets, windborne aerosol or fomite transmission (Cowled et al., 2009; Firestone et al., 2010). The result that those who experienced EI infection in their horses in 2007 are now less likely to believe in the effectiveness of biosecurity measures therefore should be considered in future communication campaigns. It may be important that those owners and managers fully understand the differences in disease transmission for different diseases and strains of the same disease and that they are reassured of the effectiveness of recommended measures. Ideally, additional strategies such as face-to-face delivery of information by a trusted communicator should be used to enhance biosecurity engagement with this group.

Premises type was also an important factor for perceptions of biosecurity effectiveness. Interestingly, owners and managers of small acreage homes with horses were most likely to believe that biosecurity measures are effective. Perceptions of lower effectiveness of respondents at equestrian centres and riding schools may be explained by their inability to control the behaviour of their clients and visitors effectively and the high levels of horse and people movements generally associated with such places. An unexpected finding was that people associated with farms were more than five times less likely than those on small acreage premises to deem measures effective. This result may be explained by a continuing need for on- and off-property livestock movement making some of the recommended measures impractical in

every-day farm life. Generally it can be expected that effectiveness perceptions are influenced by what measures were actually performed during the outbreak in 2007 and were remembered by interviewees. However, this relationship was not evaluated in these analyses.

A common limitation of epidemiological studies conducted using questionnaires is the subjective nature of the outcome and explanatory variables. To avoid resulting misclassification bias, only closed categorical responses were allowed to maximise the accuracy of the responses. Additionally, many questions included the option for interviewees to respond that they did not know the answer or that a measure was not applicable. These options were provided to address potential misclassification bias, where respondents may select an answer at random and those observations were treated as missing for the analyses. Another potential source of bias, confounding, was addressed in this study by forcing the variables age and gender of interviewees into all analyses irrespective of their *p*-values. Gender and age are identified as key determinants of health protective behaviours and are also associated with perceptions of the behaviour (Bish & Michie, 2010). Face-to-face on-farm interviews were conducted in this study to increase cooperation, rapport, consistency and reliability of the responses as well as completeness of the data. To improve recall of the time of the 2007 outbreak, open discussions led up to the questioning to stimulate memory; however some degree of recall bias is possible.

In conclusion, this study identified three factors important for EI biosecurity effectiveness perceptions of horse owners and managers two years after the first-ever outbreak of EI in Australia. Those who received infection control information from a veterinarian during the outbreak, those who did not experience EI infection in their horses and those associated with small acreage homes with horses on site were all more likely to perceive EI biosecurity measures as effective in the case of a future outbreak. These findings should be considered in the design of future infection control programs in order to alter effectiveness perceptions and to ultimately increase horse owner's and manager's biosecurity compliance.

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MULTIVARIABLE METHODS

RISK FACTORS ASSOCIATED WITH SEVERITY OF POST-WEANING MULTI-SYSTEMIC WASTING SYNDROME (PMWS) IN ENGLISH PIG FARMS

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SUMMARY

A cross-sectional study involving 147 pig farms across England was conducted in 2008-09. Farms were classified as non/slightly, moderately or highly affected by PMWS using an algorithm that combined data on post-weaning mortality, morbidity and proportion of porcine circovirus type 2 PCR positive pigs. Risk factors were identified in a multivariable ordinal logistic regression and were subsequently subjected to correspondence analysis. Results provide evidence that poor environmental conditions and management practices, such as overstocking, rearing pigs indoor or low number of diet changes, were associated with severity of the disease. In addition, this study confirms the potential involvement of infectious co-factors as evidence was found that farms negative to *M. hyopneumoniae* had higher odds of being slightly affected, while lack of several biosecurity measures increased the odds of being highly affected. Continued education of farmers about the multi-factorial nature of PMWS is necessary in order to reduce severity.

INTRODUCTION

Porcine circovirus type 2 (PCV2) has been identified as the causative agent of one of the most economically damaging disease syndromes in the pig industry worldwide in 1996 (Harding, 1996). Post-weaning multi-systemic wasting syndrome (PMWS) typically affects weaned pigs between 8 and 15 weeks old. Pigs with the disease present wasting condition and growth retardation, pallor of the skin, respiratory signs and, occasionally, jaundice and intermittent diarrhoea (Harding and Clark, 1997). In histopathology the virus has been shown to produce lymphoid depletion in several organs and tissues, indicating deterioration of the immune system in diseased pigs (Nielsen et al., 2003). In affected farms morbidity has been reported to reach up to 50-60% during the epidemic phase and decreased to levels of 1-30% in the recent common endemic situations (Quintana et al., 2001; Alarcon et al., 2011). In affected farms post-weaning mortality is a major problem, and currently is an important parameter for the diagnosis of PMWS on the farm (Segales et al., 2003), where it can rise up to 25% during outbreaks (Madec et al., 2000; Quintana et al., 2001). It is also suspected that infected farms, instead of the typical severe clinical signs, might have subclinical problems which would result in reduced productivity.

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However, very little is known yet on the mechanism and factors triggering severe disease on the farm. The virus has been shown to be highly ubiquitous and is found in diseased and non diseased animals on PMWS affected farm, as well as in most of non-affected farms. This variation in severity on farms may have hampered the investigation of the epidemiology of the disease. Several studies have found evidence of the importance of other pathogens, such as Porcine Reproductive and Respiratory Syndrome virus (PRRS), *Mycoplasma Hyopneumoniae* (M. Hyo), Porcine Parvovirus (PPV) and Swine Influenza (SI) as possible co-factors for triggering PMWS (Krakowka et al., 2000; Pogranichniy et al., 2002; Opriessnig et al., 2004; Wellenberg et al., 2004; Dorr et al., 2007). Some studies hypothesized that techniques reducing infection pressure and stress in the farm were able to protect against the disease (Madec et al., 2000; Rose et al., 2003). A sow effect was identified as an important risk factor for the development of PMWS at animal level (Madec et al., 2000). Although, the reasons behind this phenomenon have not yet been fully understood, studies suggests that piglets born from sows with negative or low PCV2 seroconversion (Calsamiglia et al., 2007) or sows of higher parities (Wieland et al., 2010a) have higher odds of developing the disease. Recently, different studies have indicated a relationship between specific breeds and PMWS susceptibility. There is evidence that some breeds, such as Landrace, were more susceptible to PMWS, while Pietrain pigs were more protected against the disease (Lopez-Soria et al., 2004; Opriessnig et al., 2009). Finally, several large scale epidemiological studies have shown evidence of the importance of biosecurity measures to prevent PMWS breakdowns (Cottrell et al., 1999; Cook et al., 2001; Woodbine et al., 2007). In summary, the majority of studies conducted so far have suggested that PMWS is a multi-factorial disease in which the presence of other pathogens, stress and/or sow/genetic factors are required.

Most studies investigating risk factors for PMWS have been based on comparison between affected and non affected farms, and the majority was conducted during the epidemic stage. Those carried out at a later stage, had difficulties in finding enough control farms to get significant results. In the current endemic stage, however, a large majority of farms are affected by the disease, with a considerable variability of severity seen across farms. This variability was shown in a cross-sectional study involving 147 English pig farms where severity of PMWS was measured and quantified for each farm (Alarcon et al., 2011).This study was able to differentiate farms that were non or slightly affected from those farms that were moderately or highly affected by the disease. Understanding the mechanism behind the severity and, therefore, understanding why some farms are more affected than others is essential for the implementation of control and preventive measures. Furthermore, although extended evidence of the need of co-infection has been obtained, evidence of environmental and genetic interactions, and thus evidence of the multi-factorial nature of the disease needs to be assessed. The aim of this study is therefore to investigate the risk factors associated with different PMWS severity levels seen on English farms in the current endemic situation.

MATERIALS AND METHODS

In a cross-sectional study, 147 farms across England were visited between April 2008 and April 2009. The geographical distribution of farms included reflects the pig density across the country, with a greater number of farms recruited in the higher pig farm density areas North-Yorkshire and East Anglia. Farms were recruited through a PCV2 vaccination programme launched by BPEX, an organisation representing the pig levy payers in England, and were visited before the implementation of PCV2 vaccination. In addition, to minimise selection bias towards PMWS problem farms, several farms not participating in the PCV2 vaccination plan

were recruited through veterinary practitioners. Details on the recruitment process and visit protocol are described in Alarcon et al. (2011) and Wieland et al. (2010b).

PMWS case definition

For this study, severity of PMWS was estimated for each farm using the protocol described in Alarcon et al. (2011). The algorithm used combines PMWS morbidity in weaner and grower age groups, post-weaning mortality and proportion of PCR positive pigs to PCV2 on the farm to quantify PMWS severity. Based on this severity score, 27 farms were classified as non or slightly affected, 58 farms as moderately affected, and 25 farms as highly affected by PMWS. For 37 farms, the presence of missing values, doubtful or incorrect estimation of PMWS morbidity by the farmers (see misclassification trees in Alarcon et al. (2011)) or low confidence on production parameters estimation hampered the calculation of severity score. These farms were removed from the analysis.

Data collection

Data were collected through an interview based questionnaire with farmers and by on-farm assessment by two researchers. The questionnaire used was tested in 5 farms at the start of the study and questions were reordered, rephrased and some of them redesigned to increase quality of data collected and to shorten the time of the interview. Closed questions were formulated to collect data on potential risk factors. In addition, 20 samples of pigs of different age groups were collected (6 samples of weaners aged between 4 and 10 weeks, 6 samples of growers aged between 10 and 14 weeks, 6 samples of finishers aged >14 weeks and 2 sows). Serological samples were tested for the presence of antibodies against *Actinobacillus pleuropneumoniae, Mycoplasma hyopneumoniae,* parvovirus, porcine reproductive and respiratory syndrome virus (PRRS), Swine influenza, and PCV2.

Hypothesis testing and statistical analysis

Data were grouped in different sections according to the topic they related to, for example health status, husbandry, genetics, etc. All explanatory variables were checked for collinearity with variables from their corresponding section and potential confounding variables such as degree of indoor rearing and number of sows. When collinearity was detected between two variables (p-value ≤ 0.01) only the variable with the highest strength of association with the outcome or presenting the most biological meaning towards explaining risk factors for PMWS severity was retained. For each section a univariate analysis was performed. Analysis of confounding by "degree of indoor" and "farm size" was done with only variables associated with the outcome, and variables completely confounded by these factors were removed from further analysis. To compare means between groups, analysis of variance was done. For non-normal distributed data, the Kruskal Wallis test was used. To identify associations between two categorical variables, normal Chi-square test and Chi-square test for trend was used. To assess association with PMWS categories, ordinal logistic regression was used.

For the multivariable analysis, all variables with p-values equal or lower than 0.20 in the univariate analysis were included. A multivariable ordinal logistic regression model was built using a stepwise forward method. The order of introduction of variables into the models was done by selecting variables from smaller to the highest p-values obtained in the univariate analysis. A variable was retained if the p-value was equal or lower than 0.05 and if the model prediction was improved, which was assessed by a likelihood ratio test, p-value <0.05. The

number of sows and degree of indoor system were forced into the model at all time to control for possible confounding effects. In addition, other potential confounders such as vaccination against specific pathogen were forced into the model if this pathogen was present in the final multivariable model. To check for the assumption of proportional odds, the command "omodel" from the stata statistical package sg79 was used.

For a graphical representation of the identified risk factors in the multivariable model and to understand their position in respect to the different PMWS severity categories, a correspondence analysis using a contingency table was performed. In this analysis, the PMWS severity category was the dependent variable (column variable). Only the variables retained in the final multivariable ordinal logistic regression were added as row variables in the contingency table used for the correspondence analysis.

Univariate analysis and multivariate ordinal and linear regression were performed in Stata 9 (Statacorp, Texas, USA). Analysis of correspondence was done using the command CORRESP in SAS 9.2 (SAS institute Inc, Cary, USA).

RESULTS

Multivariable analysis showed evidence that having growers in indoor facilities, purchasing boars onto the farm, having sick pens draining to other areas holding pigs, rearing poultry or game bird and having *M. hyopneumoniae* antibody positive pigs in the farm increased the odds of a farm in being in a higher PMWS severity category. In contrast, low stocking density in grower's pen, requiring visitors to be at least 2 days pig free and giving more than 2 diets between weaning and 14 weeks old increased the odds of a farm in being in a low PMWS severity category (Table 1). The test performed to assess the assumption of the proportional odds was not significant ($p_{model\ 1}$=0.9128). The R^2 of the model, representing goodness of fit, was 0.50.

Table 1. Risk factors identified in the final multivariable ordinal logistic regression models.

Variables[a]	OR[b]	CI[b]
>1 m^2 / Grower (baseline category is <0.5 m2/grower)[c]	0.06	0.02-0.29
Sick pen drain to other areas holding pigs (binary variable)	5.92	2.15-16.33
>2 diets to the age of 14 weeks old (binary variable)	0.17	0.03-0.84
Presence of poultry (binary variable)	5.08	1.44-17.94
Visitors pig free (binary variable)	0.19	0.06-0.62
Buying boars (binary variable)	4.97	1.79-13.76
Having growers indoor (binary variable)	16.36	3.38-79.07
M. *Hyo.*[d] Elisa positive (binary variable)	4.66	1.50-14.51

[a] Variables 'number of sows', 'vaccination against *Mycoplasma hyopneumoniae'* and 'outdoor/indoor' were forced into the model.
[b] OR=Odds ratio, CI=95% Confidence interval
[c] Variable describing the stocking density in growers' pen and is composed of three categories: (1) <0.5m^2/grower (baseline), (2) 0.5-1m^2 (not significant in the model with OR=0.36 and 95% CI=0.13 – 1.01), (3) >1m^2/grower.
[d]M. *Hyo= Mycoplasma hyopneumoniae*

Analysis of correspondence shows the position of each risk factor identified in the multivariable model in relation to each severity category (Figure 1). Two dimensions were generated; however the inertia of the first dimension was 82% and hence is the most important reference for the analysis of the graph. This dimension effectively separates non/slightly affected farms and highly affected farms, while the second dimension, accounting for 18% of the total inertia, separates to some extent moderately affected farms from the other two severity categories. Figure 1 shows how non/slightly affected farms were more closely related to growers kept indoor, low stocking density in growers and no antibodies to *M. hyopneumoniae*. Related to the highly affected severity category were drainage of sick pen into other areas, visitors being less than two days pig free, having less than three diet changes from weaning to 14 weeks old, presence of poultry or game birds and high stocking density in growers.

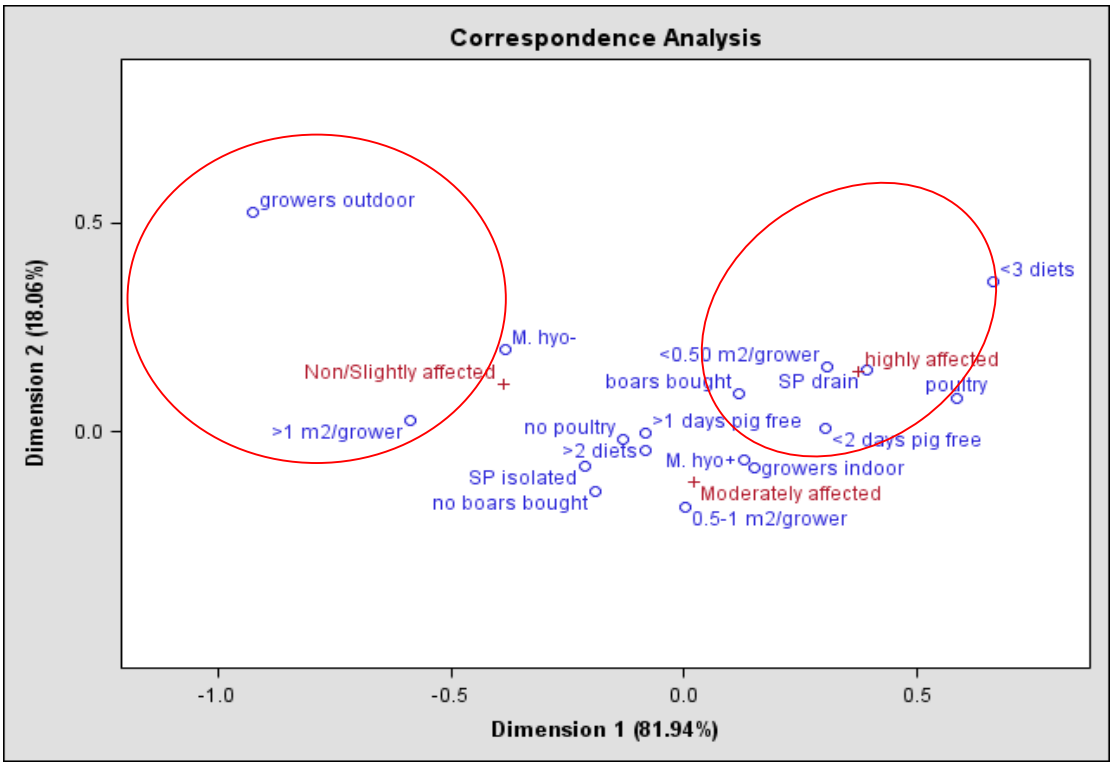

Fig. 1 Position of risk and protective factors in relation to the three PMWS severity categories from a multi-way table correspondence analysis on 110 English farms. Risk factors closely related to non/slightly or highly affected farms are circled. (SP=sick pen).

DISCUSSION

Results of this study confirm the multi-factorial character of this syndrome, in which other pathogens, environmental conditions and management practices, in particular nutrition, were found associated with severity of the disease. In addition, this study highlights the importance of adequate biosecurity measures for the prevention of PMWS on farms and identifies poultry or game birds as a possible risk factor.

Although the cut-offs used for the case definition for the PMWS severity categories were subjective, they effectively compare farms with different severity levels and these categories proved useful in the risk factor analysis. Ordinal logistic regression models assume that the odds ratios between consecutive severity categories are constant for each risk factor. However, this

assumption has been verified to some extent by the non significant results obtained in the "test of assumption of proportional odds". It is important to notice though, that in the farms used here, the categories were based on PMWS severity scores which were calculated from precise estimations for which quality was insured through a misclassification procedure. The selection criteria of farms, data gathering and protocol used during farm visits ensured the reduction of bias as much as possible. Further the number of farms in the study, the spatial distribution of the farms (data not shown), and the number of farms in each PMWS severity category indicates that the sample obtained is considered representative of the English pig farm population. The possibility of recall bias needs to be considered when interpreting the results. Because of the nature of the cross-sectional study, farms were visited once and affected farms had been suffering from the disease for a period of time. Thus some of the risk factors identified could have been introduced after an increase or decrease of PMWS severity on the farm. In addition, because of the high number of variables that were analyzed, some of the risk factors would be expected to be found to be associated due to random variation alone.

The present study indicates the protective effect of rearing pigs in outdoor facilities until the age of 14 weeks old. It was clearly shown in the correspondence analysis that these farms fell well within the PMWS non-slightly affected category and that indoor farms presented very high odds ratio in the ordinal logistic regression. The results also show evidence of the protective effect of providing sufficient space to growers and the risk of overstocking towards PMWS severity. Both factors, stocking density and indoor type, were correlated, as outdoor farms tend to provide more space to their pigs, resulting in less social and environmental stress. However, this correlation was not strong enough to be eliminated during the collinearity analysis. These findings agrees with the hypothesis that environmental stressors and infection pressure factors are required to develop the disease on the farm (Madec et al., 2000). The present study specifically revealed a protective effect when pigs were reared outdoor at least until the end of the grower's period and when growers are kept in low stocking density pens. For many pathogens, such as PCV2, it is known that maternal antibodies have waned at approximately 10 weeks of age and this can explain the need of giving a protected environment to pigs during this delicate transition period. Further research is needed to understand which specific factors or combinations of factors are needed to provide the protective effect in outdoor farms.

Farms giving only 1 or 2 diets to their pigs from weaning to 14 weeks old were identified as having higher odds of being in a more severely affected category. There is a daily change in the balance of protein to energy of a pig. An incorrect balance due to few nutritional adjustments in the first 14 weeks of the pig could lead to a protein and energy deficiency that could compromise the development of immunity of the pig or to a nutritional stress due to an excess of protein that can further cause kidney damage. Further research investigating diets compositions and its relationship with PMWS severity is required.

Three biosecurity measures were identified as risk factors for increase of PMWS severity. Two of them, not requiring visitors to be at least 2 days pig free (NPF) and buying boars onto the farm, are related to the risk of introduction of the virus. The other factor, having sick pens not properly isolated (SPdrain), is related to dissemination of PCV2 or potentially other pathogens within the farm. This could contribute to the increase of the infection pressure on the farm and, this way, to increase the risk of being more severely affected. As shown in the correspondence analysis, NPF and SPdrain were associated with highly affected farms. These results are supported by other epidemiological studies (Rose et al., 2003; Woodbine et al., 2007). Considering that PCV2 has been shown to be present in all farms, the findings of this study suggest that a continuous reintroduction of virus or introduction of possible higher virulent

PCV2 strains from other sources onto the farm could be the cause of increase severity of the disease at farm level.

Apart from *M. hyopneumoniae*, no significant association with other pathogens was found in this study. The ELISA test used to assess the presence of *M. hyopneumoniae* antibodies was not able to differentiate between antibodies originated from the use of the vaccine or due to natural infection. Vaccination against this pathogen reduces clinical signs and severity of the disease on the farm, but does not prevent colonisation or eliminate the disease (Haesebrouck et al., 2004). Therefore, possible interaction with both pathogens remains possible. Moreover, this variable remained significant after forcing vaccination against this pathogen in the final model. As a result, farms that tested negative to the blood samples could be considered as *M. hyopneumoniae* free, and were more likely to be in the non/slightly affected group. Various experimental studies successfully reproduced PMWS through co-infection of PCV2 with *M. hyopneumoniae* live pathogen or inoculation of *M. hyopneumoniae* bacterins (Opriessnig et al., 2004; Krakowka et al., 2007). In addition, another experimental study showed an increase of the quantity of PCV2 in serum and severity of lymphoid lesion when PCV2 infected pigs were vaccinated against *M. Hyo*. (Opriessnig et al., 2006). As a result, the present study supports the hypothesis that co-infection of PCV2 with *M. hyopneumoniae* increase severity of PMWS. Furthermore, the absence of *M. hyopneumoniae* could also indicate a high health farm, where in general a low level of any infectious diseases is observed.

An unexpected finding was that farms raising poultry and/or game birds were more likely to be highly affected by the disease. Viruses belonging to the genus *Circovirus* have been reported in pigeon, duck and goose, and are the cause of several diseases that have similar clinical and pathological picture to PMWS in pigs. In contrast, circovirus in poultry has not yet been identified, but instead virus from the genus *Gyrovirus* that belongs to the family *Circoviridae* are responsible for a disease known as the chicken anaemia virus (Todd, 2004). Consequently, although the transmission of PCV2 through poultry or game birds needs to be investigated, the role of these animals as an environmental stress factor for pigs should be considered.

In conclusion the investigation of risk factors associated with PMWS severity at farm level showed that farms rearing growers outdoors, providing sufficient space to growers or being *M. hyopneumoniae*-free were more likely to be less affected by the disease. Farms not adjusting diets to age of the animals, not requiring visitor to be at least 2 days pig free, not having sick pen properly isolated, overstocking their growers or rearing poultry or game birds were more likely to be highly affected by PMWS. In addition buying boars was identified as a risk factor that increases PMWS severity. Communication of these findings to pig farmers is important as it will inform PMWS control in the future and will ensure that even in the absence of vaccine, the impact of the disease can be minimised or in combination with PCV2 vaccines, how the efficiency of control measures can be improved. Impact on productivity of these factors will have to be investigated more in detail.

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ESTIMATING THE PROPORTION OF CLINICAL MASTITIS ATTRIBUTABLE TO SUBCLINICAL MASTITIS IN DAIRY CATTLE USING TWO MULTIVARIABLE STATISTICAL APPROACHES

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SUMMARY

The aim of this study was to quantify the proportion of first bovine clinical mastitis (CM) cases attributable to high composite somatic cell counts (CSCC). Cows were followed from the first CSCC measurement postpartum until CM or censoring, using survival analysis. A conditional logistic regression model was also fitted to the data with CM cows being matched to cows without CM. Both models identified high CSCC cows to have a higher risk for subsequent CM cases than low CSCC cows. The population attributable fraction was 0.22 for primiparae and 0.17 for multiparae according to the logistic regression model, while it was 0.25 in the survival analysis. The latter approach also identified that the proportion of cows without CM would increase from 89% to 93% if no high CSCC would occur in the dairy population. Both multivariable statistical approaches showed that a substantial reduction in CM can be achieved by decreasing the prevalence of high CSCC in the dairy population.

INTRODUCTION

Mastitis, the inflammation of the mammary gland, generally follows infection. When inflammation is accompanied with visible alterations of the udder and/or milk it is called clinical mastitis (CM). Subclinical mastitis is defined as a mammary gland that is inflamed, but has no visible signs. In dairy cows, both appearances are associated with economic losses (e.g., milk yield loss, antimicrobial use, culling, extra labour; Halasa et al., 2007). Bovine subclinical mastitis is generally monitored by measuring the composite somatic cell count (CSCC) during the regular test day recording (Schukken et al., 2003). High CSCC are measured before CM occurs (de Haas et al., 2002) and are therefore considered predictive for the development of CM (e.g., Rupp & Boichard, 2000; Green et al., 2004). Several statistical methods have previously been used to determine this relationship, but most investigations neither corrected for the length of the time period up to CM occurrence nor adjusted for the dynamic nature of CSCC. Intramammary infections can occur during lactation resulting in new CSCC elevations (Schukken et al., 2003).

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The population attributable fraction (PAF) is the proportion of disease events that can be prevented from the total population when a perfect intervention is applied to a certain risk factor (Dohoo et al., 2009). PAF estimation is important to determine the (economic) impact of intervention strategies on population level because, in contrast to the attributable fraction, it includes the exposed as well as the non-exposed individuals. Several techniques have been proposed to calculate PAF but only some of them adjust for other risk factors in case of multivariable modelling (Rückinger et al., 2009). Moreover, the PAF becomes dynamic with censored time-to-event data as the event rate accumulates over time and exposed subjects are more rapidly depleted from the population than non-exposed subjects (Cox et al., 2009). The PAF indicates the potential reduction in CM events in the total dairy cow population, by preventing or removing high CSCC using intervention (e.g., culling or antimicrobial treatment) in this study. The objective of this study was to estimate the proportion of CM cases attributable to high CSCC in Dutch dairy herds using two statistical multivariable approaches.

MATERIALS AND METHODS

Data

The data for this investigation were obtained from a previously described observational study on 205 randomly selected Dutch dairy herds participating in four-weekly test recording (van den Borne et al., 2010). In short, CSCC measurements of the regular test day recording and farmer-diagnosed CM cases on these herds were collected from July 1, 2004 until June 30, 2005. Test day recordings and CM cases were selected from cows that calved within the study period to evaluate the association between CSCC and the first subsequent case of CM. Recurrent CM cases were not included in the analysis, nor were CM cases before the first test day. Cows without a test day record preceding a CM case (n=892) were removed from the dataset. The first lactation was analysed when cows had two calvings within the study period (n=48 cows). This dataset comprised cow level information on stage of lactation, parity, CSCC and production parameters at each test day. Seventeen mastitis management related factors on herd level (including herd size, bulk milk SCC measurements, and factors on hygienic procedures and treatment decisions) were obtained from a questionnaire sent to the farmers before the start of the study (Jansen et al., 2009) to correct for the influence of these herd level factors on the relationship between CSCC and CM. Nine herds were removed from the data because of missing herd level data. This resulted in a dataset of 13,917 cows in 196 herds, of which 1,560 cows (11.2%) had a first case of CM after a CSCC measurement. This was the initial dataset for both analytical procedures.

Survival analysis

Cox proportional hazard regression was used to determine how the hazards for CM differed for cows with low (<200,000 cells/ml) or high (≥200,000 cells/ml) CSCC. CSCC status and other test day parameters (milk production (kg/day); protein percentage; fat percentage; and season (pasture period: May-October or housing period: November-April)) were included as time-varying predictors that could change at each test day. Each cow became at risk at the first test day after calving and its failure time was determined in each test day interval until the first case of CM or until censoring at the end of the study period (July 1, 2005) or at the last available test day if cows were culled or dried off. All available risk factors (5 time-varying predictors, parity, and 17 herd level risk factors) were tested in bivariable analyses with CSCC status forced into the model. The reduction in deviance was used to select variables with $P < 0.25$ for the

multivariable analyses, in which a stepwise backward elimination process was used to identify the variables that were significantly ($P < 0.05$) contributing to the model. A herd frailty effect was added to all multivariable models to adjust for clustering within herds. All models were checked for confounding, which was assumed to occur when estimates changed >25%. Biologically relevant interactions between CSCC status and other cow level factors were also investigated. Survival analysis was performed in R (R Development Core Team, 2009).

Conditional logistic regression

In addition to the Cox proportional hazards model, a matched case-control study design was also applied to the initial dataset in which a case was defined to be a cow with a first CM case after at least 1 CSCC measurement. CSCC status of these CM cases was determined at the last test day preceding the first CM observation (td = -1). The preceding test day was evaluated if CM occurred on a test day. A cow was considered to have high CSCC when CSCC was ≥200,000 cells/ml, similar to the definition used for the survival analysis. Test day recordings from control cows in the same stage of lactation, without CM in the study, were matched to td = -1 from case cows. Therefore, eligible control cows needed to have their calving dates within 7 days around the calving date of a case cow. Additionally, the test day recordings from control cows needed to be within 7 days of td = -1 of their case cow and were subsequently also defined td = -1. CSCC status at td = -1 for control cows was determined similar to case cows. This matching procedure adjusted for potential differences in CSCC between lactation stages. Four control cows from any herd were randomly selected to each case cow to increase precision. Control cows were matched to only 1 case cow.

All available risk factors were studied using conditional logistic regression with CM occurrence as the binary response variable. Lactation stage was included in the analysis to verify if matching did not result in confounding with the risk factor exposure (i.e., CSCC status). Analyses started with univariable and bivariable conditional logistic regression models for all eligible variables. CSCC status was forced into all models to observe the effect of the variable of interest on the parameter estimate of CSCC status. All variables with a $P < 0.25$ qualified for the multivariable analysis, in which a stepwise backwards approach was used until all variables had a $P < 0.05$ based on the reduction in deviance. Observations with missing values for some herd level variables were added to the dataset again when these variables were not included in the model anymore. All models were checked for confounding, which was assumed to occur when estimates changed >25%. Biologically relevant interactions between cow level variables were also tested. No random herd effect could be added to the model due to the matched case-control study design and the accompanying analytical procedure (i.e., conditional logistic regression). Standard errors of herd level variables in the final model may therefore be underestimated resulting in too low P values. Consequently, the herd level variables in the final model were not interpreted or further tested in interaction terms, but were assumed to adjust the effect estimates of CSCC status for a potential herd effect. Conditional logistic regression analysis was performed using proc logistic in SAS 9.2 (SAS Institute, Cary, USA).

Population attributable fraction

For the Cox frailty model, the PAF was calculated as follows (Cox et al., 2009):

$$PAF(t) = \frac{S^*(t) - S(t)}{1 - S(t)} \quad (1)$$

with $S(t) = \sum_j \ _jS_j(t)$ the survival function of the total population (both exposed and unexposed individuals) for j strata, $S^*(t) = \sum_j \ _j{}^*S_j(t)$ the resulting survival distribution for j strata when a perfect intervention of the exposure variable (i.e., high CSCC) is assumed to result in an alternative distribution of $_j{}^*$ (absence of high CSCC), and $_j$ the proportion of exposed individuals in stratum *j*.

PAF(t) indicates the reduction in diseased individuals, but interest mainly is in survival of individuals when conducting survival analysis. Cox et al. (2009) therefore introduced a new measure of association to assess the impact of interventions with survival data: the attributable survival (hereafter called the population attributable survival (PAS), in agreement with PAF). PAS represents the additional proportion of individuals in the population who survive to a given time, if a fully effective intervention to exposed individuals has been administered at t = 0. For the current study, PAS represents the additional proportion of cows without subsequent CM after a CSCC measurement when preventing or removing high CSCC from the population. PAS was calculated as follows (Cox et al., 2009):

$$PAS(t) = \frac{S^*(t) - S(t)}{S^*(t)} \tag{2}$$

Both PAF(t) and PAS(t) provide information at which moment intervention may have its largest effect as the survival function is dependent on t (Cox et al., 2009). PAF(t) and PAS(t) calculations were based on the final Cox proportional hazards model without the herd level frailty effect included.

The 'average' PAF for high CSCC, as introduced and defined by Eide and Gefeller (1995), was calculated in the unconditional logistic regression analysis, using a readily available SAS macro (Rückinger et al., 2009). Only the cow level variables and the interaction between CSCC status and parity were included in the PAF calculations because of computational capacity. The change in PAF estimate for CSCC status, however, was determined when each herd variable was added one at the time to the model.

RESULTS

Survival analysis

Survival without CM differed between parities. Survival without CM was the highest in primi- and multiparae with a low CSCC, while survival without CM was the lowest in multiparae with a high CSCC (Fig. 1).

The results from the final Cox proportional hazards model are presented in Table 1 and are based on 62,742 test-day intervals, of which 1,280 (2.0%) had a first case of CM. High CSCC cows had a 2 to 4 times higher hazard for subsequent CM than cows with a low CSCC. Multiparae with a low CSCC had a 2 times higher hazard for CM than primiparae with a low CSCC. Higher production levels were associated with increased hazards for CM, especially in high CSCC cows. The hazard in the housing period was slightly higher than in the pasture period. No herd level variables were significant in the final Cox frailty model. Variance of the herd frailty effect was 0.37 ($P < 0.0001$).

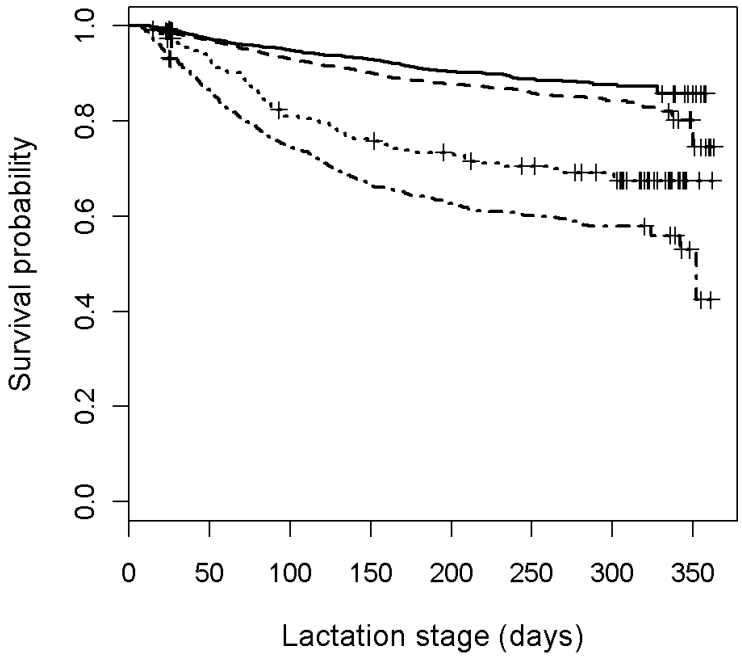

Fig. 1 Survival curves (not having clinical mastitis) for 4 groups of dairy cows, after the first somatic cell count (CSCC) measurement postpartum (from top to bottom: primiparae with a low CSCC (<200,000 cells/ml); multiparae with a low CSCC; primiparae with a high CSCC; multiparae with a high CSCC).

Table 1. Results from the final Cox proportional hazards model with time-varying predictors for the first case of clinical mastitis (CM; n=1,280) after a composite somatic cell count measurement (CSCC).

Variable	Category	HR	95% - CI
High CSCC (≥200,000 cells/ml)	No	1.0	Ref
In primiparae	Yes	4.0	2.5 – 6.5
In multiparae	Yes	2.1	1.3 – 3.6
Parity	1	1.0	Ref
For low CSCC cows	≥2	1.9	1.6 – 2.4
For high CSCC cows	≥2	1.0	0.8 – 1.3
Season	Pasture	1.0	Ref
	Housing	1.2	1.0 – 1.3
Milk production (kg/day)	Continuous		
In low CSCC cows		1.02	1.00 – 1.03
In high CSCC cows		1.04	1.03 – 1.05

HR = Hazard Ratio; 95% - CI = 95% - confidence interval. Ref = Reference category.

The PAF(t) and PAS(t) based on the final Cox proportional hazards model are presented in Fig. 2. The PAF starts at 0.29 at the beginning of the lactation and is monotone decreasing to

0.25 towards 300 days in lactation. The PAS starts at zero directly after calving and is monotone increasing towards 0.04 at 300 days in lactation. This indicates that an additional 4% of cows do not develop a first subsequent CM after a high CSCC measurement when the latter can be prevented. Hence, it results in a reduction of cows with a first case of CM after a CSCC measurement during their lactation from 11% to 7%, thereby increasing the proportion of cows without CM after a CSCC measurement from 89% to 93%.

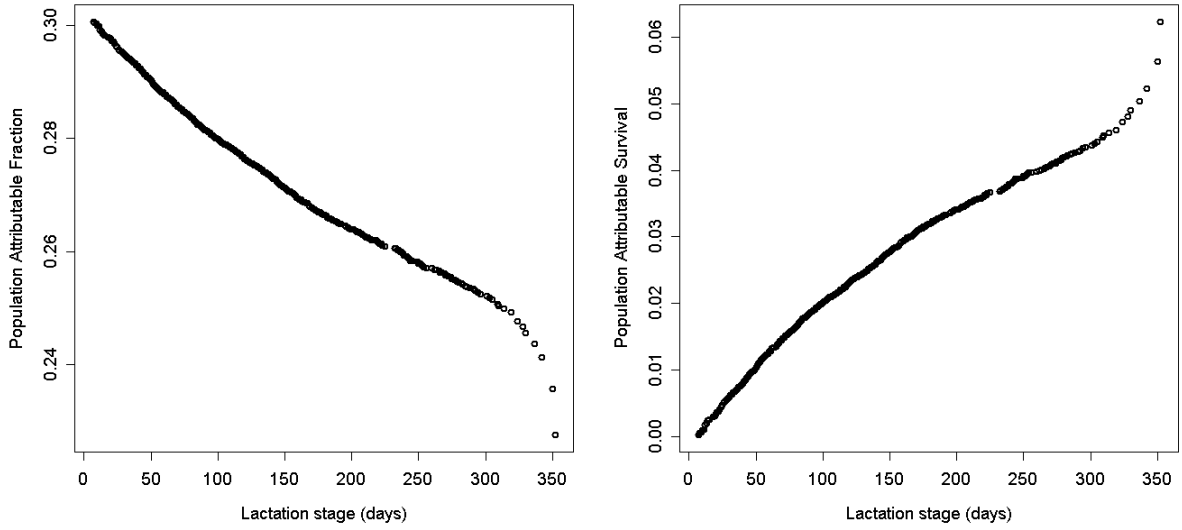

Fig. 2 Estimated population attributable fraction (left) and population attributable survival (right) for clinical mastitis in dairy cows with a high composite somatic cell count.

Conditional logistic regression

The data analyzed in the conditional logistic regression consisted of 1,560 CM cows and 6,240 selected control cows. There were no herd mates within 1,479 matched case and control sets, while there was 1 herd mate among the controls in 81 matched sets. This indicates that only a small proportion (5.2%) in matched sets came from the same herd. High CSCC at td = -1 was observed in 41.3% of the CM cows while high CSCC were observed in 15.3% of the control cows.

Results from the final conditional logistic regression model are presented in Table 2 and are based on 7,684 observations (including 1,538 cases) due to missing values for some herd level variables. All cow level variables significant in the final Cox proportional hazards model were also significantly associated with CM in the conditional logistic regression. Primi- and multiparae with a high CSCC at td = -1 had a higher odds (OR = 6 and OR = 4 for primi- and multiparae, respectively) for CM occurrence than cows with a low CSCC. CM was found more frequently in multiparae than in primiparae when they had a low CSCC (OR = 2). Crude proportions of CM occurrence were 35.5% and 42.7% in primi- and multiparae with a high CSCC, respectively, and were 8.5% and 18.3% for primi- and multiparae with a low CSCC. A linear increase in milk production was associated with a log linear increase in CM occurrence. In contrast to the Cox proportional hazards model, CM occurred slightly more frequently in the pasture period than in the housing period. Four herd level variables were significant in the final conditional logistic regression model. The number of test day recordings since calving, as a

proxy for stage of lactation, was not associated with CM occurrence, indicating proper matching of case and control cows.

Table 2. Results from the final conditional logistic regression model for the first case of clinical mastitis (CM; n=1,538) after a composite somatic cell count (CSCC) measurement.

Variable	Category	OR	95% - CI
High CSCC (≥200,000 cells/ml)	No	1.0	Ref
In primiparae	Yes	6.1	4.5 – 8.3
In multiparae	Yes	3.6	3.1 – 4.2
Parity	1	1.0	Ref
For low CSCC cows	≥2	1.9	1.5 – 2.3
For high CSCC cows	≥2	1.1	0.8 – 1.5
Season	Pasture	1.0	Ref
	Housing	0.6	0.5 – 0.9
Milk production (kg/day)	Continuous	1.03	1.02 – 1.04
Average milking herd size		NI	
Post-milking teat disinfection		NI	
Treatment of cows with a 1st high CSCC		NI	
Performing a dynamic milking test		NI	

OR = Odds Ratio; 95% - CI = 95% - confidence interval. Ref = Reference category. NI = Not interpreted. Herd level variables were not interpreted because they were assumed to correct the cow level estimates for clustering within herds.

According to the logistic regression model, the 'average' PAF for high CSCC was 0.221 and 0.166 in primi- and multiparae, respectively. The maximum change in 'average' PAF for CSCC status was 5.3% when herd level variables were added to the model one at the time, indicating a robust 'average' PAF estimate.

DISCUSSION

This study quantified the relationship between high CSCC and the first subsequent CM case using Cox proportional hazards models and conditional logistic regression models. This relationship was only based on a statistical relation, while the relationship between subclinical mastitis and CM should ideally be based on the presence of bacteria in both types of mastitis. High CSCC are a good approximation for the bacteriological status of a cow's udder but its sensitivity and specificity are not perfect (Schukken et al., 2003). Nevertheless, both approaches identified high CSCC cows to have a higher risk for developing CM than cows with a low CSCC, as did other studies (Rupp & Boichard, 2000; Green et al., 2004). The identified risk factors (parity, milk yield and season) behaved as determined earlier (Rupp & Boichard, 2000; Green et al., 2004). The opposite effect of season in the conditional logistic regression model compared with the Cox proportional hazards model was probably due to the matching procedure. Case and control cows were in the same season in 85% of the matched groups, indicating limited variation within matching groups because of overmatching (case and control cows calved within 7 days of each other).

Both statistical approaches were based on the same initial dataset but the effects estimates and the PAF estimates from the Cox proportional hazards model cannot be directly compared with the estimates from the conditional logistic regression model. There are methodological differences between both statistical approaches (e.g., matching vs no matching, time-fixed vs time-varying predictors, no corrections for clustering within herds vs random frailty effects). Effect estimates, and thus the PAF estimates, from Cox proportional hazards models are deemed more accurate and more precise compared with effect estimates from conditional logistic regression models (Green & Symons, 1983) and are therefore preferred.

Calculation of the 'average PAF' identified that a substantial reduction of 22.1% and 16.7% of first CM cases after a CSCC measurement of primi- and multiparae may be achieved by preventing, treating and/or culling of high CSCC. The 'average PAF' for CSCC status was calculated based on the unconditional logistic regression model while model estimates were obtained from a conditional logistic regression model. However, model estimates from both models were similar (data not shown), indicating that less bias in the PAF estimation for CSCC status would have been introduced by not correcting for matching. Other approaches to PAF calculations have been suggested previously and were recently reviewed by Rückinger et al. (2009). The 'average' PAF according to Eide and Gefeller (1995) was acknowledged to give the most plausible PAF estimates while adjusting for other risk factors. Less complicated formulas may overestimate the potential population effect (Rückinger et al., 2009). Those were therefore not applied in the current study. It has to be noted that, despite its superiority to other formulas, PAF interpretation is only possible for dichotomous risk factors. PAF estimates can be adjusted with non-dichotomous covariates, which was the case in the current logistic regression model that included milk production. Communicating PAF results for continuous covariates, however, may be difficult (Ruckinger et al., 2009).

Using the recently proposed formulas for PAF and PAS estimation for time-to-event data (Cox et al., 2009), PAF was 25% and PAS was 4% in the Cox proportional hazards model. This confirms the potential gain in CM in the total population when high CSCC is prevented or intervened. Both formulas assume a perfect intervention at the time of detection (i.e., td = -1 in this study), while interventions on high CSCC are neither directly applied nor perfect in dairy practice. These aspects can be taken into account using more advanced formulas (Cox et al., 2009).

In conclusion, this study quantified the relationship between high CSCC and first cases of subsequent CM. It was shown that approximately 25% of first subsequent cases of CM in dairy cows can potentially be reduced by preventing, treating or culling of high CSCC cows. This population effect is considered a substantial reduction, especially when it is considered that only first subsequent CM cases after a CSCC measurement were taken into account. Cows may develop repeated cases of CM, resulting in an underestimation of the true population effect.

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OPEN SESSION

TRANSMISSION OF LIVESTOCK ASSOCIATED MRSA IN PIGS

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SUMMARY

Data from a transmission experiment and a longitudinal field study on 4 pig farms were used to quantify transmission of Livestock Associated-Methicillin Resistant *Staphylococcus aureus* (LA-MRSA) in pigs. In an extended five-to-five experiment, 5 inoculated pigs (C0) were first mixed with 5 naïve pigs (C1) and, subsequently, the 5 C1-pigs were put together with another 5 naïve pigs (C2). All C2-pigs became MRSA-positive during the experiment, and R_0 was estimated at 3.92 (95% CI: 2.55-6.17). In a field study, sows and their piglets were sampled for about 5 weeks and 5 months, respectively. R_0 varied between 0.13 (95% CI: 0.10-0.18) where there was a relative within-pen infection pressure of 0 (compared to within-section and environmental infection pressure), no use of β-lactams and tetracyclines and where pigs were not located in the farrowing section, and 4.55 (95% CI: 1.26-16.32) in the case where risk antimicrobials were being used in pigs in the farrowing section with a maximum relative within-pen infection pressure. The results indicate that there is a high probability of having major outbreaks of LA-MRSA after introduction into a susceptible herd.

INTRODUCTION

In 2005, a distinct clone of methicillin resistant *Staphylococcus aureus* (MRSA), referred to as Livestock Associated (LA)-MRSA, was found in pigs and people in contact with pigs (Voss et al., 2005). Since then, many countries have detected LA-MRSA in pigs and other livestock, showing large variations in prevalences (Broens et al., 2008; EFSA, 2009; Smith et al., 2009; Graveland et al., 2010; Mulders et al., 2010). In order to design control programs, more insight in the transmission dynamics of LA-MRSA is needed. Transmission can be assessed quantitatively by using experimental and field data and can be expressed by the basic reproduction ratio (R_0), which is defined as the average number of secondary cases caused by one typical infectious individual during its entire infectious period in a fully susceptible population. If $R_0 > 1$, each infected animal will infect on average more than one susceptible, which might lead to a major outbreak (De Jong & Kimman, 1994). The aim of this paper is to estimate R_0 for LA-MRSA from both a transmission experiment and a field study on Dutch pig farms. Additionally, in the field study the effect of several risk factors on R_0 was studied.

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MATERIALS AND METHODS

Transmission experiment

The design of the experiment is a modification of the extended transmission experiment described by Velthuis and co-workers (2003) for *Actinobacillus pleuropneumoniae*. Three groups of 5 conventionally reared, 6-week-old castrated male pigs from a confirmed MRSA-negative farm were used in the experiment. Five experimentally inoculated pigs (C0) were placed together with 5 MRSA-negative pigs (C1) on day 0. After C1-pigs confirmed to be MRSA-positive, they were placed together with 5 MRSA-negative pigs (C2) on day 22; C0 pigs were euthanized at that day. Swabs were taken twice a week until day 70.

Field study

Five farrowing sections on 4 farrow-to-finish farms were selected. Sows (6-16 per section) were sampled nasally 3 times (1 week before and 3 days after giving birth and at weaning). Piglets from these sows were sampled nasally 5 times (3 days after birth, at weaning, 3 and 6 weeks after weaning and just before transport to the slaughterhouse). Environmental samples (4 per section) were taken at all sampling moments.

Laboratory analysis

MRSA isolation was performed on single environmental wipes and individual swabs according to the protocol described by Broens et al. (2010).

Statistical analysis

A Susceptible-Infectious-Susceptible (SIS) model was used to estimate the transmission of LA-MRSA, both in the experiment (from C1 to C2 pigs) and the field study. At each sampling moment, a positive-tested animal was assumed to be infectious and a negative-tested to be susceptible.

The log of the transmission parameter β, i.e. the number of secondary cases (C) out of a number of susceptibles (S) caused by infectious pigs (I) during each time interval (Δt), was estimated using a Generalised Linear Model for binomially distributed data with a complementary log-log link function. In the transmission experiment log ($I/N*\Delta t$) was used as an offset, where N is the total number of individuals present.

In the field study, use of risk antimicrobials (yes vs. no), location (farrowing vs. other sections) and relative proportion of infection pressure within the pen (IP; based on the total within-pen, within-section and environmental prevalence) were significant and therefore included in the model as explanatory variables; interactions were not significant. Tetracyclines and β-lactam antibiotics were defined as 'risk antimicrobials' as these antimicrobial classes select 100% for LA-MRSA. Again a Generalized Linear Model for binomially distributed data with a complementary log-log link function was used to estimate log β; here log ($IP*\Delta t$) was used as an offset.

Models were analysed statistically using SAS, version 9.1 (SAS, 2004). Subsequently, R_0 was calculated by multiplying β with 9.8, i.e. the length of the infectious period, based on the observed average duration of successive positive tests in the experiment.

RESULTS

In the transmission experiment, all C1-pigs became MRSA-positive within 2 days after mixing with C0-pigs. All C1-pigs stayed MRSA-positive until day 22; the day that the C1-pigs were mixed with the C2-pigs. Three out of five C2-pigs became MRSA-positive within 6 days after mixing with C1-pigs. Eventually, all C2-pigs became MRSA-positive at some time during the experiment. After turning MRSA-negative, recurrent infections occurred in several pigs. Transmission parameter β and reproduction ratio R_0 were estimated at 0.40 per day and 3.92 (95% CI: 2.55-6.17), respectively.

In the field study, MRSA-prevalence among sows before giving birth varied from 0.0 to 50.0%, and increased in 3 out of 5 farrowing sections after giving birth. Average MRSA-prevalence among piglets increased from ~ 50% just after birth to ~ 75% at 6 weeks after weaning. Subsequently, a slight decrease to ~ 65% during the finishing period was observed. The transmission parameter β varied from 0.014 to 0.464 per day, depending on antimicrobial use, location of pigs and proportion of relative within-pen infection pressure. R_0 varied between 0.13 (95% CI: 0.10-0.18) with a relative within-pen infection pressure of 0, no use of risk antimicrobials and not located in the farrowing section, and 4.55 (95% CI: 1.26-16.32) in the case where risk antimicrobials are being used in pigs in the farrowing section with a maximum relative within-pen infection pressure, i.e. 1 (Fig. 1).

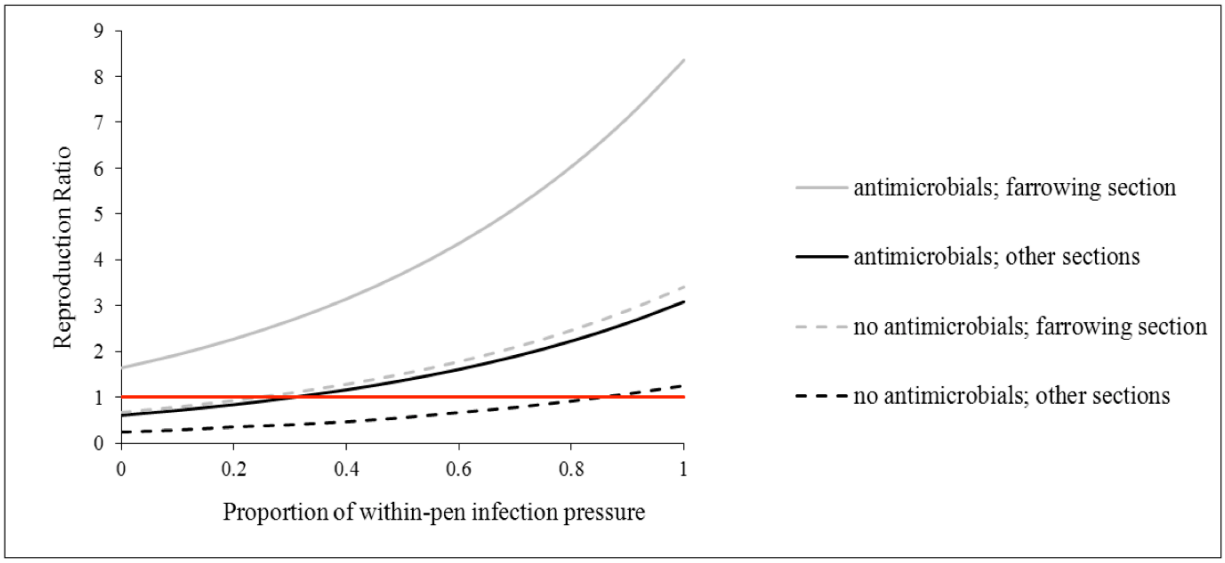

Fig. 1 Effect of relative proportion of within-pen infection pressure with regards to MRSA on the Reproduction Ratio for pigs with and without risk antimicrobial use in farrowing or other sections of a pig farm (based on data from 179 pens on 4 farms).

DISCUSSION

The results in both experimentally and naturally infected pigs indicate that MRSA is able to spread when contacts between pigs occur randomly. Even when the infectious period is only 3 days, the estimated reproduction ratio is higher than one. Therefore, there is a high probability of having major outbreaks of MRSA after introduction into a fully susceptible herd. Transmission by direct contact between pigs seems to play an important role in the spread of MRSA relative to environmental contamination within pig herd.

Defining the infection status, either susceptible or infectious, of an individual pig during the study highly affects the outcome of the study. In studies on *Staphylococcus aureus* intramammary infections, an animal was defined infectious if the bacteria were isolated from more than two consecutive samples (Lam et al., 1996). Using this definition in the experimental study, fewer cases were observed, though the observed infectious period would have been longer, resulting in a similar reproduction ratio (data not shown). In the field study, the period between sampling moments varied between 7 days and 16 weeks; so too long to use the above mentioned definition.

Use of risk antimicrobials increased the reproduction ratio, which might be explained by the selection for LA-MRSA when these antimicrobials are used. In the farrowing section, the reproduction ratio was higher than in other sections. At the first sampling in the farrowing section, the MRSA prevalence was relatively low, and thus the proportion of susceptibles and thus potentially new cases relatively high. At all sampling moments in the other sections, the MRSA prevalence was much higher, leaving fewer susceptibles to become a case. The R_0 in other sections might be as high as in the farrowing sections if the starting point is a fully susceptible pig population in these sections. On the other hand, the sow might be a primary source for the newborn piglets and young piglets might be more susceptible to infections due to an immature mucosal immune system or a greater impact of antimicrobials on their unbalanced microflora (Zoetendal et al., 2004; Bailey et al., 2005). The reproduction ratio also increased when the proportion of relative within-pen infection pressure increased compared to infection pressure from other pigs in the section or the environment, indicating that transmission by direct contact is an important route of transmission. In another study in pigs during transport to the abbatoir it was shown that environmental contamination might also be an important route for transmission of MRSA (Broens et al., 2010).

Since only 4 farrow-to-finish farms were included in the field study, the results of this study might not be representative for the Dutch pig herd population. Moreover, the observed association between explanatory variables and the reproduction ratio, e.g. antimicrobial use, might be confounded by other effects. With only 4 herds it is hardly possible to distinguish the herd effect from an un-confounded estimate of the effect of exposure. Prudence is therefore called for in drawing conclusions on these associations and studies involving larger numbers of herds are required to confirm the findings.

To summarize, introduction of MRSA in a fully susceptible population most probably leads to a major outbreak with direct contact between animals as an important route of transmission, even without use of risk antimicrobials. However, as the results in the field study indicate, transmission rates might differ depending on type of antimicrobial use, location of the pigs and proportion of within-pen infection pressure relative to other infection sources. Control programs should therefore focus on (1) prevention of introduction into a herd, and (2) prevention of transmission within a herd, e.g. by restrictive antimicrobial use.

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GOING BEYOND TRADITIONAL APPROACHES FOR MODELLING ANTIMICROBIAL RESISTANCE DATA – THE APPLICATION OF BAYESIAN NETWORK ANALYSIS

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SUMMARY

Antimicrobial resistance is a complex phenomenon for which risk factors and their inter-relationships have yet to be fully described. Methods based on generalised linear models (GLM) do not provide an optimal overview of the overall structure of the relationships between all variables together. This work was focused on the investigation of suspected resistance risk factors of antimicrobial resistance in pig farms across Canada and is used to illustrate the added value provided by Bayesian Network analysis (BNA) in complementing traditional GLM.

Binomial logistic regression results indicated a likelihood of detecting resistance to ampicillin (AMP) significantly higher when an all-in-all-out (AIAO) production system was used or when tetracycline was used in feed. In BNA, AIAO was also directly associated with AMP resistance but the effect of using in-feed tetracycline on AMP resistance appeared to be mediated through barn capacity and AIAO. Resistance to tetracycline, sulfisoxazole and streptomycin were also investigated.

INTRODUCTION

Antimicrobial resistance is a complex phenomenon for which risk factors and their interrelationships have yet to be fully described.

Most risk factor analyses are performed using generalised linear models on datasets derived from observational studies gathering a large number of often inter-related variables. While conceptual models to investigate the epidemiology/ecology of antimicrobial resistance would naturally include numerous variables and complex interactions, current methods for data analysis based on generalised linear models cannot easily handle such complexity. These generally only consider the relationship between a single response variable and all other variables (covariates). Furthermore, there is often insufficient power to retain interaction terms in the models. Consequently, these methods do not provide researchers with an optimal overview of the overall structure of the relationships between all variables together.

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Bayesian network analysis constitutes an excellent complementary approach because it is concerned with estimating relationships between multiple variables. Bayesian network analysis has been used in fields such as ecology, bioinformatics, genetics, and image processing (Borsuk et al., 2004; Kuikka et al., 1999; Rieman et al., 2001; Yan and Cercone, 2010). Very recently, Bayesian methods have been used by Denwood et al. (2010) to study the possibility of inferring genotypic information from phenotypic data in studying antimicrobial resistance in *Salmonella* Typhimurium DT104; however, Bayesian network analysis as a specific approach has received little attention in the field of veterinary epidemiology or in the study of AMR determinants. This work was focused on the investigation of suspected resistance risk factors and the objective was to illustrate the added value provided by Bayesian Network analysis in complementing traditional GLM approaches to assess associations among production practices, antimicrobial use and resistance, using observational field data from Canadian swine farms.

MATERIALS AND METHODS

Data collection

The dataset included the susceptibility to 15 antimicrobials of *E. coli* isolates from pig farms across Canada and data on a variety of farm level risk factors (e.g., herd size, all-in/all-out management, etc.) collected in 2007-8 by the Canadian Integrated Program for Antimicrobial Resistance (CIPARS) (Government of Canada, 2010b). To ensure producer anonymity, CIPARS farm sample and data collection are performed by herd veterinarians purposively selected from the five major pork producing provinces. Herds were selected by each participating veterinarian according to specified inclusion/exclusion criteria. Criteria for inclusion were validated participation in the Canadian Quality Assurance (CQA®) swine farm quality assurance program, marketing >2000 hogs/year, and that the farm be representative of the demographic and geographic distribution of the corresponding participant veterinary practice. Organic herds feeding edible residual material and pasture raised herds were excluded since they represent only a small fraction of grower/finisher operations in Canada. Most participant herds (n=143) were visited more than once during the two year period included in this analysis. The total number of visits was 453 and the number of visits per farm ranged from one to six. Pooled fecal samples were collected from close-to-market-weight (>80 kgs) finisher hogs pens. Samples were sent to the Laboratory for Foodborne Zoonoses (Saint-Hyacinthe, Quebec) for generic *E. coli* isolation and for antimicrobial susceptibility testing. Minimum inhibitory concentrations (MIC) of 15 antimicrobials were determined for each isolate using the Sensititre® broth microdilution system (CMV1AGNF panel; TREK Diagnostic Systems, Westlake, Ohio). A complete description of microbiological methods is available in the 2007 CIPARS Annual Report (Government of Canada, 2010a).

For our analysis, predicted variables comprised the presence or not (binary variable) at the farm level of resistance to ampicillin (AMPr), streptomycin (STRr), sulfisoxazole (SSSr) and tetracycline (TETr) among *E. coli* isolates from the samples collected during a herd visit. If any isolate from one visit was resistant to one of the four antimicrobial of interest, the visit was attributed a positive status regarding this specific antimicrobial. Breakpoints for the selected antimicrobials were AMP ≥32µg/mL, STR ≥64µg/mL, SSS ≥512µg/mL and TET ≥16µg/mL. Antimicrobial use data were collected using sampling day questionnaires and farm CQA® forms. From the data collected, a list of 14 predictor variables were developed, including production flow type (All-In-All-Out (AIAO) or continuous), antimicrobial use (penicillins (PEN), sulfonamides (SULF), aminoglycosides (AMINO) and tetracycline (TET)), route of

administration of the antimicrobials (via injection, in water or in feed) and barn capacity. The barn capacity variable was categorized into five discrete categories (BarnClass) using a mixture model Mclust library in R (R Development CoreTeam 2010). Apart from BarnClass, all predictors were binary variables (i.e. Use or not of AMINO via Feed during the grow-out period for the group of pigs sampled).

Methodological approach

The main analytical approach consisted of comparing the results obtained from analysing the same dataset separately with binomial logistic regression (BLR) and with Bayesian Network Analysis (BNA). The analyses were performed at the visit level to improve robustness and avoid issues of clustering within farms. This means that for each visit a farm was summarised as either negative if no resistant isolates were found or positive if at least one resistant isolate was detected among all isolates recovered. No adjustment was made for repeated measures as there were insufficient numbers of repeat visits in addition to a wide variation in the number of repeat visits per farm (48 farms had three visits or less).

More specifically, BLR was performed separately for each of four antimicrobial drugs (ampicillin, streptomycin, sulfisoxazole, tetracycline) using GLM in R (procedure: glm, package: stats). Reduced model selection was performed using forward-stepwise and backward-stepwise algorithms using the Akaike Information Criterion (AIC) to decide on addition or removal of variables. The two approaches were performed in parallel, starting from an empty and a full model for the forward-stepwise and backward-stepwise respectively. The resulting reduced optimal models were compared using the AIC and only the best model was kept. Interactions were only considered in the BNA model. Odds ratio and confidence intervals (CI) were computed for all variables present in the final reduced model with $p<0.10$.

For the BNA, the following methodological steps were performed. A single database including data for all selected resistance variables was used. A total of 140 000 searches were performed. The following search parameters were used: a limit of 5 parents for locally optimal networks was performed in R, each search starting from an initial empty network randomly permuted 20 times. The individual results of those 140 000 searches were divided in two groups and a consensus network at the level of 51% was obtained for each group. These were compared to verify that they were identical, indicating stable results. A final reduced network was produced by trimming out all variables that were not directly linked to at least one of the resistance variables. Trimming was used as it is well established that fitting Bayesian Networks using marginal likelihood – the standard approach – in common with other machine learning methods can result in substantial over-fitting (Needham et al., 2007). Relative risk (RR) and 95% CIs on RR were computed.

RESULTS

For the BLR approach, both the forward-stepwise and the backward-stepwise model selection ended up with the same best reduced model in the cases of AMP and TET resistance. For STRr and SSSr, the best-reduced models obtained from both methods were slightly different. However, the forward-stepwise approach produced a model for which the AIC value was higher and was therefore the one kept.

Models retained were:

$$\text{logit}(AMPr_i) = \beta_0 + \beta_1 AIAO_i + \beta_2 Water\_Tet_i + \beta_3 Feed\_Tet_i + \beta_4 Feed\_Sulf_i + \beta_5 Inj\_Amino_i$$

$$\text{logit}(SSSr_i) = \beta_0 + \beta_1 BarnClass_i + \beta_2 Inj\_Amino_i + \beta_3 Inj\_Tet_i + \beta_4 Water\_Sulf_i$$

$$\text{logit}(STRr_i) = \beta_0 + \beta_1 Feed\_Tet_i + \beta_2 Inj\_Amino_i + \beta_3 Water\_Sulf_i + \beta_4 Inj\_Sulf_i$$

$$\text{logit}(TETr_i) = \beta_0 + \beta_1 Feed\_Tet_i + \beta_2 Water\_Pen_i$$

where logit is the link function and $AMPr_i$, $SSSr_i$, $STRr_i$, and $TETr_i$ is the mean probability of the i^{th} visit having an isolate resistant for AMP, SSS, STR, and TET, respectively.

Results from the BLR are presented in Tables 1-3. These include the coefficient estimate, p-value, OR and 95% CIs for odds ratios. No variables could be found to be statistically associated with TETr, hence results are not presented. Results from the BNA approach are presented in one single network in Fig. 1 and include relative risks for arcs between resistance variables and predictive variable of interest.

Table 1. Binomial logistic regression results for ampicillin resistance

Variable	Coeff. estimate	S.E.	p-value	OR (95% CI)[a]
Intercept	0.46	0.16	0.004	–
AIAO	0.48	0.22	0.027	1.61 (1.30 – 2.00)
Water_Tet	15.64	708.74	0.982	–
Feed_Tet	0.56	0.24	0.020	1.74 (1.37 – 2.21)
Feed_Sulf	-0.79	0.46	0.089	0.45 (0.29 – 0.72)
Inj_Amino	-1.55	1.17	0.182	–

[a] Odds Ratios were calculated for variables with p value < 0.10 only

Table 2. Binomial logistic regression results for sulfisoxazole resistance

Variable	Coeff. estimate	S.E.	p-value	OR (95% CI)[a]
Intercept	2.94	0.40	1.49E-13	–
BarnClass	-0.40	0.12	0.001	0.67 (0.59 – 0.76)
Inj_Amino	-2.16	1.06	0.042	0.12 (0.04 – 0.33)
Inj_Tet	-0.76	0.41	0.065	0.47 (0.31 – 0.71)
Water_Sulf	14.45	717.47	0.984	–

[a] Odds Ratios were calculated for variables with p value < 0.10 only

Table 3. Binomial logistic regression results for streptomycin resistance

Variable	Coeff. estimate	S.E.	p-value	OR (95% CI)[a]
Intercept	0.99	0.14	2.02E-12	–
Feed_Tet	0.85	0.26	0.001	2.35 (1.81 – 3.05)
Inj_Amino	-2.09	1.16	0.072	0.12 (0.04 – 0.40)
Water_Sulf	14.78	720.41	0.983	–
Inj_Sulf	-0.59	0.36	0.095	0.55 (0.39 – 0.79)

[a] Odds Ratios were calculated for variables with p value < 0.10 only

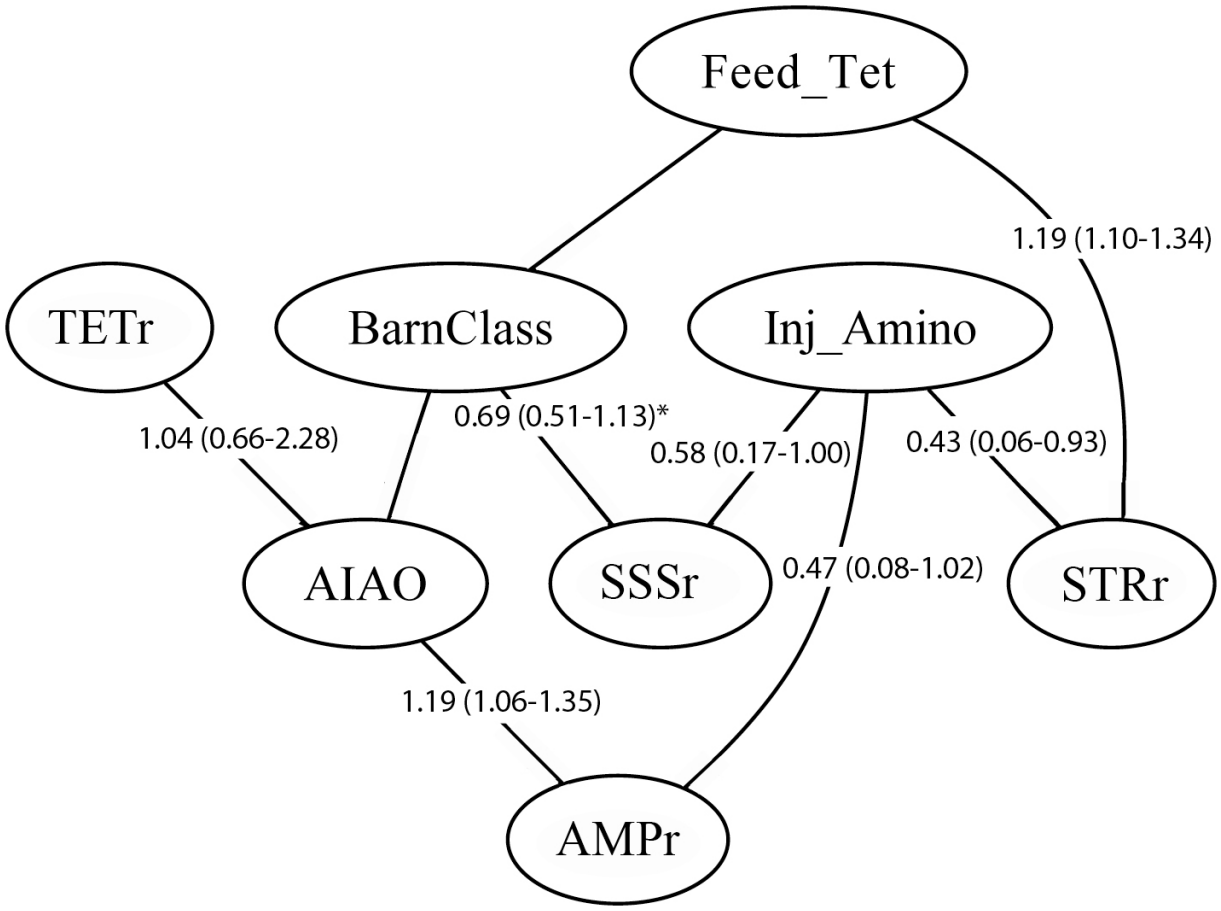

Fig.1 Final consensus network and 95% confidence intervals for relative risks obtained from a Bayesian graphical modelling approach. (* Relative risk of Barn capacity greater than 5000 pigs).

The risk of detecting AMP resistance was significantly higher in AIAO systems and when tetracycline was used in feed (Feed_Tet). In the BNA approach, AIAO was also directly associated with AMP resistance but the effect of using in-feed tetracycline on AMP resistance appeared to be mediated through the size of the barn and raising hogs in an all-in-all-out system. Regression results indicated both the barn capacity (BarnClass) and the use of injectable aminoglycosides to be statistically associated with a reduction of the risk of finding SSS resistance on the farm. In the case of BNA, the same two variables were found to be directly associated with SSS resistance. The use of injectable aminoglycosides and a high barn capacity (> 5000 pigs) were also associated with a lower risk of SSS resistance. Feeding tetracycline was found to be associated with a higher risk of finding STR resistance in both the BLR and the BNA approaches. Injection of aminoglycosides was also associated with a lower risk of STR resistance using both BLR (p= 0.07) and BNA (using consensus at the level of 51%). No significant risk could be identified for resistance to tetracycline by either method. Although there was an association found between tetracycline resistance and AIAO production by the BNA approach at the 51% consensus level, the 95% confidence intervals around the computed relative risk included 1.

DISCUSSION

The increased risk of STR and AMP resistance when tetracycline is used in feed could be explained by co-selection mechanisms (Akwar et al., 2008, Rosengren et al. 2009). On the other hand, the absence of significant association between the use of tetracycline in feed and TET resistance might be the result of the almost ubiquitous presence of tetracycline resistance in the herds included in the database (442 positives visits, 11 negative visits). Additionally, tetracycline is often used on successive lots and resistance may persist across at levels still detectable with the current sampling scheme. The observed impact of the injection of aminoglycosides is somewhat counterintuitive: results from both BLR and BNA indicated a protective effect on AMP, STR and SSS resistance, although the 95% CIs for the relative risk for AMP and SSS resistance computed after BNA included 1. In addition, in the BLR models, injectable TET use was negatively associated with SSS resistance and injectable sulphonamide use with STR resistance. Although it is typically expected that antimicrobial use selection pressure is positively associated with the occurrence of resistance this is not always the case, particularly when crossing antimicrobial classes. Similar results for the use of injectable trimethoprim-sulfonamide on ampicillin, chloramphenicol, sulfisoxazole and tetracycline resistance in nursery pigs, and of injectable oxytetracycline on streptomycin resistance in growers were reported in a study on 90 hog farms in the province of Alberta, Canada (Varga et al. 2009). Although the mechanism for a reduced risk when exposed to some antimicrobials through injection, most consistently aminoglycosides, could not be identified in this study, it could be an indication of missing variables. Herds using injectable antimicrobials might have different general husbandry procedures which in turn might have a positive impact on the reduction of antimicrobial resistance. These herds might also have reduced their quantitative use of drugs, which could in turn reduce selection pressure and antimicrobial resistance. In the current study, drug use information was only analysed qualitatively (using yes/no) and this might be an over simplification of the true ecological model. Among the participating farms, only 2 farms reported using injectable aminoglycosides (and only spectinomycin) in 2008 and one in 2007 (Government of Canada, 2009; Government of Canada 2010). Additionally, the apparent protective effect could be the result of a variety of molecular mechanisms including plasmid incompatibility, for example if injectable administration imparts a different selection pressure than in-feed or in-water use and that then results in selection for different or incompatible molecular mechanisms of resistance.

Consistent results from both methods provide more confidence in the robustness of the results. The BNA approach provides an explanation of the relationships between the predictors themselves, in addition to the relationships between predictors and the predicted variable (RA). BNAs are concerned with estimating relationships between multiple variables in a data set and allow for more than one response variable to be included in the model. In contrast, GLMs only consider the relationships between a single response (or dependent) variable and all other (independent) variables (covariates). More flexibility is needed when covariates are correlated and/or cannot be controlled and/or there are multiple "response" variables, which is where BNA approaches can be of significant added value. The relationship between the use of tetracycline in feed and AMP resistance is an example of such benefit of the BNA approach. Bayesian graphical modelling suggests the hypothesis that it is not a direct relationship but rather one that is mediated through other factors. This should be considered as a potential working hypothesis for future work.

Utilizing both regression analysis and BNA methodologies allows greater depth of exploration of the complexity of the AMR phenomenon compared to more traditional analyses.

One limit of the BNA is the difficulty of integrating hierarchical structure when exploring the data (for example the effect of herd). Adjustment for clustering, such as for example non-independence of disease status of animals on the same farm, random effects and/or over-dispersed distribution is theoretically possible in a BN but is not without significant technical and computational challenges! This is why both approaches should be considered as complementary rather than competing.

This study was a first step in exploring the benefits of BNA. Future work will potentially include using data at the isolate level to explore if this level of analysis is possible, as it would likely provide a better interpretation of cross-resistance or co-selection phenomena. Additional analysis might also include the use of quantitative measures of drug use and resistance, and exploring how data clustering could be taken into account in BNA.

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VARIANCE PARTITION COEFFICIENTS IN SAMPLE SIZE ESTIMATION

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SUMMARY

Disease cases are usually clustered within herds or in groups that share common characteristics. In the presence of clustering, sample size formulae must be adjusted for the within cluster correlation of the sampling units. Traditionally, the intra-cluster correlation coefficient (ICC), an average measure of the data heterogeneity, has been used in sample size adjustments. However, sample size estimates based on the ICC may not achieve the desired precision and power when applied to subgroups of clusters that exhibit greater or less than the average heterogeneity. In such instances, the use of the variance partition coefficient (VPC) is advocated because it measures the clustering of infection/disease for clusters with a common risk profile. Here, VPC-based methods for sample size estimation in prevalence estimation and substantiation of disease freedom surveys are proposed. An example is given for a risk factor study on *Mycobacterium avium* subsp. *paratuberculosis* infection in Danish dairy cattle.

INTRODUCTION

Disease is usually clustered within litters, pens, groups, herds (or generally, groups that share common characteristics) due to its contagious or point source nature (Carpenter, 2001) and/or the effect of managerial, environmental or nutritional factors. In the presence of disease clustering, individual sample size formulae must be inflated to adjust for the correlation of disease cases within the clusters. Traditionally, measures like the variance inflation factor (VIF) or comparable quantities (Campbell et al., 2001; Donner & Donald, 1988; McDermott et al., 1994), which are based on the intra-cluster correlation coefficient (ICC), have been used to modify sample size formulae. ICC is an average measure of clustering in the population under study. However, it would normally be expected that subgroups of clusters exposed to different risk factors have different heterogeneity patterns. Hence, sample size estimates based on the ICC may not be adequate for such subgroups with heterogeneity largely deviating from the average. For instance, a larger sample size may be required for clusters with excessive heterogeneity where ICC-based sample sizes may lead to an increased risk of type-I error. Contrarily, for subgroups of clusters with markedly less heterogeneity, a higher than needed and hence unnecessary allocation of resources occurs.

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There is an increasing need for a risk-based approach in surveillance due to the limitations in human and financial resource availability for disease surveillance and control measures (Stark et al., 2006). Thus, measures of clustering, which are specific to subgroups with a common heterogeneity pattern, should be preferred to ICC in sample size estimation in order to optimize resource allocation. The variance partition coefficient (VPC), which was initially introduced by Goldstein and co-workers (2002) quantifies the amount of clustering for individuals that share a combination of characteristics/covariates; the same covariate pattern/risk profile. The VPC estimate from an intercept-only model equals to the ICC estimate and is a measure of the heterogeneity in the whole population. Marked differences in VPCs dictate groups of clusters that exhibit markedly less or excessive heterogeneity compared to the average heterogeneity of the data (Browne et al., 2005). Such differences may be missed if the impact of the imperfect sensitivity (Se) and specificity (Sp) of disease diagnosis is ignored during VPC estimation (Kostoulas et al., 2009).

In this paper, the use of the VPC rather than the ICC for sample size estimation is advocated. Initially, a Bayesian method for VPC estimation is described, adjusting for the presence of differential test errors (i.e. Se and Sp depending on exposure status). Subsequently, methods for the use of the VPC in the estimation of sample size for (i) prevalence and (ii) disease freedom surveys are presented. Finally, an example application is given for a risk factor study on *Mycobacterium avium* subsp. *paratuberculosis* infection, in Danish dairy cattle.

MATERIALS AND METHODS

Variance partition coefficient estimation in binary data

A binary response model with two levels of hierarchy (e.g. i individuals clustered in j populations/herds) is considered. For a binary (0/1) response, the observed test outcome of the i^{th} individual, in the k^{th} factor level (e.g. age-specific categories that possess different diagnostic performance characteristics) in the j^{th} population/herd, y_{ikj}, can be assumed to follow a Bernoulli distribution with probability q_{ikj}, the probability of an observed positive test result:

$$y_{ikj} \sim Bernoulli(q_{ikj}) \quad (1)$$

$$q_{ikj} = p_{ikj}Se_k + (1 - p_{ikj})(1 - Sp_k) \quad (2)$$

where p_{ikj} denotes the probability that the ikj^{th} individual is diseased/infected, and Se_k and Sp_k are the sensitivity and specificity of the diagnostic process for category k individuals, respectively (Rogan & Gladen, 1978). A random effects logistic regression model for the probability of infection for the ikj^{th} individual can be fitted as follows:

$$\text{logit}(p_{ikj}) = X_{ikj}^{T}\beta + u_j \quad (3)$$

where X_{ikj}^{T} is a vector of known predictor variables (McCullagh & Nelder, 1989). To account for clustering within populations/herds normally distributed random effects u_j are included.

$$u_j \sim N(0, \sigma_u^2) \quad (4)$$

Prior information can be included for the regression coefficients ( ) of the covariate vector as proposed by Bedrick et al. (1996), while prior information on the sensitivity and specificity can be incorporated in the form of beta distributions, *Beta*(*a, b*).

$$Se_k \sim beta(a_{Se_k}, b_{Se_k}) \tag{5}$$

$$Sp_k \sim beta(a_{Sp_k}, b_{Sp_k}) \tag{6}$$

Subsequently, from the fitted model described in Eq. (1-6), VPCs are estimated using a predictive simulation approach (Browne et al., 2005; Kostoulas et al., 2009). Briefly, at every MCMC iteration, simulated values are drawn from the posterior predictive distribution of replicated data y_{ikj}^{rep}.

$$y_{ikj}^{rep} \sim Bernoulli(p_{ikj}^{rep}) \tag{7}$$

with

$$\text{logit}(p_{ikj}^{rep}) = X_{ikj}^{Trep}\beta + u_j \tag{8}$$

where $X_{ikj}^{Trep}\beta$ is the linear predictor for a selected covariate pattern (combination of covariates).

For each considered $X_{ikj}^{Trep}\beta$, the VPC is calculated as the fraction of the total variation that can be ascribed to the higher level of organization as follows:

1. From the fitted logistic mixed model simulate a large number (*M*) of sets of level-2 residuals (u_j), using the sample estimate of the variance σ_u^2.
2. For a particular X_{ikj}^{T*} □, compute the corresponding *M* values of p_{ikj}'s.
3. For each of these *M* values compute the level-1 variance $L1_{ikj} = p_{ikj*}$ (1- p_{ikj}). The VPC is now estimated as: $VPC = L2*(L2+L1)^{-1}$, with L2 = var(p_{ikj}) and L1 = $E(L1_{ikj})$ the mean of all the $L1_{ikj}$'s.

A detailed description and extensively explained WinBUGS codes for VPC estimation under posterior predictive simulation have been given recently (Kostoulas et al., 2009).

Sample size calculations for prevalence estimation surveys.

In the presence of clustering, sample size formulae for determining the required sample size for the detection of a minimum specified level of prevalence or difference between prevalences, must be inflated by VIF (Campbell et al., 2001; Donner & Donald, 1988; McDermott et al., 1994). Traditionally, VIF calculation is based on the ICC. It is suggested that the formula is modified to use covariate pattern-specific VPCs rather than the ICC in order to obtain sample size requirements that would be specific to the subgroups of clusters corresponding to the VPCs (VIF_{VPC}):

$$VIF_{VPC} = 1 + VPC(ms - 1) \tag{9}$$

where *ms* is the mean sample size within each cluster and VPC the covariate pattern specific VPC.

Sample size calculations for surveys to substantiate freedom of disease.

Sample size estimation to substantiate disease freedom, in a Bayesian framework, has previously been presented (Branscum et al., 2006). The authors modified the classical Beta-binomial model to allow for the probability ( ) that some herds are free of infection and for the probability ( ) that the entire region is free of infection. The notation of p_{ikj} is continued, representing the probability of individual *i* with covariate pattern *k* in herd/population *j* having the disease. Thus, following Eq. (1-2), it is allowed for the possibility of herds to be entirely free of disease by modeling p_{ikj}as:

$$p_{ikj} = \begin{cases} p^*_{ikj} & \text{with probability } \tau \\ 0 & \text{with probability } 1-\tau \end{cases} \quad (10)$$

where denotes the proportion of infected herds. The prevalences among the diseased clusters were modeled using a Beta distribution:

$$p^*_{ikj}|\mu,\psi \sim Beta(\mu\psi, \psi(1-\mu)) \quad (11)$$

where is the mean prevalence among all disease clusters in the region and is related to the variability of these prevalences with larger values of corresponding to less variable prevalences.

Finally the authors allowed for the possibility ( ) that the entire region is free of infection:

$$\tau = \begin{cases} \tau^* & \text{with probability } \gamma \\ 0 & \text{with probability } 1-\gamma \end{cases} \quad (12)$$

Subsequently, for sample size estimation, future survey data are generated under this model, assuming =0, for a given sample size of *m* herds with *n* animals sampled within each herd. That is:

$$y_{ikj} \sim Bernoulli(1 - Sp_k) \quad (13)$$

Generated data are then analyzed under the model, which incorporates prior information on the , , and . The predictive probability that =0, at a pre-specified level of confidence (usually 95%) is calculated. This procedure is repeated for different combinations of *m* and *n*, to determine the required number of herds and within herd sample size to effectively substantiate disease freedom at the specified confidence level. Branscum et al. (2006) extensively described the computational details of this approach.

Implicitly, this simulation approach assesses the required *m* and *n* to substantiate disease freedom when testing against the hypothesis that, if disease is present, it would have a distribution pattern explicitly described by the priors set on , , and . Therefore, the selected priors on and quantify the belief regarding the distribution of infection within and between infected herds. The utilization of covariate pattern specific VPCs and predicted risk of infection/disease is proposed for prior derivation on and . In this way, the required number

of clusters to be sampled and the within cluster sample size take into account the expected mean prevalence of infection and its variability between clusters, had the infection been present.

To do this the formal relationship between measures of clustering and the within- and between-herd prevalence of infection (Branscum et al., 2005) have been modified, by replacing the ICC with pattern-specific VPCs, to accommodate estimation of sample sizes specific to the corresponding sub-populations of clusters. Specifically for :

$$\psi = \frac{(1-VPC)(\mu\tau-1)}{VPC(\mu\tau-1)+\mu(1-\tau)} \quad (14)$$

which for =1 simplifies to:

$$\psi = \frac{VPC-1}{VPC} \quad (15)$$

A prior on can be directly obtained from the fitted logistic regression model and is equal to the predicted covariate pattern specific mean p_{ikj}^{rep} as calculated by Eq. (8). Detailed WinBUGS codes and R-functions for the implementation of the abovementioned models are available from the first author upon request.

Goodness of fit tests and model convergence

The fit of the model (Eq. 1-6) to the data was assessed as has been previously described (Kostoulas et al., 2009). For MCMC a combination of diagnostics plus visual inspection of the trace plots and summary statistics is recommended (Best et al., 2003). In all models, use of standard diagnostic procedures (Heidelberger & Welch, 1983; Raftery & Lewis, 1992; Gelman & Rubin 1992) and visual inspection of the autocorrelation plots and the posterior distributions of the parameters revealed no convergence problems. For the predictive model used in sample size estimation to substantiate disease freedom, convergence diagnostics were checked for a random sample of simulated data sets, as proposed by Branscum et al. (2006).

Statistical software

All models were run in the freeware program WinBUGS (Spiegelhalter et al., 2003) through R (http://www.r-project.org/) with the utilization of the R2WinBUGS R-package (Sturtz et al., 2005).

Application of the proposed models in sample size estimation for MAP infection in Danish dairy cattle.

Individual animal records on the MAP antibody milk ELISA status and age were retrieved from the Danish Cattle Database for 64,945 animals in 633 herds. Colostrum and milk feeding practices for these herds have also been recorded (Table 1). A thorough descriptive analysis of test responses by feeding practices are given by Nielsen and co-workers (2008).

The model described in Eq. 1-6 was used to assess the association between milk and colostrum feeding practices and the risk of MAP infection, adjusting for the differential (i.e. depending on the age) Se and Sp of the diagnostic process.

The Se of the milk ELISA for paratuberculosis is age dependent (Nielsen & Toft, 2006). Hence, herds with different age distribution are expected to have different overall Se and Sp.

Each herd was split into two groups; the set of animals ≤ 3 years old and the set of animals > 3 years old. Thus, the model and subsequent VPC estimation adjusted for the varying with age Se and Sp of the milk-ELISA.

Specifically, for cattle ≤ 3years old there was confidence that the most probable value for the Se was 0.15 and that it could not be less than 0.06; this translates to a *Beta* (3.04, 2.56) prior. For Sp the most probable value was set to 0.99 and it was considered certain that it was not less than 0.98, which translates to a *Beta* (560.72, 6.65) prior. Corresponding values for cattle > 3years were for Se an expectation of around 0.50 and a minimum of 0.35, resulting in a *Beta* (14.59,14.59) prior and for Sp an expectation of around 0.98 and a minimum of 0.95, resulting in a *Beta* (151.77, 4.08) prior.

From the fitted model, VPCs and corresponding predicted risks of MAP infection were obtained. Subsequently, covariate-pattern-specific VIF_{VPCs} were calculated as proposed in Eq. 9. The VIF based on the ICC of the whole data was also calculated for comparison.

Furthermore, we estimated, via the predictive simulation, the required number of herds (*m*) to substantiate freedom from MAP infection for subgroups of herds with a covariate pattern that exhibited markedly less or greater heterogeneity (dictated by the VPC). In all simulations it was assumed that a constant number of *n*=50 animals was sampled within each of the *m* herds. The covariate-pattern-specific VPC and predicted risk of MAP infection estimates were used to derive priors on the minimum expected prevalence ( ) and its variability ( ). The prior on was based on the mean predicted risk for each considered covariate pattern according to Eq. (8); priors on ( ) were derived from Eq. (15) rather than Eq. (14) because all estimates were based on a sample of herds known to be infected with MAP, thus, =1. ICC-based sampling requirements were also estimated for comparisons.

RESULTS

Medians and 95% credible intervals of the predicted risk of MAP infection and the corresponding VPCs, adjusting for the differential Se and Sp of the milk ELISA, are in Table 1. Estimated VIF_{VPCs} under the fitted model are in Table 2. The required number of herds to be sampled for each subgroup of herds with a common covariate pattern is in Table 3. Both colostrum and milk feeding practices were associated with the risk of MAP infection. Herds feeding colostrum to calves only from their own dam and/or using milk replacer possessed the lower risk of MAP infection and were of the least heterogeneous (had the lowest VPCs). The highest risk of MAP infection (and VPC) was in herds feeding colostrum from multiple cows and/or allowing suckling from foster cows.

Table 1. Estimated VPCs and predicted risk of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection for sub-groups of herds that share common characteristics – common covariate pattern, with 95% credible intervals. Estimations were based on models that adjusted for the age-varying diagnostic accuracy of the milk-ELISA for MAP

Intercept-only model			VPC	Risk
			0.114 (0.071; 0.171)	0.177 (0.132; 0.221)
Covariate patterns under the fitted model				
Source of colostrum	Milk source for heifer calves	Age		
Own dam	Milk powder	≤3yrs	0.080 (0.042; 0.139)	0.081 (0.055; 0.125)
		>3yrs	0.124 (0.078; 0.182)	0.203 (0.096; 0.392)
	Milk from cows with high SCC[a]	≤3yrs	0.094 (0.052; 0.155)	0.109 (0.060; 0.205)
		>3yrs	0.135 (0.090; 0.191)	0.261 (0.103; 0.537)
	Milk powder + milk from cows with high SCC	≤3yrs	0.113 (0.062; 0.178)	0.162 (0.066; 0.359)
		>3yrs	0.146 (0.098; 0.200)	0.357 (0.114; 0.716)
Multiple dams	Milk powder	≤3yrs	0.093 (0.051; 0.155)	0.109 (0.059; 0.203)
		>3yrs	0.135 (0.090; 0.191)	0.259 (0.102; 0.534)
	Milk from cows with high SCC	≤3yrs	0.107 (0.062; 0.169)	0.144 (0.063; 0.313)
		>3yrs	0.143 (0.098; 0.199)	0.327 (0.109; 0.673)
	Milk powder + milk from cows with high SCC	≤3yrs	0.125 (0.073; 0.188)	0.209 (0.070; 0.498)
		>3yrs	0.149 (0.104; 0.201)	0.432 (0.121; 0.817)

[a] SCC = somatic cell count

Table 2. Covariate-pattern-specific variance inflation factors (VIF_{VPCs}), assuming a mean sample size within each herd (*ms*) of 100 animals. VIF from an intercept only model that ignored the covariate pattern specific heterogeneity is also given for comparisons.

Intercept-only model			VIF
			12.286
Covariate patterns under the fitted model			VIF_{VPCs}
Source of colostrum	Milk source for heifer calves	Age	
Own dam	Milk powder	≤3yrs	3.871
		>3yrs	10.702
	Milk from cows with high SCC [a]	≤3yrs	4.663
		>3yrs	12.979
	Milk powder + milk from cows with high SCC	≤3yrs	6.742
		>3yrs	19.018
Multiple dams	Milk powder	≤3yrs	4.564
		>3yrs	12.880
	Milk from cows with high SCC	≤3yrs	5.554
		>3yrs	15.751
	Milk powder + milk from cows with high SCC	≤3yrs	8.128
		>3yrs	22.780

[a] SCC = somatic cell count

Table 3. Estimated number of herds (k) required to substantiate freedom of MAP infection for selected covariate patterns that exhibit markedly greater or less heterogeneity. A within herd sample of 50 animals was assumed in all estimations. Priors on the minimum expected prevalence ( ) were based on the mean predicted risk of MAP infection for each considered covariate pattern. Priors on the variability of the within herd prevalence ( ) were based on the corresponding VPC estimates. Estimated m from an intercept only model that ignored the covariate pattern specific heterogeneity is also given for comparisons

					m
Intercept-only model			0.177	7.772	110
Covariate patterns under the fitted model					
Source of colostrum for calves	Milk source for heifer calves	Age			
Own dam	Milk powder	≤3yrs	0.081	11.500	120
		>3yrs	0.203	7.065	
	Milk powder and milk from cows with high somatic cell count	≤3yrs	0.162	7.850	60
		>3yrs	0.357	5.849	
Multiple dams	Milk powder and milk from cows with high somatic cell count	≤3yrs	0.209	7.000	40
		>3yrs	0.432	5.711	

DISCUSSION

For clustered data, sample sizes must be adjusted to account for the correlation of the sampling units within the clusters. Here, an integrated approach is proposed based on a modification of the formula derived for a Beta binomial model (Branscum et al., 2006), which utilizes covariate-specific VPC estimates rather than the ICC in sample size estimation. VPCs are specific to subgroups of clusters of a common risk profile and, hence, optimize the balance between resource allocation and sample size demands for groups of clusters that exhibit markedly different heterogeneity. The latter is crucial due to the lessening of available resources and the subsequent increased demand for a better, risk-based resource allocation (Stark et al., 2006). Thus, it is advocated that sample size estimation, which is based on an average measure of heterogeneity like the ICC, may not be appropriate for clusters with excessively greater or less than the average heterogeneity. In these instances, sample size adjustments based on the ICC will lead to under- and over-estimation of the required sample sizes, respectively. In the latter case, unnecessary resource allocation occurs, at the required significance level and power, while in the former, a potential of increased type-I errors, beyond the minimum acceptable limit, exists.

Indeed, the estimated VIF_{VPCs} in the example (Table 2) for subgroups of clusters with a specific covariate pattern were markedly different than the VIF estimate based on the ICC (intercept-only model). Hence, for prevalence estimation surveys, VPC-specific sample size adjustments proved more efficient compared to the traditional utilization of the ICC. Accordingly, the required number of herds (m) to substantiate freedom from disease varied between subgroups of herds with different heterogeneity (Table 3). Specifically, a larger number of herds was required to prove disease freedom in the subgroups of herds that were feeding colostrum from their own dam and milk powder, while a smaller sample of herds was required from the subgroup of herds that were feeding colostrums from multiple dams and subsequently

sustained heifer calves on both milk powder and milk from cows with high somatic cell counts. These estimations used the predicted risk of MAP infection and the corresponding VPC (Table 1) to derive priors on the belief about the expected mean within herd prevalence of MAP infection ( ) and its variability ( ) among infected herds. Essentially, under the predictive simulation approach of Branscum et al. (2006), the specified priors on , , and , implicitly express the perceived risk of infection for the whole area, the proportion of the infected herds and the between and within infected herds dispersion of infection, had the infection been present. Hence, the model simulates how many data points (*m* and *n*) from an actually disease/infection free population are required to prove disease/infection freedom when testing versus a disease/infection distribution pattern described by the abovementioned prior specifications. These, explicitly fall within the basic principle of the minimum expected prevalence used to calculate sample size for disease freedom surveys. The concept of minimum expected prevalence is either based on biological grounds, like in the example, and quantifies the expected spread of disease due to its contagiousness or on the grounds that below a threshold value, it is small enough to be considered negligible or not of major concern or, for infectious diseases, to move towards extinction without intervention due to non-sustainable transmission rates (Cameron & Baldock, 1998).

VPC estimates and subsequent sample size calculations under models that ignored test errors are not presented here because the severe impact of ignoring misclassification rates has been recently demonstrated (Kostoulas et al., 2009). An analogous effect on the ICC has also been described (Branscum et al., 2005). However, previous work (Kostoulas et al., 2009) has been extended, here, to adjust for the differential misclassification rate on a specific exposure, which in the example is the different age group. Taking into account the presence of differential misclassification is crucial because a certain trend does not exist for differential errors; they can result in either over- or under-estimation of the parameters. Contrarily, non–differential misclassification (i.e. Se and Sp invariant to exposure status) always leads to the underestimation of parameters (McInturff et al., 2004). Hence, it is wrong, to use an average Se and Sp estimate for the whole data and falsely treat differential misclassification rates as invariant to exposure status. A trend differing from reality can be observed in the parameter estimates if an average non-differential Se and Sp estimate is falsely used in the estimation process.

Furthermore, comparison between the VPC of the intercept-only model (i.e. ICC) and the covariate-pattern-specific VPC's from the fitted model provided information about the portion of the heterogeneity among clusters that was explained by the inclusion of significant herd-level covariates. In the example, much of the heterogeneity in the risk of MAP infection in Danish dairy cattle remained unexplained despite the inclusion of significant herd–level predictors in the fitted model. This indicates that additional, unmeasured or immeasurable, factors exist – which operate at the herd level – that were not accounted for, thus contributing to the unexplained between herd heterogeneity of MAP infection (Browne et al., 2005; Kostoulas et al., 2009).

The use of covariate-pattern-specific VPCs rather than ICC in sample size estimation from clustered data is demonstrated. For greatly heterogeneous data, partitioning of clusters within subgroups that share a common disease/infection risk profile and subsequent VPC-specific sample size estimation would better resource allocation to achieve the required precision and power.

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DYNAMIC MODELLING

COUPLING TICK POPULATION AND PARASITE INFECTION DYNAMICS IN ORDER TO TEST SCENARIOS TO CONTROL BOVINE BABESIOSIS

T. HOCH·, J. GOEBEL, A. AGOULON AND L. MALANDRIN

SUMMARY

Tick-borne diseases can be harmful to humans and animals. As regards animal health, bovine babesiosis is a recurrent threat to cattle herds. Investigating babesiosis spread and testing control measures represents therefore an important issue which is addressed here through a modelling approach. Modelling tick-borne disease requires coupling a tick (*Ixodes ricinus*) population dynamics with a pathogen (here the protozoan *Babesia divergens*) spread model. Based on a previously developed model for the former purpose, this study focuses on the model that describes the pathogen spread in a dairy herd through the relevant processes. Simulated prevalences in ticks and cattle indicate that the model allows a realistic representation of the pathogen spread. Based on simulation findings, increasing deer densities generates a marked increase in prevalence whereas increasing cattle stocking rate has a small opposite effect. The simulated regular use of acaricides results in a strong reduction in pathogen prevalence.

INTRODUCTION

Tick-borne diseases represent a growing concern, notably as a result of changes in land use and climate. Ticks are vectors of numerous pathogens: viruses (e.g. virus responsible for tick-borne encephalitis), bacteria (e.g. *Borrelia burgdorferi*, agent of Lyme disease) and protozoa (*Babesia divergens*, agent of bovine babesiosis). *B. divergens* is transmitted by the tick *Ixodes ricinus*, which is the most common tick species in Europe. Bovine babesiosis is often asymptomatic but has a widespread occurrence with a high level of seroprevalence in dairy herds of European countries (see review of Zintl et al., 2003). Clinical cases are rare (0.4% incidence in France, according to L'Hostis and Seegers, 2002; 1.7% incidence in Ireland, according to Gray and Harte, 1985). Furthermore, *B. divergens* is also a zoonotic agent and can cause damage to splenectomised humans (Gray, 2006). Numerous recent studies have been devoted to this protozoan (see Hunfeld et al., 2008) but little is known about the effect of potential control measures (for instance the use of acaricides) on the course of the infection in dairy herds.

Modelling is an appropriate tool to test such effects. After having integrated current knowledge into a mathematical model, simulations of different scenarios can lead to relevant conclusions about the effectiveness of various control measures. In the case of tick-borne diseases, models require the coupling between a tick population dynamics model and a model

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that represents the spread of the pathogen. No such model is at the moment available to describe and simulate the spread of the infection of *B. divergens* in a dairy herd.

The objectives of this paper are to describe the model developed for this infection and thereafter to use the model to test the effects of (*i*) factors such as host densities and (*ii*) acaricides on simulated prevalence in dairy herds.

THE MODEL

As stated above, a model for the spread of a tick-borne pathogen requires the development of two models that have to be linked: (1) a tick population dynamics model and (2) a model for the spread of *B. divergens*. The former has already been published (Hoch et al., 2010) and will be only briefly described whereas the main focus will be on the latter.

Tick population dynamics model (see Hoch et al., 2010 for details)

The model for *I. ricinus* population dynamics is a discrete time deterministic model with a one-day time step. It considers four stages in tick development: egg, larva, nymph and adult. For larva, nymph and adult stages, three phases are taken into account: in development (or pre-oviposition for adult females), questing and feeding on host. The model consists therefore of 10 variables. The processes involved are the following: egg laying, hatching, development, host questing, drop-off from host and mortality during all phases. The maximal number of eggs laid per adult female varies according to the season and is higher in spring than in autumn. This maximal number is multiplied by a density-dependent fecundity rate that decreases when the number of ticks per host increases. Hatching and development are temperature-dependent processes. A diapause has been considered for individuals that have engorged after the end of the summer and for which development will take place at the next spring. Host questing is also a density-dependent process: the basal probability for a tick to find a host increases with host density. This probability is multiplied by a temperature-dependent factor by which low or high temperatures prevent questing. We assumed that larvae and nymphs fed mostly, but not exclusively on small mammals (SM), whereas adults only fed on large mammals (LM, i.e. deer and cattle). Mortality differs according to the stage. Mortality rate during development is constant for a given stage but decreases from egg to the adult stage. Mortality rate during questing is constant for all stages but varies according to the season considered with higher mortality rates during the summer period. Mortality during feeding increases with the number of ticks per host and varies also between stages and whether the considered host belongs to SM or LM. The following equation set for the nymph stage gives an example of the equation system used for this model:

$$\begin{cases} N_Q(\mathrm{t}+1) = N_Q(\mathrm{t}) + L_{D\,k>1}(\mathrm{t}) - \left(h_N.f_T + \mu_Q\right) N_Q(\mathrm{t}) \\ N_F^j(\mathrm{t}+1) = N_F^j(\mathrm{t}) + h_N^j.f_T.N_Q(\mathrm{t}) - \left(\gamma_N + \mu_{N_F}^j\right) N_F^j(\mathrm{t}) \\ N_D(\mathrm{t}+1) = N_D(\mathrm{t}) + \gamma_N.N_F(\mathrm{t}) - N_{D\,k>1}(\mathrm{t}) - \mu_{N_D}.N_D(\mathrm{t}) \end{cases} \quad (1)$$

where L and N represent larvae and nymphs (expressed in number of individuals per 100 m^2), respectively, and D, Q and F the development, the questing and the feeding phases, respectively. $L_{D_{k>1}}(t)$ represents the larvae that have achieved their development. μ is the mortality rate and the drop-off rate. $h_N^j.f_T$ is the product of basal host finding probability with

temperature effect on questing. Superscript *j* represents the type of host (*j=SM* or *LM* for small or large mammals, respectively).

The relevance of the model was assessed first by analyzing qualitative outputs of the model (see Hoch et al., 2010). For instance, survival rates (around 12 and 20% from larva to nymph, and from nymph to adult, respectively) and life cycle duration (between two and three years) were consistent with current knowledge. Second, we compared questing nymph densities with literature data and showed that the model generates realistic simulations of the overall tick dynamics.

This model has been applied to a heterogeneous area in order to represent different connected habitats. We considered a woodland connected with a meadow through an ecotone at the interface. This theoretical landscape consists of two squares (100 m x 100 m) for woodland and meadow, assuming that ecotones are a 2 m band inside the meadow. These habitats represent different biotopes between which host densities and tick mortality rates vary. Connections between habitats are realized through the migration of wild hosts (small mammals, deer). For instance, the equation for migration acts on nymph density in the woodland as follows:

$$N^j_{Fw}(t+1) = N^j_{Fw}(t) + h^j_N.f_T.N_{Qw}(t) - (\gamma_N + \mu^j_{N_F}).N^j_{Fw}(t) + M^j_{ew}.N^j_{Fe}(t) - M^j_{we}.N^j_{Fw}(t) \quad (2)$$

where *M* represents migration rate (in d^{-1}), the subscripts *w* and *e* refer to woodland and ecotone (and *m* for meadow), and the superscript *j* represents the type of host.

We assumed continuous mixing of cattle between ecotone and meadow. Therefore, at each time step, the number of ticks feeding on cattle in the ecotone and the meadow are summed and then distributed according to the respective areas of both habitats.

This spatialized model is called multi-habitat model.

Pathogen spread model

Models for *Babesia* sp. spread have been already developed but none of them was dealing with *Babesia divergens*. Smith (1983, 1991) developed a simulation model for the spread of *B. bovis* and its transmission by the vector *Boophilus microplus*. Randolph (1995) dealt with the parameters involved in the transmission of *B. microti* by the tick *I. trianguliceps*. Although pathogens differ, concepts, equations and parameters applied to these species could be useful in the present study.

The main features of the model are described on Fig. 1 flow chart. Ticks and cattle are considered either susceptible or infectious and the dynamics of *B. divergens* are not explicitly accounted for. To facilitate interpretation, tick population dynamics are not represented in this figure, but infection status (susceptible or infectious) is taken into account for each of the ten variables involved in the dynamics. The spread model involves therefore 22 variables (20 for ticks and 2 for cattle). We considered that infection by the protozoan does not impact tick population dynamics. Among vertebrates, only bovines are assumed to become infected with *B. divergens* and infectious. No infection for this protozoan has been reported in rodents, and even though deer have been identified as hosts of *B. divergens*–like species, the babesias involved are probably not conspecific with this cattle parasite (Gray et al., 2010).

The processes considered in the present model are dealing either with the transmission and the acquisition or with the loss of the pathogen.

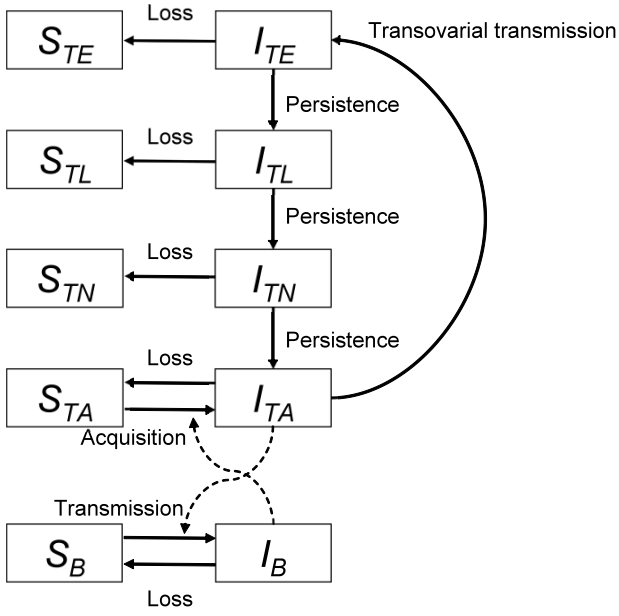

Fig. 1 Flow chart of the pathogen spread model. *I* and *S* represent susceptible and infectious individuals, respectively. *T* and *B* subscripts are for ticks and bovines, respectively and *L*, *N* and *A* represent larval, nymphal and adult stages for ticks, respectively.

Transmission and acquisition of the pathogen: The transmission process describes the flow of bovine individuals from the susceptible to the infectious status. Owing to longer feeding durations and much larger blood meal, we considered adult ticks as only transmitting the protozoa and thus assumed a negligible role for larva and nymphs. The daily probability for a bovine to become infected is calculated through the following equation:

$$P_T = 1 - e^{-\beta_T \frac{I_{T_F A}}{B}} \tag{3}$$

where $_T$ represents the transmission rate which is multiplied by the number of infected adults feeding on a bovine. This number is obtained by the ratio $\frac{I_{T_F A}}{B}$, where *B* corresponds to the stocking rate.

The acquisition process refers to the flow of ticks from the susceptible to the infectious state. Similar as for transmission, only adult ticks are taken into account as it is the only stage able to acquire *B. divergens* (Donnelly and Peirce, 1975). The probability for a tick to acquire the protozoa is expressed as follows:

$$P_A = 1 - e^{-\beta_A \frac{I_B}{B}} \tag{4}$$

where $_A$ represents the acquisition rate and I_B the density of infected bovine.

Loss of the pathogen: We assumed that individuals lose the pathogens through two processes: first, a continuous elimination or death of the parasite in its hosts with time, second,

through a discontinuous loss in ticks which determines transovarial transmission and transstadial persistence at time of laying, and hatching or moulting, respectively.

In the former case, a daily probability of loss is applied for ticks and bovine. Considering an exponential distribution for the survival duration of the pathogen in an individual, for instance for ticks (subscript *T*) this probability is calculated as follows:

$$P_{LT} = e^{-\frac{1}{\gamma_T}} \quad (5)$$

where γ_T represents the survival duration of the protozoan in a tick, with γ_B equivalent for bovines.

As for the discontinuous loss, the efficiency of transovarial transmission of the pathogen between tick adult female and eggs is not maximal and this was expressed by a probability for adult females to infect eggs. Similarly we also modulate the efficiency of the transstadial persistence between the different tick stages by a factor.

Parameters

Parameters for the tick population dynamics model are the same as those used in Hoch et al. (2010). The spread model has 8 parameters: transmission and acquisition rates, survival duration of the pathogen in tick and bovine, which correspond to continuous loss terms and four parameters for transovarial and transstadial transmission, with three transitions considered in the latter case.

According to Randolph (1995), the between stage transmission of *B. microti* by *I. trianguliceps* is high and a value of 0.9 for the probability of transovarial and transstadial transmission was therefore chosen. We considered a value of 300 days for the survival duration of the protozoa in ticks and cattle. The value for cattle is derived from experimental studies (Malandrin et al., 2004) whereas the duration in ticks allows the parasite to survive one year in some individuals (Donnelly and Peirce, 1975). Transmission and acquisition rates were calibrated using knowledge and literature data. High transmission rates are reported (Randolph, 1995) but they referred to the transmission of *B. microti* between *I. trianguliceps* and voles and may not be relevant for our study. To our knowledge (Becker, 2009), acquisition of the pathogen by ticks seems a much more efficient process than transmission. Values of 6.10^{-4} and 1 d^{-1} were chosen for β_T and β_A, respectively.

The multi-habitat model applied to a heterogeneous area was used for the tick population dynamics in order to simulate realistic tick per host evolution. As in the original model, rodent densities are 60, 90 and 30 individuals per ha (ind.ha^{-1}) in woodland, ecotones and meadow, respectively. Deer density is constant among habitats and equals 0.1 ind.ha^{-1}. A value of 1.3 bovine.ha^{-1} in the meadow and the ecotone, which is consistent with observations in extensive dairy farming systems, has been assumed for the stocking rate.

Simulations

The herd size is constant in the model and neither renewal nor culling was considered. Ten year simulations were performed so that results for the last year were not influenced by initial conditions. The infection was initiated by considering a prevalence of 30% in the dairy herd at

time 0. A theoretical temperature cycle was modeled with values of 10°C for the mean and the amplitude which roughly correspond to a temperate climate.

Results from the simulations were compared with data from the literature concerning the number of ticks per host, the prevalence of *B. divergens* in cattle. Thereafter, the model was used to test several scenarios that could change the course of the infection. First, we performed simulations in order to assess the effect of variations in host densities. The influence of cattle stocking rate was assessed by testing values of 1.2 and 1.4 bovine.ha^{-1}. Moreover, there is a growing concern for a potential increase in deer densities owing to land use changes and decreased hunting pressure. The test of the consequences of a doubling in deer density (from 0.1 to 0.2 ind.ha^{-1}) on *B. divergens* prevalence in cattle was thus carried out. We considered two cases: (1) the cattle stocking rate remains unchanged, (2) the total LM density is kept constant and stocking rate equals 1.2 bovine.ha^{-1} for a doubled deer density.

The model was also used to test the influence of acaricide treatment on *B. divergens* prevalence in cattle. Acaricides are widely used in cattle herds. This test was performed by considering a single treatment occurring at the beginning of June on each bovine. The efficiency of acaricides, *i.e.* the effect on tick survival, was considered optimal at the beginning, i.e. 90%, but we assumed that this efficiency was decreasing with time through a logistic equation:

$$E(t) = E_0 . \left(1 - \frac{1}{1 + e^{-\alpha . (t - hl_E)}} \right) \qquad (6)$$

E_0 represents initial efficiency level, hl_E corresponds to the time when initial efficiency is halved and is a parameter determining the inflexion of the curve. Figure 2 depicts the evolution of the efficiency level considered in the model. This evolution is consistent with our knowledge on the initial efficiency level and the efficient action duration of commercial acaricides (Anonymous).

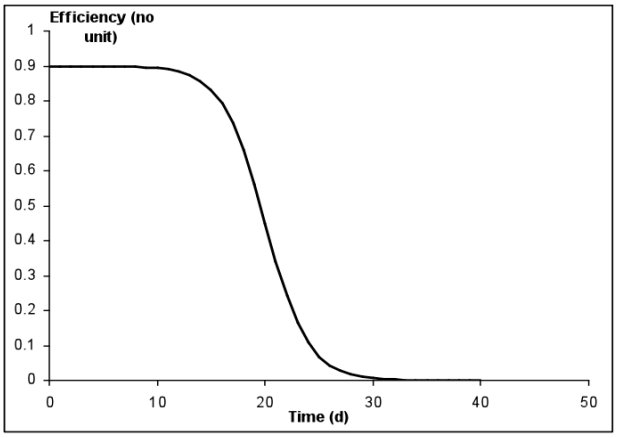

Fig. 2 Evolution of acaricide efficiency from the time of application onwards.

The model was developed and run using SciLab© (http://www.scilab.org) software.

RESULTS

Dynamics of ticks per host

Figure 3 shows the yearly evolution of adult tick numbers on cattle. The dynamics are consistent with the simulated evolution of questing nymphs (see Hoch et al., 2010), with a spring peak simulated at the beginning of June. The number of ticks per cattle remains at a high level until the beginning of October before it declines. A small autumn increase is simulated in relation with a simultaneous increase in questing tick densities in temperate areas. Data based on an exhaustive collection of ticks on cattle are scarce. L'Hostis et al. (1994) reported an average adult tick burden of approximately 15 individuals. Data collection shows high variability since lower (Gern and Brossard, 1986) or higher (Evans, 1951) densities may be encountered. Simulated values can be considered consistent with these observations.

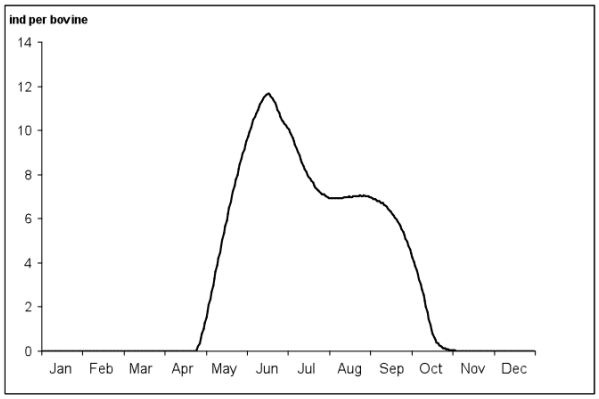

Fig. 3 Evolution of the number of adult ticks per bovine.

Evolution of *B. divergens* prevalence in cattle

The prevalence in cattle shows variation on an annual basis (Fig. 4). Minimum values are simulated in winter. Maximum prevalence occurs in summer, after the spring peak in tick density, on account of the combination between high values in tick density and prevalence in questing ticks. Taylor et al. (1982) and Devos and Geysen (2004) reported seroprevalence average values of 31.8% and 20% for *B. divergens* prevalence in cattle, respectively. The maximum simulated values fall well within this range. This simulation (thick line in Fig. 4) will be referred to as reference scenario.

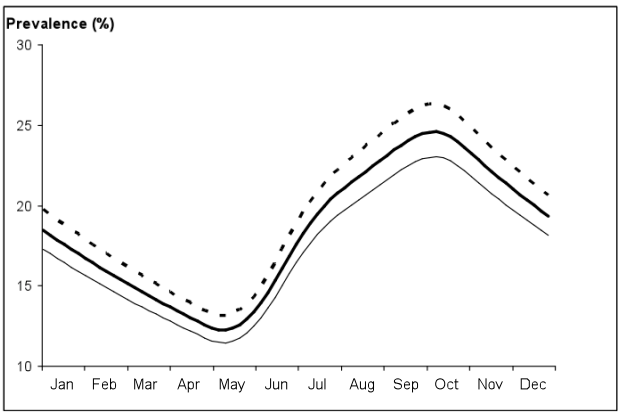

Fig. 4 Evolution of *Babesia divergens* prevalence (in %) in cattle for different values of the cattle stocking rate (in bovine.ha^{-1}): 1.3 (thick line), 1.2 (dotted line) and 1.4 (thin line).

Using the model to test scenarios

Interestingly, decreasing stocking rate generates an increase in *B. divergens* prevalence in cattle (Fig. 4). An increase in stocking rate has the opposite effect.

A doubling in deer density generates a symmetric increase in *B. divergens* prevalence in cattle, which suggests a strong influence of these hosts on infection dynamics (Fig. 5). This effect is almost the same whether we assume that variation in deer density does not affect stocking rate or a constant density for Large Mammals. In this latter case, a slight increase in prevalence is even simulated, in line with the influence of the stocking rate.

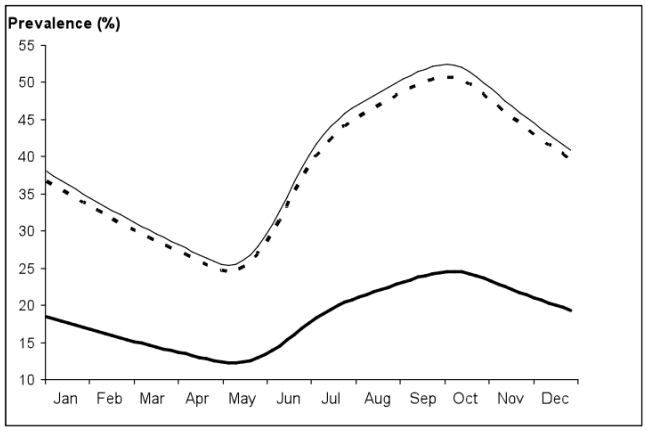

Fig. 5 Influence of deer density on *Babesia divergens* prevalence (in %) in cattle: nominal curve (thick line), with a doubled deer density (from 0.1 to 0.2 individual per ha) and either no change (1.3 bovine.ha^{-1}, dotted line) or a value of 1.2 bovine.ha^{-1} for the cattle stocking rate (thin line).

The use of acaricides has the opposite influence on *B. divergens* prevalence in cattle since results show a halving in prevalence throughout the simulation (Fig. 6), with no difference in annual variation. However, the protozoan is still spreading within the herd after a ten-year simulation.

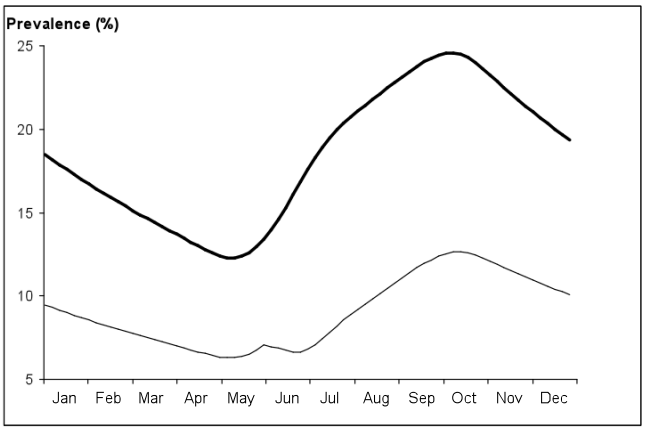

Fig. 6 Influence of acaricide use on *Babesia divergens* prevalence (in %) in cattle: nominal curve (thick line) and with a use of acaricide (thin line).

DISCUSSION

This article presents the first model of *B. divergens* spread in a dairy herd. The model is based on existing knowledge on the parasite dynamics. The coupling between a vector population dynamics model and a pathogen spread model leads to an increase in model complexity, linking ecological and epidemiological processes. This model allows a realistic simulation of both tick and parasite dynamics. Even though large variation of situations is reflected in the literature, results from the model are consistent with existing data, whether they relate to tick population dynamics or *B. divergens* prevalence.

Whether increasing non-competent host density generates a decrease (dilution effect) or an increase (amplification effect) in tick-borne disease prevalence represents a major issue. Ogden and Tsao (2009) showed, using a model for Lyme disease, that dilution or amplification could occur depending on competition between hosts, host contact rate with ticks and acquired host resistance to ticks. Our model suggests that increasing deer density would lead to a drastic increase in *B. divergens* prevalence. The influence of deer density on tick-borne disease upsurge has already been reported by Rizzoli et al. (2009) for tick-borne encephalitis. In our simulation study, the amplification effect occurs whatever the assumed type of competition, i.e. either without competition, where deer density does not depend on the stocking rate, or if we assume a constant density for large mammals, mimicking between host competition for the habitat. *B. divergens* prevalence in cattle increases with deer density as a result of an increase in tick density and consequently in the density of infected ticks, namely the acarological risk.

The amplification effect, due to deer density, on *B. divergens* prevalence is even greater when the total LM density is assumed to be constant. In this case, cattle density is reduced and *B. divergens* prevalence increases on account of an increased number of ticks per host and because cattle density has little effect on tick density (Hoch et al., 2010). More generally, cattle density has the opposite effect on prevalence compared with deer density. However, the number of infected individuals, obtained by multiplying prevalence in cattle by their density, is a more relevant variable for quantifying pathogen spread: it increases very slightly with the stocking rate (0.298 vs 0.294 animal per ha are infected yearly, for respective values of 1.3 and 1.2 animal per ha for the stocking rate).

The use of acaricides generates a strong reduction in *B. divergens* prevalence in cattle, yet only reduces the spread of the pathogen. Moreover, with a doubling in deer density, the use of acaricides could not prevent an increase in *B. divergens* prevalence, compared with the reference scenario (results not shown). The model suggests therefore that a potential increase in wild animal densities would counteract the effect of acaricides.

In addition to the first modelling study applied to *B. divergens* spread, the model described here provides a framework to be applied to other tick-borne pathogens. Even though for other pathogens different parameter values for transmission, acquisition and persistence should be used, such a model would generate new knowledge about other tick-borne diseases, like Lyme borreliosis.

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KEY SHEDDING FEATURES OF *COXIELLA BURNETII* IN CATTLE AND IMPLICATIONS FOR WITHIN-HERD CONTROL

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SUMMARY

The control of *C. burnetii* in livestock is crucial for limiting both the economic consequences of the infection and the zoonotic risk. Identifying key factors of the infection dynamics is a precondition for implementing efficient control strategies. A model representing the spread of *C. burnetii* within a dairy cattle herd taking into account the heterogeneity of shedding was built, and the key parameters whose variation highly influences the infection dynamics were identified through a sensitivity analysis. These key parameters were the levels of shedding and the characteristics of the bacterium in the environment. To illustrate the implications of such a study for control, the effectiveness of a vaccination programme was explored by simulation. Contrary to a vaccination programme not impacting the shedding levels, a vaccination programme with a vaccine preventing high levels of shedding was very effective, even when the probability of infection for vaccinated animals was assumed to be high.

INTRODUCTION

Q fever is a worldwide zoonosis due to *Coxiella burnetii*. This intracellular bacterium infects a wide range of animals. In ruminants, reproductive disorders such as abortions or metritis are frequent signs of infection (Marrie, 1990) and can impact production and economic efficiency of the farm. Besides, human infections occur mainly after inhalation of aerosols generated from excreta of infected livestock (parturition products, faeces, urine, milk – Angelakis & Raoult, 2010). This was recently experienced in the Netherlands where more than 3,000 human cases were reported since 2007 (Van der Hoek et al. 2010). Q fever is then an issue in both public and animal health. The control of this infection in ruminants is therefore crucial for limiting the production losses in livestock as well as the zoonotic risk.

In *C. burnetii* infections, a large heterogeneity between shedders has been described (Arricau-Bouvery et al., 2003; Guatteo et al., 2006; Rodolakis et al., 2007): the shedding duration, level (i.e. the quantities of bacteria shed) and routes are variable between animals. Infected animals can indeed shed bacteria through birth products, urine, vaginal mucus, faeces, and milk (Rodolakis, 2008). Amongst the three latter, no predominant route was identified in 242 dairy cows from 31 herds in which abortions due to *C. burnetii* were reported (Guatteo et al., 2006). Besides, in the same study, only 35% of the shedder cows shed by several routes at

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the same time. The shedding duration and levels are also variable between animals: cows can shed from a sporadic to a persistent way and the concentrations of bacteria shed in vaginal mucus or milk can vary from less than 100 bacteria/g to more than 1,000,000 b/g (Guatteo et al., 2007). Moreover, the shedding can be intermittent (Rodolakis et al., 2007). The intrinsic heterogeneous characteristics of populations of individuals (e.g. variability in age, contact structure, infectiousness, etc…) are known to affect infection dynamics in many diseases. As an example, a model assuming that all farms and all animals are governed by the same underlying dynamics was unable to explain the highly overdispersed distribution of prevalences of *Escherichia coli O157* shedding on Scottish farms (Matthews et al., 2006). The best fit to the prevalence data was obtained when incorporating individual variability in transmission. For *C. burnetii* infections, the influence of this heterogeneity of shedding on the infection spread has not been evaluated yet. However, the identification of the mechanisms influencing the infection process the most is a precondition to a comprehensive description of the infection dynamics and is required to propose effective control strategies specifically targeting these key mechanisms.

The spread of *C. burnetii* within a herd is a complex process: the infection risk is linked to the contamination of the environment, therefore to bacterial shedding and survival in the environment. As shedding routes are various and viable bacteria in the environment can not be easily quantified, the monitoring of the infection spread on the field is difficult. The objective of this study was then to build a dynamic model representing the spread of *C. burnetii* within a dairy cattle herd, taking into account the heterogeneity of shedding and to identify the key parameters whose variation highly influences the infection dynamics, through a sensitivity analysis. In order to illustrate the worth of such a study for defining potentially effective control measures, we simulated the infection spread during a vaccination programme, assuming (i) that the vaccine impacts parameters identified as most influential, and (ii) that the vaccine does not influence those key parameters.

MATERIALS AND METHODS

Dynamic model

Based on the available knowledge concerning the clinical and epidemiological aspects of Q fever and on a previous work (Courcoul et al., 2010), an epidemic model describing *C. burnetii* spread within a dairy cattle herd was built. It is a more sophisticated variant of the SIR (Susceptible-Infectious-Removed) basic model: as *C. burnetii* spread within cattle herds is a complex process, the general SIR framework had to be modified accordingly. In field data (Guatteo et al., 2006), different shedding routes and ranges of shedding levels were observed among animals. To take into account this variability and the real epidemiological contribution of each shedder, three types of shedders with different shedding routes and levels distributions were distinguished: shedders without antibodies, shedders with antibodies and milk persistent shedders (this latter are shedders with antibodies which always shed in milk, for a longer period and at higher levels than other shedders with antibodies). For each of these three types of shedders represented in the model, three different shedding route categories (shedding in (i) milk only, (ii) vaginal mucus and/or faeces, (iii) milk and vaginal mucus and/or faeces) were considered. Shedders without antibodies were assumed to have the possibility to clear the infection and to return to an apparently susceptible health state. The shedding was assumed intermittent: transitions in both directions between shedders with antibodies and non-shedders with antibodies were allowed. The antibodies were assumed to have long but limited life duration: non-shedders with antibodies had a chance to lose their antibodies. As Q fever is an

airborne disease, the probability of infection was linked to the environmental bacterial load. This load was assumed to depend on the quantity of bacteria shed by shedders and reaching the environment as well as on the mortality rate of the bacterium in the environment. For a shedder, the quantity of bacteria shed and reaching the environment was assumed to be function of (i) the shedding level (three categories of shedding levels were represented: low, moderate and high level shedding) and of (ii) the impact of the shedding on the environment. Shedding in milk was indeed assumed to have a lesser impact on the environmental bacterial load than shedding in vaginal mucus or faeces, (i.e. a lower proportion of the bacteria shed in milk was supposed to arrive into the environment of the herd, because most of the milk is directly sent to the bulk tank).

To summarize, as shown in Fig. 1, each cow was in one of the six mutually exclusive health states at a given time: *S* (susceptible, non-shedder without antibodies), *I-* (shedder without antibodies), *I+* (shedder with antibodies), $I^{+\ milk\ pers}$ (milk persistent shedders), *C+* (non-shedder with antibodies), *C-* (non-shedder without antibodies which was infected and had antibodies in the past). All shedding cows *I* were subdivided according to their shedding routes: (1) I_1, milk only, (2) I_2, vaginal mucus and/or faeces, (3) I_3, both. The probability of infection *p* (transition probability from *S* to *I-*) was expressed at each time step as $1 - \exp^{(-E_t)}$ where E_t is the quantity of bacteria in the environment of the herd at time *t*.

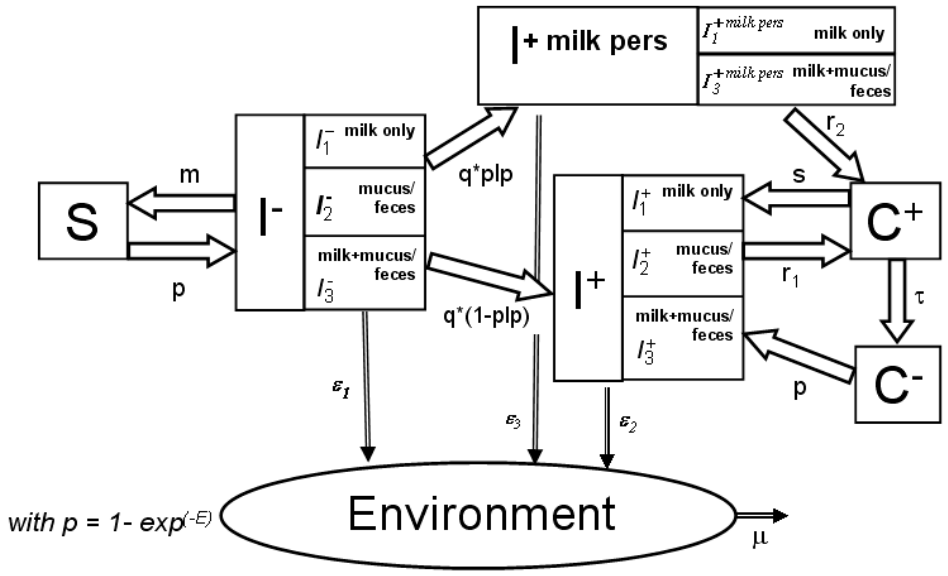

Fig. 1 Flow diagram describing the modelled spread of *C. burnetii* within a cattle herd. *p*, *m*, *q*, *s*, r_1, r_2 and τ are parameters of transition between health states. *pIp* is the proportion of cows going from *I-* to *I+* and becoming $I^{+\ milk\ pers}$. $_1$, $_2$ and $_3$ are the quantities of bacteria shed during a time step by an individual *I-*, *I+* and $I^{+\ milk\ pers}$ respectively and contaminating the environment. These quantities are the sum of all quantities of bacteria shed by the shedder through all the shedding routes, times ρ the fraction of bacteria shed reaching the environment of the herd. μ is the mortality rate of the bacterium in the environment.

This epidemic model was coupled with a model of herd demography. Only cows (neither heifer nor calf) were represented in the model. For each animal, the stage of lactation, stage of gestation, lactation number, health state towards *C. burnetii* infection, shedding route(s) and

shedding level(s) (if the cow is shedding) were accounted for in the model. Abortions due to Q fever and culling were also considered. The model was a stochastic individual-based model using discrete time with a time step of one week.

Sensitivity analysis

A sensitivity analysis was conducted to identify the parameters that contributed most to the output variability. Various scenarios were run, each of them being characterized by a specific combination of parameter values, in order to relate the variability obtained for the outputs to that induced by the input parameters. Four outputs were considered: *(i)* the environmental bacterial load, *(ii)* the prevalence of milk shedders, *(iii)* the prevalence of mucus/faeces shedders, *(iv)* the prevalence of persistent milk shedders. All these outputs were computed weekly over a 5-year period. Parameters related to the herd demography were fixed since demography and herd management processes are considered as well understood. The sensitivity of the model outputs was evaluated for the 19 epidemiological parameters. A fractional factorial experiment design of resolution V (allowing exploration of main effects and two-factor interactions) was used, with four parameter values (called levels) per factor relating to the shedding and two levels for the other parameters. As the model is stochastic, it was run 30 times for each combination of factor levels.

In order to compare the influence of the factors on the temporal outputs, a method developed by Lamboni et al. (2009) was applied to the mean of the 30 repetitions of each scenario. This method allows simultaneously analyzing correlated variables (here the successive time points of a given output). It consists of two main steps. First, a principal component analysis (PCA) was performed in order to provide orthogonal component variables (or principal components) of the model output variables (here the weekly values of a model output for a given scenario) explaining maximum high proportion of inertia (i.e. variability) between scenarios. Only the first three principal components (PC) were kept since they represented most variability (more than 99% for all outputs) amongst simulations. The PCA generated for each scenario a score for each component. The second step involved an ANOVA, including the main effects and the two-factor interactions for all factors and carried out on the scores of each of the principal components considered. Sensitivity indices (SI), corresponding to the main effect or to interactions, and total sensitivities (TS), corresponding to the sum of the main effect and the interactions, were calculated for each factor and for each component.

Simulation of a vaccination of both heifers and cows for 10 years specifically targeting or not the most influential parameters

In order to illustrate the worth of such a sensitivity analysis for defining potentially effective control measures, the effectiveness of a vaccination programme with a vaccine influencing the most influential parameters, i.e. the shedding levels, or not, was simulated.

When incorporating vaccination in the model, different assumptions were made: all the animals were assumed seropositive but 2 types of I and C were still represented: I- and I+ became I_1 and I_2, respectively, (the first had the possibility to clear the infection, the second did not), C+ and C- became C_1 and C_2, respectively (a difference in the cellular immunity between these 2 health states was assumed). Based on Guatteo et al. (2008), the vaccine was assumed to be effective only when applied to non-pregnant uninfected individuals. Thus, in the epidemic model, non-pregnant S and C_2 individuals become partly protected when vaccinated and move to the 'vaccinated in an effective way' (Ve) states. For pregnant S and C_2 when vaccinated, as well

as for all I_1, I_2 and C_1, nothing changed. The susceptible *Ve* animals (*SVe*) had a decreased probability of becoming infected *IVe* (i.e. a decrease in susceptibility to infection). Besides, the *Ve* cows were assumed not to abort. Except for these differences, the *Ve* animals could evolve through the same health states with identical transition rates as the non *Ve* animals.

The temporal dynamics of the shedders' prevalence and environmental bacterial load were simulated for two different scenarios: considering a decrease of the shedding level (and thus of the infectiousness) for the *Ve* cows or not. When a decrease of infectiousness was assumed, no *Ve* animal could be a high level shedder. When no decrease of infectiousness was assumed, the *Ve* animals had the same shedding level distributions as the non *Ve* ones. These simulations were performed considering 3 levels of susceptibility (i.e. 3 values for the probability of transition from *SVe* to I_1Ve). The whole herd (heifers and cows) was assumed to be vaccinated for 10 years after the occurrence of three abortions due to Q fever within the herd. 100 runs were performed.

RESULTS

Identification of the parameters having strongest influence on model outputs

Since the first PC explained more than 90% of the global variability for each of the model outputs, only these results are presented. For the mean environmental bacterial load, the factors *Q1* (probability distribution of shedding levels for all *I-* and for some *I+* shedding in mucus/faeces), μ (mortality rate of *C. burnetii*) and ρ^{mf} (proportion of bacteria shed through mucus/faeces reaching the environment compartment) were the most influential ones. For the mean prevalences of mucus/faeces (Fig. 2) and milk shedders, the most sensitive factors were *q* (transition probability from *I-* to *I+*), *s* (transition probability from *C+* to *I+* representing the intermittency of shedding) and *Q1*, whereas the mean prevalence of milk shedders in a persistent way was mostly impacted by *pIp* (proportion of cows changing from *I-* to *I+* and becoming $I+^{milk\ pers}$), *q* and r_2 (the transition probability from $I+^{milk\ pers}$ to *C+*). Globally, the most sensitive two-factor interactions (with a SI higher than 5%) were $Q1{:}\mu$, $\rho^{mf}{:}Q1$, $\rho^{mf}{:}\mu$ on the variability of the environmental bacterial load and $q{:}pIp$, $q{:}r_2$, $pIp{:}r_2$ on the variability of the prevalence of persistent milk shedders ($I+^{milk\ pers}$).

Simulation of a vaccination programme with a vaccine specifically targeting the shedding levels of vaccinated cows or not

Consistent with the results of the sensitivity analysis (i.e. shedding levels were identified as being most influential parameters), a vaccination programme with a vaccine preventing high level shedding is effective in reducing both shedders' prevalence and environmental bacterial load (Fig. 3), regardless of the susceptibility level of the vaccinated animals. If the vaccine does not influence the infectiousness of effectively vaccinated cows, the vaccination programme is effective only when their susceptibility is strongly reduced compared with non-vaccinated cows.

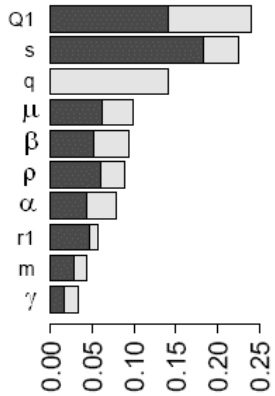

Fig. 2 Sensitivity analysis for mean prevalence of mucus/faeces shedders over time: results of the ANOVA performed for the first component (inertia: 93.9%). Loadings defining the principal component for each time variable (in abscissa) and total sensitivities for the 10 most influential factors ranked in descending order. Sensitivities are split into main effects (black) and two-factor interactions (grey). *Q1* is the probability distribution of the shedding levels for all the *I-* and for some *I+* shedding in mucus/faeces, s, q, r_1 and m are transition parameters (see Fig.1), μ is the mortality rate of the bacterium in the environment, α, β and γ are shedding route distributions for respectively *I-*, *I+* and $I^{+\,milk\,pers}$ and ρ is the proportion of bacteria shed through mucus/faeces reaching the environment.

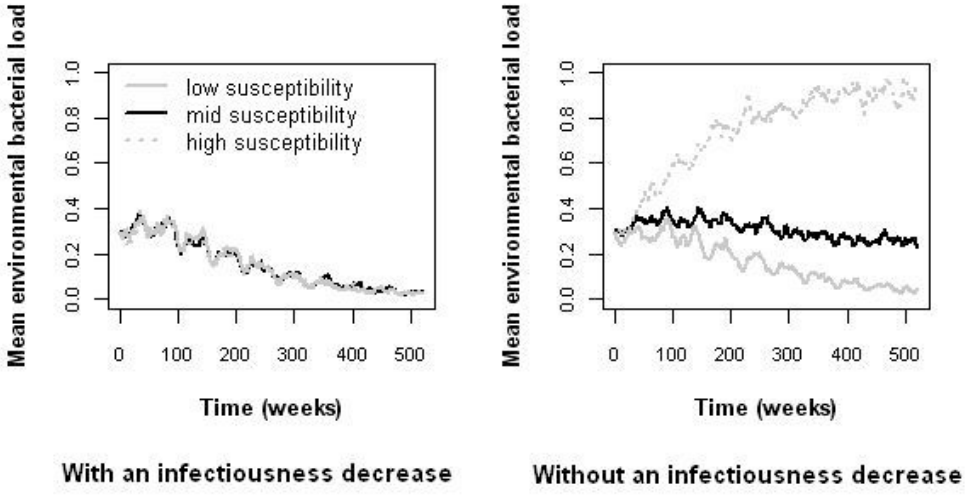

Fig. 3 Dynamics of the mean environmental bacterial load with a decrease in infectiousness (no high level shedding for *Ve* animals) or without such a infectiousness decrease (same shedding level distributions for *Ve* and non *Ve* animals) for different levels of susceptibility. The low, mid and high susceptibility corresponds to an infection probability for susceptible effectively vaccinated (*SVe)* animals equal to 5%, 21% and 90% of the infection probability for susceptible non vaccinated animals, respectively. These values are based on Guatteo *et al.* 2008.

DISCUSSION

The aim of this study was to build a dynamic model representing the spread of *C. burnetii* within a dairy cattle herd, to identify through a sensitivity analysis the parameters whose variation strongly influences the infection dynamics, ,and to explore the effectiveness of

vaccination targeting these key mechanisms. The individual variability of the shedding routes, levels and duration was taken into account as heterogeneity in a population was expected to have an impact on infection dynamics. According to the results of this study, representing the shedding routes and levels in the model seemed worthy as shedding levels were identified as highly influential parameters.

The most influential parameters were indeed associated with the probabilities governing the levels of shedding, especially for mucus/faeces shedders and to the characteristics of the bacterium in the environment. Some physiological parameters relating to the intermittency of shedding or to the transition from one type of shedder to another also played a non-negligible role. These parameters have to be precisely assessed to improve the model accuracy and the understanding of the infection spread. Besides, interventions impacting those key parameters would be of great interest. As an example, the parameter s (transition probability from $C+$ to $I+$) is linked to the intermittency of shedding and it is biologically plausible to assume that a control measure such as vaccination could decrease this transition probability and then have an impact on the prevalence of shedders. In the same way, r_2 (transition probability from $I^{+milk\ pers}$ to $C+$), which rules the shedding duration of persistent milk shedders, could probably be modulated by control strategies. According to Astobiza et al. (2009), an oxytetracycline treatment would not limit the duration of bacterial excretion in a dairy sheep flock; however further studies are needed in order to determine if vaccination could decrease the duration of the shedding period. It seems also possible to influence μ, the mortality rate of the bacterium in the environment, by implementing environmental control measures such as increased cleaning of the farm.

To illustrate the worth of the identification of the most influential parameters to define effective control measures, the prevalence of shedders and environmental bacterial load were monitored when implementing a vaccination programme (i) with a vaccine targeting the infectiousness (i.e. the shedding levels) of vaccinated animals, and (ii) with a vaccine which does not target the infectiousness of vaccinated animals. Consistent with the results of the sensitivity analysis, a vaccination programme with a vaccine preventing high levels of shedding was effective at decreasing the prevalence of shedders and environmental bacterial load, independent of the susceptibility level of vaccinated animals. For a vaccine which does not impact the shedding levels, the reduction in the susceptibility has to be high for the vaccination programme to be effective. This finding has also been described by Lu et al. (2009) who showed that, to reduce Salmonella prevalence in the long term, highly effective vaccines lowering infectiousness would be a better choice than highly effective vaccines reducing susceptibility. Therefore, control measures should aim at reducing the proportion of high shedders in mucus/faeces in *C. burnetii* infections, such as phase I vaccines seem to do. In Rousset et al. (2009), the vaccine was effective at reducing massive bacterial shedding from a heavily infected goat herd. Similarly, Hogerwerf et al. (in press) found that in uterine fluid, vaginal mucus and milk of vaccinated dairy goats, both the prevalence of shedders as well as the concentration of bacteria were reduced. However, further studies are needed to determine if the decrease of infectiousness is a realistic assumption for all vaccinated animals or only for the effectively vaccinated ones and to quantify this decrease for all shedding routes.

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USING SIMULATION TO ESTIMATE THE POWER OF A BADGER VACCINE TRIAL

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SUMMARY

The aim of this study was to estimate the power of a badger vaccine field trial using simulation techniques. The effects of sample size, sensitivity and specificity of the diagnostic test, transmission rate between unvaccinated badgers, Vaccine Efficacy for Susceptibility (VE_S) and Vaccine Efficacy for Infectiousness (VE_I) on study power were determined.

The most striking result was the large effect of the specificity of the diagnostic test on study power. Sample size had a small effect on power. Study power increased with increasing transmission rate between non-vaccinated badgers. Changes in VE_S had a higher impact on power than changes in VE_I.

In summary, study power in group randomized trials depends not only on sample size but on many other parameters. In the current vaccine trial, power was highly dependent on the specificity of the diagnostic test. Therefore, it is critical that the diagnostic test used in the badger vaccine trial is optimized to maximise test specificity.

INTRODUCTION

Badgers *(Meles meles)* are an important reservoir of *Mycobacterium bovis* for cattle in Ireland and the United Kingdom and as a result, eradication of bovine tuberculosis (bTB) becomes likely impossible without measures to prevent cattle-to-badger transmission (More, 2009). In a recent Irish study, 36% of badgers were found to be infected with bTB (Murphy et al., 2010). In 2001, Ireland initiated a 10-year work programme investigating the use of Bacillus Calmette-Guerin (BCG) vaccine in badgers as a medium-long term strategy to assist with national bTB control and eradication (Corner et al., 2007; Lesellier et al., 2009). Based on a series of initial experimental studies in captive badgers, BCG vaccination in badgers was associated with a reduction in both the number and size of gross histological lesions (Corner et al., 2008[·], Corner et al., 2010). These pen-based studies are currently being extended, with the design and implementation of a field trial in Ireland to evaluate vaccine efficacy in wild badger populations (Aznar et al., 2011).

In traditional vaccine field trials, individuals are randomly allocated (individual randomization) to either a vaccine or a placebo treatment and the relative risk of acquiring infection is determined by comparing infection rates in vaccinated and non-vaccinated individuals. This design is appropriate for non-communicable diseases because the probability that an individual will become infected only depends on their susceptibility. Individual

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randomized trials allow the estimation of vaccine effects that reduce the susceptibility of an individual to infection or VE_S (Vaccine Efficacy for Susceptibility). This is also known as the direct effect of vaccination (Halloran et al., 1999). When dealing with infectious diseases, however, the likelihood that an individual will become infected depends not only on its susceptibility but also on the infectivity of surrounding individuals. The reduction in infectivity achieved by vaccination is known as Vaccine Efficacy for Infectiousness, (VE_I) and is the result of the indirect effects of the vaccination in vaccinated and non vaccinated individuals. With infectious diseases, group randomized trials are the design of choice, allowing estimates of both the reduction in susceptibility (VE_S) and infectiveness (VE_I) (Riggs and Koopman, 2005), with herd immunity being the most important indirect effect. In a field trial to evaluate BCG vaccine efficacy in badgers, Aznar et al. (2011) outlined the use of group randomization to provide estimates of both VE_S and VE_I based on incidence data from three badger populations vaccinated with BCG at different levels of vaccination coverage: 100%, 50% and 0%. VE_I can be estimated using this design by varying the proportion of susceptible individuals across populations (Longini et al., 1998). In the badger vaccine trial, estimates of VE_I may be particularly important, given the reported reduction in gross histological lesions (and, potentially, reduced infectiousness) in vaccinated badgers (Hayes et al., 2000, Corner et al., 2008). Aznar et al. (2011) have previously outlined how the combined effects of VE_S and VE_I can be summarized using the Basic Reproduction Ratio (R_0) estimated as a function of vaccination coverage (R(p)).

It is essential to estimate study power to determine whether a vaccine field trial design is sufficient to detect a difference in outcome of a particular size or larger between the vaccinated and non-vaccinated group (Riggs and Koopman, 2004). As outlined by Charvat et al. (2009), power calculations based on the comparison of two independent binomials in which indirect effects are not taken into account, can largely overestimate study power. The same study refers to these calculations as 'naive'. In recognition of this concern, there have been recent changes both to trial design and to methodologies that are used to estimate sample size and power in these studies. In group randomized trials, where direct and indirect vaccine effects are each important, power depends on a range of factors. Riggs and Koopman (2004, 2005) looked at some of these factors including unit size (size of the groups), contact rate, external force of infection and infection duration. Computer simulation techniques are now frequently used to address study power estimation (Joines et al., 2000; Walters, 2004).

The aim of this paper is to estimate the power of a group randomized badger vaccine field trial designed to assess the effect of vaccination on *M. bovis* transmission in badgers using simulation techniques. The vaccine trial started in September 2009 and it will run for four years. The effects of sample size, sensitivity and specificity of the diagnostic test, transmission rate between badgers prior to the start of the trial, VE_S and VE_I on study power are determined. Although sample size was determined prior to the start of the study based on logistical issues without potential for further expansion, power calculations are still relevant as other parameters affecting power, such as Se and Sp of the diagnostic test, could potentially be adjusted to optimize study power.

MATERIALS AND METHODS

Vaccine trial design

The vaccine trial area comprised about 750 square kilometres and it was divided into three zones North to South: A, B and C with similar characteristics in terms of size, number of main badger setts, cattle herds, cattle and land classification type. Three vaccination levels: 100%, 50% and 0% were allocated to zones A, B and C in a way that a gradient of vaccination coverage North to South was achieved. The vaccination trial started in September 2009 and three trappings have been already carried out in the trial area. The middle zone (Zone B) has been vaccinated at 50% coverage, while Zones A and C were randomly allocated to a 100% and 0% vaccination coverage. Vaccination within Zone B has and will be done randomly at an individual level (Aznar et al., 2011). Badgers have been trapped twice a year since the beginning of the trial. The first time badgers are trapped, they are allocated to a vaccine /placebo treatment. The treatment will be repeated on a yearly basis and the trial will run for four years since the start in 2009.

The model

The total number of expected newly infected vaccinated and non-vaccinated badgers (E(C)) at the end of every time interval (Δt or time between two subsequent trappings) were simulated using the cumulative binomial distribution with parameters (s, p), where s is the total number of susceptible badgers caught in each of the trial zones at the beginning of each time interval Δt, and p is the probability that each of these badgers will become infected during that time interval. E(C) was simulated by drawing a random number (between 0 and 1) from a uniform distribution and next, using the random number, s and p, the smallest integer E(C) is determined such that the binomial cumulative distribution function evaluated at E(C) is equal to or exceeds that random number (this is implemented as the BIN.INV function in Excel 2010 and as the CRITBINOM function in earlier versions). This procedure assures a random draw from a binomial distribution with parameters s and p. The probability of each of the badgers becoming infected (p) was dependent on the transmission parameter β, the fraction of infected vaccinated badgers (Fv) and the prevalence (Prev) of infection in the area where each badger was located during each time interval (Aznar et al., 2011).

Model parameterisation was determined based on expert opinion and/or data available at the time of model construction (see Table 1), as follows:

- Three hundred badgers were used in the simulation model including 120, 60 and 120 in zones A, B and C, respectively, based on the figures obtained during the first trapping exercise (120, 64 and 115 in zones A, B and C, respectively).
- The percentage of badgers re-trapped was set at 70%. No data was available at the time of the start of the study on re-trapping as only one trapping exercise had been carried out in the vaccine trial area. The figure of 70% was based on expert opinion based on previous trappings in other areas.
- The initial prevalence for the three trial zones was set at 30%. In zone A, where no vaccination will be implemented, the prevalence was assumed to remain constant until the end of the trial. In zone B, where 50% vaccination will occur, prevalence was expected to reduce to 20% at the 7^{th} and 8^{th} trapping. In zone C, where we assumed all animals were vaccinated, prevalence was set to 20% at the second trapping, and to 10% and 5% at the fourth and seventh trappings, respectively.

- The fraction of susceptible and infected badgers vaccinated was set to 0% in zone A. In zone B, this fraction was increased from 30% to 40% at the 5th trapping. In zone C, this fraction was increased from 60% to 70% in the 5th trapping, and then to 80% in the 6th and subsequent trappings.

Based on the simulated dataset and using the methodology described by Aznar et al. (2011) to estimate the four transmission parameters, β_{UU}, β_{VV}, β_{UV} and β_{VU}, the transmission rate between non-vaccinated badgers was estimated to be β_{UU}=0.1.

The simulation process

The badger vaccine trial was designed to run for four years with two trapping exercises per year. Therefore, a dataset of 42 records was generated (7 subsequent trappings x 3 trial zones x 2 vaccine status), comprising the total number of newly infected vaccinated and non-vaccinated badgers in each of the three trial zones at the end of each subsequent trapping. The final dataset was read into SAS (SAS 9.1, SAS Institute Inc., 2005) and a simulation process was set up by means of a macro. We performed 1,000 simulations for each set of conditions (see following section). In each simulation, a new random number was drawn and a t-test was used to test for the null hypothesis of the transmission parameters between vaccinated (β_{VV}) and non-vaccinated badgers (β_{UU}) being equal. The power of the study was then estimated as the fraction of simulations in which the null hypothesis was rejected at a 0.05 level of significance.

The scenarios tested

Se and Sp of the diagnostic test: The power of the study was estimated for all combinations of sensitivity (Se) between 0 and 100%, and specificity (Sp) between 98 and 100% at increments of 5% and 0.2%, respectively, assuming an initial transmission between non-vaccinated badgers of β_{UU}=0.1 and a vaccine effect on susceptibility (VE_S) and infectivity (VE_I) of 80%. The same simulations were repeated assuming VE_S and VE_I were each 40%.

Sample size: The study sample size was determined by both the expected number of trapped badgers and by re-trapping percentages. We explored the effects of sample size on study power by modifying both parameters so that the total number of re-trapped badgers varied from 10% to 100% of the expected number of badgers (120/60/120). We assumed an initial transmission between non-vaccinated badgers of β_{UU}=0.1(this is the figure obtained based on the simulated data), a Se of 40% and a Sp of 99.9%. Vaccine effect on susceptibility (VE_S) and infectivity (VE_I) was set at 80% (we assumed that vaccination conferred protection against the infection to 80% of the vaccinated badgers and infectiousness of vaccinated badgers was reduced by 80%). The same simulations were repeated assuming VE_S and VE_I equal to 40%. To investigate the decrease in power observed for larger samples sizes, the same simulations were run assuming a perfect test with 100% Se and 100% Sp.

Initial transmission: We estimated power using a fixed Se and Sp of 40% and 99.9%, respectively, for vaccine effects (VE_I and VE_S) of 80% and 40%, and for β_{UU} values of 0.3, 0.2, 0.1, 0.005 and 0.025. We aimed to see the effect of variations in the β_{UU}=0.1 obtained from the simulated data on study power.

Table 1. Table showing simulated dataset consisting on: trapping exercise number, zone of the vaccine trial, fraction of susceptible vaccinated badgers (fs), fraction of infected vaccinated badgers (fi) and prevalence used during the model simulations.

Trapping	Zone	Vaccine Status	fs*	fi**	Prevalence
2	A	0	0	0	0.3
3	A	0	0	0	0.3
4	A	0	0	0	0.3
5	A	0	0	0	0.3
6	A	0	0	0	0.3
7	A	0	0	0	0.3
8	A	0	0	0	0.3
2	B	0	0.3	0.3	0.3
3	B	0	0.3	0.3	0.3
4	B	0	0.3	0.3	0.3
5	B	0	0.4	0.4	0.3
6	B	0	0.4	0.4	0.3
7	B	0	0.4	0.4	0.2
8	B	0	0.4	0.4	0.2
2	C	0	0.6	0.6	0.2
3	C	0	0.6	0.6	0.2
4	C	0	0.6	0.6	0.1
5	C	0	0.7	0.7	0.1
6	C	0	0.8	0.8	0.1
7	C	0	0.8	0.8	0.05
8	C	0	0.8	0.8	0.05
2	A	1	0	0	0.3
3	A	1	0	0	0.3
4	A	1	0	0	0.3
5	A	1	0	0	0.3
6	A	1	0	0	0.3
7	A	1	0	0	0.3
8	A	1	0	0	0.3
2	B	1	0.3	0.3	0.3
3	B	1	0.3	0.3	0.3
4	B	1	0.3	0.3	0.3
5	B	1	0.4	0.4	0.3
6	B	1	0.4	0.4	0.3
7	B	1	0.4	0.4	0.2
8	B	1	0.4	0.4	0.2
2	C	1	0.6	0.6	0.2
3	C	1	0.6	0.6	0.2
4	C	1	0.6	0.6	0.1
5	C	1	0.7	0.7	0.1
6	C	1	0.8	0.8	0.1
7	C	1	0.8	0.8	0.05
8	C	1	0.8	0.8	0.05

fs*=Fraction of susceptible badgers that are vaccinated
fi**= Fraction of infected badgers that are vaccinated

Vaccine effects on susceptibility and infectivity: Using a Se of 40% and a Sp of 99.9% and fixing VE_I at 80%, power was estimated for different β_{UU} values (0.3, 0.2, 0.1, 0.005 and 0.025) and two values of Vaccine Efficacy for Susceptibility: VE_S=80% and VE_S=40%. The same simulations were done but in this case we fixed VE_S at 80% and looked at the effect of modifying VE_I from 40% to 80% on study power when β_{UU} values varied from 0.025 to 0.3.

Outputs

Two dimensional graphs were built using the software Mathematica® 6.0 (Wolfram Research, Champaign, IL). To obtain the function that best fitted our data, we used Stata® version 10 (Stata Corp, College Station, TX, USA) to fit a generalized linear model (GLM) with different links of the binomial family. The link with the lowest AIC was selected. Line graphs were built using Microsoft Excel 2003 (Microsoft Corporation, Redmond, WA, USA).

RESULTS

Study power for different sensitivity and specificity of the diagnostic test

The study power for different Se and Sp values of the diagnostic test are shown in Figures 1 and 2. A decrease in Sp has much bigger impact on power than a decrease in Se. In Figure 1 it can be seen that the power of the study decreases sharply to around 5% when the Sp is 98.0% independently of the Se of the test. The effect of the Se on study power is much lower and given a Sp of 98.8 remains above 50% even when the Se is 40%. A VE_S and VE_I of 80% were assumed in this graph. Similar results were obtained when we assumed a VE_S and VE_I of 40%. This is shown in Figure 2 where for a Sp of 99.8% and a Se of 40%, study power was 45%.

Study power for variations in sample size

The effect of a decrease in the expected number of total badgers re-trapped (120/60/120) is presented in Figure 3. Assuming a Se of 40%, a Sp of 99.9% and a transmission parameter for non-vaccinated badgers β_{UU}=0.1, the study power was determined for two scenarios: both VE_S and VE_I equal to 40% and 80%, respectively, when the percentage of total badgers re-trapped varied from 10% to 100%. Figure 3 shows an increase in study power as sample size increases from 10% to 30% and then a slow and small decrease as sample increases up to 100%.

To investigate reasons for the slow decrease in the study power observed as sample size increases, the same simulations were repeated using a perfect diagnostic test with 100% Se and 100% Sp. Then, it is shown that study power increases when sample size rises from 10% to 20% and remains constant up to 100% of the expected sample size (Figure 4). Assuming a perfect test, the study power varied from 79.8% to 85.9% when sample size increased from 30 to 300 badgers and VE_S and VE_I were equal to 40%. When VE_S and VE_I were equal to 80%, the study power varied from 91.2% to 95.8% as sample size went from 30 to 300 badgers.

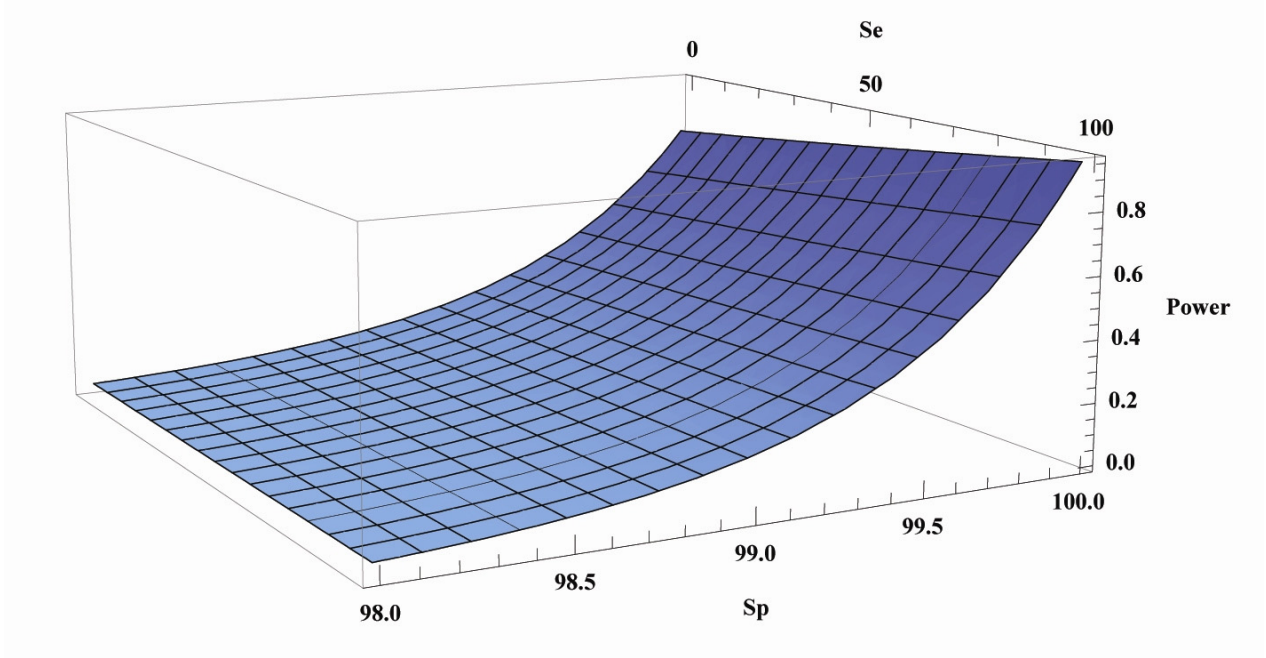

Fig. 1 Three dimensional plot showing the power of the badger vaccine trial as a function of the sensitivity (Se) and specificity (Sp) of the diagnostic test. β_{uu} was set at 0.1, VE_I and VE_S at 80%.

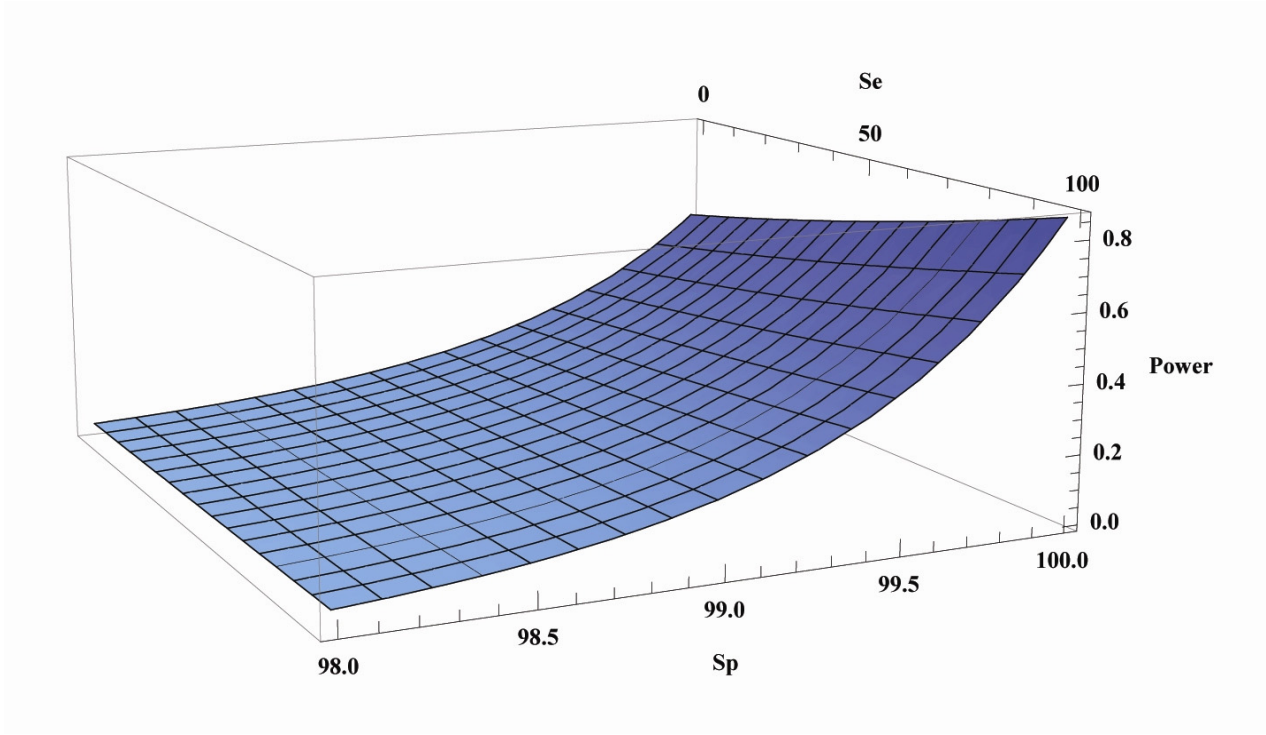

Fig. 2 Three dimensional plot showing the power of the badger vaccine trial as a function of the sensitivity (Se) and specificity (Sp) of the diagnostic test. β_{uu} was set at 0.1, VE_I and VE_S to 40%.

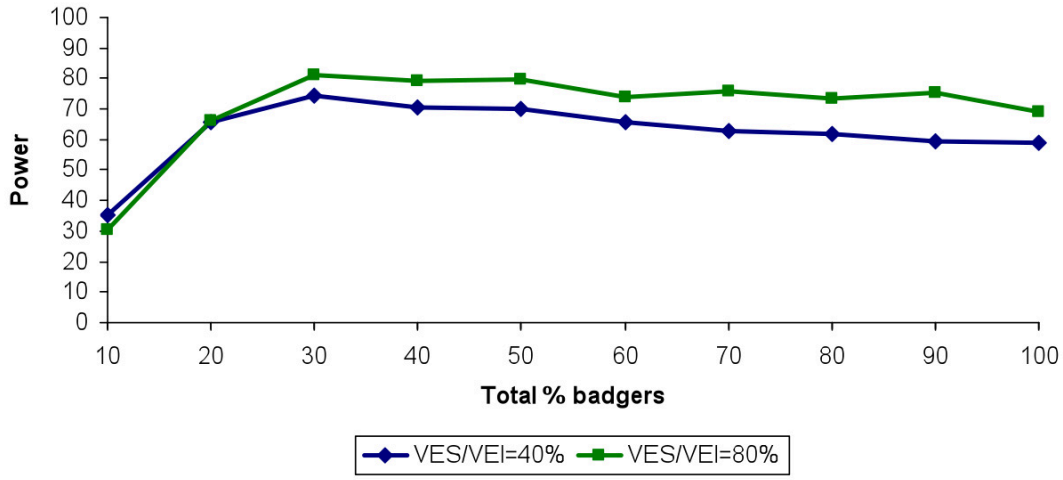

Fig. 3 Graph showing the study power as function of the total percentage of badgers re-trapped and variation in vaccine efficacy. β_{uu} was set at 0.1, VE_I and VE_S to 80% (or 40%), Se at 40% and Sp at 99.9%.

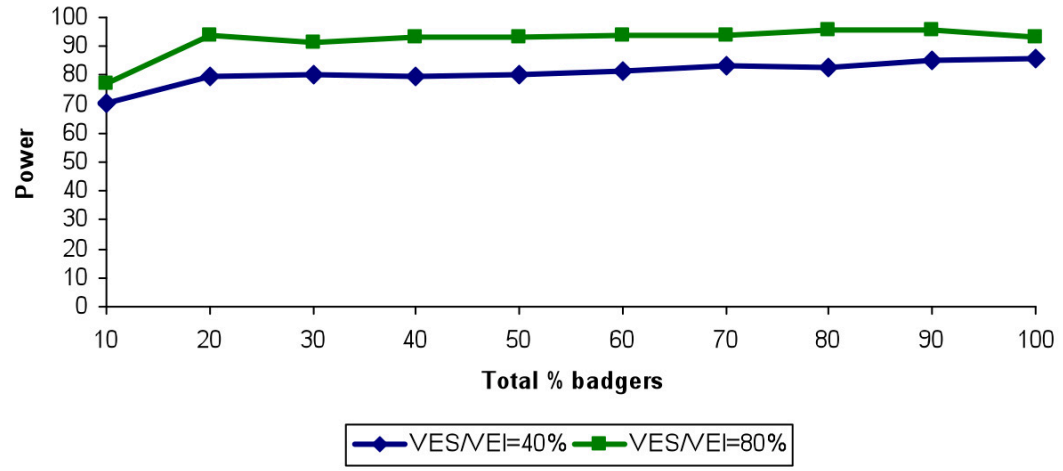

Fig. 4 Graph showing the study power for variations in the total percentage of badgers re-trapped. Sensitivity and specificity of the diagnostic test was set at 100%. Transmission between non-vaccinated badgers was 0.1%. The simulation process was carried out for both VE_S and VE_I equal to 40% and VE_S and VE_I equal to 80%.

Study power for variations in the initial transmission

A transmission value of 0.1 was used in all previous calculations of study power. In order to see the effect of deviations from this value on study power, we ran our macro keeping all other parameters fixed except β_{UU}. Figure 5 illustrates how the transmission rate between badgers prior to the start of the vaccine trial (β_{UU}) affects study power. Assuming Se=40%, Sp=99.9% and VE_S and VE_I=80%, the study power increases from 77% to 80% when β_{UU} increases from 0.1 to 0.3 and decreases to 0.63 when transmission is four times smaller (0.025). Under the same conditions but assuming a VE_S and VE_I of 40%, power varies from 0.57 to 0.70 when β_{UU} goes from 0.025 to 0.3. The power obtained when assuming an 80% reduction in susceptibility and infectivity due to vaccination were on average 73.4% versus an average of 63.4% when the

vaccine effects reduction was 40%. In both cases, the power decreases as the transmission rate between non-vaccinated badgers decreases.

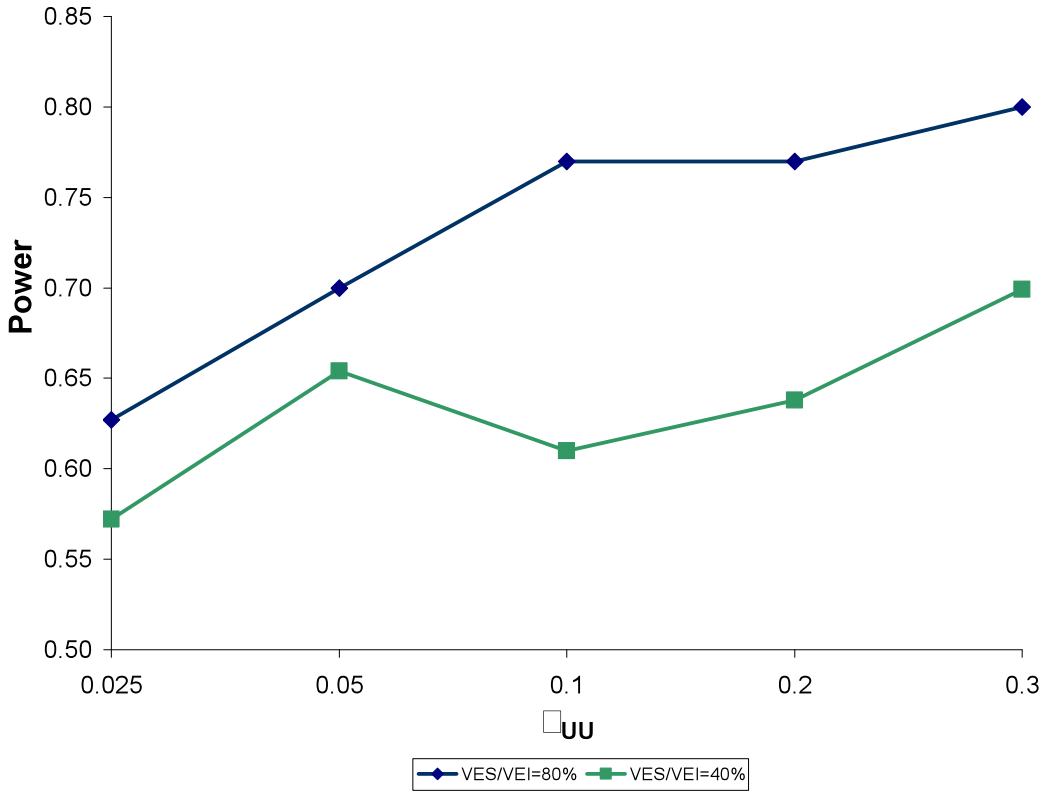

Fig. 5 Graph showing the power of the badger vaccine trial by transmission rate between non-vaccinated badgers (βuu) assuming a sensitivity and specificity of the diagnostic test of Se= 40% and Sp=99.9%.

Study power for different vaccine effects on susceptibility and infectivity

To illustrate whether changes in the vaccine effect on susceptibility had higher, lower or equal impact on power than changes in the vaccine effect on infectivity, we ran our simulation process for the same range of β_{UU} values as in the previous section but keeping VE_I =80% (Figure 6) or VE_S =80% (Figure 7) while varying the opposite vaccine effect. Both graphs then show the study power when VE_S and VE_I both equal 80% in a dark blue line (solid triangles) compared to the study power values when only one of the vaccine effects is 80% and the other is set at 40% (green line with solid squares).

The average study power when VE_I is 80% and VE_S is 40% is 65% (green line with solid squares in Figure 6) compared to an average of 72.2% (showed by a green line with solid squares in Figure 7) when VE_I is 40% and VE_S is 80%. If we compare these two averages with the average obtained when both vaccine effects are set to 80% (average power=73.4%), we can see then that changes in VE_S have a much higher impact on power than changes in VE_I. This is also observed by looking at the proximity of both lines in Figure 7 compared to Figure 6.

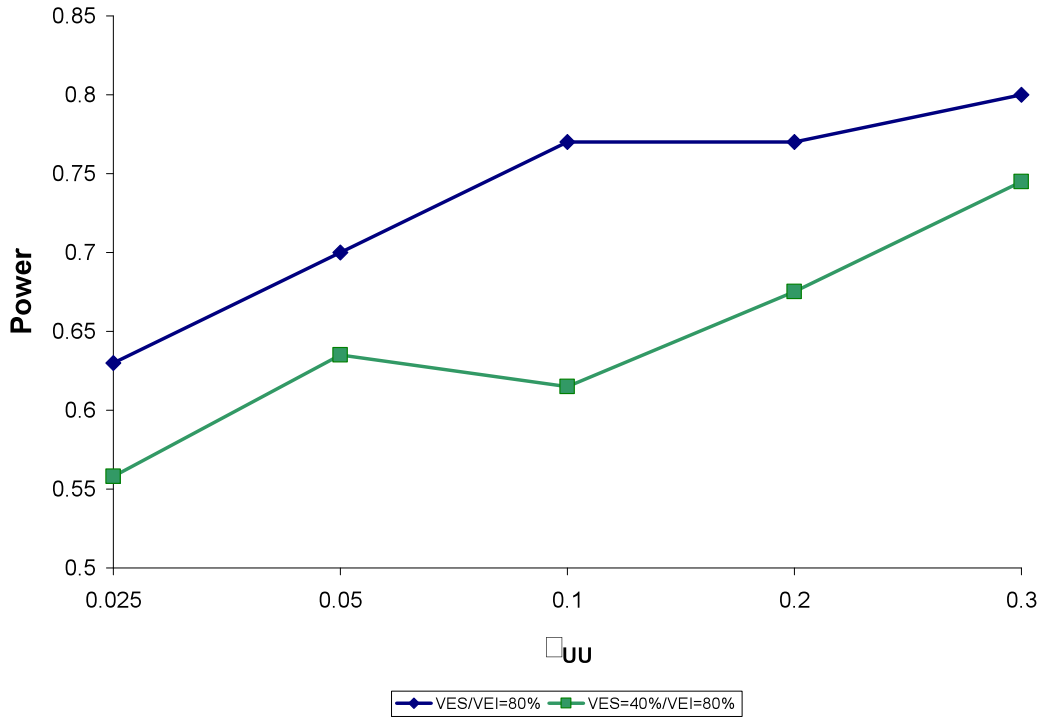

Fig. 6 Graph showing the power of the badger vaccine trial by transmission rate between non-vaccinated badgers (β_{UU}) assuming a sensitivity and specificity of the diagnostic test of Se= 40% and Sp=99.9% and an effect of vaccination in infectivity of 80%; % and 40%, respectively.

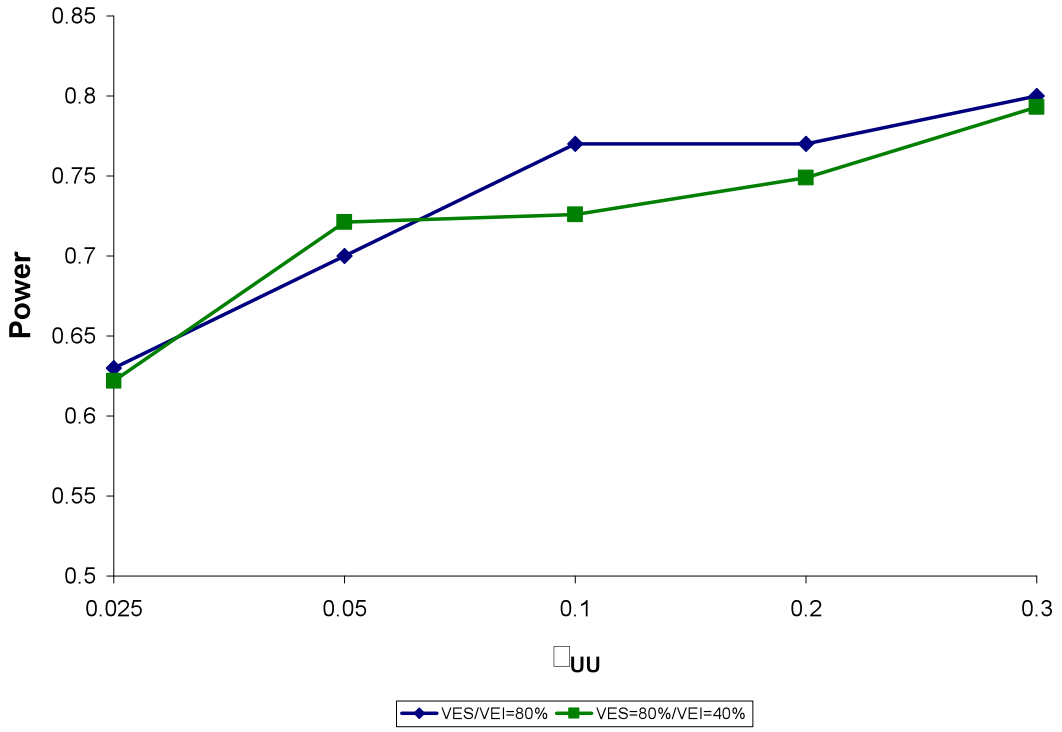

Fig. 7 Graph showing the power of the badger vaccine trial by transmission rate between non-vaccinated badgers (βuu) assuming a sensitivity and specificity of the diagnostic test of Se= 40% and Sp=99.9% and an effect of vaccination in susceptibility of 80%.

DISCUSSION

Scientific evidence obtained from vaccine field trials has played an important role in public health, helping governments to plan successful vaccination programs. Estimating statistical power in a vaccine trial is of great importance. If done *a priori* (before the trial starts), the optimal sample size to be used in the trial can be estimated. Using this sample size can give assurance of having sufficient statistical power to detect a pre-determined vaccine effect if it does indeed exist, whilst avoiding any unnecessary waste of resources. In the badger vaccine trial the maximum sample size was determined based on logistical issues without potential for further expansion of the vaccine trial area. Nonetheless, power calculations were still relevant as other parameters affecting power, such as Se and Sp of the diagnostic test, could potentially be adjusted to optimize study power.

A simulation approach was used to estimate the power of the trial where the following assumptions were made:

- The contact function is frequency rather than density-dependent. Riggs and Koopman (2004) show how in density-dependent models sample size has a much higher impact on study power than in frequency-dependent models. This is in agreement with the results obtained in our simulations. Power remained relatively constant, varying from 70% to 87% when sample size increased from 30 to 300 badgers (assuming a perfect diagnostic test). De Jong et al. (1995) explain how for most animal diseases, a frequency-dependent contact rate function fits the data better than a density-dependent function. Therefore, our assumption seems reasonable.
- The badger prevalence in the trial area was assumed to be 30%. A recent study carried out in Ireland, where comprehensive bacteriological culture methods had been used, detected a prevalence of 36.3% in badgers (Murphy et al., 2010).
- The expected number of badgers being trapped in each trapping exercise was set to 120, 60 and 120 for zones A, B and C, respectively. Although the three zones in the vaccine trial area were selected to have a similar number of main badger setts, trappings conducted prior to the start of the trial revealed a lower number of badgers in Zone B compared to zones A and C.
- Data on the re-trapping percentage and the fraction of susceptible (fs) and infected (fi) vaccinated badgers were based on expert opinion. Although a sensitivity analysis was carried out to see the effect of changes on the re-trapping percentage, slower or faster changes in fs and fi were not explored.

The most striking result obtained during the simulations was the large effect of the specificity of the diagnostic test on study power. The minimum specificity required to achieve a power above 60% was 99.8%. The effect of the sensitivity on power was much smaller. Assuming both VE_S and VE_I=80% and given a specificity of 99.8%, the power remained above 50% even when the sensitivity was 40%. These results have substantial implications in terms of the optimization of the diagnostic test to be used in the vaccine trial, showing that although specificity needs to be very high, there is some degree of flexibility in terms of the sensitivity of the diagnostic test. The effect of sample size on study power was deemed to be very small. The small decrease observed on power as sample size increased (Fig. 3) disappeared when a 100% Se and Sp test was assumed (Fig. 4). The decrease in power observed was proven to be an artefact.

Decreasing VE_S and VE_I both had a negative effect on power, but changes in VE_S had a larger impact on power than changes in VE_I. For a β_{UU}=0.1, a reduction in VE_I of 50% (from

80% to 40%) resulted in a reduction in power from 77% to 73%, while the same reduction in VE_S led to a higher reduction in power, going from 77% to 62%. The reduction in power is not considered high in either case, nonetheless the effect of VE_S in power is something to consider because of the uncertainty around the biological mechanisms in which BCG vaccine works in badgers. Using BCG vaccine by the subcutaneous or mucosal routes in badgers, Corner et al. (2008) demonstrated a reduction in histological lesions in vaccinated badgers compared to non-vaccinated badgers but did not show individual protection against infection. This lack of protection has to be interpreted with caution as the challenging doses used during the experiments might not be representative of the natural infection dose. However, if the results obtained in the study are indicative of the real VE_S, study power could be somewhat compromised.

In summary, it can be concluded that study power in group randomized trials depends not only on sample size but on many other parameters. In the current vaccine trial, power was highly dependent on the specificity of the diagnostic test. Therefore, it is critical that the diagnostic test used in the badger vaccine trial is optimized to maximise test specificity.

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EPIDEMIOLOGICAL TOOLS

ESTIMATING SPATIAL AND TEMPORAL VARIATIONS OF THE REPRODUCTION NUMBER FOR HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1 EPIDEMIC IN THAILAND

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SUMMARY

Since 2003, Highly Pathogenic Avian Influenza (HPAI) H5N1 virus has, caused a pandemic with serious economic consequences and public health implications. Quantitative estimates of the spread of HPAI H5N1 are needed to adapt control measures. This study aimed to estimate the variations of the reproduction number *R* in space and time for the HPAI H5N1 epidemic in Thailand. Transmission between sub-districts was analyzed using three different and complementary methods. Transmission of HPAI H5N1 was intense ($R(t)>1$) before October 2004, after which the epidemic started progressively to fade out ($R(t)<1$). The spread was mainly local, with 75% of the putative distances of transmission being less than 32 kilometres. The map of the mean standardised ratio of transmitting the infection (*sr*) showed sub-districts with a high risk of transmitting infection. Findings from this study can contribute to the discussion regarding the efficacy of control measures and can help with targeting surveillance programmes.

INTRODUCTION

Since the disease first emerged in China in 1996, outbreaks of Highly Pathogenic Avian Influenza (HPAI) H5N1 virus have occurred worldwide, causing important losses in animal production (Kilpatrick et al., 2006). The HPAI H5N1 crisis appears to represent the greatest threat to animal health that the veterinary community has ever been called to face, with very serious implications for public health (Capua, 2007). Controlling the spread of the disease in poultry should contribute to limiting the number of human HPAI cases. Prevention and control measures targeting HPAI outbreaks in poultry are mainly based on the prohibition of movement, pre-emptive culling, and vaccination around infected areas (Tiensin et al., 2009a). However, the temporal and spatial scales used for the implementation of control measures often rely on empirical knowledge of disease spread mechanisms. The occurrence of recent outbreaks indicates that the disease remains difficult to control despite the range of strategies that have been implemented.

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Mathematical modelling provides quantitative estimations of the main parameters that characterize the spread of infectious diseases in space and time. The reproduction number is a key notion of modelling in epidemiology. Previous studies on the dynamics of the 2001 foot-and-mouth-disease epidemic in Great Britain (Haydon et al., 2003) showed how informative estimations of the reproduction number can be for policy-making in veterinary public health. The "basic reproduction number", R_0, is the expected number of secondary infections caused by a primary case in a completely susceptible population (Diekmann et al., 1990). If $R_0>1$, the infection is expected to spread, whereas if $R_0<1$, it should die out. By definition, R_0 is an idealized quantity that is virtually impossible to observe directly but can be estimated indirectly (Mishra et al., 2011). On the other hand, the effective reproduction number, R_t, is defined as the actual mean number of secondary cases per primary case at time t, in a population in which not all hosts are susceptible. The value of R_t is typically smaller than the value of R_0. Time variations of R_t can be assessed from epidemiological data. The recent HPAI H5N1 epidemic was analysed in previous studies using methods that enabled estimations of the within-flock basic reproduction number R_0 (Tiensin et al., 2007; Soares Magalhaes et al., 2010).

Thailand was infected by HPAI H5N1 during the first and second wave of influenza (2004-2005), with 2045 outbreaks reported. Sporadic outbreaks have been reported since 2006. In the absence of vaccination, control measures rely mainly on pre-emptive culling of poultry and control of movements within a given perimeter around outbreaks. The aim of the present study was to estimate the variations of the basic reproduction number (R_0) in space and time for the HPAI epidemic in Thailand. The unit of interest was the sub-district, a small administrative area used as the base unit in the design of surveillance and implementation of sanitary control measures (Paul et al., 2010). Findings from this study should facilitate further discussions on the efficiency of control measure policies.

MATERIAL AND METHODS

Data

Data on HPAI H5N1 outbreaks in Thailand have been collected since January 2004 by official veterinarians of the Department of Livestock Development (DLD, Bangkok, Thailand). The DLD is in charge of the surveillance and monitoring of Avian Influenza in poultry (Paul et al., 2010). The presence of the H5N1 virus has been confirmed in sick and dead birds and cloacal samples from poultry by diagnostic laboratories using reverse-transcriptase polymerase chain reaction and virus isolation (Tiensin et al., 2009a). The DLD outbreaks database includes for each H5N1 outbreak the following data: the detection date, location (code of sub-district infected) and species of dead or sick poultry (Tiensin et al., 2009a). The present study is restricted to outbreaks in poultry which occurred during the second wave of HPAI in Thailand (July 2004 to May 2005). During this period, both passive and active surveillance systems were implemented to detect the disease.

A week number was assigned to each outbreak, with week 1 corresponding to 1-7 January 2004. The DLD outbreaks database included records of 1,314 laboratory-confirmed cases (flocks of affected poultry) dating from 3 July 2004 (week 27) to 12 April 2005 (week 67). The epidemiological unit of interest for the study was the sub-district ('*Tambon*' in Thai) having a mean area of 69.7 km^2. The few outbreaks reported in the sub-districts located in the south of Phetchaburi province (on the Kra isthmus of Thailand) were excluded from the analysis as they were assumed to play a minor role in disease transmission because of their remote location and

little geographical connection with the main part of the country. As a result, 6,277 out of the 7,408 sub-districts of Thailand were considered for statistical analysis.

An outbreak was defined as a sub-district with at least one laboratory-confirmed case during a given week. Two confirmed cases that occurred in the same sub-district and during the same week were considered as one outbreak at the sub-district level. In the period of interest, 1,208 outbreaks were defined. Successive confirmed cases that occurred within the same district with more than a one week but less than a two or three weeks interval were, according to the hypothesis of the infectious period, considered as a persistent outbreak. Otherwise they were considered as new outbreaks.

Estimation of the basic reproduction number (R_0) from the intrinsic growth rate

The basic reproduction number R_0 was estimated for the early phase, during which the epidemic expands in absence of interventions and effects of depletion of susceptible animals are small. An initial exponential growth is usually assumed for this phase, which is a characteristic for most infectious diseases (Anderson and May, 1991). The initial exponential-growth rate ( ) was first estimated using a linear regression model. With the logarithm of the cumulative number of cases as the dependent variable and time as the predictor variable, the parameter corresponded to the slope of the straight regression curve. The regression should only be calculated during the best length of exponential phase (Chowell et al., 2007; Vynnycky et al., 2007). Secondly, was also estimated during the same exponential growth period by plotting the mean number of new cases per week against the cumulative number of cases. The slope of the corresponding straight regression curve was the initial exponential-growth rate (Favier et al., 2006).

The exponential phase of the curve was assumed to end at the culmination point of the epidemic, which corresponded to the period from week 16 to week 41 (i.e., with 141 new outbreaks for a refractory period of one week and 122 new outbreaks for a refractory period of two weeks). The refractory period represented the time period following a case notification and during which a new notification in the same sub-district was not considered as a new outbreak, but as a persistence of the previous outbreak.

The reproduction number then was computed by substituting the estimate for into an expression derived from the linearization of the SIR deterministic epidemic model (Chowell et al., 2007). The method used here was adapted from (Degallier et al., 2005; Favier et al., 2006):

$$R_0 = 1 + \lambda/\gamma = 1 + \lambda \times T \quad (1)$$

With γ = recovery rate (at the sub-district level); = initial exponential rate, T= length of the infectious period (at the sub-district level), and $T \leq$ refractory period.

Spatial scale of spread: distance to the nearest infectious neighbour

The distribution of the distance between two successive outbreaks, as defined in the data section, located in different sub-districts was analyzed. The package" fitdistrplus" of R software was used to find the best fit for this distribution (Pouillot and Delignette-Muller, 2010).

Estimation of the time variations of the effective reproduction number

First, the transmission dataset was constructed on the basis of back-calculation (Tiensin et al., 2007; Ward et al., 2009). Assuming that all newly infected sub-districts were infected by another infectious sub-district during this phase of the epidemic, a simple deterministic SI model (Anderson and May, 1991) was used to describe the transmission of HPAI H5N1 between sub-districts. The transmission rate of HPAI H5N1 between sub-districts ( ) was estimated from the relationship between the number of newly infected sub-districts (*C(t)*) each week, the number of infectious sub-districts (*I(t)*) per week and the number of susceptible sub-districts (*S(t)*) per week. At the sub-district level, it would be irrelevant to consider a recovered category (*R*) in the model.

A latency period of one week was used to take the time interval between the real date of infection for a sub-district and the notification date registered in the database into account. This latency period corresponded to the delay for the expression of clinical signs in the poultry flocks, added to the delay before declaration or discovery of the outbreak by the surveillance system. As a result, with no restriction in the scale transmission, *C(t)*, was equal to the number of infectious sub-districts in the following week.

According to the model, susceptible sub-districts in contact with the virus enter the infectious class at the rate **I(t)/N(t)*, where is the transmission rate; *I(t)* is the number of infectious sub-districts at time *t*; and *N(t)=S(t)+I(t)* is the total number of sub-districts exposed to the risk of H5N1.

By calculating *C(t)*, *I(t)*, *S(t)* and *N(t)* each week *t*, the transmission rate β could be deduced from the following equation (Ward et al., 2009):

$$=C(t)\times N(t)/(S(t)\times I(t)) \quad (2)$$

Where is the transmission rate; *C(t)* is the number of new cases at the week *t*; *S(t)* is the number of susceptible to the infection at the week *(t)*, *I(t)* is the number of infected at the week *t*;

$$N(t)=S(t)+I(t) \quad (3)$$

The effective reproduction number of the epidemic was obtained from following equation (Anderson and May, 1991; Tiensin et al., 2007):

$$R= \quad \times T \quad (4)$$

Where *T* is the duration of the infectious period.

The calculation of the number of sub-districts categorised as infectious at each week (*I(t)*) depends on the duration of the infectious period. The calculation was performed for 2 hypotheses: an infectious period of one week or an infectious period of two weeks.

The calculation of *N(t)* also depended on epidemiological features, especially the geographical scale of the HPAI transmission between sub-districts. Two hypotheses were explored: 'national' versus 'local' transmission. A national transmission could occur between two sub-districts even when the two sub-districts were far apart. Consequently, the number of exposed sub-districts *N(t)* is equal to the overall number of sub-districts included in the study. Indeed, all sub-districts in Thailand were assumed to have poultry and thus to be at risk of an

HPAI H5N1 outbreak. As suggested by Souris et al. (2010), a local transmission, between two sub-districts could occur only when the distance between sub-district centroids was less than a distance *d.* In this case, the number of exposed sub-districts *N(t)* is equal to the number of sub-districts in Thailand for which distance between the centroid of the exposed sub-district and the centroid of an infectious sub-district is less than *d*. To determine the distance *d* that should be considered to assess the local spread of the disease, the 80% and the 90% quantiles of the fitted distribution of the distance to the nearest infectious neighbour for each sub-district were used.

As a result, the model was run for a national transmission and two different radius of local transmission. The curve representing the time variation of the effective reproduction number was drawn for the different hypotheses.

Estimation of the spatial variation of the transmission of HPAI in Thailand

Despite the absence of detailed information concerning the infectious network, it was possible to reconstruct an epidemic tree from the notification data. The nearest infectious neighbour method, a deterministic method adapted from Haydon et al. (2003) and Ward et al. (2009) was used to build the infectious network. With this method, a putative source of infection (referred to as a "parent") was attributed to each outbreak.

For each outbreak, previously reported outbreaks within the infectious period (for example, up to one week before for a one-week infectious period) were selected. Based on the results of Ward et al. (2009) (one week infectious period at the village level for HPAI H5N1 in Romania) and Souris et al. (2010) (three week infectious period at the sub-district level), the model was tested for both a one-week and a two-week infectious period.

The closest outbreak (Euclidian distances between sub-district centroids) within the last or last-two weeks was selected as the putative parent of the outbreak, until each outbreak had a putative source. Then the number of putative daughter outbreaks different from itself (persistent outbreaks) arising from each outbreak at each week was deduced. This number, corresponding to the local effective reproduction rate, was called $R_i(t)$, *i* referring to the sub-district source of the infection and *t* referring to the week considered. This $R_i(t)$ is a very local and temporal estimation of the transmission, varying with sub-district and week.

It then was possible to calculate a weekly mean *R(t)*, by averaging the local $R_i(t)$ of each week:

$$R(t) = \left(\sum_{i=1}^{n_t} R_i(t)\right)/n_t \quad (5)$$

With $R_i(t)$ representing the number of putative daughters outbreaks different from itself arising from sub-district *i* at week *t*, n_t representing the number of outbreaks (sub-districts) identified as the putative source of infection at week *t* (outbreaks of week *t*-1 or outbreaks of week *t*-1 and of week *t*-2, depending on the duration of the infectious period of one week versus two weeks). This value *R(t)* corresponds to a mean value of the effective reproduction number for all of the areas where the epidemic occurs at week *t*.

A transmission ratio was standardised according to time. For each sub-district *i,* identified as a potential parent, a weekly standardised ratio of transmission $sr_i(t)$ was calculated. It was the ratio of the value the $R_i(t)$ on the weekly mean *R(t)*, defined as:

$$sr_i(t) = R_i(t)/R(t) \quad (6)$$

Finally, for each sub-district i a global standardised ratio sr_i was calculated by averaging the $sr_i(t)$ over the whole epidemic course, defined as:

$$sr_i = \left(\sum_{t=1}^{k_i} sr_i(t) \right) / k_i \quad (7)$$

In this equation, sr_i represents the mean standardised ratio of transmission for the sub-district i over the entire period of interest. The term k_i represents the total number of weeks during which the sub-district i was infectious (during which this sub-district was identified as the putative source of infection for other infected sub-districts). The term sr_i is a local measure of the transmission at the sub-district level over the entire period of interest. The mean standardised ratio sr_i was mapped using geographical information systems (ArcGIS© software).

RESULTS

Basic reproduction number

Two methods and two assumptions were used to estimate the basic reproduction number R_0 during the second wave of the epidemic, based on the data of the epidemic curve. Depending on the assumption that is used, the estimated value of the R_0 during the second wave of the HPAI H5N1 epidemic in Thailand ranges from 1.27 to 1.6 (Table 1).

Table 1. Estimation of the R_0 during the initial exponential-growth phase of the second wave of the HPAI H5N1 epidemic in Thailand, using two different methods adapted from literature

	Refractory period of 1 week		Refractory period of 2 weeks	
	Method adapted from (Vynnycky et al., 2007)	Method adapted from (Favier et al., 2006)	Method adapted from (Vynnycky et al., 2007)	Method adapted from (Favier et al., 2006)
Initial exponential-growth rate (SE)	=0.267 (SE=0.045)	=0.310 (SE=0.038)	=0.262 (SE=0.044)	=0.299 (SE=0.039)
R_0 [95% CI]	R_0=1.267 [1.180-1.355]	R_0=1.310 [1.234-1.385]	R_0=1.526 [1.352-1.670]	R_0=1.597 [1.444-1,750]

Spatial scale of spread: distance to the nearest infectious neighbour

This distribution of the logarithm of the distance to the nearest infectious neighbour is very well fitted with a gamma distribution. The distribution is very similar indifferent of the length of the infectious period considered (one or two weeks). The distribution for a one week infectious period is shown in Figure 1.

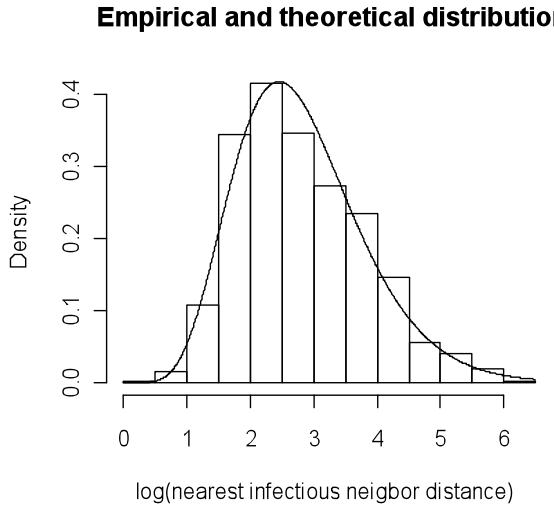

Fig. 1 Distribution of the logarithm of the distance between an outbreak and the nearest infectious neighbour, for a one week infectious period. The histogram represents the empirical distribution and the curve represents the density of probability of the theoretical fitting distribution (gamma distribution with a shape parameter of 7.54 and a scale parameter of 2.74).

The nearest infectious neighbour was located on average at a distance of 29.9 km (for an infectious period of one week) or 25.9 km (for an infectious period of two weeks) from an outbreak. More than 75% of the outbreaks had a parent outbreak within a 32.1 km range.

Temporal variation of transmission of HPAI

Results of estimations of *R(t)* were very similar, whatever the hypothesis made regarding the length of the infectious period (one or two weeks). Figure 2 presents the output of the SI model for a one-week infectious period, with the time variations of *R(t)* according to different hypotheses concerning the scale of the spread (local versus global).

The variation of *R(t)* was the same regardless the considered spatial scale of the spread of HPAI. However, the effect of long-distance jumps was visible on the spread at the beginning and end of the epidemic course. In these parts, a national transmission, allowing long-distance jumps between outbreaks, increased the value of *R(t)* compared to more local transmission hypothesis (Fig. 2).The course of the epidemic can be graphically divided into two sections. Before week 40, *R(t)* was on average high with several peaks. In contrast, after week 40, *R(t)* remained almost steadily under the epidemic threshold.

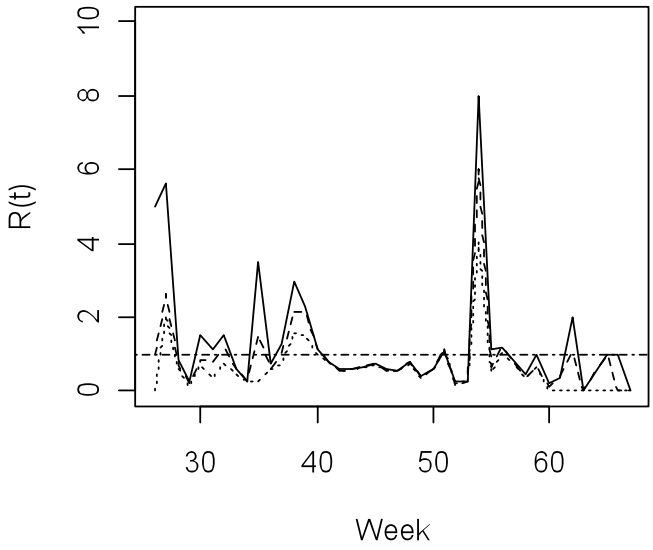

Fig. 2 Time variation of the effective reproduction number of the HPAI H5N1 epidemic in Thailand, for a one-week infectious period: graph corresponding to a local spread, over the whole territory of Thailand (—); graph corresponding to a local spread, within a radius of less than 90% quantile of the nearest infectious neighbour distance distribution (- - -); graph corresponding to a local spread, within a radius of less than 80% quantile of the nearest infectious neighbour distance distribution (·····); line corresponding to the epidemic threshold (-·-).

Spatial variation of HPAI transmission

Distribution of $R_i(t)$: during the second wave of HPAI H5N1 in Thailand, 1,208 outbreaks (sub-districts with at least one case notified during a given week) occurred. For a one week infectious period, the reconstruction of the infectious network showed that out of these 1,208 outbreaks, the sub-district was infectious the previous week for 199 outbreaks (outbreaks persisting for at least 2 weeks) and 1,009 outbreaks were new outbreaks (Table 2).

Table 2. Distribution of *Ri(t)*, the number of putative daughter outbreaks

		Infectious period	
		1 week	2 weeks
Number of outbreaks (a sub-district infectious for a given week)	new outbreaks	1009	918
	persistent outbreaks	199	209
All Ri(t)	Min	0	0
	Max	25	13
	Average	0.83	0.41
Ri(t) >0	Min	1	1
	Max	25	13
	Average	1.86	1.51

The standardised ratio (sr_i): was mapped using a choropleth map with categorization at three levels of the risk: high ($sr_i>1.3$), intermediate ($0.7<sr_i<1.3$) and low standardised ratio ($sr_i<0.7$) (Fig. 3). A standardised ratio equal to one ($sr_i=1$) corresponds to a risk of transmission from a specific sub-district *i* during the study period, equal to the mean risk of transmission between all sub-districts in the same period.

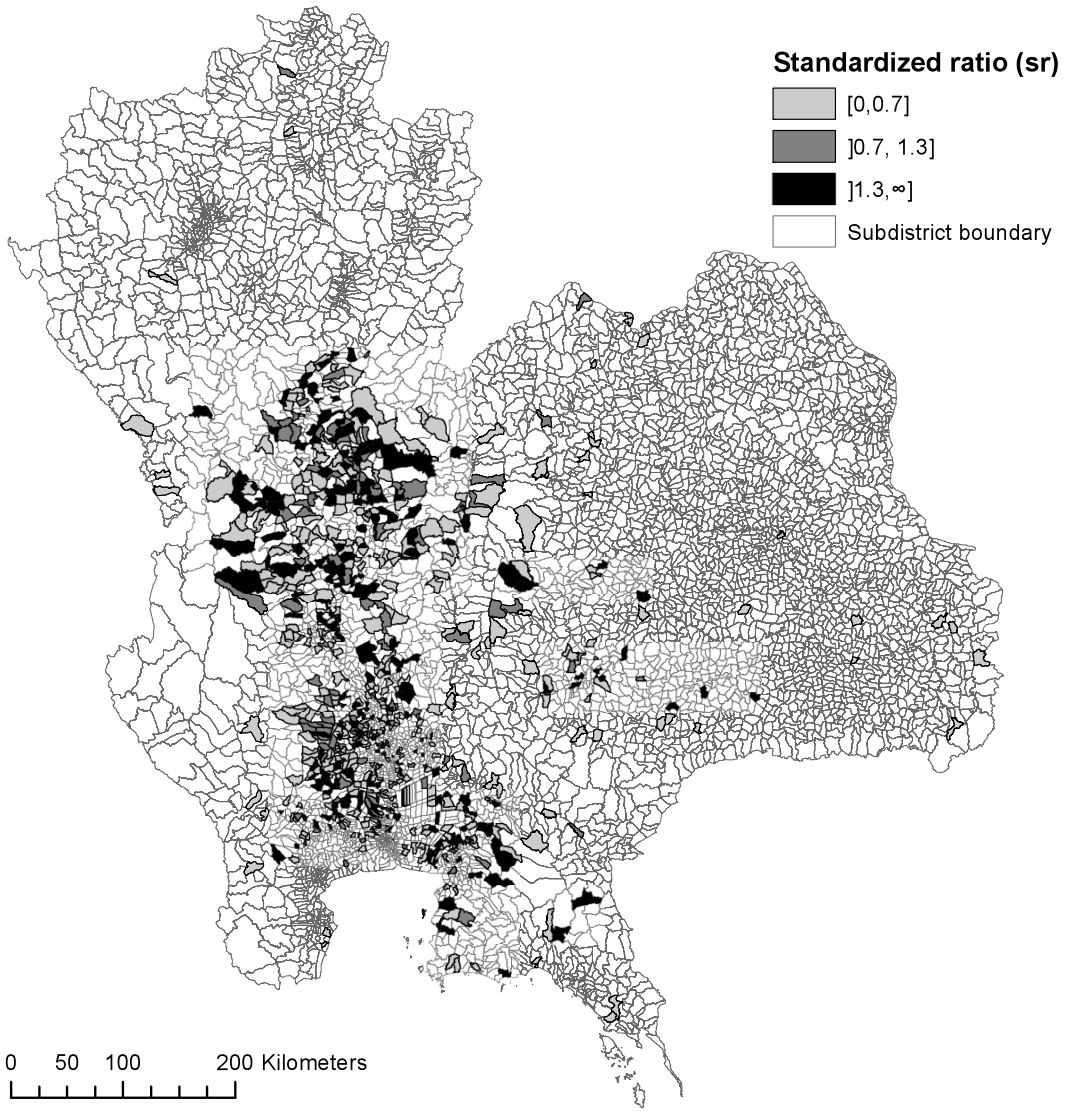

Fig. 3 Map of the transmission standardised ratio at the sub-district level during the second wave of the HPAI H5N1 epidemic in Thailand, for a 1-week infectious period

DISCUSSION

In this paper, variation of the key parameters of the HPAI H5N1 dynamics during its second wave of the epidemic in Thailand were analysed in time and in space. The use of several methods provided complementary information to characterise the main features of the spread of HPAI from one sub-district to another from July 2004 to April 2005. It should be noted that the main methodological assumption underlying this work involved the length of the Infectious Period at sub-district level. In accordance with previous work (Ward et al., 2009; Souris et al., 2010), the sensitivity of our model to the length of the infectious period was tested using two infectious periods of one and two weeks that gave similar results. Another important assumption, one common to all methods adapted from the nearest neighbour method, was that proximity in both time and space are the strongest factors driving the spread of a contagious disease such as HPAI. However, the same patterns can arise from the presence of other risk factors that are spatially correlated. This study also implicitly assumed that all outbreaks were detected and reported. This assumption was reasonable as the two complementary surveillance systems

(passive surveillance, and a reinforced, nationwide, active monitoring program called, "X-ray survey") (Tiensin et al., 2007; Paul et al., 2010) were implemented during the study period and ensured the detection of outbreaks (Tiensin et al., 2009).

The basic reproduction number R_0 at the beginning of the epidemic was estimated to be between 1.27 and 1.60 depending on the method of estimation and the hypothesis of infectious period used. It is noteworthy that, for both methods, values of R_0 always were significantly higher than the epidemic threshold value of 1 (none of the CI included the value 1). These estimated values for R_0 were slightly lower than the reproduction number R calculated by Ward (1.95–2.68) for the epidemic of HPAI H5N1 in Romania (Ward et al., 2009). This may reflect a difference in the pattern of HPAI spread in Thailand compared to Romania. The difference could also be explained by the different in intensity of the epidemics or by the difference in the size of the epidemiological units.

This study showed that the spread of HPAI during the second wave of the epidemic in Thailand was predominantly a local process, with a mean distance between the centres of two successively infected sub-districts of 29.9 km for an infectious period of one week. These results are similar to those of Ward et al., who found a mean distance between outbreaks of 23.36 km (95% CI 18.19–28.53) (Ward et al., 2009). In the present study, 75% of the outbreaks were located at a distance of less than 32 kilometres from the closest outbreak. This is consistent with previous work concerning the H5N1 epidemic in Thailand (Souris et al., 2010), which estimated 60 km as the maximum distance for the transmission process, with rare long range transmission jumps. Given the short distances involved in transmission, control measures that reduce the transmission between neighbouring areas are very important.

The number of putative daughter outbreaks ranged from 0 to 25 for a one week infectious period, and from 0 to 13 for a two week infectious period. These results are very similar if they are calculated on the overall infectious period. The mean number of putative daughter outbreaks each week was 0.83 and 0.41 respectively for an infectious period of one week or two weeks. This corresponds to an estimation of the mean effective reproduction number (0.83 for an infectious period of one week, and 0.82 (=0.41×2) for an infectious period of two weeks). These values are lower than the value of basic reproduction number. This is logical, as the effective reproduction number was estimated from an average of the values of $R(t)$ over the epidemic curve while the basic reproduction number was estimated from the early epidemic phase only, when few control measures were in place.

Moreover, the outputs of a deterministic SI model gave a time variation of $R(t)$. A peak was observed in week 54, but it would be excessive to conclude that there was a very high spread at this period of time. It is noteworthy that week 40 (30 September 2004-6 October 2004) corresponded to the beginning of the first X-ray survey, designed to reinforce the surveillance of HPAI in Thailand with door-to-door surveys carried out by 990,000 volunteers. Results from our study suggest that the considerable efforts made to control the disease were fruitful as the transmission parameters decreased remarkably at that time. Soares Magalhães et al. (2010) observed the same tendency in the temporal variations of R at the flock level as the one we evidenced at the sub-district level.

The standardised ratio (*sr*) we produced showed the spatial variations of the capacity of each sub-district to transmit HPAI H5N1 to other sub-districts. The implementation of control measures in the field may have differed between sub-districts, and may have contributed to spatial variations of the sub-district reproduction number. However, the characteristics of these

'hot-spots', i.e. sub-districts with a high potential for transmitting the disease to another, should be explored. Intrinsic features such as elevation, rice cultivation, presence of free-grazing ducks or proximity to main transportation axes have been found to influence the spatial distribution of HPAI H5N1 outbreaks (Gilbert et al., 2006; Tiensin et al., 2009; Paul et al., 2010). It is natural to ask whether the risk factors associated with transmission between sub-districts would be the same.

This study illustrates how complementary methods can be used to study the temporal and spatial pattern of disease spread. With regard to the spread of HPAI between sub-districts in Thailand during the second wave, the variation in time of the reproductive number likely was associated with the efficacy of disease control measures that were implemented at that time. Control measures may also partially explain the spatial variation of transmission capacity that was highlighted in our study, but further work is needed to precisely identify which factors underlie the geographical pattern of transmission.

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META-ANALYSIS OF DIAGNOSTIC TEST PERFORMANCE AND MODELLING OF TESTING STRATEGIES FOR CONTROL OF BOVINE TUBERCULOSIS IN GB

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SUMMARY

The British Government spends over £100 million per annum on the control of bovine tuberculosis (bTB). Improvement in the control through targeted use of diagnostic tests is one focus of eradication plans. The aims were: a) through systematic literature review identify primary research with estimates of sensitivity (Se) and specificity (Sp) for diagnostic tests for bTB in cattle b) conduct a statistical meta-analysis to estimate test performance, and c) using the estimates, model and compare different testing strategies. Of 9782 references reviewed, only 261 met agreed criteria and contained performance estimates for one or more of 14 diagnostic tests. The performance of bTB surveillance systems using the estimates of test performance was affected by the historical probability of herd freedom and the risk of introduction of infection. Where the probability of introduction of infection was high, it was difficult to achieve a high target probability of herd freedom from infection.

INTRODUCTION

Bovine tuberculosis (bTB) is a notifiable infectious disease and its control and eradication in cattle herds is mandatory in the European Union (Anon, 1964; de la Rua-Domenech, 2006). Before effective statutory eradication programmes had been instituted, bTB was an important zoonotic infection in Europe and other economically developed nations (Waddington, 2004). Several EU countries have controlled and eradicated bTB using programmes based on the intradermal injection of tuberculin, removal of TB test reactors and restrictions on the movements of cattle from infected herds. However, bTB continues to have an important impact upon the British cattle industry due to the endemic nature of the infection in large tracts of England and Wales associated with a wildlife reservoir and the rising costs of control with over 6.5 million cattle tuberculin skin tests carried out in 2009.

The primary test currently used for bTB surveillance in GB is the Single Intradermal Comparative Cervical Tuberculin test (SICCT). The frequency of SICCT herd surveillance is determined by the EU Council Directive 64/632/EEC (as amended) and based upon a calculation of historical incidence of herd breakdowns with post-mortem (PM) evidence of bTB infection

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within a defined area (http://www.legaltext.ee/text/en/PH0123.htm). A herd is identified as 'infected' and put under movement restrictions by the detection of SICCT positive animals (reactors) or tuberculous carcases at routine meat inspection.Thereafter a series of herd-level bTB control actions are implemented to prevent spread and restore the official TB free status of the herd. Herd-level bTB control measures include the removal, slaughter and post-mortem examination of animals at slaughter, the imposition of herd movement restrictions, and tuberculin test of the herd at short intervals with removal of any reactors until a number of successive herd tests are negative. In some circumstances, ancillary diagnostic tests such as the interferon-gamma blood assay (IFN-γ) are used in conjunction with the SICCT in known infected herds to enhance the Se of the bTB testing regime. Post-mortem examination, microscopic examination of tissues and bacterial culture of test reactors are also undertaken to assess the severity of infection, characterise the molecular type of the causative bacterium to support epidemiological investigations and can further influence decisions regarding the bTB control measures applied in the herd. Accurate detection and diagnosis is critical for facilitating effective control and research continues into the development of new and improved tests, the better targeting of tests (and combinations thereof) to maximise performance.

A systematic literature review attempts to bring together all work in a subject area, to provide an impartial and objective assessment of findings. The methodology is used extensively in conjunction with statistical meta-analyses to estimate the efficacy of medical treatments in randomised controlled trials by Cochrane and others (Greenland, 1987; Bero et al., 1998). More recently the methodology has been used to obtain pooled estimates of diagnostic test performance (EFSA, 2008a; Leeflang et al., 2008; More et al., 2009).

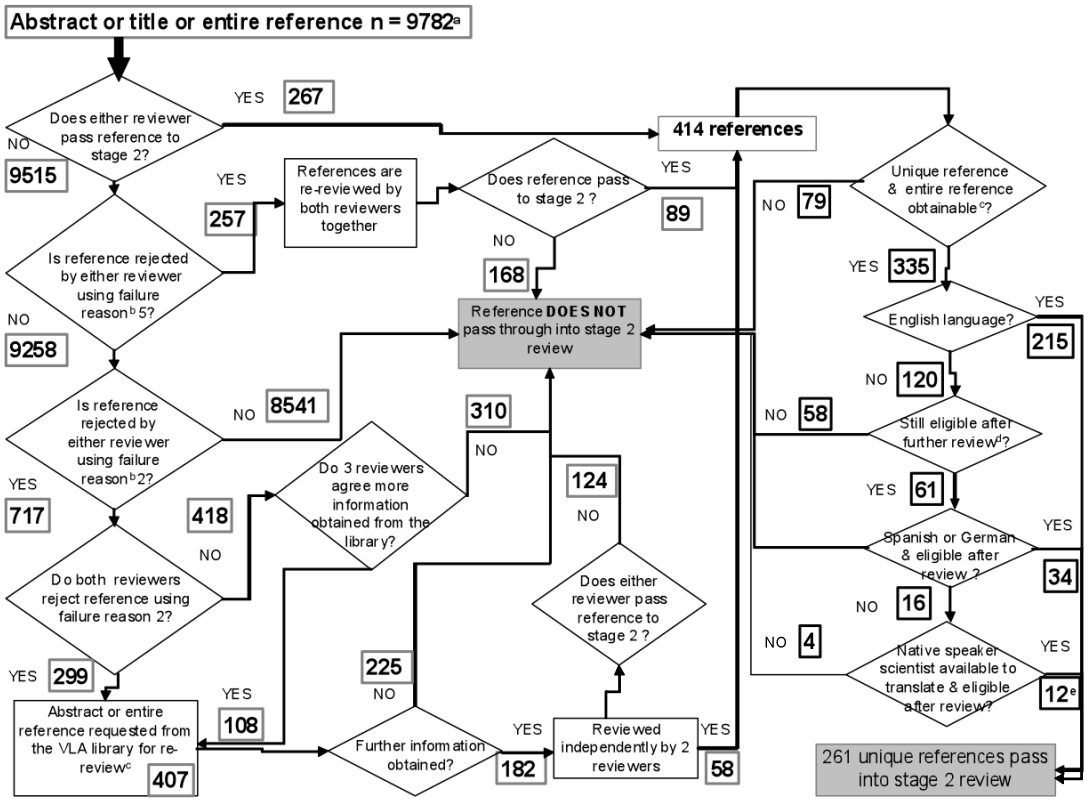
The current project had three aims: a) identify primary research sources for estimates of sensitivity (Se) and specificity (Sp) for diagnostic tests for bTB using standardised systematic review methodology b) conduct a statistical meta-analysis to obtain combined estimates of test performance, and c) input the distributions for the estimates obtained through the meta-analysis into a model that estimated the probability that a herd was free from infection in order to compare different testing strategies in the control of bTB.

MATERIALS AND METHODS

Systematic review

The Working Group (WG) for the project included 18 reviewers with a scientific expertise ranging from bTB immunology to pathology and laboratory culture of *Mycobacterium bovis* to veterinary epidemiology and implementation of bTB control programmes. The process of the review was discussed and agreed upon at WG meetings and standardised at every stage. The methodology was adapted from an approach taken previously whilst reviewing the performance diagnostic tests for bTB in deer (EFSA, 2008a).

The WG members each contributed to a two-stage review of relevant literature. The stage 1 review was conducted with only an abstract or title available. Stage 2 was a more detailed review, conducted after reading the entire reference selected in stage 1 and led to the extraction of Se and Sp and other data from eligible references (Figure 1).



[a] Identified through searches of electronic bibliographies and other sources
[b] Failure reasons 1: Does not contain performance information for diagnostic test for bTB in bovines (wrong subject material) 2. Insufficient information in record 3: Surveillance or prevalence report 4: Review not primary study 5: Not possible to calculate either Se or Sp
[c] By VLA library using reasonable means, cost and effort
[d] By three WG members in the light of stage 2 exclusion criteria
[e]French, Chinese, Polish, Dutch and Italian references reviewed by scientific staff at the VLA

Fig. 1 Systematic review of references at stage 1

Stage 1 review

a. Overview: A comprehensive search strategy was developed (see b. below) to identify abstracts that were then each reviewed by two WG members against predetermined inclusion and exclusion criteria (see c. below).

b. Source of references and search strategy:

Sources for references included:

- Electronic databases including:
    - Web of Knowledge (includes Web of Science 1995- Current Contents 1998-, CAB Abstracts 1910-, Medline 1950-);
    - Dialog (includes Embase 1974-, Agricola 1970-, Agris 1975-);

- Unpublished data and reports identified through contacting research institutions and laboratories (grey literature);
- References known to members of the Working Group;
- Review of bibliographies of reports and papers.

c. Inclusion and exclusion criteria at stage 1:

Inclusion criteria:

- The reference related to primary research.
- The reference included either report(s) of Se and/or Sp of a diagnostic test for bTB, or provided data enabling the statistics to be calculated.
- The diagnostic test performance was measured on cattle.

Exclusion criteria:

- The Se estimates were from studies where cattle had been experimentally infected with *M. bovis*.

A test type was excluded by the WG from further review at stage 2 where it was agreed that the test in question was not currently in use, was impracticable or unlikely to be used or developed in the foreseeable future.

Stage 2 review

a. Overview: The entire reference was obtained for abstracts that passed through the stage 1 review and references written in English were randomly allocated to WG members whilst references written in other languages were allocated to native speakers. Each reviewer was asked to evaluate each reference against agreed inclusion and exclusion criteria (see b. below). Where the reference passed through the stage 2 review, data relating to the population, test characteristics, characteristics of the reference standard and the data required to calculate test performance were entered into a purpose-built database.

b. Inclusion and exclusion criteria at stage 2:

Inclusion criteria for Se estimates:

- Se could be calculated.
- The bovine population had been naturally exposed to bTB.
- Each study animal had been individually examined using one of the following positive reference tests: post mortem examination (PM) (meat inspection or detailed laboratory inspection), culture, microscopic inspection (histology or histopathology), SICCT.

Exclusion criteria for Se estimates:

- The study population had been experimentally infected with *M. bovis*
- The definition of "infected" was based on a "group" level inference (such as a sample of animals in the study population being positive for culture of *M. bovis*)

Inclusion criteria for Sp estimates:

- Sp could be calculated.
- There is good evidence that the bovine population was free from infection with, and exposure to, *M. bovis*, including herds with Officially Tuberculosis Free (OTF) status, herds from an OTF area or OTF country, herds from a non-endemic bTB area where the authors stated that the area has been free of TB for several years, or herd that in the authors' opinion was tuberculosis-free and had been free for several years.

Exclusion criteria for Sp estimates:

- Any other evidence of lack of exposure to bTB.

c. Resolution of differences: The data entered into the stage 2 database by the two reviewers for each reference were compared using a query system and a hierarchical process was followed to resolve inconsistencies. Each reference was randomly assigned one lead reviewer from the pair who had reviewed the reference. After resolution, a revised corrected database was returned by each reviewer to the Veterinary Laboratory Agency (VLA) data management team. Remaining disagreements between data were resolved by cross-checking related fields within data entries, harmonising data recorded in free text fields and harmonising thresholds by scaling estimates reported in different units.

Meta-analysis

The statistical meta-analysis was conducted to pool independent estimates of for diagnostic performance obtained through the systematic review. As a result of the inclusion and exclusion criteria at stage 2 of the systematic review, the estimates for Se and Sp were derived from different populations.

The analysis faced several challenges. In the first place, there were relatively few eligible performance data for most tests. Secondly, references often contained several estimates of performance for the same test which were not independent because they were derived from the same population. Thirdly, test performances for the IFN-γ and ELISA tests were interpreted at different thresholds and on different scales in different references.

To address the varying number of records per reference, population and test type, the number of "test positives" and "true positives" were weighted as inversely proportional to the number of records from the same reference, population and test type. After this, one random-effect term was incorporated into the meta-analysis to account for differences between references where there were more than one reference with eligible estimates for a test type. To address the problem of combining estimates derived from different thresholds and scales, counter parameters for Se and Sp were extracted from references that contained these data for IFN-γ and/or the ELISA blood tests and included as covariates.

Binary logistic regression models were used to explore potential confounding factors (covariates). Categorical variables were assigned dichotomous coding using 1 for the baseline category (chosen to match GB conditions) and 0 for the complement set of categories. Although the selection of covariates for which data were collected in the systematic review had been made *a priori*, the final set of covariates that were modelled in order to estimate Se and Sp was data driven from the set of covariates that had been collected. Stepwise logistic regression (with fixed effect) for each test type was conducted. Covariates that remained in the models after the stepwise procedure were used in the logistic regression modelling with a random-effect.

The form of the model was

$$logit(Se)=z+\theta X$$

where

$$z=N(\mu,\tau) \text{ and } \mu=N(0,\sigma),$$

τ and σ are the variance within and between studies, θ is the vector of parameters and X the set of covariates.

The model was run in a Bayesian framework implementing the Monte Carlo Markov Chain (MCMC) technique provided by the BRugs package for R (Thomas et al., 2006). The methodology had already been successfully used for the estimation of diagnostic test performance for other conditions and species (EFSA, 2008b; Greiner et al., 2009). The posterior distribution of Se (Sp) calculated was based on the baseline categories for the set of covariates X. To measure the effect of a baseline category for a particular covariate X_i in the final estimate, the following odds ratio was used:

$$\frac{Se/(1-Se)}{Se_{\beta_i}/(1-Se_{\beta_i})} = \exp(\beta_i)$$

where Se_{β_i} is the Se estimated using the same model but excluding covariate X_i.

For tests with sparse data, a fixed effect model using MCMC was fitted to generate posterior distributions. The final outputs were probability distributions for the test performance parameters (Se and Sp).

Modelling different bTB surveillance and control regimes

The objective was to evaluate the performance of both current and alternative bTB surveillance and control system components (SSCs) which make up an overall bTB surveillance system. A stochastic scenario tree model approach described and applied elsewhere (Martin et al., 2007a; Martin et al., 2007b; Berg et al., 2009; More et al., 2009) was further developed. The freedom from infection (FFI) model developed was then used to estimate the probability that a herd with specified characteristics and bTB surveillance history was free from infection. Using the model, a range of potential future surveillance options, based on test Se, Sp, cost and time required to achieve free status could be compared.

The model was used to estimate the probability of freedom from bTB infection in herds with specified characteristics in GB for specified Surveillance System Component (SSC). An individual SSC may comprise a single diagnostic test or involve the application of multiple tests before the status of a herd or animal is determined. The model required several inputs including evidence from previous herd surveillance, the prior probability that the herd was infected, the distributions of animal-level Se and Sp of diagnostic tests (derived from the meta-analysis) and the risk of introduction of infection into the herd. In absence of other information, the prior probability that a herd was infected was estimated using the prevalence of bTB in the local region of the herd. The values for the probability of introducing infection in the model were derived from the observed incidence of bTB in GB four scenarios representing different levels of risk from new infection: high risk/endemic areas; edge of high risk/endemic areas; newly infected areas outside endemic areas; and clean areas.

The FFI model was used to calculate the surveillance Se required to meet a target probability of freedom from infection and thereby identify SSC with adequate Se. The choice from the SSC that provided adequate Se was then based on Sp (expected number of false positives), time to freedom, and therefore cost. In this analysis, target probability of freedom for a herd or group of herds has been arbitrarily determined but should, in practice, be determined by consideration of the consequences of infection in the herd.

RESULTS

Systematic review

In total, 9,782 references from all sources were reviewed by two reviewers as part of stage 1. Figure 1 shows the review process that led to 261 references passing through to stage 2. Over 85% of references excluded by reviewers at stage 1 were excluded on the basis that the references would not contain information about the performance of diagnostic tests in cattle (wrong subject material); between 5% and 7% of references were not immediately passed because there was insufficient information with which to determine eligibility; between 0.5% and 1.5% of references were excluded on the basis that the report was a prevalence or surveillance study, between 3% and 5% of references were rejected on the basis of being a review and not a primary study of test performance, and both reviewers rejected 2% of references because it seemed unlikely that the reference would contain enough data to calculate test performance.

Table 1. Number of references and records with eligible estimates for sensitivity or specificity for diagnostics tests for bTB by year of publication

	Number of				Percent of references by year[a]									
	references		records		1931-69		1970-79		1980-89		1990-99		2000-09	
	Se	Sp	Se	Sp	Se	Sp	Se	Sp	Se	Sp	Se	Sp	Se	Sp
SICCT[b]	15	8	46	18	13	0	20	13	13	0	20	38	33	50
SIT[c]	8	4	18	10	50	25	25	0	0	0	13	0	13	75
Caudal fold	17	2	75	3	12	0	24	50	12	0	35	50	18	0
IFN-γ[d]	27	19	172	145	0	0	0	0	0	0	41	26	59	74
Elisa	23	12	62	27	0	0	0	0	26	25	48	50	26	25
Multiplex immunoassay	1	1	5	4	0	0	0	0	0	100	0	0	100	0
Post mortem	8	1	14	3	0	0	13	0	25	10	0	0	63	0
Culture	8	1	16	1	0	0	25	0	38	0	0	0	38	100
Microscopic examination	13	1	21	1	15	100	15	0	15	0	8	0	46	0
PCR	12	4	25	5	0	0	0	0	0	0	25	50	75	50
Fluorescence PA[e]	2	0	2	0	0	0	0	0	0	0	0	0	100	0
Glutaldehyde	1	0	1	0	0	0	0	0	0	0	100	0	0	0
Latex bead AA[f]	2	1	3	1	0	0	0	0	0	0	0	0	100	100
Serological lateral flow	0	2	0	3	0	0	0	0	0	0	0	0	0	100
Combination tests														
SICCT + IFN-γ	1	0	1	0	0	0	0	0	0	0	0	0	100	0
SIT + IFN-γ	1	0	1	0	0	0	0	0	0	0	100	0	0	0
Caudal fold + ELISA	2	0	8	0	0	0	0	0	50	0	50	0	0	0
Caudal fold + IFN-γ	5	0	8	0	0	0	0	0	0	0	100	0	0	0
Caudal fold + SICCT	1	0	15	0	0	0	0	0	0	0	0	0	100	0
Culture+ Microscopic examination	1	0	2	0	0	0	0	0	0	0	0	0	100	0
IFN-γ+IFN-γ	0	1	0	1	0	0	0	0	0	0	0	0	0	100

[a]Percent reported within each performance indicator (Se or Sp) [b]Single Intradermal Comparative Cervical tuberculin Test [c]Single Intradermal cervical Test [d]Interferon gamma blood test [e]Fluorescence polarization assay [f] Latex bead Agglutination Assay

Following the stage 2 review, 160 of the 261 references were accepted by at least one reviewer. Following the resolution process between paired reviewers 41 references were rejected. Of the 119 references with eligible test performance estimates 100 were in the English language, 10 in Spanish, four in German, one in Chinese, one in Dutch, one in French, one in Italian and one in Polish). The stage 2 review included 14 eligible test types ranging from tuberculin skin tests to blood tests to post mortem (see Table 1). The number of records is also shown since the results from studies on more than one population or by more than one threshold was often reported in the same reference.

Meta-analysis

Probability distributions for Se and Sp were obtained for each test and for modifications of the test where data existed. There were relatively few data per test (see Table 1) which limited the power to detect differences related to (confounding) factors suspected of affecting test performance. For many of the tests pooled estimates were similar to the estimates after modelling and the credible intervals for test performances were wide.

The distributions of test performance resulting from the modelling were input into the FFI model. Table 2 shows the distributions of test performance for the SICCT, Single Intradermal skin Test (SIT), IFN-γ, meat inspection and the multiplex immunoassay after running the FFI model.

Table 2. Test performance of surveillance system components (SSC) evaluated in the freedom from infection model

SSC Code	SSC description	Combination Type	Percentile distributions of herd-level sensitivity[a]			Percentiles of false positives[a] for herd size=100		
			5th	50th	95th	5th	50th	95th
SSC1	SICCT-4[b]	single test	0.29	0.51	0.73	0	0	1
SSC4	IFN-γ BA[c]	single test	0.52	0.67	0.80	2	4	8
SSC15	Meat Inspection	single test	0.44	0.70	0.91	[e]	[e]	[e]
SSC16	Multiplex immunoassay	single test	0.38	0.73	0.94	2	14	46
SSC24	SIT[d]	single test	0.68	0.93	1	1	8	38
SSC26	SIT + IFN-γ BA	retest negatives	0.89	0.98	1	4	12	36
SSC27	SIT + Multiplex	retest negatives	0.90	0.98	1	6	25	57

[a] Derived from 1000 Monte Carlo Markov Chain samples of probability distributions of test performance from meta-analysis [b] Single intradermal comparative cervical tuberculin test at standard interpretation (A "standard" positive result in GB herds is recorded when the bovine reaction after 72 hours is 4mm or more greater than the avian reaction or local clinical signs are present at the bovine site) [c] interferon-gamma assay based on the comparative response to bovine and avian purified protein derivative (PPD) [d] Single Intradermal cervical test [e] No estimates available

Demonstrating and maintaining freedom from bTB infection: Examples using the FFI model

The examples below demonstrate the impact of a range of factors on the ability to demonstrate freedom from infection from GB herds. The model assumes the following conditions for these examples: bTB surveillance history of the herd is available from 01/01/2006 to 01/11/2010, modelling is conducted with time steps of 1 month and the historical probability of freedom calculation is made after 60 months. The number of animals in the herd is assumed to be relatively constant across the time periods although this is likely to fluctuate in reality.

Example 1 is a dairy herd of 100 animals located in an area of high incidence of bTB where the probability of introduction of infection into the herd is approximately 10%. The herd is located in an area that has been subject to annual whole herd skin tests. The farmer sends approximately 7% of the herd to slaughter each year where animals are subject to meat inspection. Given the herd characteristics, testing history and the risk of introduction of infection into this herd, the historical probability of freedom calculated by the model on 01/12/2010 is 0.81 (81%). A summary of the probability distribution for this herd being free from infection is shown in Figure 2. Examination of the historical probability of freedom in the herd shows that it degrades immediately following an annual whole herd test using the SICCT because the probability of introduction of new infection is so high (see Figure 3).

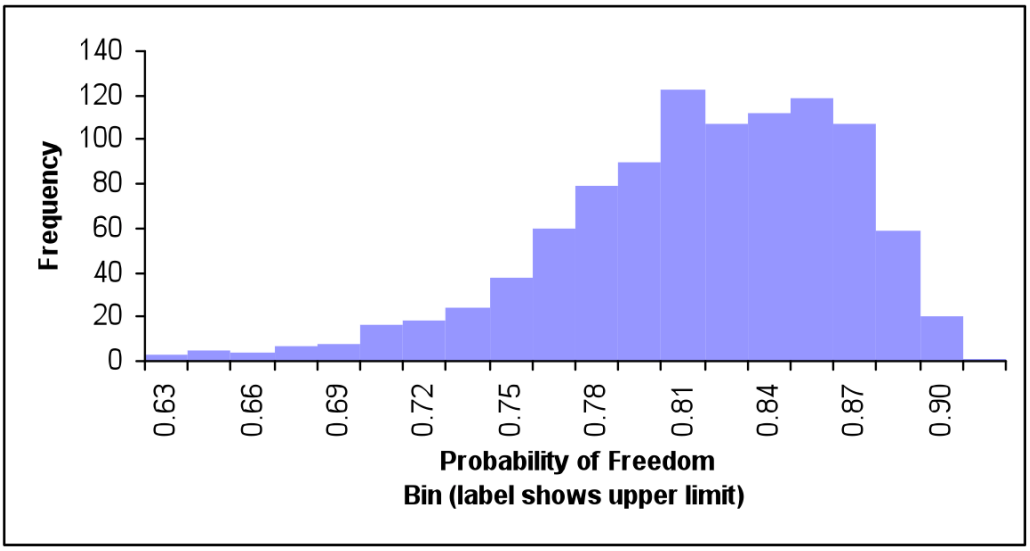


Fig. 2 Probability of freedom distribution for a DAIRY herd of 100 animals in a high bTB incidence area

A target probability of freedom for this herd was set at 98%. The model shows that the SSC Se required to achieve the target freedom of 98% equals 0.91. Using the information on the performance of diagnostic tests in the model, it was apparent that none of the single herd tests were sufficiently sensitive to achieve a probability of freedom of 98%. However the required Se could be achieved through the application of the single intradermal skin test with IFN-γ (SSC26) or the single intradermal skin test followed by multiplex immunoassay (SSC27) (see Table 2), where further positives can be identified through retesting negatives of the other test in the combination.

Figure 4 shows the impact of an increased risk of introduction of infection to 0.4 as a result of poor biosecurity, exposure to infection in local wildlife and neighbouring herds on the DAIRY herd. This figure shows that given a heightened risk of introduction of infection the probability of freedom degrades rapidly between tests over time and the target of 98% probability of freedom from infection cannot be demonstrated or maintained.

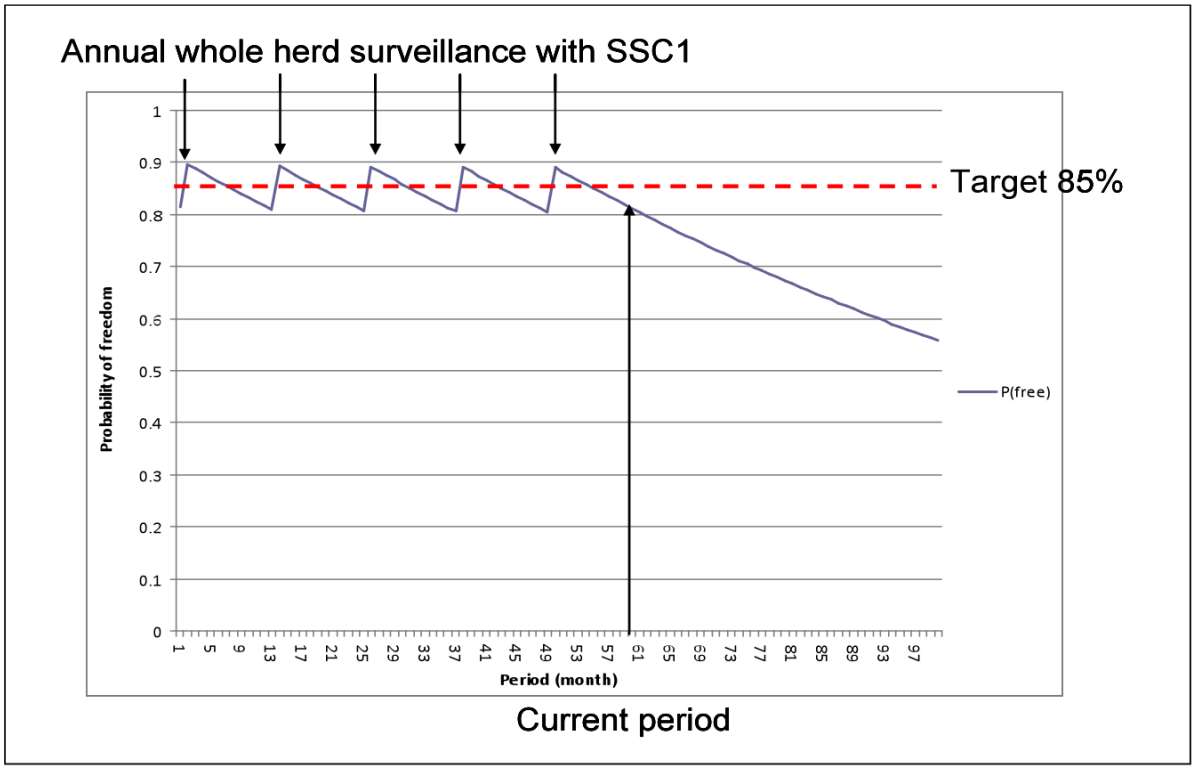


Fig. 3 Historical probability of freedom for a dairy herd of 100 animals in a high bTB incidence area

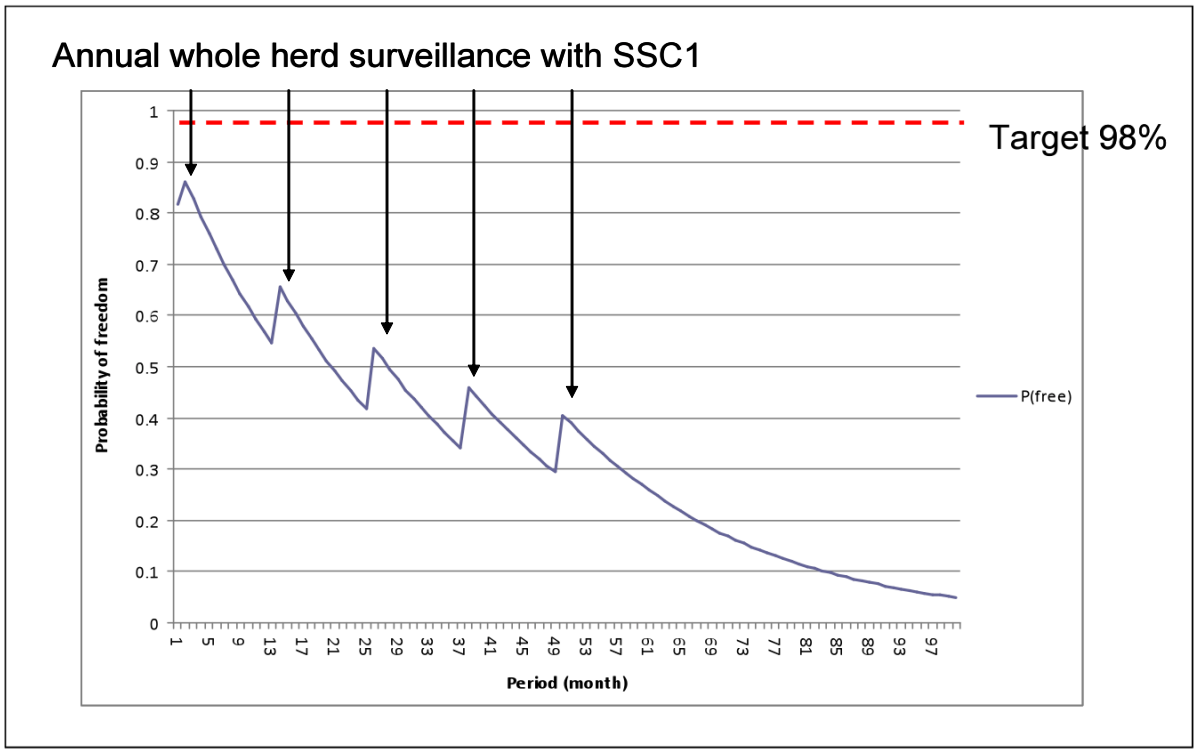


Fig. 4 Historical probability of freedom from bTB for a DAIRY herd of 100 animals with a risk of introduction of infection of 0.4.

The second example is a beef herd of 100 animals located in an area of low incidence where the risk of introduction of infection in to the herd was low (approximately 0.004). The herd was located in a parish testing interval 4 and so was subject to whole herd skin tests every four years. The herd sends approximately 30% of the animals to slaughter each year where they are subject to meat inspection. The target of freedom for this herd was set at 98% since (a) the herd exports animals to other herds and (b) preventing the infection of the local wildlife populations in disease scenario 4 was high priority.

Given the herd characteristics, testing history and the risk of introduction of infection into this herd, the calculated historical probability of freedom = 0.99 (99%). As a result of the low risk of introduction of infection into this herd the low level of surveillance using meat inspection (see table 2) on this herd was adequate to maintain the probability of freedom above 98%. In fact even if this was a dairy herd sending only 7% of the herd to the slaughterhouse per year this freedom from infection target would be met.

DISCUSSION

A comprehensive literature search was conducted to identify primary studies that had measured Se and Sp of diagnostic tests for bTB in cattle. Fourteen different types of tests that would be practical for use in cattle in the present day were identified but the data available per test in order to estimate performance were relatively sparse. The probability distributions of Se and Sp of the different tests were then used in models to show how combinations of test can provide differing levels of evidence that a herd is free from infection with *M. bovis*. These models showed that setting a reasonable target probability of freedom and the risk of introduction of infection into a herd are crucial influences on the success of a control strategy. Where the risk of introduction is high, the current surveillance systems in GB may be inadequate to maintain freedom over time.

We designed a sensitive search for references in order to reduce selection bias as far as possible (Saveleva and Selinski, 2008). Despite an extensive search, comparatively few studies were found with eligible performance data for each test type. At stage 1 almost 10,000 abstracts were reviewed although less than 300 were actually accepted for the stage 2 review. Other work has shown that the terms describing test performance such as “sensitivity” and “specificity” are inconsistently applied and referenced in electronic databases and that it is not possible to design specific searches for test performance given the way these references are currently indexed (Leeflang et al., 2008).

There were relatively few data per test which limited the power to detect differences related to factors suspected of affecting test performance. Of the tuberculin skin tests, the SIT had the highest median value for Se followed by the caudal fold and the SICCT. Test performance of the SICCT in GB was derived from references in the mid 1970s (Lesslie et al., 1975; Lesslie and Hebert, 1975). These studies date from the period before the epidemic of bTB in GB that started in the mid 1980s (de la Rua-Domenech, 2006) and the validity of the results may need re-assessing due to the various changes that have taken place, since then (e.g. changes in quality of testing, tuberculins used, biology of the organism and changes in the cattle industry, for example in the distribution of cattle breeds). However, the estimates were consistent with more recent studies in Ireland and estimates derived from whole herd slaughters.

Using the FFI model it was possible to show that a combination of tests is required to achieve evidence of freedom from infection in herds located in areas of high bTB incidence. Results from the Randomised Badger Culling Trial show that badgers significantly contribute to the incidence of bTB in GB and may contribute to as much as 40% of new herd breakdowns in areas of England and Wales where bTB is endemic (Jenkins et al.; Bourne, 2007). With this level of risk of introduction of infection, tests such as the single intradermal tests and multiplex immunoassay, that are currently not part of routine surveillance in GB, may provide the necessary Se in order to achieve a 98% probability that a herd is free from infection.

However, the Sp of a SSC, designed to maximise Se also means that a relatively large proportion of the herd would falsely test positive. Therefore it is likely that, even if uninfected, the herd would become 'restricted' as a result of this combination of tests which has significant economic consequences. In a herd of 1000 the 50th percentile for false positives is even higher (117 and 249 for SSC26 and SSC27 respectively). However, should the risk of infection of onward spread from this herd be low, for example if animals are sent to slaughter only, it may be justifiable to reduce the target freedom to a more achievable 85% and a wider range of tests would be available. A consequence assessment is required to inform this target setting. Those herds identified as low risk may require less testing or testing with less sensitive tests and avoid the occurrence of false positives from low Sp tests. A herd level risk-based approach would allow the targeted and appropriate allocation of resources to disease surveillance and control.

In conclusion, there are relatively few studies with robust data on the performance of diagnostic tests for bTB. The ability of different surveillance system components and systems to demonstrate and maintain a target probability of freedom from infection varied in different scenarios and was affected by both the historical probability of herd freedom and the current risk of introduction of infection. Under some of the simulated scenarios assumed in the FFI model, where the probability of introduction of infection was high, it was difficult to achieve a high target probability of herd freedom from infection within a short time frame even with a high surveillance system component Se combined with immediate elimination of infected animals from a herd. Strict adherence to the minimum herd testing requirements for bTB prescribed in the EU legislation may not always achieve acceptable probability of freedom from infection. Using the FFI model has the potential to tailor bTB cattle controls to the herd level.

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DOES VIRULENCE DECLINE BY TIME IN WILD BOAR POPULATIONS INFECTED BY CLASSICAL SWINE FEVER VIRUS (CSFV)?

M. LANGE*, S. KRAMER-SCHADT AND H.-H. THULKE

SUMMARY

Classical Swine Fever (CSF) is an endemic viral disease in European wild boar populations causing high economic impact to the pig farming industry. Virulence is a crucial factor determining persistence in local wild boar populations. We considered shift in virulence on the time scale of recent outbreaks using an individual-based spatially-explicit model which represents knowledge on wild boar ecology and CSF virus epidemiology. Two alternative scenarios were supposed as reasonable mechanisms that shift virulence pattern: 1) evolution of pathogen's virulence and 2) selection for host resistance. With both processes we found a possible short term shift to lower virulence. Pathogen evolution, however, resulted in faster decline down to a threshold level, while host selection resulted in slower but continuous decline of virulence. Both mechanisms promoted disease persistence.

INTRODUCTION

Classical Swine Fever (CSF) is a viral disease of *Suidae*, affecting wild boar (*Sus scrofa*) and domestic pigs (Depner et al. 1995; Laddomada 2000; Kaden et al. 2004). The disease is notifiable to the World Organisation of Animal Health (OIE). CSF has serious economic implications for domestic pig farming due to preventive culling and trade restrictions (Meuwissen et al. 1999). Wild boars are assumed to act as reservoir for Classical Swine Fever Virus (CSFV), and 60 % of the primary outbreaks in domestic pig farms are expected to be caused by direct or indirect contact with wild boar (Moenning et al. 1999, Fritzemeier et al. 2000).

Nowadays, CSF occurs worldwide and became endemic in wild boar populations in several European countries. A decrease in virulence during the last decades is assumed to be a crucial reason for disease persistence (Kramer-Schadt et al. 2009). Case mortality, being an expression of virulence, is reported to vary from high to very low, depending on the virus strain (Kaden et al. 2000) and age class of the infected host (Moenning et al. 2003).

Observations from past outbreaks suggested that the case fatality of infected hosts decreases within years or even months of an epidemic in wild boar (Artois 2002; Ruiz-Fons et al. 2008). Virus isolates of recent outbreaks were associated with the moderately virulent genotype subgroup 2.3 (Kaden et al. 2004, Pol et al. 2008). Highly virulent genotypes of group 1 were

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restricted to older isolates (Greiser-Wilke et al. 1998, Fritzemeier et al. 2000) which might indicate a pathogen evolution towards reduced virulence. On the other hand, it is discussed whether case mortality is attributed to the condition of the hosts (Depner et al. 1997). Considering those facts, emergence of moderately virulent strains during the time span of a particular outbreak in wild boars could be reasonable.

We tested temporal dynamics of CSF case fatality under two alternative hypotheses: 1) evolution of pathogen's virulence and 2) selection on host resistance. To that end, a spatially-explicit, individual-based wild boar demography model incorporating social behaviour was coupled with a CSFV model attending individual disease courses (Kramer-Schadt et al. 2009). In the model, case mortality and mean infectious period of lethally infected hosts together represent the virulence pattern of a particular CSFV and are subject to mutation (in pathogen) and selection (in host). Results show that both scenarios – host resistance selection as well as pathogen virulence evolution – are able to decrease virulence pattern over the short term of a particular outbreak.

MATERIALS AND METHODS

Model description

The model is based on the approach by Kramer-Schadt et al. (2009). It is described following the ODD protocol (Overview, Design, Details; Grimm et al. 2006, 2010). Sub-models are only partially presented in case they are essential to understand the applied model. For a complete documentation we refer to the online repository (http://ecoepi.eu/CSFWB).

Overview: The CSF wild boar model is a compilation of a spatially explicit, stochastic, individual based demographic model for wild boars (*Sus scrofa*), and an infection and disease course model for CSFV.

Purpose: The model serves for testing the hypothesis of a decreased virulence by either host selection or pathogen short-term evolution in outbreaks.

State variables and scales: The model is composed of two major components, 1) a wild boar demography model considering seasonal reproduction, dispersal and mortality, and 2) a CSF virus model operating on the wild boar population. Wild boar population density and structure are affected by the disease via virus-induced mortality and litter size depression.

All processes take place on a raster map, where each cell represents a functional classification of a landscape into breeding capacity, denoting habitat quality by the number of female boars that are allowed to have offspring. Thereby, density is regulated in the model. The model landscape is represented by a grid of square cells, each representing 4 km² and encompassing a home range of a group of wild boars (Leaper et al. 1999).

The crucial model entity is the individual wild boar, characterised by age in weeks (where one week represents the approximate CSF incubation time; Artois et al. 2002, Moenning et al. 2003), resulting in the age classes piglet (< 8 months ± 6 weeks), yearling (< 2 years ± 6 weeks), and adult. Each host has a location, which denotes its home range cell on the raster grid as well as the individual's family group. Further state variables of the host are the demographic status (disperser or not) and the epidemiological status (susceptible, transiently infected, lethally infected with varying survival times or immune by surviving the infection, vaccination or

maternal antibodies). Each host has a probability of virus-induced death following infection (*M*) and a 'mean' infectious period conditioned on lethal infection ( ).

Process overview and scheduling: The model processes in weekly time steps. Processes of each time step are: infection, wild boar group splitting, reproduction, death and ageing, executed in the given order. In the first week of each year, mortality probabilities are assigned stochastically to represent annual fluctuations in wild boar living conditions, and they are assigned to breed or not, according to the carrying capacity of their home range cell.

Design concepts: Wild boar population dynamics emerge from individual behaviour, defined by age-dependent seasonal reproduction and mortality probabilities, and age- and density-dependent dispersal behaviour, all including stochasticity. The dynamics of the infection emerge from within- and between-group virus transmission, boar dispersal, individual stochastic disease courses, and infectious periods for infected wild boars. The parameter which reflects host mortality induced by infection is subject to alterations according to the simulated scenarios (see below).

Stochasticity is included by representing demographic and behavioural parameters as probabilities and probability distributions, respectively. Annual fluctuations of living conditions are accounted for by varying mortality rates. Course and duration of infection are modelled explicit and stochastic, since the variation in disease outcome between individuals was identified as essential for virus endemicity without reservoirs (Kramer-Schadt et al. 2009).

Initialisation: The model landscape represents 200 km by 50 km of connected wild boar habitat without barriers. The 2,500 grid cells are initialised randomly with uniformly distributed integer breeding capacity values $C_{ij} \in \{1, 2, \ldots, 9\}$. Thus, the mean breeding capacity is 5 females per cell, resulting in approximately 20 boars per cell or a host density of 5 boars per km² (Howells & Edwards-Jones 1997). One wild boar group is released to each habitat cell and group size was three times the local breeding capacity. Initial age distribution is taken from the results of a 100 years model run, conducted by Kramer-Schadt et al. (2009).

Input: The applied model setup does not include any external inputs or driving variables.

Sub-models: In this section we describe sub-models crucial for the simulation experiments. For detailed sub-model documentation we refer to the authors' online repository (http://ecoepi.eu/CSFWB).

Transmission:

Transmission is modelled stochastically. Parameters determine the weekly probability to receive an infection from an infectious group mate $P_{\mathrm{inf}}^{(i)}$ and the probability to receive an infection from an infectious animal in a neighbouring group $P_{\mathrm{inf}}^{(e)}$. For each susceptible animal the probability to become infected accumulates over all infectious animals within the group and in the neighbourhood. Infection might be translocated within the host population during dispersal of sub-adult females.

The transmission parameter was reversely fitted to recorded disease spread velocity of approx. 8 km per quarter (Rossi et al. 2010). The resulting parameter values were assigned constant as $P_{\mathrm{inf}}^{(i)} = 2.08 \cdot 10^{-2}$ within and $P_{\mathrm{inf}}^{(e)} = 2.08 \cdot 10^{-3}$ between groups.

Disease course:

The disease course sub model is described by two parameters: individual case mortality M and , the mean infectious period of lethally infected hosts. On infection, the host is stochastically assigned either as lethally infected (with probability *M*) or as transiently infected (1-*M*). *M* applies unchanged for yearlings $M^{(y)}$, is decreased for adults to $M^{(a)} = M^2$ and increased for piglets to $M^{(p)} = \sqrt{M}$ to represent age-dependent disease outcomes (Dahle & Liess 1992). Transiently infected wild boars are infectious for one week and turn immune three weeks later (Artois et al. 2002; Moenning et al. 2003). Differing from the approach of Kramer-Schadt et al. (2009), the infectious period (in weeks) of lethally infected hosts is drawn from an exponential distribution with mean . Lethally infected hosts remain infectious until death.

Highly-lethal CSFV outbreaks are expected to coincide with short mean infectious period of lethally-infected hosts while longer mean infectious period of lethally-infected hosts should coincide with moderate case-fatality (Day 2003). In the model this qualitative correspondence is represented ad hoc according to:

$$\mu = (1 - M)^x \cdot s + 1. \qquad (1)$$

In these *M* – – relations exponent *x* and scaling factor *s* are chosen to represent linear or quadratic relations (Fig. 1 a) (see section 'Simulation experiments'). In the simulation scenarios, i.e. pathogen's virulence evolution and host resistance selection, case fatality *M* is modulated directly and the mean infectious period indirectly via Equation (1).

Host selection:

Each individual host inherits its case mortality value $M_{offspring}$ from the mother (M_{sow}). In case of lethal infection, the individual's infectious period is drawn from the exponential distribution with mean according to Equation (1), inserting $M_{offspring}$. At beginning of a simulation run, individuals' case mortality *M* is drawn individually from an inverse beta distribution 1–*Beta*(,), i.e. the probability of transient infection is distributed as *Beta*(,). Parameters and were selected to achieve mean case mortality of all yearlings of $M^{(y)} = 0.8$. Case mortality of adults $M^{(a)}$ and piglets $M^{(p)}$ is determined according to section 'Disease course'. The resulting average case mortality over the entire population and one simulated year was 0.76.

Pathogen evolution:

At infection, each individual host inherits case mortality $M_{inherited}$ from the infecting host ($M_{transmitted}$). Mutation is introduced by a randomised shift of case mortality:

$$M_{inherited} = M_{transmitted} + r(b_{virus}) - \frac{b_{virus}}{2} \qquad (2)$$

where b_{virus} is twice the maximum shift and $r(b_{virus})$ is a uniformly distributed random number drawn from $U(0, b_{virus})$. In case of lethal infection the corresponding mean infectious period is derived from eq. (1) using $M_{inherited}$.

Case mortality of the initially released virus is $M = M^{(y)} = 0.8$. Case mortality of adults $M^{(a)}$ and piglets $M^{(p)}$ is determined according to section 'Disease course'. The resulting average case mortality over the entire population and one simulated year was again 0.76.

Parameters, simulation experiments, analysis

Independent variables: The primary independent variables of the study are the virulence evolution scenarios (i.e. host selection versus pathogen evolution), the M – – relation, the mutation strength of the virus b_{virus} for pathogen evolution, and the initial distribution of the expected case mortality (i.e. parameters and of the beta distribution) for host selection.

Dependent variables: The average case mortality over the entire population of hosts and over each simulated year was measured. Moreover, the time point of each virus extinction event was recorded.

Simulation experiments: Evolution scenarios were either host selection or virus evolution. Both scenarios were simulated for linear and quadratic M – – relations (Fig. 1 a). Parameters of Equation (1) are:

- Linear relation: $x = 1$, $s = 10$
- Quadratic relation: $x = 2$, $s = 16.875$

where s was chosen to achieve for both scenarios equal maxima of the effective mean infectious periods $T_{inf} = 3.5$ weeks over all infected hosts (Fig. 1 b).

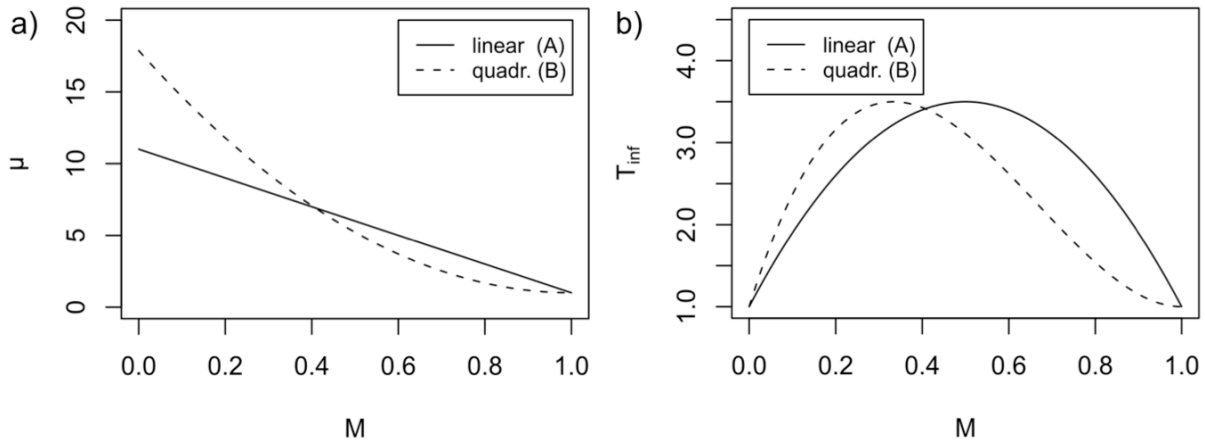

Fig. 1 a) Mean infectious period μ of lethally infected hosts, and b) effective mean infectious period T_{inf} over all hosts, as function of average case mortality M for different M – – relations $\mu = (1 - M)^x \cdot s + 1$. Solid: $x = 1$, $s = 10$ (linear), dashed: $x = 2$, $s = 16.875$ (quadratic).

For reference, simulations were performed with linear and quadratic M – – relation but without evolution or selection.

For pathogen evolution, different realisations of the mutation strength parameter were simulated: $b_{virus} \in \{0.0, 0.01, 0.05, 0.1\}$.

For host selection, two alternative beta distributions were used for initialisation of individual host's case mortality values: $1 - Beta(0.5, 2)$ (solid in Fig. 2) and $1 - Beta(2, 8)$ (dashed in Fig. 2). For both scenarios, however, average case mortality over the entire initial population was kept as 0.76.

All simulations were performed for 40 years or until host or virus went extinct. The virus was released to the boar population in a random week of the sixth year by infection of one randomly selected boar individual.

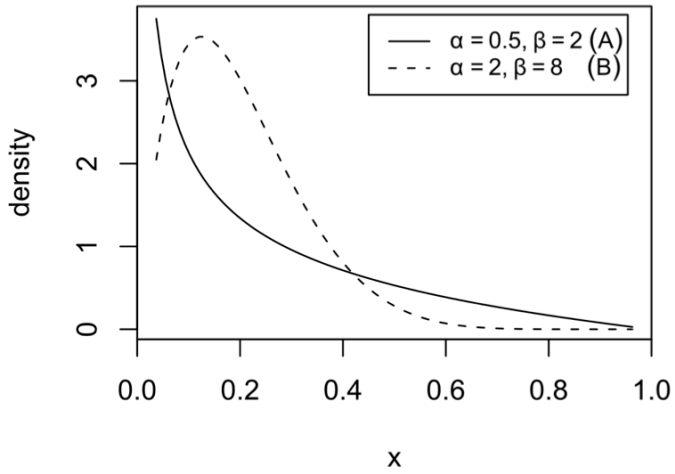

Fig. 2 Probability density functions of beta distribution as used for initialisation of individual's case mortality parameter in the host selection scenario.

For each scenario, 500 model runs were conducted to achieve minimum precision of $\pm 5\%$ with 95% confidence for proportions.

Analysis: Aggregated case mortality values, $\overline{M}$, were calculated over 500 repetitions over the 52 weeks of each year, weighted by the number of hosts receiving an infection in the particular week. The mean infectious period after lethal infection and the effective mean infectious period T_{inf} over all hosts infected in a particular week were calculated from aggregated case mortalities $\overline{M}$ (Fig. 1).

Survival curves of the virus in the host population were determined by calculating the proportion of runs in which the virus was not yet extinct until the particular time step.

Analysis was performed using GNU R 2.9.2 (R Core Development Team), plots were created with SigmaPlot® 10.0 (Systat Software Inc.).

RESULTS

Fig. 3 shows the simulation output in terms of the evolving $\overline{M}$, i.e. aggregated case mortality, during simulation for the two scenarios, being pathogen evolution and host selection, and the two M – – relations (linear and quadratic). According to Fig. 1 b the maximum effective infectious period T_{inf} (i.e. 3.5 weeks) was expected to be achieved with $\overline{M} \approx 0.5$ for the linear M – – relation and $\overline{M} \approx 0.33$ with quadratic M – – relation. Next we explore the simulated dynamics of $\overline{M}$ values over the time course of 35 years and the two evolution scenarios.

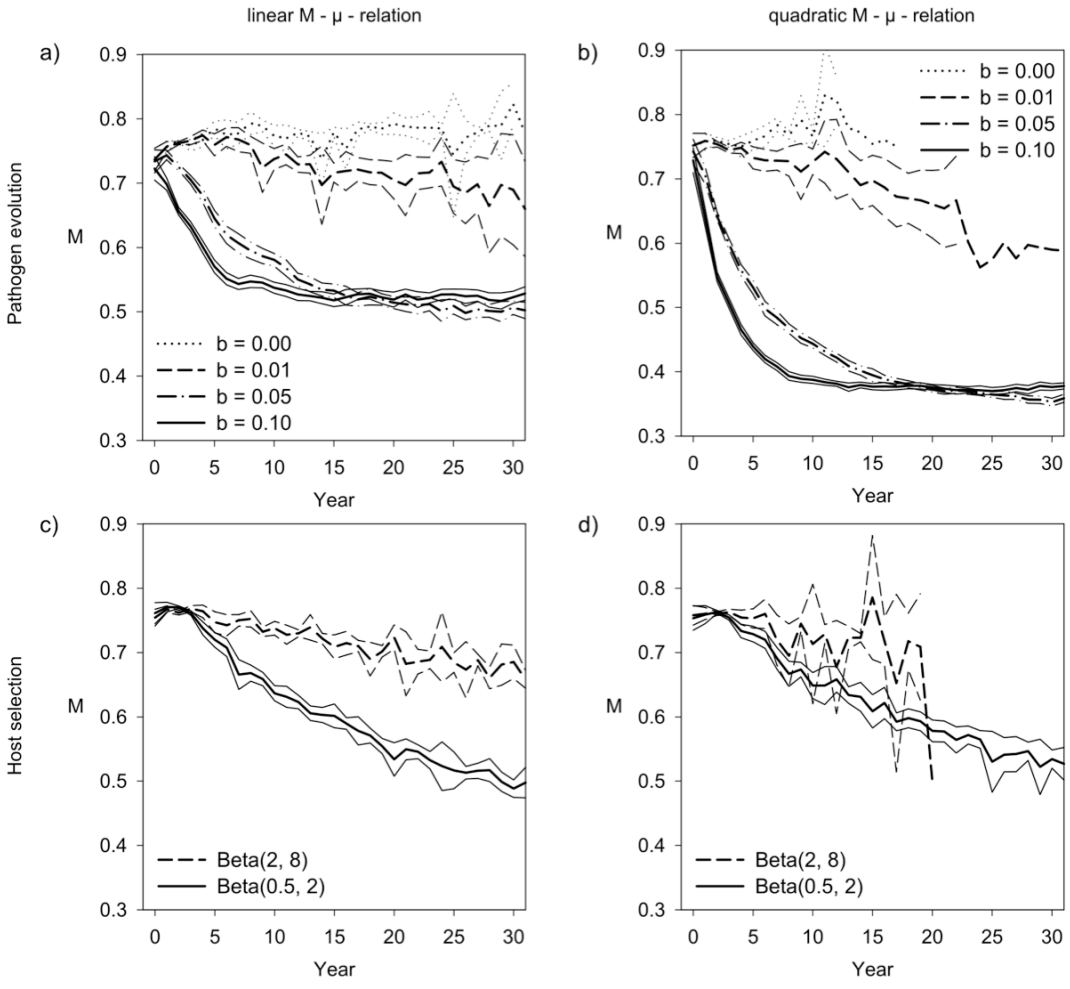

Fig. 3 Mean (thick) and 95 % confidence intervals (thin) of aggregated case mortality $\overline{M}$ for a), b) pathogen evolution, c), d) host evolution with a), c) linear, b), d) quadratic M – – relation.

Pathogen evolution

For pathogen evolution, aggregated case mortality $\overline{M}$ decreased immediately after virus release. The speed of decline depended on mutation frequency, i.e. parameter b_{virus} (Fig. 3 a or b). With high mutation force, $b_{virus} = 0.1$, aggregated case mortality $\overline{M}$ rapidly converges to the threshold value, i.e. 0.5 for the linear and 0.33 for the quadratic relation, which results in a maximum mean infectious period $T_{inf} = 3.5$ weeks. With the linear M – – relation $\overline{M}$ became

less than 0.55 after 7 years of virus perpetuation (solid in Fig. 3 a), and falls below 0.4 after 8 years of virus perpetuation with the quadratic relation (solid in Fig. 3 b).

For an intermediate mutation force b_{virus} = 0.05, virulence shift was slowed down, but the case mortality corresponding to the maximum effective infectious period was still reached within the simulated 35 years after virus release (dash-dotted in Fig. 3 a and b; $\overline{M} < 0.55$ after 11 years with the linear M – – relation, and $\overline{M} < 0.4$ after 15 years with the quadratic relation). For even lower mutation force b_{virus} = 0.01, shift in $\overline{M}$ values was very slow and the maximum effective infectious period was not achieved within the simulated time span (dashed in Fig. 3 a and b).

Host selection

Under the host selection scenario, a 3 to 4 years phase of unchanged aggregated case mortality $\overline{M}$ with decreasing variation between runs was followed by continued decrease of aggregated case mortality values ($\overline{M}$) (see Fig. 3 c and d) independent of the applied M – – relation.

The temporal dynamics of $\overline{M}$ were strongly influenced by the distribution of M in the initial population (see Fig. 2):

When initial case mortalities were drawn from the beta distribution *Beta*(2, 8), the fraction of "resistant hosts", i.e. hosts with low case mortality value, was very small which resulted in a very slow decrease of $\overline{M}$ (dashed in Fig. 3 c and d).

When initial values of M were drawn from the beta distribution *Beta*(0.5, 2), the larger fraction of hosts with a low value of M allowed for faster decline of $\overline{M}$ (solid in Fig. 3 c and d). The value of $\overline{M}$ that corresponds to the maximum effective infectious period T_{inf} = 3.5 weeks (i.e. $\overline{M}$ = 0.5, see Fig. 1 b) was achieved by year 29, assuming the linear M – – relation (solid in Fig. 3 c), and not approached ($\overline{M}$ = 0.33) for the quadratic relation (solid in Fig. 3 d).

Persistence

The survival curves of the simulated combinations of scenarios and M – – relations showed a similar extinction pattern (Fig. 4, selected scenarios). Within the first year after virus release, the virus extincted in a noticeable proportion of simulations (30 – 40 % for linear M – – relation, about 50% for all scenarios with quadratic relation). The initial phase was followed by about 3 years of stability, i.e. very few virus extinctions, designating the epidemic phase of the outbreak (EFSA 2008). From year 4 to year 8, extinction probability was high again, denoting the transition between the epidemic and endemic phase. In the model the virus had achieved the edges of the simulation area and could no longer conquer unaffected regions. If the transition to the endemic phase had happened, only stochastic extinction events determine the progress of the survival curves (May 1976).

Both evolution scenarios increased the probability of long-term persistence of the virus in the simulated population compared to the reference scenarios (dotted lines in Fig. 4). Only pathogen evolution reduced the case mortality sufficiently fast to efficiently increase disease persistence beyond year 4 (dashed in Fig. 4). Especially in the transition between epidemic and endemic phase (approx. 4^{th} – 8^{th} year in Fig. 4), where the disease fades out with high

probability (solid and dotted lines in Fig. 4), pathogen evolution strongly increased virus survival, since case lethality already had decreased and hence the effective mean infectious period had been maximised during the preceding phase.

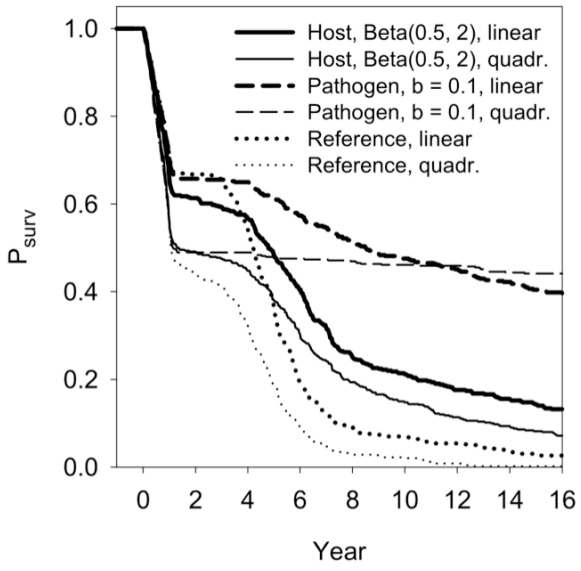

Fig. 4 Virus extinction curves.

DISCUSSION

The perception of decrease in severity of CSF outbreaks in wild boar was investigated. Such an alteration of severity is usually associated with fewer animals that are dying due to a CSFV infection. Decrease in severity may either be related to one outbreak, i.e. a very rapid dynamic (Artois et al. 2002; Ruiz-Fons et al. 2008); or over many outbreaks, i.e. a slow dynamic continued over several populations (Greiser-Wilke et al. 1998). Rapid decrease in severity may be associated with reducing virulence of the virus (Leifer et al. 2010) or with increasing host resistance (Depner et al. 1997). How far these changes could contribute to a perceived decrease in case mortality and a dramatically prolonged persistence of virus in natural populations as observed in the last decades in Europe (Rossi et al. 2005; EFSA 2008; von Rüden 2008) remains an important issue.

Severity or virulence evolution via host selection or pathogen evolution was investigated using an established epidemiological model (Kramer-Schadt et al. 2009). The analysis focussed on the evolution of virulence in terms of case mortality *M*, the dependent mean infectious period of lethally infected hosts (Day 2003), and the impact of virulence evolution on possible long-term disease persistence. The stochastic, individual-based, spatially-explicit modelling approach was chosen to cope with individual variability, stochasticity, and space as these were foreseen to influence disease dynamics. The model is able to reflect individual disease courses, ecological traits of the host and density dependent disease and population dynamics based on existing knowledge, e.g. Artois et al. (2002), EFSA (2008) or Kramer-Schadt et al. (2007).

The infection of individual wild boar with CSFV is well known to vary with regard to the disease outcome (EFSA 2008). Transient infections with subsequent recovery after short infectious periods are reported as well as lethal infections that results in animal dying quickly

within 4 weeks, or chronic which allowing long infectious periods of up to 120 days (Kramer-Schadt et al. 2007). The feature is represented in the model by stochastic case mortality (parameter *M*) and stochastic life-expectancy of lethally infected hosts (parameter as mean). In the field the actual case mortality will be the only pattern to be recorded and, hence, the pattern that determines perceived severity of an outbreak. In order to mimic the perception of reduced disease severity, we incorporated mechanisms that modulated case mortality *M* in the model version. The modulation was driven by two processes that reflect either pathogen evolution (i.e. selection on *M* due to the dependent infectious period) or host selection (i.e. selection on *M* due to survival of the infected hosts). To underpin the temporal dynamic of the two processes, alternative mutation forces and initial distributions of the characteristic *M* in the host population were considered without being exhaustive.

The approach to model and parameterise selection or adaptation was considered as plausible but ad hoc and served as a pathway to understand the quantitative effect on case mortality and persistence in a qualitative relationship. The model does not consider the relationship between virulence and infectiousness, which could slow down virulence shifts in an advanced stage of an outbreak.

Evolutionary scenarios

Assuming appropriate parameters, host selection and pathogen evolution show an efficient decline in virulence over the period of a particular outbreak. Evolution of the pathogen occurs much faster than host selection due to shorter "generation times". The generation time of the pathogen which is decisive for evolution equals the time span from infection of the host till infection caused by this particular host. The mean generation time is thus even shorter than the mean infectious period T_{inf}. For host selection, the evolutionary decisive generation time equals the generation time of the host, i.e. at least 6 months.

Starting with high virulence, i.e. high case mortality and a short infectious period after lethal infection, host selection as well as pathogen evolution could decrease virulence with both *M* – – relations. Pathogen evolution with strong mutation forces b_{virus} caused a rapid virulence decrease already in the epidemic phase (1^{st} – 4^{th} year in Fig. 3 a and b). Host evolution, on the other hand, caused a slower virulence shift which starts with the endemic phase (Fig. 3 c and d) as during the epidemic phase only parts of the population were affected where no selection took place yet.

Evolution of the pathogen is driven by the mean infectious period T_{inf}. The 'virus strain' with the longest mean infectious period has the highest rate of secondary infection, i.e. the highest basic reproduction number R_0, and can thus reproduce most successfully. Simulations start with one 'defined virus', so the rate of virulence shift over time is determined by the mutation force parameter b_{virus}. A higher b_{virus}, results in a faster virulence shift. Pathogen evolution works towards the case mortality value where the infectious period achieves its maximum of T_{inf} = 3.5 weeks. The quadratic *M* – – relation thus causes convergence to a lower mortality level (*M* = 0.33), compared to the linear relation (*M* = 0.5) (see Fig. 1 b)).

Host selection is driven by the survival probability of infected hosts. Hosts that are more resistant have a higher probability to survive infection, thus have a greater reproductive success. The rate of virulence shift over time is determined by the distribution of potential case mortality in the initial host population. A higher fraction of hosts with a low potential case mortality, i.e. the more "resistant" hosts, results in a faster shift in virulence. Host resistance selection favours

hosts with a low probability of virus-induced death. It thus works towards continuously lowering case mortality.

Persistence

Virus survival curves revealed the characteristic pattern of invasion, epidemic, and endemic phase (EFSA 2008; Kramer-Schadt 2009). After virus release a high proportion of simulated outbreaks faded out within the first year. For the quadratic M – – relation, the probability of early virus extinction was much higher (50%) compared to the linear relation (30 – 40%). The difference is caused by a higher mean infectious period T_{inf} in the linear case, causing a higher probability of secondary infections. Obviously this dynamic is mainly independent of the assumed evolutionary scenario as the selection processes were not functioning while the outbreak had already faded out.

From years 1 to 4 after virus release when, in the epidemic phase, the infection wave progresses through the naïve population, rather few extinctions occur. This time, which is basically defined by the spatial extent of the wild boar population (not varied in our simulation), is available for the selection mechanisms, i.e. towards host resistance or low virulence (less lethal).

During the transition to the endemic phase that followed, extinction probability rose again. As Kramer-Schadt et al. (2009) have shown, only outbreak simulations with moderate virulence could survive the transition towards the endemic phase, hence getting long-term persistent as observed in France and Germany. Virus persistence in our simulations was thus determined by the successful evolution towards reduced virulence features, i.e. lower case fatality. Persistence was increased by host selection as well as pathogen evolution where suitable parameters (= 0.5, = 2 and $b_{virus} \geq 0.05$, respectively) allowed a fast decrease of virulence. Pathogen evolution exhibits a faster decline in mortality towards maximum effective mean infectious period T_{inf} (Fig. 1 b), resulting in higher virus persistence probability in the endemic phase.

When comparing linear versus quadratic M – – relations, the probability of virus presence with the quadratic relation exceeded the one for the linear relation after 12 years, although showing a lower value at the start of the endemic phase. This phenomenon can not be explained by the effective mean infectious period T_{inf}, since its maximum value, as the target of pathogen evolution, is equal for both scenarios. Instead the further reduction of case mortality, M and the resulting higher population densities for the quadratic M – – relation decreased the extinction probability in the endemic phase.

The evolutionary gain driving a virulence decrease in the short term of a particular outbreak induces an advantage for the virus in the long term too. Prolonging the mean infectious period of affected hosts, the decline in virulence promotes maintenance of the infection chain and thus facilitates disease persistence. A virus strain persisting in a local population has an increased chance to cause further outbreaks in neighbouring or remote areas.

Conclusions

The perception of declining case fatality in the course of an outbreak would have been possible with both mechanistic scenarios, although the smooth dynamics with host selection might be less apparent in the field. Both host mediated and pathogen mediated mechanisms producing lower case fatality result in an increased probability of persistence. However, the shorter generation time of pathogen mediated selection enables a rapid dynamic that might be

sufficient to reach an endemic situation within reasonable time of a CSFV outbreak in wild boar, e.g. about 8 months in the French Vosges mountains or German Pomerania. Future research is required to investigate the implication of both assumptions with regard to the apparent picture if the virus had invaded new, naïve populations in the past, and how long highly virulent situations were actually observed.

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NETWORK MODELLING

ADDING THE SPATIAL DIMENSION TO THE SOCIAL NETWORK ANALYSIS OF AN EPIDEMIC

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SUMMARY

Equine influenza spread rapidly through the horse populations of two Australian states in 2007; the first reported outbreak in Australia. Over 80% of this epidemic's geographic spread occurred during the first two weeks, through the movement of infected horses prior to implementation of movement controls. This study demonstrates a novel network analysis framework that integrates contact-tracing and spatial data enabling investigation of transmission through a network followed by secondary local spread into spatially defined clusters.

Combined analysis of spatial and contact network data demonstrated that local spread emanated outwards from the small number of infected premises in the contact network, up to a distance of around 15 km. Spread was classified as 36% 'network-associated' and 64% 'local spatial spread', leading to the formation of 44 clusters. This novel method provides a tool for describing an epidemic and defining spatial clusters when a contact network structure underlies spread.

INTRODUCTION

Emerging infectious disease outbreaks often spread undetected for a period of weeks. Initial spread is commonly through contact networks, seeding clusters of cases that are soon detected in widespread locations. These contact networks often follow major transportation or trade routes between large cities, or between gatherings of animals such as markets. Several recent epidemics in both humans and livestock have followed this paradigm, with their subsequent size and spatial pattern driven by network-based spread prior to their detection. For example, the index cases of foot-and-mouth disease (FMD) in the 2001 United Kingdom outbreak were detected in south-east England nearly a week after infected sheep had entered the market chain in the north-east of the country, leading to a widespread epidemic (Gibbens & Wilesmith, 2002). The aim of this study is to demonstrate the utility of a novel network analysis framework that combines contact-tracing and spatial data to enable investigation of infectious disease spread through a network followed by secondary local spread into spatially defined clusters.

Contact-tracing involves interviewing known cases to establish their links to other individuals during the time periods when they were most likely infected and infectious. The aim is to identify other cases through tracing-backwards to uncover the source, and tracing-forwards to identify subsequent cases. Successful contact-tracing provides an incomplete but nonetheless

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useful overview of the epidemic, and defines the population at risk, enabling the implementation of appropriate and targeted interventions.

Empirical research into the spread of H5N1 avian influenza (Small et al., 2007) has shown that when a complex contact network structure underlies an epidemic, traditional approaches may be insufficient to appropriately describe the spatiotemporal pattern of the epidemic and estimate key parameters. For example, estimation of the effective reproductive rate (R) relies on the assumption of mean field spread (i.e. random mixing), however the underlying contact network structure constrains spread such that this assumption is violated. To this end, social network analysis (SNA) has been used to study contact-tracing data from a range of epidemics (Shirley & Rushton, 2005; Small et al., 2007).

A network, social or otherwise, is a set of connections amongst a set of nodes. Networks can be used to represent the patterns of connectivity of populations, and therefore describe aspects of disease transmission that depart from the mean field model. Depending on the disease context, a network can be constructed from contact-tracing data representing different units of interest. Nodes can represent either individual animals or people, or collectives such as farms, offices, schools, nursing homes or hospitals. The connections can represent a wide range of context-dependant relationships or links including actual physical contact, movement of a resource from one node to another, or the sharing of a space at a given point in time. The links thus formed may be either undirected or directed depending on whether a resource can or cannot travel in both directions between the pair of nodes. Undirected links can be represented in directed networks by two directionally-opposed directed links. Links may also be either valued or dichotomised, depending on whether or not the magnitude of flow is contextually important.

In 2007, equine influenza A/H3N8 spread rapidly through the horse populations of two Australian states; the first reported outbreak in Australia. Only small numbers of imported horses had been recently vaccinated, leaving almost the entire horse population susceptible. During the ensuing four-month epidemic approximately 70,000 horses were infected on over 9,000 premises. Most of the geographic spread of this epidemic occurred undetected during the first ten days, through the of movement of infected horses prior to the complete implementation of movement restrictions (Cowled et al., 2009).

Clinical signs were first observed in a horse in quarantine 40 km west of Sydney, then five days later in two horses kept in a large horse agistment and training facility in central Sydney (Centennial Parklands Equestrian Centre). These horses had attended an equestrian event on the previous weekend (17–19 August 2007). Contact-tracing revealed numerous infected premises linked to horses returning from two equestrian events (Callinan, 2008). Movement restrictions were progressively implemented from 25 August 2007; subsequent spatial spread was believed to be driven by local spread mechanisms (direct contact, fomite and windborne transmission) from those premises infected in the first weeks of the epidemic.

This study describes the application of social network analysis to investigate the early spread of the 2007 outbreak of equine influenza in Australia, identifying spread through a contact network of infected horse movements and secondary local spatial spread. The methods used may be applied to contact-tracing data from other epidemics where disease transmission occurs initially through major transportation networks followed by local spread to adjacent farms or other similarly structured groupings.

MATERIALS AND METHODS

Networks were constructed based on epidemiological data collected during the outbreak: a 'contact network' of horse movements between all premises infected in the first ten days of the outbreak; a 'proximity network' based on a distance matrix between these early IPs; and a 'contact-and-proximity network' constructed by combining the contact network with the proximity network. Characteristics of these networks were analysed and compared to identify key characteristics of the spread during the early phase of this epidemic.

The networks were constructed from three datasets provided by the New South Wales (NSW) and Queensland state governments: a contact-tracing dataset, an infected premises dataset and an all-horse-premises dataset. The contact-tracing dataset included 1,052 reported horse movements onto and off premises investigated by NSW and Queensland animal disease authorities during the outbreak. Each movement record included the date of the movement, the addresses and unique identifiers of the origin and destination premises. Most of these records were retrospectively traced backwards by these authorities during interviews of horse owners and managers whilst attempting to establish the source of infection and implement quarantine and horse movement restrictions.

The infected premises dataset included attribute data such as address, geocoded coordinates (based on property centroid), number of horses, and date of onset of first clinical signs in the first horse affected ('onset date'), and was linked to a database of laboratory testing records. Premises were defined as infected if they held horses that had been observed with the classical clinical signs of equine influenza (cough, elevated temperature, nasal discharge and lethargy) and if the diagnosis had been confirmed by laboratory testing based on real-time reverse transcription polymerase chain reaction assay (Foord et al., 2009). The all-horse-premises dataset included similar data on all non-infected horse premises from outbreak affected areas and when combined with the infected premises attribute dataset could be considered a quasi-census of these regions in 2007. All data were collated into a relational Microsoft Access 2007 database (Microsoft Corporation, Redmond, WA, USA) and then exported for further analyses into the R statistical package version 2.12.0.

Network construction

In all networks, the nodes represented all premises holding horses infected in the first ten days of the epidemic. These early IPs were selected from the infected premises dataset, allowing for the one to three day incubation period of equine influenza (Myers & Wilson, 2006) and a one day margin of error in observation (i.e. IPs with an onset date prior to 31 August 2007, the fourteenth day of the epidemic).

The contact network of infected horse movements was constructed by adding directed links representing horse movements originating from infected premises until day ten of the outbreak. As data was not available on actual numbers of horses moved, these links were binary in that movement of a single horse was treated equivalently to several horses moving between two premises. The date of movement was included as an attribute of all such contact links to maintain the temporal dimension. All of the back-traced horse movements occurred prior to disease detection, with infected horses being moved whilst incubating the disease. Infected horses can shed virus as early as 24 hours after infection, and one day before developing clinical signs (Myers & Wilson, 2006). Consequently, movements were only included if they occurred on or after the day before clinical signs were first observed on the originating premises, and if

clinical signs were observed at the destination premises on or after the day of movement. Premises were considered infectious for up to ten days (Myers & Wilson, 2006). A one day margin of error was again included in these calculations to allow for inconsistent observation and reporting practices.

The proximity network of infected premises in space was constructed based on a matrix of the distances between each pair of early IPs in the contact network, using the spatial coordinates of the centroid of each premises. Pair-wise distances between premises were calculated using the SPATSTAT library in R, the resulting distance matrix was dichotomised at a distance cut-off used to represent an assumption of the maximum distance over which local spread might have occurred. A 5 km cut-off was selected for initial analyses based on previous empirical research which suggested that local transmission occurred over approximately 5 km (Firestone et al, in press). Two directed links (in opposing directions) were added to the proximity network between nodes representing premises separated by less than the respective distance cut-off. Spatial coordinates were included as node attributes in all networks to maintain the spatial dimension.

To produce the contact-and-proximity network, first, the contact and proximity networks were transformed into valued networks by nominally weighting their links. When combined, this allowed differentiation in the contact-and-proximity network between pairs of nodes that were connected by contact only ('1'), proximity only within the respective distance cut-off ('2') or contact and proximity ('3'). The contact-and-proximity network was therefore valued and directed, with proximity (within the distance cut-off) represented by two opposing edges between a pair of nodes.

Network analyses

All network analyses were conducted in R using the STATNET library. Networks were described in terms of their size, centrality and cohesion and graphs were constructed for each network incorporating temporal and spatial dimensions. Two classes of network sub-structures were identified highlighting important movements and premises: components and cutpoints; a component being a subset of nodes connected to each other, and cutpoints being nodes that if deleted would fragment the network into a larger number of smaller components (Wasserman & Faust, 1994). In directed networks, two types of components may be defined: 'strong components' where every node within the subset can be reached from every other node obeying the direction of links, and 'weak components' where the directions of the links are disregarded (Wasserman & Faust, 1994). From random network theory, it is known that if links are added randomly to an empty network a tipping-point is rapidly reached above which one very large ('giant') component is created that is much larger than the next largest component (Erdős & Rényi, 1961). The relative proportions of premises infected by horse movements (as described by the contact network), local spatial spread (described by the contact network), and unexplained (isolates in the contact-and-proximity network) were estimated by calculating the number of nodes in the giant weak component (GWC) of each network.

The centrality of each individual node, and its potential importance to disease flow within each network, was analysed by calculating the betweenness, in-degree, out-degree and reach of each node. The betweenness of a node is the frequency that it lies along the shortest path (the 'geodesic') between other nodes in the network (Freeman, 1979). The degree of each node, being the number of connections incident upon that node, was also differentiated into 'in-degree' and 'out-degree'. Two nodes are 'reachable' if there is a set of connections between

them, and the reach of a node is the longest geodesic distance to another reachable node. 'Reach-to' and 'reach-from' each node was calculated as the three networks were directed. Heterogeneity of total degree is important epidemiologically in estimation of the effective reproductive rate of the epidemic (R). Centralisation of degree was further investigated by calculating the coefficient of variation (CV_{deg}) as the ratio of the standard deviation of degree to the mean degree amongst all nodes with degree≥1 (i.e. non-isolates). In the initial phase of an epidemic, the small fraction of immune individuals in the population exerts negligible constraint on R. It therefore approximates the basic reproductive rate (R_0), defined as the average number of secondary infections produced when one infected individual is introduced into a host population where the entire population-at-risk is susceptible (Anderson & May, 1991). R_0 is commonly estimated assuming homogenous (random) mixing of individuals. However, this assumption is violated by the heterogeneous contact network structure. Therefore, to estimate R at the herd-level for the early phase of this epidemic, we corrected the previous estimates of R_0 (Cowled et al., 2009) calculated assuming random mixing ($_0$), using the following formula:

$$R = \ _0 (1 + [CV_{deg}]^2) \qquad \text{(Anderson \& May, 1991)}$$

Sensitivity analysis

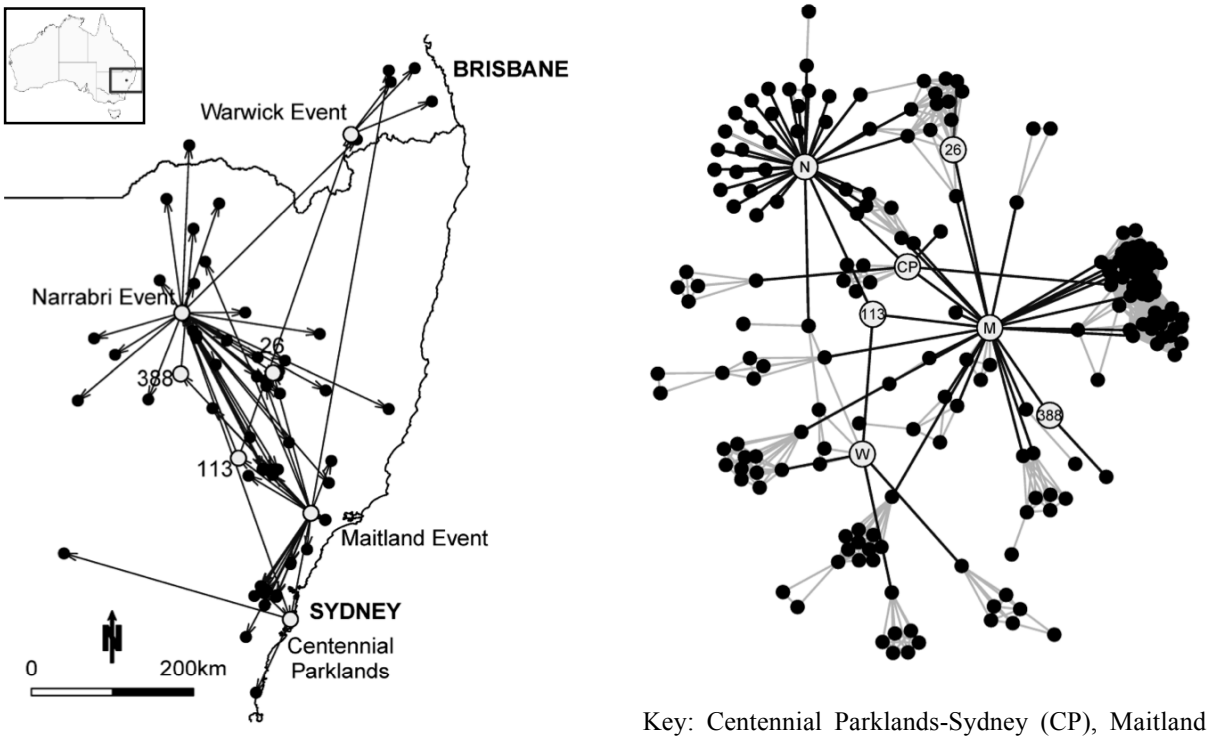
The distance cut-off used to dichotomise the proximity networks can be considered an assumption of the distance over which local spread of equine influenza was occurring. As the distance cut-off is increased, fragmentation in the proximity network is reduced, leading to fewer isolates in the combined contact-and-proximity network. A sensitivity analysis was conducted to test the influence of varying the distance cut-off on the number of nodes included in the largest weak component of the contact-and-proximity network. The point when further increases in the distance cut-off did not lead to inclusion of further isolates was considered to approximate the maximum distance at which local spread was likely to have occurred.

Cluster description

To describe spatial clusters of infected premises whilst also considering network topology, the proximity and contact-and-proximity networks with distance cut-off equal to the estimated maximal distance of local spread were selected and analysed using block-modelling. This technique involves grouping nodes into 'blocks' (also termed 'clusters') based on some similarity (Wasserman & Faust, 1994) and reconstructing networks representing the relationships between these groups. In this analysis, nodes were grouped into 'clusters' according to the GWCs in the maximal proximity network, then the maximal contact-and-proximity network was block-modelled. Such analysis emphasised movements that introduced infection into new spatial clusters.

RESULTS

In the first ten days of the equine influenza outbreak in Australia, horses on 197 premises were infected. A total of 978 horse movements were reported in the first 10 days of the epidemic, only 70 of these recorded horse movements were between early IPs with appropriately corresponding dates of movement and onset on originating and destination premises. These infected horse movements were all backwards traced, and covered the ten days prior to the complete implementation of movement restrictions.



Key: Centennial Parklands-Sydney (CP), Maitland event (M), Narrabri event (N), Warwick event (W), numeric labels are unique premises identifiers.

Fig 1. (a) Infected horse movements and (b) the combined network of contact-and-proximity relationships (with distance cutoff of 15 km) between premises holding horses infected in the first ten days of the equine influenza outbreak of 2007 in Australia.

The contact network, comprising 197 nodes and 70 infected horse movements (Fig. 1a), was dominated by the equestrian events at Maitland and Narrabri. Over 85% of the infected horse movements originated from these two events where disease transmission is known to have occurred. Only seven nodes (including these two events) served as the origin of infected horse movements (out-degree ≥1, large labelled circles in Fig 1.), each functioning as a potential cutpoint. A single node (113) with high betweenness was intermediate to the Maitland and Narrabri events and to a third event across the Queensland state border in Warwick. Horses moving from the Maitland event to the Warwick event were held midweek at premises‘113’. In contact horses from this premises travelled to the event at Narrabri on the following weekend, thereby introducing infection to that event. Overall, the contact network was sparsely connected with negligible network density and clustering (Table 1). It consisted of a single weak component, including 36% of the nodes, and 127 unconnected premises (these isolates are excluded from Fig. 1a). The heterogeneous structure of this network, dominated by several nodes with relatively high out-degree, led to a seven-fold increase in the estimated effective reproductive rate of this epidemic from 2.0 (calculated assuming random mixing) to 14.6.

Table 1. Network parameters during the first ten days of the 2007 equine influenza outbreak in Australia.

Parameter	Contact network	Proximity network[a]	Combined network[a]
Network size			
Number of nodes	197	197	197
Number of directed links	70	2518	2586
Number of isolates	127	24	0
Average path length	1.80	1.45	1.92
Network diameter	3	4	6
Network cohesion			
Density	0.00	0.07	0.07
Clustering coefficient	0.00	0.91	0.88
Network sub-structures			
Number of weak components	1	20	1
Nodes in largest component	70	58	197
Number of cutpoints	7	6	19

[a] Constructed based on a distance cut-off dichotomised at 5 km.

The proximity of infected premises in space was highly fragmented irrespective of the distance cut-off applied. With the distance cut-off dichotomised at 15 km, the proximity network comprised 20 relatively small components and 24 isolated premises. Within these highly connected components clustering was very high (clustering coefficient =0.91). Each component represented several premises infected in the same short time period and tightly grouped in space ('a cluster'). The six cutpoints in this proximity network were different from those in the contact network, each representing a premises located spatially within 15 km of two non-adjacent premises (more than 15 km apart). Compared to the contact network, the proximity network contained vastly more links, and these links were more evenly distributed across the network (in-degree and out-degree centralisation indices=0.07).

The combined contact-and-proximity network (Fig. 1b) displayed how early IPs were connected through the movement of infected horses and spatial proximity. Light thin links represent the movement of infected horses, dark thick links represent spatial proximity with distance cut-off set at 15 km. This network comprised a single component containing all 197 nodes, thus it had no isolates (Table 1). This network displayed several features characteristic of a small world network: short average path length, a high degree of clustering, and sparse long-range contacts between clusters.

The proportion of early IPs included in the GWC of the contact-and-proximity network varied according to the distance cut-off (Fig. 2). With the distance cut-off set at zero, local spread was not described; the 36% of nodes included in the GWC of this network were those nodes explained by the structure of the contact network alone. When the cut-off was increased to

5 km, 85% of nodes were included in the GWC and when the cut-off was increased to 15 km (corresponding to the network in Fig. 1b). All of the remaining isolates were included in the GWC. Therefore, all of the early epidemic spread was 'explained' with a cut-off distance of 15km, providing an estimate of the maximum distance over which local spread (through direct contact, fomite and windborne transmission) occurred during this epidemic. Further, 36% of the spread of this epidemic could be classified as 'network-associated' and 64% as 'local spatial spread' (within 15 km of those IPs identified in the contact-network).

Forty-four clusters were identified based on the maximal contact-and-proximity network applying a distance cut-off of 15km. The largest cluster included 58 of the premises infected in the first ten days of the outbreak. Over the entire four month epidemic, 82.6% of infected premises were contained within the boundaries of these 44 clusters. Of the 70 infected horse movements, only 43 movements introduced infection into a new spatial cluster. Fifteen of these originated from the Maitland event, and 21 from the Narrabri. The remaining movements came from the five other contact network cutpoints.

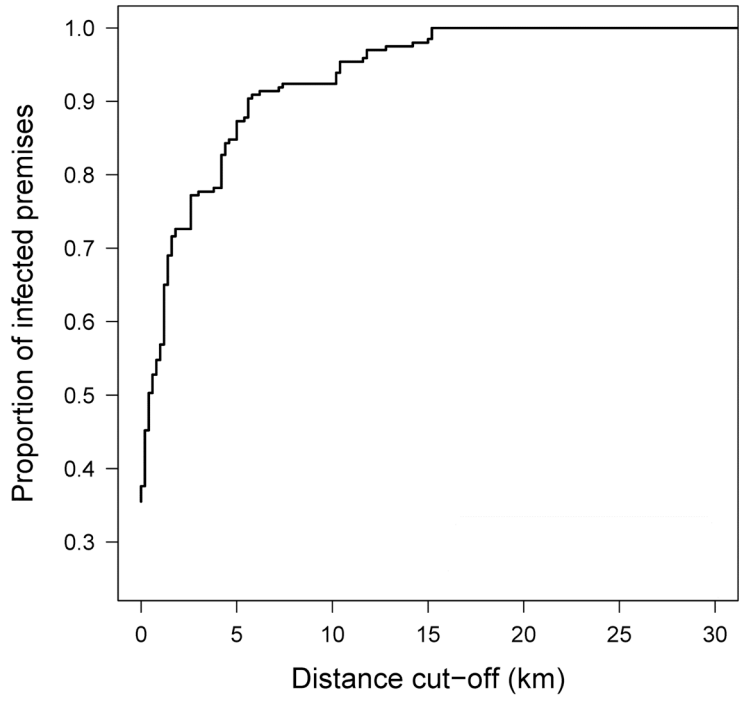


Fig 2. The proportion of nodes (infected premises) included in the giant weak component of the contact-and-proximity network when the distance cut-off is varied.

DISCUSSION

Social network analysis is a valuable tool for describing epidemics with underlying contact network structure. This paper presents a novel SNA framework, whereby spatial relationships between infected premises are incorporated into the analysis of early epidemic spread through a contact network. As the contact and proximity networks are similarly constructed they are easily combined to describe contacts between and within clusters. This method provides a clear picture of how the traced contacts lead to the formation of clusters, and improves the overall description of the dynamics of the outbreak in space and time.

The network of infected horse movements during the first ten days of the 2007 equine influenza outbreak in Australia dictated the spatial extent of this epidemic. Spread to 36% of early IPs could be explained by the contact network structure, however, a large proportion of early spread required further investigation. Certain movements introduced infected horses into new regions, thereby initiating new clusters of infection in highly susceptible populations. Within these clusters, local spread may have occurred through a variety of mechanisms including: direct contact or droplet transmission between horses on adjacent premises, and through windborne transmission and spread on fomites over longer distances. Windborne spread of aerosolised equine influenza virus up to 8 km has been reported during a previous outbreak in a naive population (Huntington, 1990). Our combined approach identified the specific contacts, in this case infected animal movements that introduced disease into new spatial clusters. This enabled estimation of the maximum range of local spread and the effective herd-level reproductive rate correcting for the topology of the underlying contact network. Over the entire geographic extent of the outbreak, 85% of early IPs were within 5km of an IP described by the contact network; 100% were within 15 km.

By constructing a maximal proximity network based on our estimate of 15 km for the maximum distance of local spread, spatial clusters of IPs were identifiable as this network's weak components. Each cluster represents the upper bound of premises that may have been infected through local spread in that small area. Further analysis into the spatial pattern of local spread within individual clusters would be useful for zoning and outbreak management in the event of future outbreaks.

The maximal contact-and-proximity network was highly clustered, had short average path length, and possessed a heterogeneous degree distribution. Each of these characteristics has important implications for infectious disease spread and the design of appropriate control strategies. Several are characteristics of small world networks. Small world networks are comprised of clusters of connected individuals with few connections between distant clusters. Spread of disease from one small world to another may be prevented by targeting the links that connect clusters. The low density of links also implies that surveillance and disease strategies built upon random sampling will be inefficient compared to those targeted towards certain classes of premises, such as those with high degree. Having prior knowledge of the underlying contact-structure of the Australian horse industry would therefore be very useful for appropriately targeting surveillance and interventions in future outbreaks.

The reachability of nodes and component structure of the maximal contact-and-proximity network are important in determining whether infection is limited to particular components. When studied alone, the proximity networks of early IPs in space were found to be highly fragmented. The movement of infected horses connected the spatial clusters leading to most nodes being reachable within a few geodesic steps (low average path length). It has been shown that clustering in such networks lowers the epidemic threshold, allowing a disease to infect all nodes in a small world very rapidly (Newman, 2003). However this may lead to localised depletion of susceptible individuals within clusters, and fewer individuals overall being infected (Keeling, 1999). In the outbreak studied, the contact network had effectively no clustering, and a heterogeneous out-degree distribution. Therefore the estimated effective reproductive rate was substantially increased. Interestingly, the combined contact-and-proximity network was highly clustered owing to the spatial structure of the data, so once movement restrictions were implemented, at the end of the study period, the epidemic could be expected to burn out over time in numerous spatially isolated clusters.

We acknowledge that our methods are sensitive to the completeness of the contact-tracing data. Despite considerable resources being devoted to contact-tracing early in this outbreak it is likely that some infected horse movements were missing from the dataset. Clusters whose contact links were missing would be connected to the GWC of the maximal contact-and-proximity network through spatial proximity to premises in the next nearest cluster, rather than through a contact link. Any such missing data might have obscured an important contact-network link thereby slightly inflating our estimates of local spread distances. The date of onset of clinical signs in the first horse affected on a premises was important in the definition of both premises and infected horse movements in this analysis. Premises were considered to be early IPs only if their onset date was within the first two weeks of the outbreak. Also, links representing infected horse movements were only added if the date of movement and the onset date on the originating and destination premises were in a logical sequence with respect to the incubation and infectious periods of equine influenza. These criteria were set quite conservatively, including only a one day margin of error. Premises with inaccurately reported onset dates, and horse movements from or to such premises, would have been excluded from this analysis. If these selection criteria were relaxed to further account for such reporting errors this would have increased the likelihood of misclassifying the mechanism of spread to certain premises and clusters. We also purposefully excluded several records of potential fomite spread from this analysis as routine reporting of such movements between infected premises was not conducted during the outbreak. The inclusion of these records in the contact-network structure may have slightly reduced our estimates of the distance of local spread but introduced a lot of uncertainty.

This social network analysis provides a complete description of early spread during the 2007 outbreak of equine influenza in Australia, capturing both the spatial relationships and contact patterns between infected premises. Our novel method provides a tool for defining spatial clusters whilst incorporating contact-tracing network topology and could be easily applied to data from outbreaks of other diseases where a contact-network structure is believed to be underlying early epidemic spread.

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CONTACT NETWORKS AMONGST DOMESTIC SHEEP: THE POTENTIAL FOR DISEASE TRANSMISSION IN FLOCKS

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SUMMARY

Successful control of livestock diseases requires an understanding of how they spread amongst animals and between premises. Here the contact structure of and potential transmission between domestic sheep is investigated. Three observational studies were carried out on conventionally managed flocks: one with newborn lambs; one with nearly weaned lambs; and one with ewes only. All direct physical and proximal contacts were recorded and adjacency matrices constructed. While proximity networks are dense, physical contacts have a more complex structure. There is a significant difference in the level of physical contact within those flocks with lambs and a clear distinction between ewes and lambs. Mathematical analysis and stochastic simulation of a multiple-class SIR epidemiological model suggest important seasonal difference in the basic reproduction ratio, with results presented for a viral disease with a fast recovery rate (foot-and-mouth disease) and a bacterial disease with a slow recovery rate (brucellosis) amongst sheep.

INTRODUCTION

Livestock diseases are socioeconomically important both in industrialized nations (e.g. Paarlberg et al., 2008) and the developing world (Perry and Grace, 2009; Rushton, 2009). The control of such disease is vital for global food security, while up to three quarters of emerging infectious diseases (EIDs) appear to be zoonotic (Taylor et al., 2001; Jones et al., 2008). Effective disease control and eradication is based upon knowledge of transmission and spread, and for many pathogens transmission between individuals is relatively well understood, either from observations or small-scale transmission experiments, while the transportation of infected animals between premises can also be studied, and modelled, using livestock movement data and holding records. Epidemiological models have already been applied to a number of important diseases, such as foot-and-mouth disease (e.g. Ferguson et al., 2001; Keeling et al., 2001; Keeling, 2005; Tildesley et al., 2006; summary in Schley, 2007; Jewell et al., 2009), Bluetongue (Gubbins et al., 2008; Hendrickx et al., 2008), brucellosis (England et al., 2004) and classical swine fever (Backer et al., 2008). To understand the dynamics of such a system as a whole, however, calls for an accurate description of what happens amongst animals, within farms and markets etc. It is fallacious to assume that groups of animals will respond in the same way; key temporal factors such as the latent, incubation and infectious periods are likely to be different for herds and flocks than for individual animals, with group level dynamics driven by the pattern of interactions between susceptible and infectious hosts, as well as intrinsic properties of the pathogen.

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Probably the least intensively farmed (and thus homogeneously mixed) of the major European livestock species are domestic sheep. The size of flocks can vary considerably, and will typically consist of breeding ewes of varying ages. In the UK, which has Europe's largest sheep industry with approximately 36 million sheep (Defra, 2007), average stocking density on lowland farms is about 13 ewes per hectare, though this does vary throughout the year and can increase to 40 ewes plus their lambs per hectare (Fisher and Matthews, 2001).

There exist a number of detailed studies on the nature and composition of flocks – for a review see for example Fisher and Matthews (2001) and Nowak et al. (2008). To understand the potential for disease transmission, however, it is important that the strength and frequency of contact can be quantified. Here the rate of physical contact between individual members of a flock, and their proximity structure, are determined from field observations, and subsequently studied using social network analysis and epidemiological modelling.

MATERIALS AND METHODS

Observational studies

Three separate observational studies were carried out on approximately thirty Dorset and Dorset cross sheep, since this is a popular choice of breed and a typical flock size in conventional farming, with breeding groups comprised mothers and daughters over several generations, as is conventional (Hunter and Milner, 1963). Initially ewes and lambs were placed in the paddocks within one week of lambing in, with fallen stock not replaced. The flock was observed for fifteen days between 2nd and 18th December 2009, starting when most lambs were no more than a couple of weeks old; and then again for fifteen days between 27th January and 22nd February 2010, at a time when ewes were starting to wean their lambs. The third trial consisted of mature ewes only, observed for fifteen days between 22nd July and 11th August 2010.

Table 1. Observable contacts recorded during field trials (no other contact types were observed at any time).

CONTACT TYPE		DESCRIPTION
Proximity	Very close	within 1m of another sheep: approx. length of a sheep
	Close	within 2m of another sheep
Physical	Sniff	usually along the back or around the head
	Head-butt	only counted if physical contact made;
	Suckling	between a lamb and ewe (not necessarily mother)
	Suckle attempt	if ewe immediately walks
	Mounting	(lamb) mounts/attempts to mount another (lamb)
	Kicking	only counted if physical contact made
	Pawing	(lamb) scratching its foot down the side of another
	Standing	(lamb) standing on top of (ewe)
	Rubbing	with any part of the body
	Resting	Mainly lying down; (lamb) resting head on another

An initial pilot study identified all potential observable contacts, as detailed in Table 1. These are consistent with previous studies (Sachs and Harris, 1978; Hass and Jenni, 1993; Fisher

and Matthews, 2001), and no additional activities were observed during the actual studies. For the studies every animal was observed for ten minutes each day at a different randomly allocated time during daylight hours (8am and 5pm). The number of occurrences of every physical contact type was recorded, together with which other individual was involved in each case, and the close proximity of other sheep recorded in binary format.

Pairwise comparisons confirmed that there was no discernable effect from which individual was under observation upon the resultant physical contact frequency or proximity counts between individuals ($p \geq 0.07$-0.78). Consequently adjacencies matrices were symmetrised, treating any observed contact between two individuals as reciprocal and equally relevant to both (Martínez-López et al., 2009). Kaplan-Meier survival estimates for a random sample of ewes and lambs showed no difference for either group in contact patterns for the morning and afternoon.

Model

The standard (SIR) model for the dynamics of susceptible/uninfected (S), infected/infectious (I) and recovered/no longer infectious (R) individuals assumes that all individual are equal and that contact is homogeneous. This is clearly not appropriate for breeding sheep flocks, where ewes and lambs display clear differences in their within-group and between-group contact rates Instead, a contact-network based formulation of a preferential mixing model (Kiss et al., 2009) has been adapted to represent the two distinct groups: ewes and lambs. Defining the force of infection as: the product of the rate of contact an individual has with members of a group (k); the probability that such a contact is made between a susceptible individual and an infectious individual – here assumed to be frequency dependent (i.e. equal to I/N); and the probability (p) that such a contact successfully results in transmission; (see for example Begon et al., 2002 for an explanation of terms) the model takes the form:

$$\frac{dS_e}{dt} = -p\left(k_{ee}\frac{I_e}{N_e} + k_{el}\frac{I_l}{N_l}\right)S_e$$

$$\frac{dS_l}{dt} = -p\left(k_{le}\frac{I_e}{N_e} + k_{ll}\frac{I_l}{N_l}\right)S_l$$

$$\frac{dI_e}{dt} = p\left(k_{ee}\frac{I_e}{N_e} + k_{el}\frac{I_l}{N_l}\right)S_e - \gamma I_e$$

$$\frac{dI_l}{dt} = p\left(k_{le}\frac{I_e}{N_e} + k_{ll}\frac{I_l}{N_l}\right)S_l - \gamma I_l$$

$$\frac{dR_e}{dt} = \gamma I_e$$

$$\frac{dR_l}{dt} = \gamma I_l$$

where the subscripts e and l refer to the ewe and lamb populations and k_{ee}, k_{el}, k_{le} and k_{ll} are the average contact rates between a ewe and other ewes, a ewe and lambs, a lamb and ewes and a lamb and other lambs respectively. In the absence of one or other population the system collapses down to the standard SIR model, with transmission rate $(pk/N)IS$.

RESULTS

There was a clear observable difference in the level of interaction between flock members as a result of lambs – especially young ones – being present, as shown in Figure 1. While ewes significantly reduced their contact while suckling young, the high level of interaction amongst lambs, and between lambs and ewes, more than compensated for this.

Suckling was found not to be exclusive to mother-offspring pairs: between birth and weaning each lamb suckled or attempted to suckle between one and four ewes (mean/std of 1.5±0.8), while ewes were subject to attempts by up to five lambs (mean/std of 2.5±1.2); the giant component of the suckling (attempted and successful) network across the first two field trials consisted of 19/29 members (66%).

The close proximity networks for all three flocks were each almost complete, with a density of 99.5%, 99.3% and 100% for ewes with newborn lambs, ewes with nearly weaned lambs and ewes only respectively (see Figure 1); thus over the observation period nearly all animals were observed within 2m of each other animal at some point

Physical contact rate

The rate of physical contacts amongst ewes, between ewes and lambs and amongst lambs were significantly different ($p<0.01$) in all the flocks, and between all three flocks ($p<0.001$) with the exception of ewe-to-ewe contact in the presence of offspring. Lambs have fewer physical contacts as they get older, but do discriminate between other lambs and ewes in how much contact they have (although parental ties are obviously stronger). Key centrality measures for each network are given in Table 2, broken down by group.

Table 2. Key measures for the three physical contact networks shown in Figure 1, giving mean ± standard deviation. Weighted Degree is the number of contacts an individual node has, weighted by the strength of those contacts. Closeness is a measure of the shortest path lengths between an individual and every other member of the group.

FLOCK	GROUP	WEIGHTED DEGREE	CLOSENESS
Ewes with newborn lambs	Ewes	904.2±476.8	75.2±3.1
	Lambs	1297.1±224.5	66.1±3.4
	Combined	1148.0±386.6	69.6±5.5
Ewes with nearly weaned lambs	Ewes	267.8±106.9	75.4±6.9
	Lambs	383.4±109.8	66.3±3.8
	Combined	337.2±121.0	70.0±6.8
Ewes only	Ewes	83.8± 52.5	88.5±8.2

Basic reproduction ratio R_0

Following the methodology of Van den Driessche and Watmough (2002) the basic reproduction ratio for the two-class model is:

$$R_0 = \frac{p}{\gamma}\frac{k_{ee} + k_{ll} + \sqrt{\left(k_{ee} - k_{ll}\right)^2 + 4k_{el}k_{le}}}{2}$$

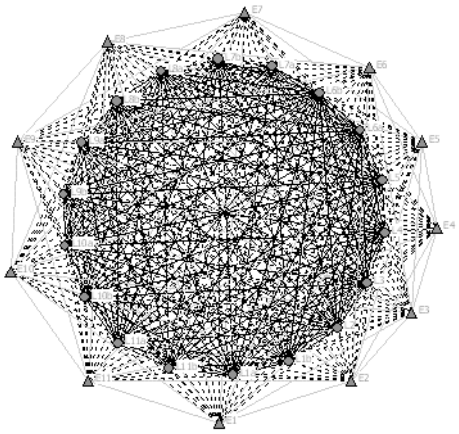
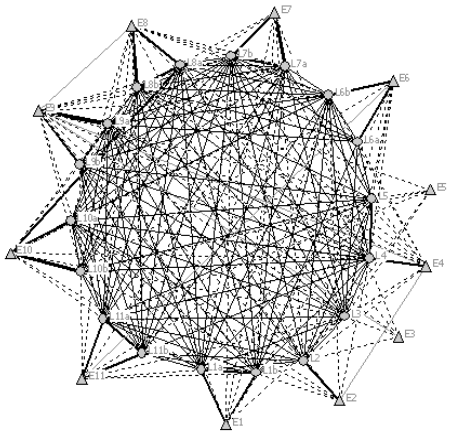
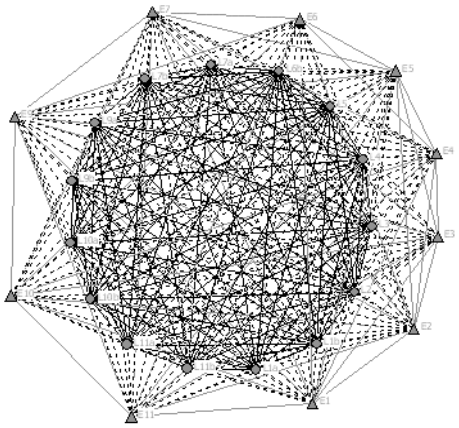
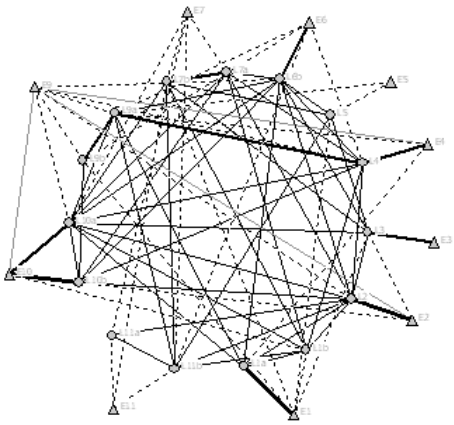
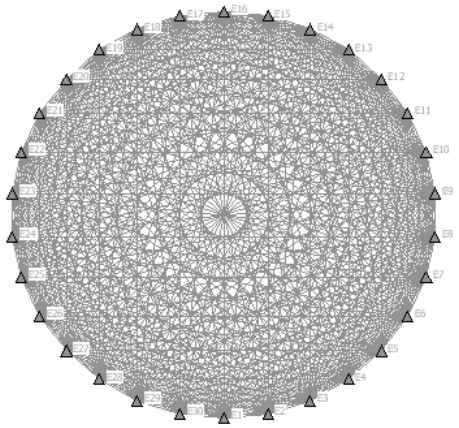
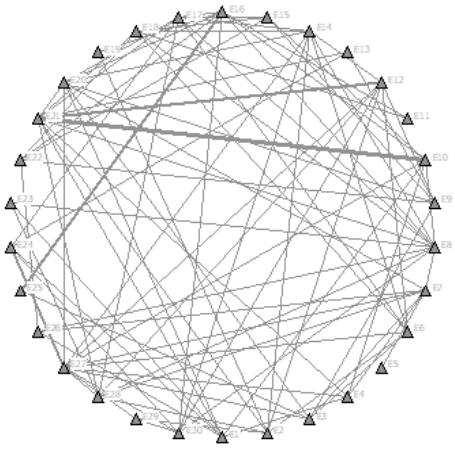
	Close proximity	Physical contact
Ewes with newborn lambs		
Ewes with nearly weaned lambs		
Ewes only		

Fig. 1 Sheep flock contact networks. Nodes represent ewes (triangles – labelled E and numbered) or lambs (circles – labelled L with same number as mother and additional letter if one of siblings) with contacts given by lines (weighted by strength of contact for physical interactions) for ewe-ewe (solid grey), ewe-lamb (dashed) or lamb-lamb (solid black) interactions.

as opposed to $R_0 = p/\gamma k$ in the conventional model. The relative magnitude of the basic reproduction number within the flock at different times of the year shows that the presence of lambs significantly increases physical contacts and that this is worst when lambs are young; explicitly:

$$\frac{R_0^{\text{with newborns}}}{R_0^{\text{ewes only}}} = 16.1, \quad \frac{R_0^{\text{with weaned}}}{R_0^{\text{ewes only}}} = 4.6.$$

Note that using the conventional (single class) form for R_0 produces smaller estimates of these ratios.

Final epidemic size, epidemic length and peak infectiousness

The final epidemic size r^∞ is the proportion of the flock that become infected i.e. does not remain in the susceptible population. For the mixed-model this is given implicitly by:

$$r^\infty = \frac{N_e}{N}\left(1-\exp\left(-\varphi_e^\infty\right)\right)+\frac{N_l}{N}\left(1-\exp\left(-\varphi_l^\infty\right)\right)$$

where

$$\varphi_e^\infty = \frac{p}{\gamma}\left(k_{ee}\left(1-\exp\left(-\varphi_e^\infty\right)\right)+k_{el}\left(1-\exp\left(-\varphi_l^\infty\right)\right)\right)$$
$$\varphi_l^\infty = \frac{p}{\gamma}\left(k_{le}\left(1-\exp\left(-\varphi_e^\infty\right)\right)+k_{ll}\left(1-\exp\left(-\varphi_l^\infty\right)\right)\right)$$

While for the ewe only flock the standard implicit formula: $r^\infty = 1-\exp\left(-\frac{p}{\gamma}kr^\infty\right)$ holds. The final epidemic size as a consequence of physical contact, as a function of the probability of successful transmission p is shown in Figure 2 for a disease with a fast recovery rate similar to FMD (Alexandersen et al., 2002) and one with a slower recovery similar to brucellosis (The Center for Food Security and Public Health, 2009). Results indicate that an epidemic is much more likely to take off in a flock with lambs, increasingly so with younger lambs, and that only in an all adult flock is it likely that some individuals will remain uninfected. Note that unless the recovery rate γ is exceptionally high, only a very low probability of successful transmission per contact is required to sustain the disease.

Epidemic length t^0 is the time of recovery of the last case, since the start of the first case, while the time of peak infectiousness t^* is defined as the time when the largest number of animals are infectious: these may be extracted from simulations by considering the duration for which $I = I_e + I_l \geq 0$ (or, more precisely for the continuous model, some strictly positive threshold value) and the time when $\max_{t\geq 0}\{I\}$ are attained respectively. Differences in the rates of contact within each flock impact on the length of the outbreak, although the interaction between the probability of infection and the rate of recovery is perhaps more significant. For flocks with lambs infection will only die out without a minor outbreak (or full epidemic) if the probability of

successful transmission on contact p is very low, while for the ewe only flock the parameter landscape is dominated by self-limiting outbreaks unless the disease recovery rate is very slow.

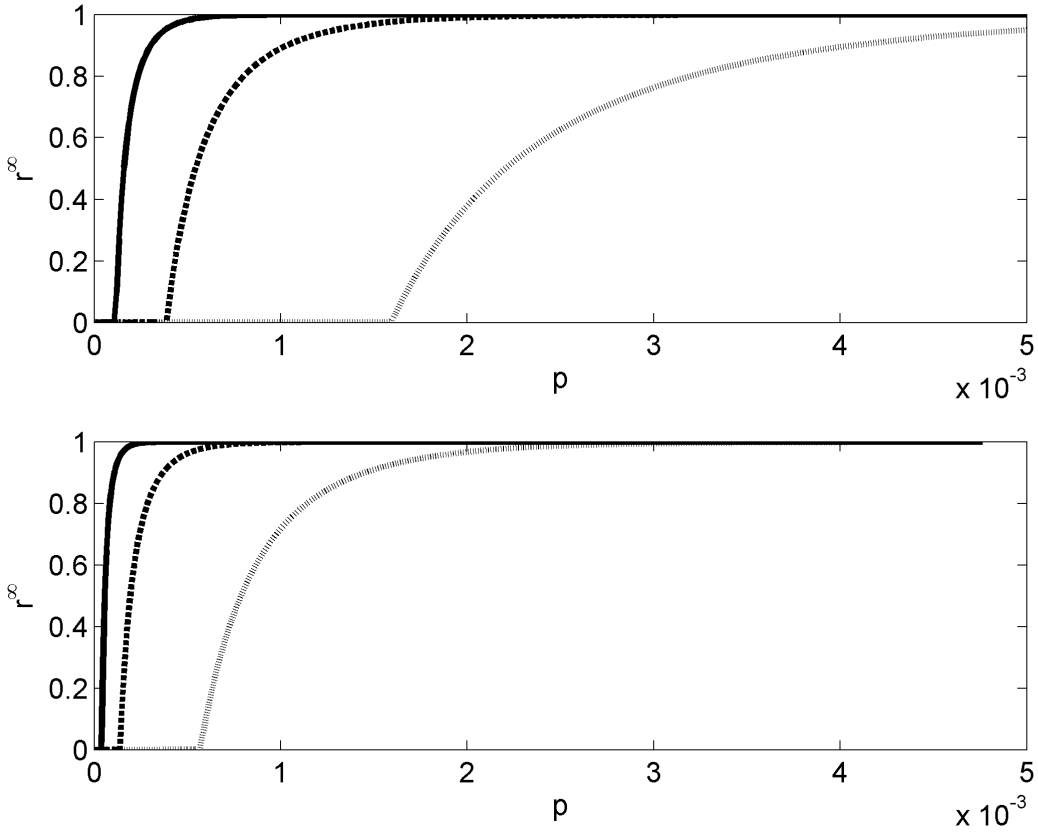


Fig. 2 Final epidemic size r^{∞}, as a function of the transmission probability p for physical contacts amongst a flock with (top) $\gamma = 2/15$ and (bottom) $\gamma = 1/30$ for: ewes with newborn lambs (solid), ewes with nearly weaned lambs (dashed) and ewe only (dotted) flocks, in a conventional group with two lambs per ewe on average.

Stochastic realizations & full network simulations

To quantify the effects of stochasticity on results in a conventional sized flock (since the underlying assumptions of the deterministic model are only truly valid in the limit $N \to \infty$) the system was simulated using the Gillespie algorithm (Gillespie, 1977), and results from 5000 replicates compared with the deterministic output.

Numerical results indicate that the deterministic solution is a good approximation to the average (median) stochastic realization if the force of infection in sufficiently high: in this case the probability of transmission on contact and/or the rate of contact between individuals. The contact between the ewe and lamb groups is sufficiently strong that there is no significant difference in results, neither for deterministic or stochastic simulations, on whether it is a ewe or lamb that is initially infected. Note that an epidemic may result even if k_{ll}, k_{ee} or both are zero, provided that $k_{el} > 0$ (and hence $k_{le} > 0$), since this is sufficient to connect all members of the flock. Results for smaller and larger flocks (e.g. 6 to 300 animals) remain consistent with these

conclusions. Transmission through the full contact adjacency matrix for each flock type was simulated, to better understand the potential loss of information resulting from the application of approximate models. Only the full network includes zero contact rates, making stochastic die-out of the disease more likely, but otherwise dynamics are not dissimilar for sheep contacts.

DISCUSSION

Sheep are known to be gregarious and form reasonably stable social groups, with relatively small social distances (Fisher and Matthews, 2001). Although communication between sheep is considered to occur primarily through olfactory, visual and auditory means, with tactile means of lesser importance outside of the breeding season (Fisher and Matthews, 2001), results here show a significant level of physical contact at three different times of year. The density of the proximity network shown here proves that there is significant potential for spread of airborne pathogens, and furthermore, that groups may be considered as homogeneous with regard to this form of transmission.

Results of observational studies indicate a significant difference in the level of physical contact within those flocks with lambs and those without (and to a lesser extend between flocks with younger lambs and those with older lambs) consistent with existing data on age-dependent activity levels of sheep (Shackleton and Haywood, 1985). A clear reduction in between-ewe contact was observed amongst individuals with young, with suckling ewes avoiding one another. It is known that ewes separate from the flock to give birth but soon return (Fisher and Matthews, 2001), and this avoidance behaviour is observed here. The lack of exclusivity in suckling between ewes and their own lambs, however, has the potential to increase the transmission of a disease via milk, skin or the oral-faecal route. Although the former is likely to be mediated by the success and length of feeding, which we expect to be correlated with paternity offspring, the latter is less so and both could therefore contribute to infection of a larger proportion of the flock.

When ewes and lambs are considered as separate classes then physical contact may be considered a reasonably homogeneous experience, at least in the sense of equivalent local structure and rates as defined by Begon et al. (2002). In contrast, the proximity network is also homogeneous in the traditional sense of individual possessing approximately equal connectivity (Begon et al., 2002). Application of field data to epidemiological models indicates that R_0 increases dramatically in the presence of lambs, an effect that decreases with age as might be expected. Furthermore, it has been shown that, for realistic contact rates based on field observations, this increase is significantly underestimated if a conventional (single class) formulation is used, as opposed to a multiple-class model.

Group size is not considered to have a tremendous impact on social behaviour, once a flock is larger than two or three sheep, unless space is restricted and resources are limited (Fisher and Matthews 2001). Sheep have been found to be gregarious when grazing, placing social bonds above food preferences (Sibbald et al., 2008), and studies of grazing behaviour have found that this is generally independent of group size (Penning et al., 1993). Distance between individuals is not correlated with range size, given sufficient grazing (Crofton, 1958), and social dominance is not an issue unless animals are confined (Nowak et al., 2008). The results presented here can therefore be generalised, and should be applicable to flocks of different sizes and those in different sized pastures (as long as they are not restrictively small), although some modification may need to be made for substantially different breeds (Dwyer and Lawrence, 2008). The limits

of deterministic and/or state-transition models to capture the stochastic dynamics of small and/or inhomogeneous networks can be determined in terms of the transmission properties of a specific disease, most notably the probability of successfully transmission between infected and susceptible individuals when a contact takes place.

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ROLE OF THE TRADING NETWORK IN THE DIFFUSION OF NEWCASTLE DISEASE IN THE LAKE ALAOTRA REGION, MADAGASCAR: A SOCIAL NETWORK ANALYSIS.

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SUMMARY

First reported in 1946, Newcastle disease (ND) is one of the major constraints of poultry farming in Madagascar. The trading network is thought to be the major pathway for transmission of this disease. This study aimed to describe the poultry commercial network in the Lake Alaotra region and assess the potential role of its components and its structure in the diffusion of ND virus. Several methods were combined to acquire data: classical survey, participatory epidemiology and disease surveillance. Social network analysis methods were used to analyze data. Network topology was scale-free, with 347 nodes and 1448 links. Hierarchical clustering showed six classes of nodes which were associated with ND outbreaks (p=0.004).

The originality of this study was having an almost complete network in developing countries with a measure of diseases. This is the first step of analysis, further studies would concern modelling the dynamics of ND within network taking into account virus strains which circulate.

INTRODUCTION

Newcastle disease (ND) is an infectious and highly contagious disease due to Newcastle disease virus (NDV) namely avian paramyxovirus type 1 (APMV-1) which belongs to the family of *Paramyxovirdae* and of genus *Avulavirus*. It affects several wild and domestic bird species but chickens are among those which are most sensitive. It is still one the major constraints to the development of poultry farming in a developing country.

The poultry industry holds an important place in Madagascar. According to Food and Agriculture Organization of the United Nations, there are 34.4 million domestic poultry (FAOSTAT, 2008), most of them being in smallholder production systems representing two-thirds of the rural population (Ocean Consultant, 2004). ND was first reported in Madagascar in 1946 (Rajaonarison, 1991). Well controlled by vaccination in industrial production, it causes high mortality in smallholder production systems. A study undertaken in 1999 in the peri-urban area of Antananarivo, the capital, showed that ND was responsible for 44% of poultry mortality

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(Maminiaina et al, 2007). Besides ND, two avian pathogens are known to circulate in Madagascar: *Pasteurella multocida,* the agent responsible of fowl cholera (FC) and avian influenza virus (AIV). A study undertaken by Porphyre (1999) determined that 14.9% (n=204) of chickens and 2.9% (n=175) of palmipeds showed serological evidence of AIV circulation. The same study (Porphyre, 1999) determined a seroprevalence rate of FC at 70.6% (*n=187*) in chickens and 25% (*n=140*) in palmipeds. FC and ND are registered as priority diseases by the national veterinary services. Leading to high morbidity and mortality rates with very similar clinical signs, these three diseases may be easily confounded in the field and the respective clinical impact of these 3 diseases remain unevaluated.

In Madagascar, the poultry industry related to smallholder production systems is complex, involving different types of actors in relation with each other: farms, collectors, live-birds markets and consumers. Farms can be classified according to their commercial practices (contact with collectors and markets). Some collectors are linked to farms where they buy poultry, collecting point and/or markets. Markets could be classified according to their size which is closely related to their administrative level (village market, municipal market, regional market and market in the capital). Generally, locally bred poultry are slaughtered at home by the consumer who buys directly from a market or eat their own birds.

The Role of the trading network or one of its components (e.g. market) in spreading AIV (Kung et al, 2003; Liu et al, 2003; Senne et al, 2003; Trock et al, 2003; Webster, 2004; Garber et al, 2007; Amonsin et al, 2008; Dent et al, 2008; Van Kerkhove et al, 2009; Yee et al, 2009; Soares Magalhães et al, 2010) and NDV (Kung et al, 2003; Sánchez-Vizcaíno et al, 2010) has already been established. In Madagascar, it is empirically hypothesised by poultry stakeholder that commercial network is the major pathway for transmission of ND and/or FC. In Madagascar, a typology study performed in the Lake Alaotra region (data not published) showed that farms could be described and classified according to a combination of risk factors including commercial practices, breeding types and environmental vicinity and that the risk of infection by NDV was statistically linked to some of these factors.

The aim of this study is to describe the poultry commercial network in Lake Alaotra region, analyze its structure and assess the potential role of its components and this structure in the diffusion of NDV.

MATERIALS AND METHODS

Study area

The study area is a landlocked region in the middle-eastern Madagascar. This is the basin of Lake Alaotra, the largest wetland area in Madagascar. It is contained in the centre, at 750 meters above sea level, 23.000 hectares (ha) of swamps, over 70.000 ha of rice paddies and 20.000 ha of open water (Ferry et al, 2009). The whole area is surrounded by hills reaching over 1300 metres of altitude. In the east it is bordered by the rainforest and in the west there are vast sparsely populated plateaus. The main channel of communication with the outside is the main road that connects with Andilamena in the north and with other regions of the island in the south. However, the region is densely populated because of the importance of agriculture and livestock.

The poultry population is high, estimated at 1.260.000 in 2001 (Andilamena included), with the largest population of domestic geese in Madagascar (UPDR/MAEP. 2003). Poultry flows are

important within the region. Exchanges with the outside are formed mainly by the supply of the largest port city on the island (Toamasina) and the capital (Antananarivo). Trade with Andilamena in the north, which is also a very isolated area, and the east and west could be considered as negligible. Finally 35 municipalities in Ambatondrazaka and Amparafaravola districts were included in the study area. Only two municipalities of Amparafaravola (Tanambao-Besakay and Soalazaina) were excluded because they were far from other municipalities and have negligible connections with them.

Network Data collection

Two methods have been combined to collect data and to get a trading network as complete as possible. Firstly, a questionnaire survey involving professional traders was carried out from December 2009 to July 2010. Professional traders buy and sell poultry permanently (throughout the year) or temporarily. This group includes collectors (or middlemen) and stallholders in markets. A list of all known live poultry markets and collection points was established and all of them were visited. In each market or collection point, all identified traders were included in the survey. The questionnaire included questions about the origin and/or destination of poultry, frequencies of activity, number of birds treated and flow variation during year.

In a second step, a participatory approach (Jost et al, 2007) was conducted from December 2009 to November 2010. This survey involved the community animal health worker (CAHW) of the region and the chiefs of fokontany. Fokontany are administrative units constituted by one or some neighboring villages. The CAHW are farmers elected by the members of their village and trained for several weeks by "Agronomes et Vétérinaires Sans Frontières" (AVSF, a nongovernmental organization) for basic care, to help veterinarians providing a local service to farmers. Each of them is in charge of two or three fokontany. However, the network of CAHW has worked with only 60% of existing fokontany. To complete data and to compare answers, chiefs of fokontany in every municipality - the municipality being the administrative unit constituted by several fokontany- were asked to participate to the survey. Meetings were organized with them in collaboration with the local officials such as Mayors. For every municipality and CAHW's association, two meetings were conducted. Network data targeted concerned villagers trading practices: the places where they usually buy and sale their poultry. After announcement of general subject i.e. trading network and poultry diseases, meeting always began with an open discussion. Individual semi-structured interview (SSI) and focus group were organized after the open discussion to focus on targeted data. Before its validation, all information collected by individual SSI was discussed again in focus groups. Our knowledge of the study area combined with direct observation and secondary data from literature were also used. At the final stage, triangulation was done to validate each data point. It consisted of allowing time for collecting all information from different sources (informants, literature and direct observation) and cross-checking them, at final stage, to correct any uncertainty or variations amongst responses.

Disease occurrence

Disease occurrence was recorded using two different methods: the above mentioned participatory epidemiology (Jost et al. 2007) and the second was a classical disease surveillance network. The same outbreak definition, established at the beginning of the study, was used for both methods. The targeted disease of interest was ND so "outbreak" declaration was validated when the following conditions were met: in at least two farms in a village there was an acute mortality (more than 30% of birds in flocks), or continual mortality (at least one bird per day

during three days), with nervous signs (e.g. torticolis) and/or respiratory or gastrointestinal signs (diarrhoea). However, this case definition cannot differentiate ND from FC and avian influenza so RT-PCR was performed from brain and/or cloacal and tracheal swabs. Fokontany were chosen as the epidemiological unit so each fokontany was considered infected if there was at least one village within it where an outbreak occurred during the study period.

Poultry diseases were the second topic during participatory meetings. Participants described: (i) occurrence of poultry diseases in their fokontany since December 2009 and (ii) the clinical signs associated with each disease. All declarations were registered but an assessment of correspondence with outbreak definition was done after each meeting before validating each declaration as an outbreak.

The disease surveillance network of ND was implemented in collaboration with local veterinary services. The surveillance started in December 2009. CAHW or the chief of each fokontany declared, by phone, when there was outbreak. Correspondence with outbreak definition was assessed and a mobile team went to collect data and samples when a declaration was validated. Sample size targeted within each outbreak was at least 10 birds to obtain an absolute accuracy of 5% with a threshold prevalence rate of 30% (Thrusfield et al, 2001). During sampling, priority was given to diseased or recently deceased birds (less than one day). Implementation of surveillance network depended on collaboration of CAHW and chiefs of fokontany. It started in December 2009 with only CAHW on the south and east sides of the lake. It covered the whole study area in July 2010 after several meetings to convince the chiefs of fokontany and the other CAHW.

Data analysis

A social network analysis method (Wasserman and Faust, 2009) was used to describe and analyze the network in R 2.12 software (R Development Core Team, 2010). As data directions of links were known, a network with directional relations was computed. Outbreaks detected by participatory epidemiology were used in statistical analysis and proportion of outbreaks detected by the classical disease surveillance and confirmed by laboratory analysis was used to investigate specificity of declaration from fields.

Definition of network elements: Farms were grouped per village because poultry within villages share same environment and contact with trading network. According to our field knowledge, it was assumed that people in neighboring villages (fokontany) go to the same places to buy or to sell poultry. As it was impossible to get data for every village, fokontany were chosen as the epidemiological unit and node. Villages which were far from the other villages of its fokontany were considered as an independent fokontany or were put into the nearest fokontany. Ties were all movements (human with poultry) connecting fokontany. In addition to poultry farms, there were, live birds market inside some fokontany (with a maximum of one market per fokontany). Farms located within these fokontany were likely to be more exposed to commercial exchanges. This characteristic was used as an attribute of nodes. The occurrence of an outbreak within fokontany was considered also as an attribute. Destination, or only receiver nodes, which were located outside the study area were withdrawn from the analysis.

Network parameters, topology and outbreak occurrence: To describe the network, centrality parameters (degree, betweenness), clustering coefficient and density were first calculated. Their values and the distribution of degree explained the topology of the network. Simplified

definitions of these parameters, in accordance with those already given in literature (Martínez-López et al, 2009; Wasserman and Faust, 2009), could be given:

(i) Degree is a measure of centrality of each node. It represents the number of ties connected with node. For directed network, there are three kinds of degree: the indegree is the number of ties received by node; the outdegree is the number of ties sent by node and the freeman degree is the sum of both in and out degree.

(ii) Betweenness is another centrality measure of node. It represents a measure of how a node lies in the middle of two other nonadjacent nodes. It means that a path which connects the two nonadjacent nodes has to pass through the node between them.

(iii) Clustering coefficient is an indicator of the importance of clusters present in network. It measures the sum of the proportion of nearest neighbouring nodes that are directly connected.

(iv) Density measures the proportion of observed contacts compared with all possible contacts among nodes. It indicates how the network is connected.

To assess the potential link between the occurrence of an outbreak and the centrality parameters of nodes, generalized linear models were implemented where the dependant variable was the occurrence or not of an outbreak in the nodes and explanatory variables were the centrality parameters. Since nodes were not independent, each parameter was tested individually. To assess the significance of model coefficients, random permutation tests were performed. Several random permutations of the matrix were done and coefficients of models were calculated for each permutation. The proportion of coefficients which were higher than for the observed matrix was calculated. The coefficient was considered as significant when this proportion was smaller than 5%.

Positional analysis and outbreak occurrence: Positional analysis consists on simplifying the network data set. This simplification consists on classifying nodes within positions identified by their structural equivalence. Two nodes are structurally equivalent if they are identically tied to and from all other nodes in the network (Wasserman and Faust. 2009). The following steps were performed for positional analysis and its association with outbreak occurrence:

(i) Measure of structural equivalence with euclidean distance.

(ii) Representation of network positions which consisted on partitioning nodes into classes where each class was constituted by structurally equivalent nodes. Partition of nodes was performed using hierarchical clustering of matrix of euclidean distances calculated above. Ward's algorithm was used to aggregate nodes into class thus minimizing within-class dissimilarity and maximizing between-classes dissimilarity. The output of hierarchical clustering was a dendrogram or tree diagram which represents the series of partition. A cut-off level within the tree diagram was chosen to get interpretable and realistic classes. Description of obtained classes was done using values of centrality parameters and attributes within each class.

(iii) The association among nodes characteristic (according to clustering) and occurrence of outbreak was assessed by comparing the number of outbreak among classes using chi squared test.

RESULTS

The participatory approach concerned 40 CAHW and 35 municipalities in the study area. Two-hundred and thirty-one traders were interrogated on 21 markets and 20 collection points. Disease surveillance network recorded and investigated 35 outbreaks in 27 fokontany but the participatory approach revealed 134 outbreaks. Up to now, samples from 17 fokontany were assessed in the lab and 15 fokontany were confirmed to be infected with ND which corresponded to 18 outbreaks confirmed out of 24 assessed.

Network topology

Three-hundred and forty-seven nodes and 1448 links were identified. Fig 1 shows the network structure. There is no isolate node and some nodes are more connected than others, so it is a connected and heterogeneous network, meaning that the majority of nodes are weakly linked and few nodes are highly linked with others. The distribution of degrees follows a power law distribution (Fig 2). Table 1 shows network parameters and a clustering coefficient that is low (0.11). This network heterogeneity with the power law distribution of degrees and the low clustering coefficient confirmed that the avian commercial trade network of the Lake Alaotra region is a scale-free network (Barabási and Albert, 1999).

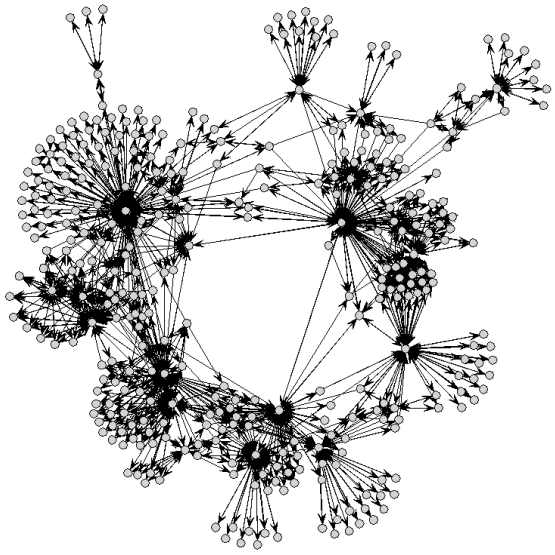

Fig. 1 Structure of the network of poultry commercial trade in the Lake Alaotra region. Madagascar

Table 1. General parameters of network

PARAMETERS	VALUES
Number of nodes	347
Number of links	1448
Clustering coefficient	0.11
Density	0.01
Average degree	8.35
Average betweenness	775.97

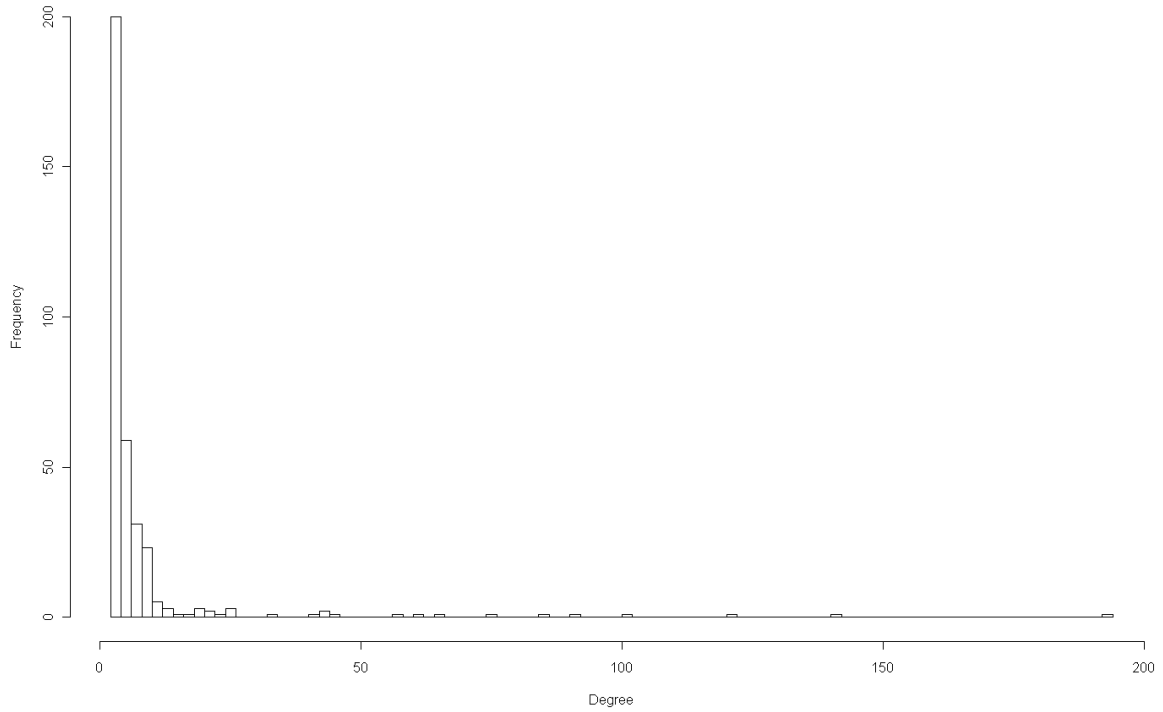

Fig. 2 Distribution of degree per nodes

Centrality measures and outbreak occurrence

Table 2 shows coefficients of each centrality parameter from binary logistic models and the related p-values from permutation tests. None of the parameters were significant indicating that occurrence of outbreak was not linearly associated with the logit of centrality parameters.

Table 2. Centrality parameters coefficients from logistic models

PAREMETERS	COEFFICIENTS	P-VALUE
Freeman degree	0.005	0.18
Indegree	0.008	0.24
Outdegree	0.015	0.12
Betweenness	$< 10^{-4}$	0.29

Hierarchical clustering and outbreak occurrence

Fig 3 shows the dendrogram from hierarchical clustering according to structural equivalence which was measured by euclidean distance. The horizontal line indicates a cut-off level putting in the same cluster all branches below. Six clusters were retained and table 3 shows their description in terms of different parameters.

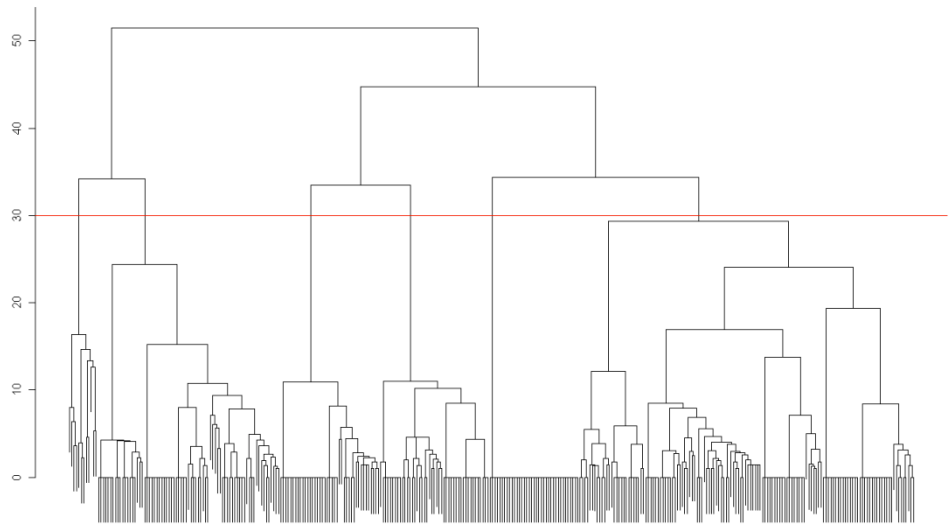

Fig. 3 Dendrogram of nodes representing assembling nodes by structural equivalence

Table 3 shows the description of clusters according to centrality parameters and attribute values. According to centrality parameters there was a hierarchy of their values among classes. Class 4 included the most connected nodes with highest betweenness and degree. All of them had big market (market 1) inside explaining this intensity of poultry commercial exchanges. It was the smallest class in terms of number of nodes inside but they were the hubs of the network. The second most connected class was class 6. There were some big markets and this class ranked second with 74 nodes. These two classes were those where outbreaks occurred more frequently during the period of study. Third place in terms of centrality was taken by class 3. It is the most common class with 138 nodes. 12% of them had small market inside. Frequency of outbreak was lower than the two former classes but it was still high with 41% of nodes infected. In terms of centrality, class 1 and class 5 were comparable. However, although there were more nodes having a big market inside in class 1, the frequency of outbreaks seemed to be lower than class 5. The last and the most peripheral class was the class 2. Its average betweenness was 0 meaning that none of the nodes of this class were necessary to connect two other nonadjacent nodes. However, the frequency of outbreak was higher than in class 1.

Chi-squared test comparing occurrence of outbreak, recorded by participatory epidemiology, among classes was highly significant (p= 0.004). It means that occurrence of outbreak was associated with position of nodes within the network.

Table 3. Cluster centrality parameters and outbreak occurrence

CLASS	NUMBER OF NODES	BETWEENNESS	FREEMAN DEGREE	INDEGREE	OUTDEGREE	FREQUENCY OF MARKET 1*(%)	FREQUENCY OF MARKET 2*(%)	FREQUENCY OF OUTBREAK (%)
1	41	55.6	5.5	2.6	2.9	5	7	22
2	37	0.0	2.5	1.5	1.0	0	5	27
3	138	203.0	5.0	2.3	2.7	3	12	41
4	12	17384.8	89.3	47.8	41.5	100	0	50
5	45	34.4	4.6	2.1	2.5	0	7	29
6	74	389.2	8.2	4.0	4.2	8	3	54

*Market 1: Biggest markets with regular presence of poultry trading.
*Market 2: Small markets with irregular presence of poultry trading.

DISCUSSION

In recent years, network analysis has increased its relevance in studying disease spread within complex inter-connected structures (Berthélemy et al, 2004; Berthélemy et al, 2005; Eames et al, 2008). There is also an increase in interest for this method in veterinary epidemiology (Martínez-López et al, 2009) and there were several studies about avian influenza and ND which used this method (Dent et al, 2008; Van Kerkhove et al, 2009; Soares Magalhães et al, 2010). However, in contrast to developed countries where there are generally good registration systems, there is a lack of data in developing countries making acquisition of network structures difficult. Consequently, most studies are based on a small sample of the population with resultant questions about validity of results. The originality of this study is that it was undertaken in an isolated area where it was possible to get almost complete network data combined with disease measures.

The combination of participatory approach and classical survey allowed the collection of a large set of data. It is safe to assume that almost 100% of existing nodes of this network were identified. As mentioned above. the study area is landlocked. Connections that directionally link the network with nodes located outside of the considered area as an output were withdrawn from analysis since it was assumed that they did not influence the circulation of a given pathogen. Other connections with external area could be considered as negligible because they were rare and included isolated villages outside the study area (i.e. in north. west and east). Apart from vaccination against ND and FC, which are facultative, there were no official control measures taken against these diseases even if an outbreak occurred. So, there was no modification of the network structure during an outbreak. Also, a survey of professional traders revealed that the flows of poultry they sell and frequency of activity change within year but places where they buy or sell poultry do not change. As the network was not valued, i.e. flows or poultry quantity exchanged were not considered, these cited modifications do not affect the network structure. This suggests that the structure of the network is stable considering the one year time-scale of study.

Results of this study show an important occurrence of poultry disease outbreak confirming their economic impact. The number of outbreaks declared corresponds to 37% of fokontany. But it should be remembered that there were several villages in each fokontany and it was declared as infected as soon as one village became infected. It means that at least 132 villages were infected.. It should be noted that the type of network identified (i.e. scale-free) is favourable for spreading pathogens (Barthélemy et al, 2004; Barthélemy et al, 2005).

Participatory epidemiology and formal disease surveillance network did not detect the same number of outbreaks. This is likely to be due to sequential implementation of the formal disease surveillance network. Furthermore, several meetings were necessary to encourage network compliance and to improve outbreak declaration from the observation posts. In addition, as field agents who declared cases were the same as those implicated in participatory approach, all outbreaks declared in formal surveillance were also declared during participatory meeting. Confidence was obtained after several meetings, during the participatory approach, so they could provide a complete list of outbreaks. The problem was that as diseased birds were not always seen by the mobile team, validation of outbreak declaration depended on clinical signs declared by Chiefs of fokontany or CAHW. Even if field's agents had a good knowledge of these diseases, it was impossible to distinguish ND, FC and avian influenza on the sole basis of clinical signs. Laboratory confirmation was necessary but this was only possible for samples from the formal disease surveillance. Thus, there was a lack of sensitivity for formal disease

surveillance and a lack of specificity for participatory surveillance. A total of 15 fokontany out of 17 were confirmed infected after laboratory analysis implying that field agents had a good knowledge of disease and the outbreak definition was good. Without having means to get perfect data, both methods were complementary because participatory surveillance gave the overall importance of the three diseases and formal surveillance provided information on ND virus outbreaks. This study confirmed the relevance of using participatory epidemiology in developing country, as already shown elsewhere (Jost et al, 2007).

Results from logistic models and comparison of outbreaks within classes seem to be a paradox because none of the coefficients from logistic models was significant but the number of outbreaks was significantly different among classes. However, logistic models have just considered the centrality position of nodes without taking into account the way in which each node was connected to others nodes in the network. It appeared that this was not sufficient to explain the differences among nodes. Justly, hierarchical clustering was based on structural equivalence of nodes i.e. nodes that held the same position in the network (Wasserman and Faust. 2009) were classified in the same cluster. These nodes were interchangeable because they were linked with the same other nodes. However, description of classes revealed that there was a hierarchy of values of centrality parameters among clusters. Finally, it appeared that there was a highly significant association between positions of nodes in the network and occurrence of outbreak. Centrality parameters were not linearly correlated to the outbreak occurrence (more precisely to its link function) but these were still important indicators when coupled with the positions of network in the network.

This study is the first step of analyzing these data. Further analysis would permit the setting up of targeted disease surveillance by identifying the appropriate nodes. Furthermore, integration of values of links, virus strains and temporality of events (outbreaks and links) within each year would enable the modelling of diseases dynamics within the network and to simulate the effects of realistic control measures.

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SCIENCE AND POLICY

BOVINE TUBERCULOSIS IN BELGIUM: OFFICIALLY FREE BUT STILL SPORADIC OUTBREAKS! EMPIRICAL APPROACH FOR A RISK BASED SURVEILLANCE PROGRAM

S. WELBY·, M. GOVAERTS, L. VANHOLME, J. HOOYBERGHS, K. MENNENS, L. MAES AND Y. VAN DER STEDE

SUMMARY

Belgium gained the bovine tuberculosis (bTB) officially free (OTF) status in 2003. The present study was carried out in order to evaluate the different surveillance components of the current bTB surveillance program and to estimate how this program could be optimized in accordance with European legislation. Separate scenario trees were designed for each component of the surveillance program. Different stochastic simulations were carried out to measure the impact of modifications in each surveillance component, regarding the diagnostic test used towards the component level sensitivities (ModelRisk). The sensitivity (mode) for the following 4 surveillance components was respectively 0.83 for testing cattle during the winter screening, 0.85, 0.99 and 0.99 for testing imported, slaughtered and purchased cattle respectively. Large variations around the average values were observed. The sensitivity analysis showed that the most influential parameter explaining this variability came from the uncertainty distribution around the diagnostic process parameter.

INTRODUCTION

Though several EU Member States (MS) have achieved the officially bovine Tuberculosis (bTB) free status (OTF) (<0.1% annual herd prevalence) (EC Decision 2003/467/EC), a possible re-emergence of bTB cannot be excluded (EFSA, 2009). Belgium, like other European Union Member States, has maintained the OTF status for bTB (herd prevalence <0.1%) since 2003 (EC Decision 2003/467/EC). Yet, sporadic outbreaks do still occur, as has recently been the case in Germany and in the Netherlands (Hooyberghs, personal communication, Probst et al., 2010; Vanholme, 2009). In 2003 Belgium had 7 breakdowns, 8 in 2004, 5 in 2005, 8 in 2006, 5 in 2007, 12 in 2008 and 2 in 2009. In 2010, no cases were detected (Vanholme, personal communication).

The current Belgian official surveillance program for tuberculosis in cattle consists of the following components, in accordance with the guidelines laid down in the Council Directive 64/432/CEE and the Royal Decree MB 17.10.2002:

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- Imported cattle, except young fattening calves (FC) for veal production, are tested by intradermal injection of bovine tuberculin (Single skin test (SST)) at import (IMP surveillance component)
- Post-mortem visual inspection at slaughterhouse of all slaughtered cattle (SLGH surveillance component)
- Purchased cattle, except FC for veal production, are tested with a SST (PUR surveillance component)
- During the winter a screening with SST is carried out (WS surveillance component) on the following:
    - all cattle older than 6 weeks from herds considered as neighbour or contact herds of a suspected or confirmed bTB positive herd, after tracing-on and tracing-back investigation (TRAC)
    - all female cattle older than 24 months that belong to on-farm 'milk selling' herds (VD)
    - all imported cattle, except FC for veal production, from non OTF Member States, are tested by means of a SST for 3 consecutive years, during the winter period (TRIM)

A country or region is considered OTF by the EU if for 6 consecutive years, 99.9% of the herds have been tested and found negative. The annual herd prevalence must be <0.1% (Council Directive 64/432/CEE and Royal Decree MB 17.10.2002).

The Belgian Federal Agency for the Safety of the Food Chain (FASFC) ordered a study on their national bTB surveillance program. The aim of this study was to evaluate the sensitivity of the current surveillance system for bTB in Belgium, and simulate the impact of changes in the different surveillance components on the respective component (CSe). The sensitivity is defined here by the probability of detecting one infected animal given the country is infected at the set design herd prevalence of 0.1%.

MATERIALS AND METHODS

For the purpose of this study, 4 separate scenario trees (Fig. 1), as described by Martin et al. (2007a), were designed for each surveillance component, namely the IMP, SLGH, PUR and WS surveillance component.

The choice and the sequence of the nodes representing these components in the scenario tree were done following a review of literature, and expert opinion using Belgian experts (Humblet et al., 2009). The nodes and node's branches of these trees were similar for each component except for the detection node.

The following risk category nodes were retained: imports from non OTF MS (yes/no), past bTB status (positive/negative), animal movement rate (low/high), herd type (fattening calves (FC) for veal production or other bovines (B)) and herd size (low, medium, high). Population proportion (PPr), sampled population proportion (SPr) and the relative risk (RR) were the parameters that enabled the categorization of the whole herd population in Belgium. The following nodes were the infection nodes: animals and herd status with their respective parameters, the average within herd prevalence (PA) calculated on data of the historical registered bTB outbreaks (2005-2009) and the legal herd design prevalence (PH).

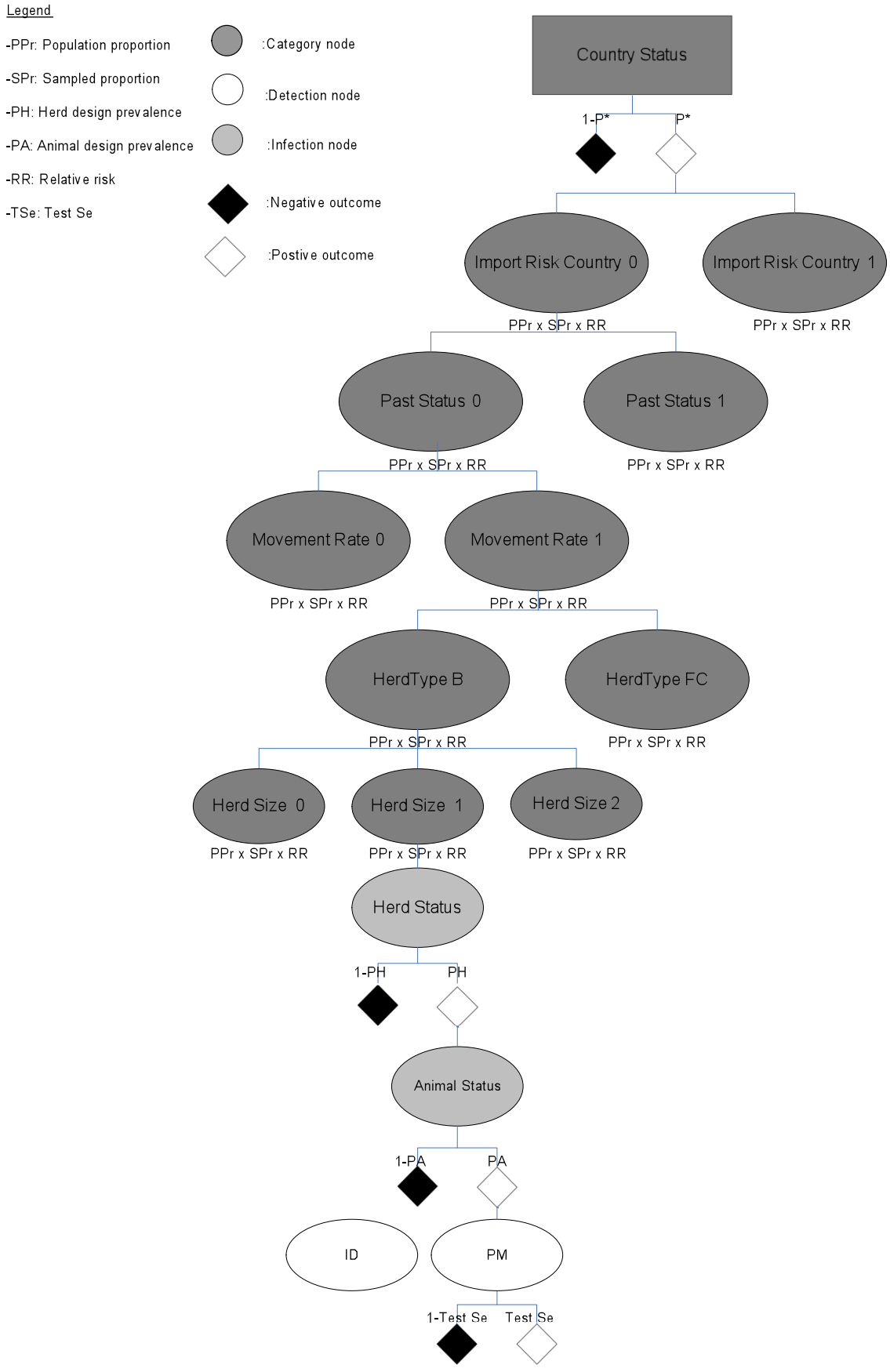

Fig. 1 Scenario tree illustrating the sequence of events from infection to detection of bTB in Belgium

These parameters enabled the computation of the effective probability of infection of an animal (EPIA) and herd (EPIH). In turn, these EPIH and EPIA allowed the computation of the animal sensitivity (ASe) for each limb of the tree, defined by the combination of each category node's branch, as well as the herd sensitivity (HSe), according to the different diagnostic processes methods sensitivities (TSe) applied to each limb of the tree (post mortem inspection (PM) or Intadermal SST test (ID)) and to the number of animals sampled. These individual herd sensitivities, for each limb of the tree, allowed the computation of the CSe for each component under study (IMP, SLGH, PUR, WS).

For each herd active in 2009, data regarding imports, purchases by national trade, herd structure and bTB status over the past 5 years (2005-2009) were collected from the Belgian animal identification system (SANITEL). In total 158,810 herd records represented all historical data from 2005 to 2009 for the 36,057 herds still active in 2009.

Cut-off values enabling the categorization in the different risk category node branches, of animal population proportion (PPr), and sampled population proportion (SPr), were determined following separate univariate analysis (SAS 9.2.). To estimate the relative risk (RR) of each branch of the risk category nodes, a risk factor analysis was carried out in SAS 9.2. to model the probability of a herd of being bTB positive given the category node branch of interest. A pert distribution was fitted around the average value of the RR estimates for each category node branch, the minimum and the maximum values being the confidence interval limits. Literature review and bTB expert opinion were used to estimate the different diagnostic test sensitivities (Cousin and Florisson, 2005).

Spreadsheets were created in Excel 2007 to represent each surveillance component investigated (IMP, PUR, SLGH, WS). Distributions were fitted on each input variable taking into account the variability, as well as the uncertainty of the key parameters. Stochastic simulations of the following scenarios were carried out (10,000 Iterations/Simulation) (ModelRisk 3.0, Vose Consulting):

- In which risk group category node would it be more efficient to sample?
- What is the CSe value for each component?
- What is the impact on Cse, of setting the diagnostic test sensitivity to a fixed value (0.50, 0.75 or 0.99)?

A sensitivity analysis was carried out for this scenario tree model to determine what input parameter was most influential on the output parameters' CSe.

In order to validate the output of this model, a generalized estimating equation (GEE) model was built in parallel to investigate the probability of detecting a positive animal given the reason for testing for bTB (investigation motive). For this purpose the data regarding the outbreaks detected during 2005-2009 were used. A Poisson distribution was used to model the probability of having positive animals given the investigation motive; the clustering effect at herd level was taken into account by using a repeated statement (SAS 9.2).

RESULTS

Two significant clusters were found: one in the province of Antwerp (Relative Risk (RR) of 19 with p-value of 0.001) and one cluster covering the provinces Liege, Limburg and Flemish Brabant (RR 8.5, p-value 0.033). However it was decided not to take into account spatial aspects

in the final scenario tree, because it would be difficult to implement and justify different target sampling in those provinces compared to others.

Table 3 summarizes the result of the risk factor analysis, only values of RR with a p-value below 0.05 being retained.

Table 3 RR values for each risk category node of the tree

Category risk node	Branches	Value of RR
Import from risk country	0	= VoseUniform(1; 1; 1)
	1	=VoseUniform(1; 1; 1)
Past status	0	=VoseUniform(1; 1; 1)
	1	=VosePERT(30.7;30.7;30.7)
Movement rate	0	=VoseUniform(1; 1; 1)
	1	=VosePERT(2.7;4.9;10.5)
Herd type	FC	=VoseUniform(1; 1; 1)
	Beef	=VosePERT(100; 100; 100)
Herd size	0	=VoseUniform(1; 1; 1)
	1	=VoseUniform(1; 1; 1)
	2	=VosePERT(0.09; 0.32; 1.05)

The preliminary results showed that according to the individual HSe obtained in each component for each limb of the tree, the most efficient sampling was obtained when target sampling was done in adult bovine herds of large size, where movement rate was high, where no bTB infection was observed previously, and where no imports from risk countries were registered (0.99 (0.87-0.99)).

The mode (50% percentile) across 10,000 iterations for the CSe of the following 4 surveillance components, was 0.83, 0.85, 0.99, 0.99 for testing only during the winterscreening with a TSe equal to ID (WS ID Se), only imported cattle with a TSe equal to ID (IMP ID Se), only purchased cattle with a TSe equal to ID (PUR ID Se), or only slaughtered cattle, respectively. Slaughter CSe remained 0.99, using a TSe equal to the PM TSe (SLGH PM Se), or TSe set to 0.5, 0.75, 0.99 (SLGH 0.5 Se, 0.75 Se, 0.99 Se), but the range around the CSe was wider with a TSe set to 0.5%. Figure 2 displays box-plots for the 0.1, 0.25, 0.5, 0.75 and 0.99 percentile of the CSe iterations of each simulation.

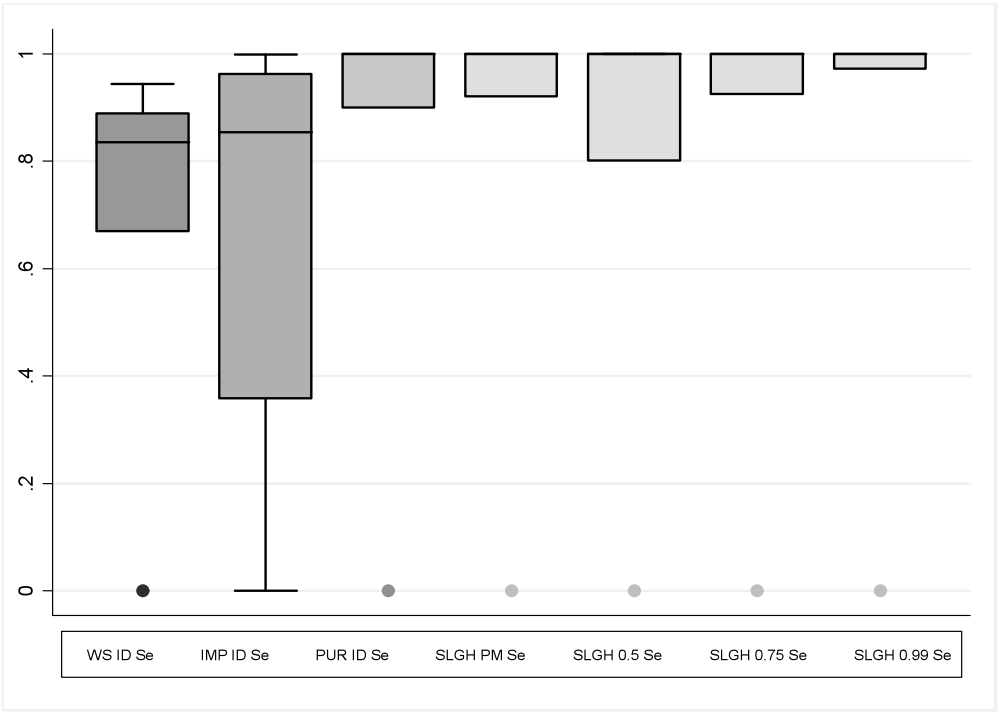

Fig. 2 Summary results of iterations for each simulated scenario

The sensitivity analysis of this model showed that the diagnostic method (TSe) was the most determinant factor on the variation of the output, followed by the within-herd prevalence.

The GEE model investigating the outbreak detection methods showed that the most significant surveillance method was post-mortem examination at slaughter, followed by tracing-on and tracing-back which confirmed the findings of the scenario tree model.

DISCUSSION

When implementing a surveillance system or evaluating different alternatives, scenario tree methodology is an interesting tool, as proven in the past (Frossling et al., 2009; Hadorn et al., 2002, 2008; Knight-Jones et al., 2010; Martin et al., 2007a,b; Racloz et al., 2008, Stark et al., 2006; Welby et al., 2009, 2010; Welby in press). Nevertheless, a limiting factor of this methodology is the access and availability of reliable/validated data regarding key parameters, such as the RR or PPr for the risk node categories of interest (Dohoo, 2009). The values for these parameters are often derived from expert opinion or literature, which could introduce bias. In the present study, an empirical method was used to estimate the key parameters, based on the available data of outbreaks in the past in Belgium.

The output of this study has underlined interesting features of the program, such as the importance of slaughterhouse surveillance (SLGH). It can be concluded that SLGH provides the best CSe. Nevertheless, in 25 % of the iterations the sensitivity of this component was below 0,80. In other words, this means that considering the different input parameters (TSe, n animals sampled, N animals in the population, RR), an infection present at 0.1 % design prevalence in a herd could remain undetected if only slaughterhouse surveillance was carried out. The same can be seen in the other components when looking at the 25th percentile of the iterations summary.

One possible explanation for the high CSe at slaughterhouse is the large sampling coverage for this component over the whole population. However, the efficiency of this component is highly dependent on individual visual inspection sensitivity (during meat inspection), as seen in the results of the sensitivity analysis. Variability in visual inspection sensitivity is also mentioned in the literature (Cousin and Florisson, 2005; Ryan et al., 2006). The GEE model, developed for validation purpose, supports this observation. In the past, slaughterhouse surveillance has been proven to be effective at national level as in some other MSs as well (Ebel et al., 2008; Collins, 2006; Probst et al., 2010; Van Asseldonk et al., 2005).

Though the WS has low CSe, partly due to the poor coverage of the population and the fraction of population sampled within this component, tracing-on and tracing-back is one of the key elements to identify the source of infection, as proven in the GEE model. Therefore, this component must not be removed from the current surveillance program. Furthermore, TSe attributed to this component was TSe of SST (ID). As a matter of fact the TSe of the WS could be assigned a higher value because it could be expected that in circumstances of outbreak investigation, the vigilance or the awareness of the veterinarians carrying out the SST will be higher compared to the common routine practices in normal circumstances, for instance testing at purchase.

Keeping good traceability of all animal movements is crucial in order to enable efficient tracing-back and tracing-on of infected herds. In a context where no more tests at purchase would be done, this traceability would be the only way to detect the route of dissemination of a bTB infection (Collins, 2006; Probst et al., 2010). Indeed in the context of freedom of disease of a MS, it can be assumed that the main route of (re-)infection or bTB dissemination would be by trade of infected animals (Gilbert, 2005; Probst, 2010) or the presence of a bTB wildlife reservoir, as in other MSs (Abernethy et al., 2006; More and Good, 2006). The latter is not the case in Belgium, so far.

The reason and the source of the spatial clusters identified in Belgium was unclear as it might be that either a true spatial cluster was linked to an environmental reservoir that gave a higher risk of infection in those areas, or that the level of disease awareness was higher in those areas, thus enhancing the number of cases found. Similar findings have been seen in other publications (Humblet et al., 2010). Because it was difficult to understand the nature of these clusters, zones were not taken into account in the scenario tree analysis.

It has been documented that concomitant use of SST and IFN-γ can help to increase the diagnostic sensitivity (Ebel et al., 2008; Collins et al., 2006; More and Good, 2006; Van Asseldonk et al., 2005; Vanholme, 2009). Persistence of infection on holdings that have carried out total sanitation by depopulation has been demonstrated in the past (Collins et al., 2006; Humblet et al., 2009, Ramirez-Villaescusa, 2010; Walravens et al., 2006; Wolfe, 2010). Therefore, it is crucial to maintain a certain amount of vigilance in those holdings. Implementing the concomitant use of SST and IFN-γ only in those holdings which had infected animals in the past 3 years could be an interesting alternative to increase the sensitivity in high-risk holdings.

Obtaining empirical figures for the test sensitivities would allow an increase in the validity of this model. Nevertheless, the simulations carried out in this study provided good insight into the key elements to be considered when implementing or revising the bTB surveillance program in Belgium such as required by European legislation and International standards.

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RISK OF INTRODUCING AFRICAN HORSE SICKNESS INTO THE NETHERLANDS BY IMPORTATION OF EQUINES

C.J. DE VOS·, C.A. HOEK AND G. NODELIJK

SUMMARY

African horse sickness (AHS) is a vector-borne viral disease of equines that is transmitted by *Culicoides* spp. Mortality in horses can reach 95%. The emergence of bluetongue in North-western Europe, another orbivirus of the family *Reoviridae*, and the potentially severe consequences of AHS have resulted in increased awareness of the risk of AHS to the Netherlands. The goal of this study was to provide more insight into (a) the regions and equine species that contribute most to this risk and (b) the seasonal variation in the risk. Model calculations indicated that the risk of introduction of AHS into the Netherlands by importation of equines was low with a median value of 5.1×10^{-4} per year. The risk was highest in July and August. Equine importations in the period October till March posed a negligible risk. Importations of donkeys and zebras constituted the highest risk from endemic areas, while international movements of competition horses constituted the highest risk from areas currently free from AHS.

INTRODUCTION

African horse sickness (AHS) is a vector-borne viral disease of equines that is transmitted by *Culicoides* spp. The main field vectors of AHS virus (AHSV) are *C. imicola* and *C. bolitinos* (Coetzer & Guthrie, 2004). *Culicoides sonorensis* has been proven to be a competent vector of AHSV in experimental settings (Mellor & Hamblin, 2004). African horse sickness virus affects all equine species including horses, donkeys, mules, hinnies and zebras. Morbidity and mortality rates vary between species, horses being most susceptible to the virus. Mortality in horses can reach 95%, whereas infections in zebras are mostly subclinical (Coetzer & Guthrie, 2004). AHSV is an orbivirus belonging to the family *Reoviridae*, which also comprises bluetongue virus (BTV). Nine different serotypes of AHSV are known, with some cross protection between serotypes (Coetzer & Guthrie, 2004). African horse sickness is endemic in sub-Saharan Africa, where it is thought to persist due to the presence of zebras acting as a permanent virus reservoir (Mellor & Hamblin, 2004). Incursions of AHSV into Northern Africa, the Near East and the Iberian Peninsula have occurred, but the virus was not able to persist for more than three to four years at most in these non-endemic areas.

Importation of infected equines is considered an important introduction route for AHSV (Wilson et al., 2009). Several incursions of the virus outside its endemic region could be traced to translocation of sub-clinically infected equines. For example, the outbreaks of 1965 in

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Northern Africa were initiated by movement of infected donkeys across the Sahara from West Africa, while in 1987 AHSV was introduced into Spain by importation of subclinically infected zebras from Namibia (Mellor, 1993; Mellor & Hamblin, 2004; Wilson et al., 2009). The subsequent outbreaks gave evidence that Palaearctic species of *Culicoides*, especially *C. pulicaris* and *C. obsoletus* may have contributed to transmission of the virus in Spain (Mellor et al., 1990). The emergence of BTV serotype 8 in North-western Europe in 2006 demonstrated that these *Culicoides* species can indeed be competent vectors for orbiviruses, even under less favourable climatic conditions (Meiswinkel et al., 2008; Wilson & Mellor, 2009; MacLachlan & Guthrie, 2010). Future incursions of AHSV in regions where *C. pulicaris* and *C. obsoletus* are abundant might thus result in epidemic spread of the disease. Recent studies in the Netherlands found that over 90% of *Culicoides* found on horse were *C. obsoletus* (Van der Rijt et al., 2008; Sloet van Oldruitenborgh-Oosterbaan, 2009). The possible role of local *Culicoides* species in disease transmission, in combination with the potentially severe consequences of the disease, has led to increased awareness of the risk of AHS to the Netherlands. International transport of equines is one of the pathways along which AHSV could be introduced into the Netherlands.

To evaluate the risk of AHSV introduction into the Netherlands by importation of equines, a risk model was constructed. The aim of this model was to provide more insight into (a) the regions and equine species that contribute most to this risk and (b) the seasonal variation in this risk. More insight into the relative risk of risk regions and groups of equines will help decision-makers in prioritizing preventive measures.

MATERIALS AND METHODS

To analyse the risk of AHS to the Netherlands by the importation of equines, an import risk assessment was conducted with emphasis on the release and exposure assessment (OIE, 2010a). Both the probability of successful release of AHSV in the Netherlands and the probability of subsequent spread by local vectors to local hosts were calculated. Countries worldwide were grouped into three risk categories: (1) high risk regions, i.e., those countries in which the virus is presumed to circulate, (2) low risk regions, i.e., those countries that have experienced outbreaks of AHS in the past and/or where the main vector of AHS, *C. imicola*, is present, and (3) very low risk regions, i.e., all other countries (Fig. 1). Importations of equines were grouped according to species: (1) horses, (2) donkeys, mules and hinnies (in the remainder of the text called donkeys), and (3) zebras. Horses were subdivided into three categories based on purpose of importation: (1a) permanent importation, (1b) temporary importation of horses participating in equestrian events in the Netherlands, and (1c) re-importation of Dutch horses that participated in equestrian events abroad.

A scenario tree was constructed to outline all steps required for successful release of AHSV in the Netherlands and subsequent exposure and spread of disease (Fig. 2). This scenario tree was the basis for a stochastic risk model to quantify the contribution of risk regions and groups of equines to the risk of AHSV introduction into the Netherlands. Probabilities were calculated on a monthly basis to take into account seasonal fluctuations in AHS prevalence in the risk regions and the seasonal effect of temperature on vector abundance and vector biology in the Netherlands. Model calculations were performed in Microsoft Office Excel 2003 and @Risk 4.5.3 (Palisade Corporation, 2004), running 10,000 iterations for each scenario. The sensitivity analysis tool in @Risk was used to evaluate the impact of uncertain and variable input parameters on model results.

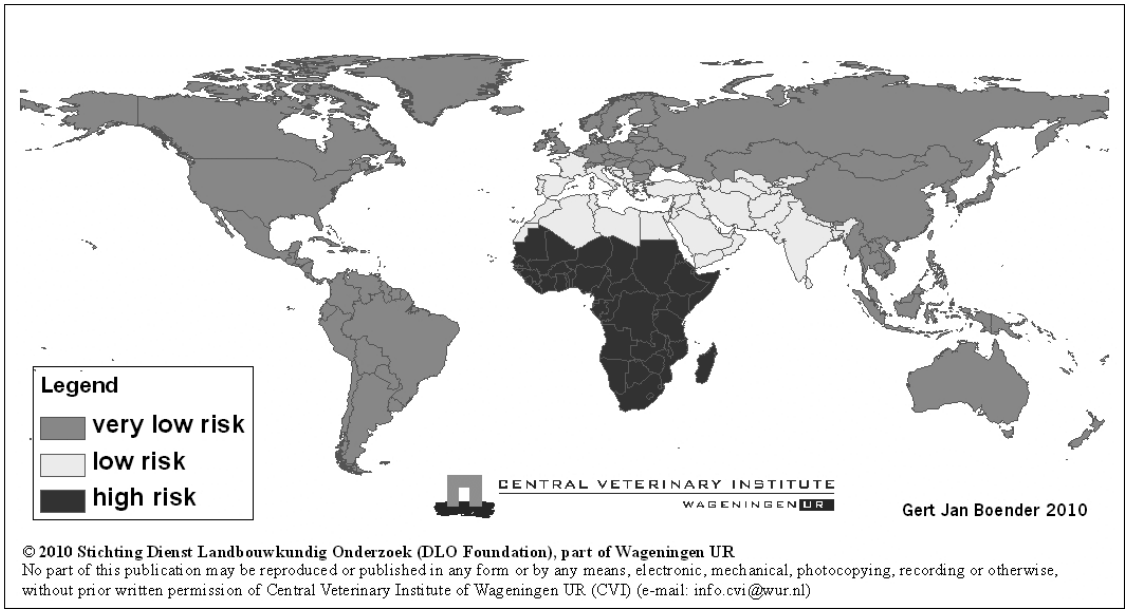

Fig. 1 Countries of the world classified as high risk, low risk and very low risk regions for African horse sickness occurrence

Model calculations

Probability of release: The model steps to evaluate the probability of release were (a) selection of an infected equine for importation, i.e. an equine in the incubation period or viraemic stage of disease, (b) detection of the infection in the risk region, and (c) detection of the infection during transport. The probability of AHSV release per imported equine ($P\_rel_{ij,m}$) was calculated as[1]:

$$P\_rel_{ij,m} = P\_\inf_{ij,m} \times P\_vir_{ij} \times (1 - P\_CF_{ij}) \times (1 - P\_C_{ij}) \times (1 - P\_T_{ij}) \quad (1)$$

where $P\_inf_{ij,m}$ is the probability that an equine of species i selected for importation from risk region j in month m is infected with AHSV, P_vir_{ij} is the probability that the equine is in its incubation or viraemic period at the moment of importation, P_CF_{ij} is the probability that the equine is detected by testing based on serology (here: complement fixation (CF) test), P_C_{ij} is the probability that the equine is detected by clinical inspection, and P_T_{ij} is the probability that the equine is detected during transportation.

$P\_inf_{ij,m}$ was calculated by multiplying the probability that the risk region experienced an outbreak (PO_j) and the monthly cumulative incidences ($CI_{ij,m}$). If quarantine was applied, the probability of infection during the quarantine period was reduced by the protective effect of quarantine (Pv_{ij}). Only AHSV-infected equines incubating the disease or in the viraemic period

[1] Explanation of subscripts:
i = equine species, where 1 = imported horses, 2 = imported donkeys, mules and hinnies, 3 = imported zebras, 4 = temporary importation of horses participating in international events in the Netherlands, 5 = re-importation of Dutch horses that participated in international events abroad
j = risk region, where 1 = high risk, 2 = low risk, 3 = very low risk
m = month, where 1 = January, 2 = February, …, 12 = December

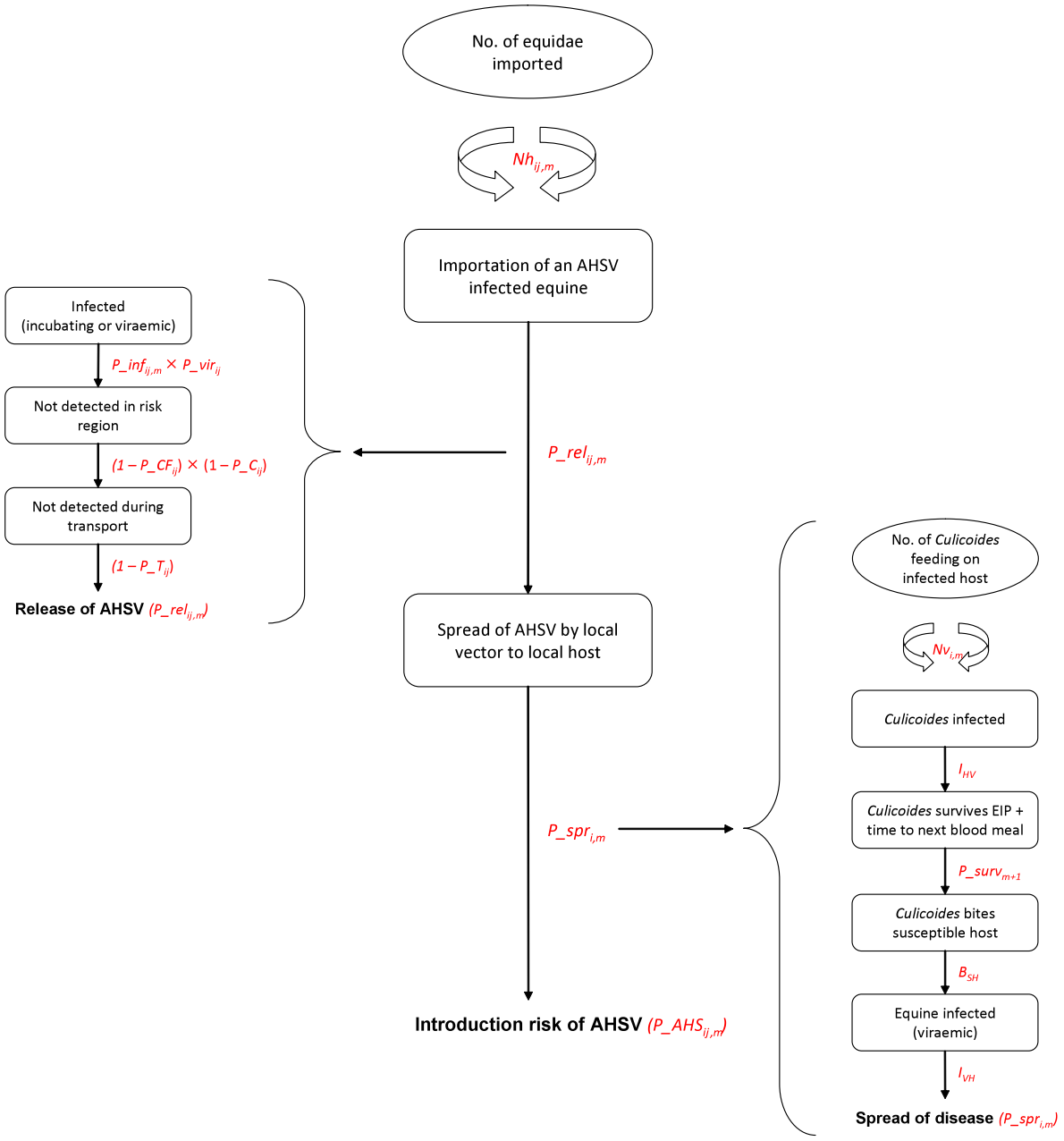

Fig. 2 Scenario tree for the introduction risk of AHSV to the Netherlands indicating the steps required for successful release of AHSV and subsequent spread of disease

pose a risk to the importing country. P_vir_{ij} was calculated taking into account the moment at which the equine was infected, the length of the incubation period (I_i) and the viraemic period (V_i), and the transportation time (T_{ij}).

Only equines that have seroconverted can be detected by the CF test. P_CF_{ij} was calculated taking into account the moment at which the equine was infected, the time needed to seroconvert after infection (S_i), and the sensitivity (Se_{CF}) and specificity (Sp_{CF}) of the CF test. Clinical inspection can only detect those equines that exhibit clinical signs. Only animals in the viraemic stage of infection were assumed to show clinical signs. P_C_{ij} was calculated taking into account

the moment at which the equine was infected, the length of the incubation (I_i) and viraemic (V_i) period, and the sensitivity of clinical inspection (Se_C). Specificity of clinical inspection was assumed to be 1. Detection during transport was assumed to be only possible if the equine exhibited clinical signs at the end of the transportation time (T_{ij}). Calculations for P_T_{ij} were similar to those for P_C_{ij}.

Probability of exposure and spread: Onward transmission of AHSV after importation of an infected equine is only possible if competent vectors are present. Model calculations assumed that AHSV will replicate in local *Culicoides* species in the Netherlands after uptake of an infectious blood meal and that these *Culicoides* can subsequently transmit the virus to susceptible equines. Calculations for exposure of local hosts to the virus comprise four steps: (a) number of vectors feeding on an imported infected equine during its viraemic period ($Nv_{i,m}$), (b) probability that a vector feeding on an infected equine gets infected (I_{HV}), (c) probability that the vector survives the extrinsic incubation period (EIP) and completes a gonotrophic cycle after becoming infectious, i.e., will at least take one blood meal while being infectious (P_surv_m), and (d) probability that the infectious vector bites a susceptible host (B_{SH}). Exposure only results in spread of disease if the latter step results in infection, which is a chance process with probability I_{VH}.

$Nv_{i,m}$ was calculated as:

$$Nv_{i,m} = Vh_m \times V_i \times 1/GC_m \tag{2}$$

with Vh_m is number of vectors per equine host in month m, V_i is the length of the viraemic period of host species i, and GC_m is the temperature-dependent length of the gonotrophic cycle of the vector in month m (reciprocal of the biting rate). P_surv_m was calculated as:

$$P\_surv_m = \exp^{-(n_m \times GC_m \times MR_m)} \tag{3}$$

where n_m is the number of gonotrophic cycles that needs to be completed to take a blood meal while being infectious and MR_m is the temperature-dependent mortality rate of *Culicoides* spp. in month m. B_{SH} was modelled as (Gubbins et al., 2008):

$$B_{SH} = 1/\left(V_{pref} \times R_{re} + 1\right) \tag{4}$$

with V_{pref} is the vector preference for animal species ($V_{pref} < 1$ if *Culicoides* feed preferentially on equines) and R_{re} is the ratio of ruminants to equines. I_{HV} and I_{VH} were both modelled by Beta distributions with parameters based on experimental data (Vose, 2008). I_{HV} was modelled as a Beta(171,3918) distribution (Paweska et al., 2003; Venter & Paweska, 2007) and I_{VH} was modelled as a Beta(6,2) distribution (Baylis et al., 2008).

The probability of spread of disease after introduction of AHSV by one infected equine ($P\_spr_{i,m}$) was calculated assuming a binomial process:

$$P\_spr_{i,m} = 1 - \left(1 - I_{HV} \times P\_surv_{m+1} \times B_{SH} \times I_{VH}\right)^{Nv_{i,m}} \tag{5}$$

The probability that a vector survives the EIP and the time to its next blood meal were based on the values in the month after introduction, i.e. P_surv_{m+1}, since it is likely that the time span to complete the EIP and/or the next gonotrophic cycle will extend into the next month.

Risk estimate: The risk of AHSV introduction into the Netherlands by equine species *i* originating from risk region *j* in month *m* ($P\_AHS_{ij,m}$) was calculated taking into account $P\_rel_{ij,m}$, $P\_spr_{i,m}$ and the number of equines of species *i* imported from region *j* in month *m* ($Nh_{ij,m}$):

$$P\_AHS_{ij,m} = 1 - \left(1 - P\_rel_{ij,m} \times P\_spr_{i,m}\right)^{Nh_{ij,m}} \quad (6)$$

Input data

Vertebrate hosts: Model input parameters for vertebrate hosts are the length of the incubation (I_i) and viraemic (V_i) period, time till seroconversion (S_i) and the case fatality rate (cf_i). I_i was modelled by a Pert(2,6,10) distribution for all equines (Coetzer & Guthrie, 2004; Mellor & Hamblin, 2004). V_i is longer for surviving animals than for animals succumbing from disease and differs per equine species. V_1 is on average 4.5 days for dying horses and 6 days for surviving horses, V_2 is 12 days for dying donkeys and 28 days for surviving donkeys, and V_3 is 28 days for dying zebras and 40 days for surviving zebras (Barnard et al., 1994; Fassi-Fihri et al., 1998; Mellor & Hamblin, 2004; MacLachlan & Guthrie, 2010). Time until seroconversion was modelled by a Uniform(10,14) distribution for all equines (House et al., 1992; Laegreid, 1994). The case fatality rate (cf_i) was set at 0.7 for horses, at 0.1 for donkeys, and 0.01 for zebras (Coetzer & Guthrie, 2004; Wilson et al., 2009).

Vector biology: Model input parameters for the vector, *Culicoides* spp., vary over the seasons and mainly depend on temperature values. Monthly temperature values (°C) in the model (T_m) were based on observed monthly temperatures in De Bilt, the Netherlands over the last 30 years (1979-2008) (Fig. 3). Temperature values were simulated using a truncated normal distribution with the average temperature in the last 30 years as μ and its standard deviation as σ, and the 1st and 99th percentile values of the observed temperatures as lower and upper bounds, respectively.

Mortality rate of the vector in month *m* (MR_m) was modelled as (Backer & Nodelijk (2011) based on data from Wittmann et al. (2002) for *C. sonorensis*):

$$MR_m = 0.015 \times e^{(0.063 \times T_m)} \quad (7)$$

The length of the gonotrophic cycle in month *m* (GC_m) was modelled as (Wittmann et al., 2002):

$$GC_m = -1.98 + 0.07217 \times T_m + 2516.65 / T_m^{\,2} \quad (8)$$

The length of the EIP in month *m* (EIP_m) was modelled as (Wittmann et al. (2002), AHSV serotype 4 in *C. sonorensis*):

$$EIP_m = 1/\left(0.0085 \times T_m - 0.0821\right) \quad (9)$$

Abundance of vectors is also temperature-dependent, although factors like humidity and biotope also play an important role. In 2007, in the Netherlands *Culicoides* were caught with black light traps during the whole year at a weekly interval at 21 locations. These data were used to calculate the number of *Culicoides* caught per trap in each month which was used as a proxy for the numbers of *Culicoides* per equine in month *m* (Vh_m) (Fig. 4). Vh_m was modelled using a truncated normal distribution with average monthly values and their standard deviations as μ and σ, and the 5th and 95th percentile values as lower and upper bounds, respectively.

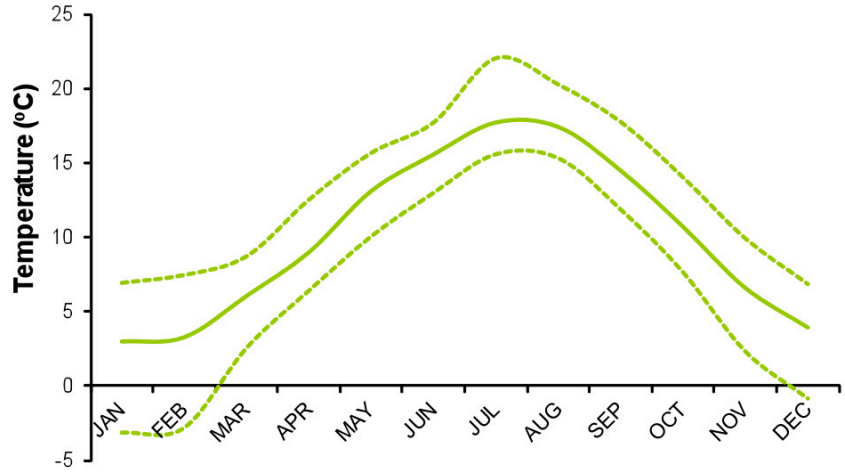

Fig. 3 Average monthly temperatures (°C) in De Bilt, the Netherlands over the period 1979-2008 (solid line) and 1st and 99th percentile values (dashed lines). Source: KNMI (2009).

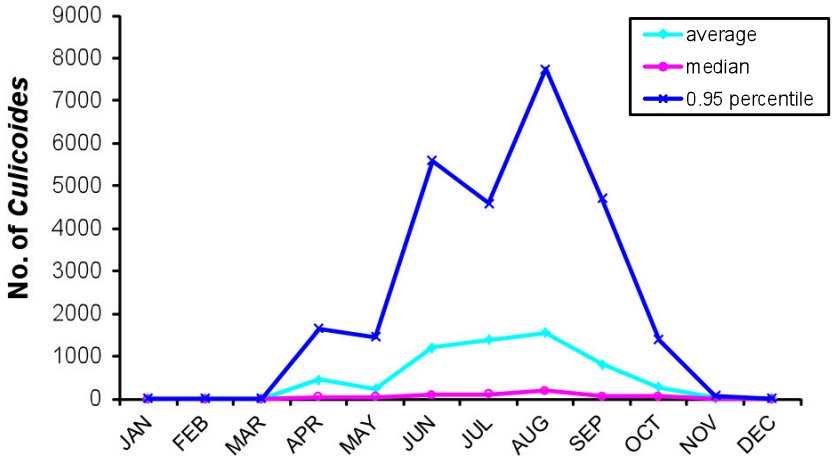

Fig. 4 Number of *Culicoides* caught per trap in the Netherlands in 2007. Average, median and 95th percentile values of black light traps at 21 different locations. Source: VWA.

Risk regions: In high risk regions, AHSV was assumed to be endemic and the probability of AHSV presence (PO_I) was set to 1 in the model calculations. For low and very low risk regions, PO_j was based on historical evidence of AHS occurrence in these regions over the last 60 years. Furthermore, the length of the expected high risk period (HRP_j), i.e. the period from first infection till first detection of disease, was taken into account, since importations of equines from these regions will only be possible during the high risk period. In the period 1950-2009, AHSV was present during 15 years in low risk regions (Mellor, 1993; Wilson et al., 2009; OIE, 2009; MacLachlan & Guthrie, 2010), while no AHS outbreaks have ever been observed in very low risk regions. Based on the outbreaks in Spain in the period 1987-1990, incursions of AHSV in low and very low risk regions were only simulated for the period July-December (Rodriguez et al., 1992; Mellor, 1993). HRP_j was calculated assuming that the first case of AHS in a country will not be diagnosed and that detection will be in second generation infected equines. The

calculated average value of HRP_j was 26 days for low risk regions and 77 days for very low risk regions based on temperatures of 18°C and 12°C, respectively. This is in accordance with the high risk period observed in the 1987 AHS epidemic in Spain, where the first clinical signs were detected 26 days after arrival of the infected zebras (Mellor, 1993).

Cumulative incidence of AHSV in horses in high risk regions was based on the maximum values of the monthly cumulative incidences reported for South-Africa over the period 2005-2008 (OIE, 2009; FAO, 2009). Cumulative incidence of AHSV in donkeys and zebras in high risk regions was calculated taking into account the foaling season and the rate of seroconversion in foals assuming a 95-100% seroconversion at the age of one year (Mattioli et al.,1992; Barnard, 1993). Cumulative incidence of AHSV in all equine species during an outbreak in low or very low risk regions was based on the number of horses reported death and destroyed and the number of horses vaccinated ('at risk' population) in the AHS outbreaks in Spain in 1987-1990 (Rodriguez et al., 1992).

Preventive measures: Preventive measures in the model were based on national and international regulations for importations of equines. For high risk regions, a 40-day quarantine period preceding exportation, a serological test (CF test) ten days before transportation, and clinical inspection on the day of embarkation were assumed (CEC, 1992; OIE, 2010a; OIE, 2010b). Equines originating from low and very low risk regions were assumed to only have been clinically inspected two days before transportation in accordance with regulations for intra-EU trade (CEC, 1990). Furthermore, competition horses going to or coming from low risk regions were assumed to be kept in restricted conditions being half as effective as quarantine.

The protective effect of quarantine (Pv_{ij}) was simulated by a uniform (0.5,0.9) distribution (EFSA, 2008). Experimental data were used to estimate sensitivity (Se_{CF}) and specificity (Sp_{CF}) of the CF test (House et al., 1990). Se_{CF} was modelled by a Beta(60,4) distribution with a mean value of 0.94 and SpCF was modelled by a Beta (62,2) distribution with a mean value of 0.97. The sensitivity of clinical inspection (Se_C) was set equal to the case fatality rate (cf_i). Specificity of clinical inspection was assumed to be 1. The sensitivity of detection during transport was set equal to Se_C.

Number of equines imported: The average number of equines imported in the Netherlands from the different risk regions was estimated using several databases on equine importations. Import figures of horses and donkeys (including mules and hinnies) were based on trade statistics over the period 1999-2008 (Eurostat, 2010). Import figures of zebras were derived from the International Species Information System (ISIS) over the period 2000-2007 (pers. comm. L. Versteeghe, Safaripark Beekse Bergen). The numbers of competition horses travelling to and from the Netherlands were derived from lists of international equestrian events given by the Royal Dutch Equestrian Federation (KNHS, 2010) and results of international horse events (Paardensport.nu, 2010; FEI, 2010). All calculated numbers are given in Table 1.

Table 1. Calculated annual number of equines imported into the Netherlands from high, low and very low risk regions[a].

Equine species	High	Low	Very low	Total
Imported horses	6.2	129	1626	1761
Imported donkeys	0.10	1.8	36	37
Imported zebras	0.13	0.63	4.6	5.4
Temporary importation of horses participating in equestrian events in NL	25	1857	4707	6590
Re-importation of Dutch horses that participated in equestrian events abroad	2.0	1225	4795	6022
Total	34	3214	11168	14416

[a] Numbers in rows and columns do not always add to the total number due to use of rounded values

RESULTS

Figure 5 gives the seasonal risk of AHSV introduction into the Netherlands. From November to February, the risk is zero because onward spread is not possible in these months. In March and October, there is only a remote probability with a mean value $< 10^{-8}$. From April to June, the median risk varies between 1×10^{-5} to 3×10^{-5} and is only due to equine importations from high risk regions. The median risk is highest in July (2.3×10^{-4}) and August (1.6×10^{-4}), when outbreaks of AHS are deemed to be possible in low and very low risk regions. The median annual risk is 5.1×10^{-4}, i.e., introduction of AHSV resulting in epidemic spread in the Netherlands is to be expected approximately once every 2000 years, with 5th and 95th percentile values being 2.5×10^{-4} to 9.4×10^{-4}, respectively.

Although the number of equine importations into the Netherlands coming from high risk regions is very low (only 0.2% of all equine importations), these regions contribute 31% to the introduction risk of AHSV to the Netherlands. Low risk regions account for 22% of equine importations and contribute most to the introduction risk of AHSV to the Netherlands with 53%. Although very low risk regions account for 77% of equine importations, these regions only contribute 16% to the introduction risk of AHSV to the Netherlands.

International movements of competition horses constitute the vast majority (87%) of all equine importations into the Netherlands and, in general, contribute most to the introduction risk of AHSV to the Netherlands with 67%. Donkeys and zebras contribute, however, most (90%) to the risk that AHSV is introduced from high risk regions although the numbers imported are extremely low (<1%).

The sensitivity analysis tool in @Risk was used to calculate the correlation coefficients of all uncertain and variable input parameters with the annual introduction risk of AHSV to the Netherlands. Results are displayed in Fig. 6 for input parameters for which correlation was $\geq |0.1|$. Variability in the length of the incubation period in equines (I_i) and uncertainty regarding the occurrence of AHS outbreaks in low risk regions (PO_2) had most impact on the calculated introduction risk of AHSV to the Netherlands.

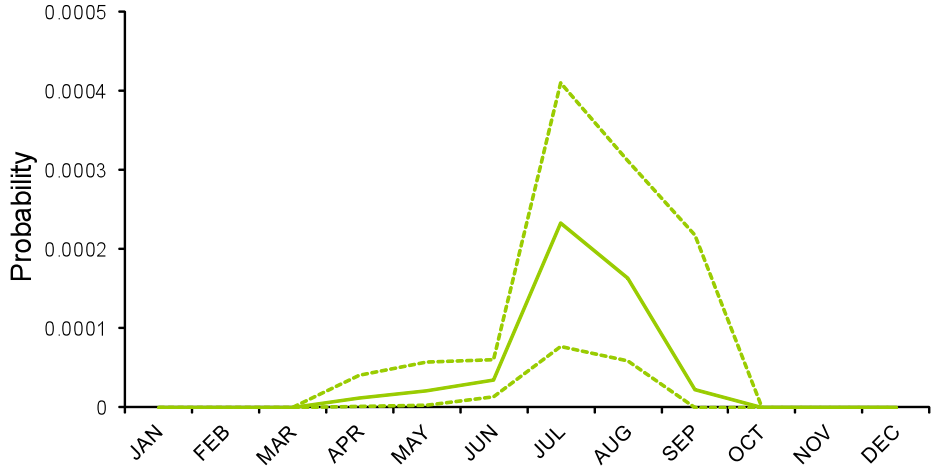

Fig. 5 Median (solid line) and 5th and 95th percentile values (dashed lines) of the seasonal risk of AHSV introduction into the Netherlands.

DISCUSSION

Model calculations indicated that the risk of AHSV introduction into the Netherlands by importation of equines is very low with a median value of 5.1×10^{-4} per year. Not only the probability of release was taken into account when estimating the risk, but also the probability of spread by local vectors to local hosts. Under the assumption of a competent local vector, establishment of the virus is possible from April till September with probabilities being highest in the period from June till August. Importations of AHSV infected equines in the period October till March pose a negligible risk unless the virus is able to survive in either vector or host till temperatures rise. The introduction risk of AHSV to the Netherlands is highest in July and August (Fig 5). This is explained by the favourable temperatures in these months and the possible presence of non-notified AHS in low and very low risk regions in the period from July till December. Furthermore, international movements of competition horses peak in May, June and July.

High and low risk regions contribute most to the introduction risk of AHSV to the Netherlands with 31% and 53%, respectively. Only few equines are transported from high risk regions to the Netherlands (Table 1), precisely because the probability of AHSV release per animal is relatively high. Although most equines imported into the Netherlands come from very low risk regions, probabilities of release per equine are much lower than for equines from high and low risk regions explaining the relatively low contribution of very low risk regions to the introduction risk of AHSV to the Netherlands (16%).

For high risk regions, importations of donkeys and zebras constitute the highest risk of AHSV introduction into the Netherlands, although the average number of donkeys and zebras imported annually is very low with an expected value of 0.10 for donkeys and 0.13 for zebras. The high risk posed by these equines is explained by the relatively high expected seroprevalence in donkeys and zebras in these countries, their prolonged viraemic period, and the often subclinical presence of disease. For low and very low risk regions, international movements of

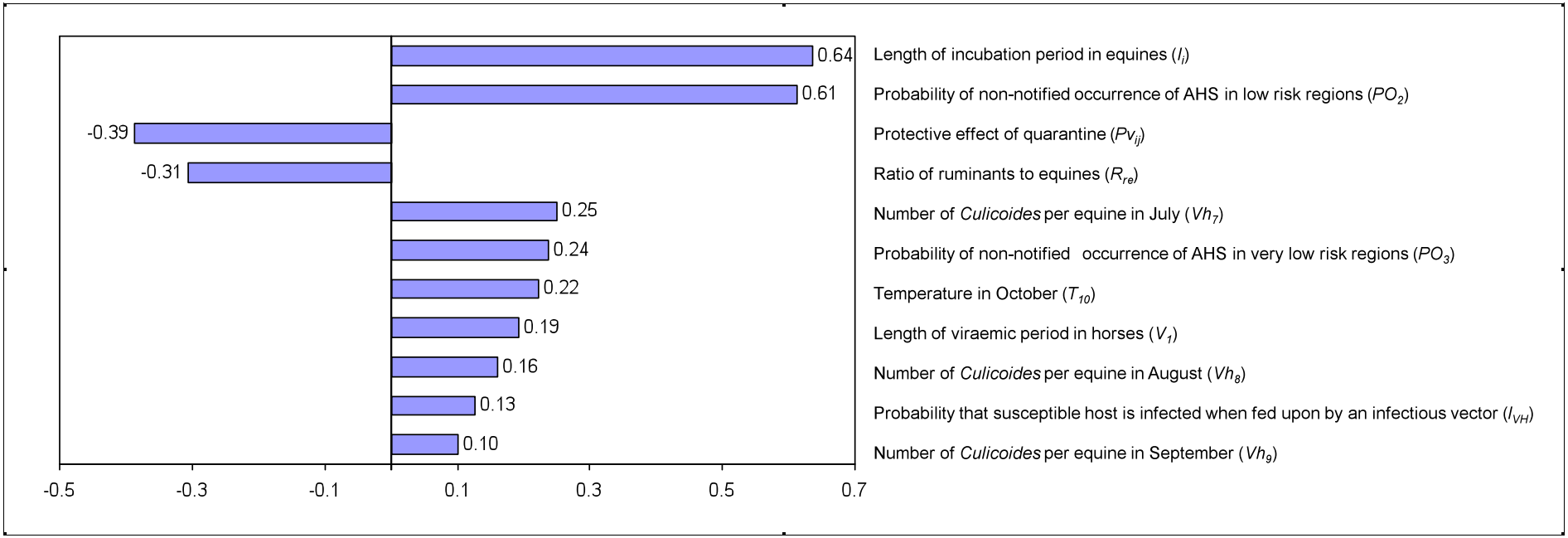

Fig. 6 Correlation of model input parameters with the annual risk of AHSV introduction into the Netherlands. Only input parameters with a correlation ≥ $|0.1|$ have been included in the tornado chart.

competition horses constitute the highest risk. This is explained by the high number of competition horses moved into the Netherlands (Table 1).

Absolute values calculated with the model cannot be considered true values because of the large uncertainty involved in estimating model input parameters. The sensitivity analysis indicated that model results were especially sensitive to the probability of non-notified presence of AHS in low and very low risk regions (PO_2 and PO_3), the protective effect of quarantine (Pv_{ij}), and the vector-host ratio (R_{re} and Vh_m). Model results were also correlated with the length of the incubation period in equines (I_i), the length of the viraemic period in horses (V_1) and monthly temperature values (T_m). The input distributions for these parameters represent, however, variability rather than uncertainty. The numbers of equines imported into the Netherlands were not considered in the sensitivity analysis, since single input values, not distributions, were used for these input parameters in the model calculations. However, the numbers of equines imported into the Netherlands are also highly correlated with the introduction risk of AHSV to the Netherlands.

The probability of non-notified presence of AHS in low and very low risk regions was based on historical data of AHS occurrence in these regions and the expected length of the high risk period in both regions. Historical reports of disease are, however, usually not a good predictor of future occurrence of disease. The likelihood of AHS incursion into these regions might have changed over time due to, for example, increased international trade and globalization or climate change. An increased probability of presence of AHS in low and very low risk regions will result in a strong increase of the introduction risk of AHSV to the Netherlands.

The number of equines imported into the Netherlands is not easy to trace. For example, intra-EU movements of registered horses are not registered in the TRACES (Trade Control and Expert System) database of the EU. Furthermore, trade statistics proved by Eurostat (2010) were not deemed reliable for 2007 given the huge increase in horse importations in comparison to previous years. Besides, international movements of competition horses were estimated using participant lists of international equestrian events and match results. Although these lists do provide the nationality of the horse rider, this is not always the country where the horse and its horse rider resides. Last, but not least, illegal equine importations were not included in the model, but can never be excluded.

Vector-host ratios were estimated using data from *Culicoides* catches in 2007 assuming that the average number of *Culicoides* caught per trap equals the number of *Culicoides* feeding on one equine. These data only give an estimate of vector abundance for 21 locations in the Netherlands and model calculations do not take into account that the presence of *Culicoides* might show large variance within short distance. Furthermore, the exact number of horses in the Netherlands and their locations is not known, impeding the estimates of the vector-host ratio and the ratio of ruminants to equines.

The risk model described in this paper only addressed the risk of AHSV introduction into the Netherlands by equine importations. Other pathways might be as important in releasing the virus into the country, e.g., entry of infectious vectors (*Culicoides*) by active flight or passive flight (Mellor et al., 2000; Baylis et al., 2008), travelling on aircraft, (livestock) trucks or vessels, or lifting on non-susceptible animals, humans or plant materials. Furthermore, importations of live animal products (genetic material, serum, plasma) or use of live modified vaccines might result in release of the virus into an area free of disease, although transmission of AHSV by genetic material has not been documented.

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THE ECONOMICS OF DISEASE MITIGATION: A CASE STUDY OF BOVINE VIRAL DIARRHOEA

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SUMMARY

The aim of this study was to conduct an economic evaluation of the Swiss national mitigation programme for Bovine Viral Diarrhoea Virus (BVDV). The ongoing eradication phase comprises testing and slaughtering of all persistently infected (PI) animals found. After eradication, a surveillance programme will be implemented to document freedom from disease. Cumulative eradication costs and benefits were estimated to determine the break-even point of the eradication programme and the residual amount (RA) justifiable for surveillance expenditures after eradication. Further, costs of four putative surveillance strategies based on antibody testing in blood and/or milk and the net benefit of the mitigation programme were estimated. The break-even point was estimated to be reached in 2012 and the RA for surveillance at 63.15 m CHF (90% confidence interval, CI: 53.72-72.82 m CHF). Discounted surveillance cost for all surveillance scenarios were found to be smaller than this RA, which means that the mitigation programme overall is expected to produce a net economic benefit.

INTRODUCTION

People gain economic value from the consumption of goods or services created in animal production systems by the transformation of scarce resources into products. Animal disease reduces the quantity of outputs available for people's consumption, which represents a decrease in people's potential economic well-being. To counter such negative disease effects additional surveillance and intervention resources are expended for disease mitigation, which reduces prevalence or incidence in the population. Surveillance helps to offset negative disease effects by promoting successful interventions. The scale and ratios in which the two elements are combined affect the efficiency of mitigation, its costs, benefits, and thus net effect on society's well-being. For economic appraisal it is indispensable to investigate and understand the technical and economic relationships between surveillance, intervention and mitigation. Resources for surveillance and intervention should be combined both at least cost and at levels consistent with the scale of output losses avoided that results in maximum net benefits overall.

The Swiss surveillance and intervention programme for BVDV was used as a case study to investigate the technical and economic relationships of elements of disease mitigation. Economic costs to farmers due to BVDV accrue from reduced conception rates, abortions and stillbirths, reduced milk yield, premature culling, reduced weight gain, and increased veterinary treatment costs (Bennett, accessed November 2010). Moreover, animal welfare may be affected

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by stress and pain associated with mucosal disease (Lindberg et al., 2006) and consumer welfare by increased commodity prices (Gunn et al., 2005). However, it is difficult to calculate these disease costs exactly, because of the variable infection dynamics in a cattle population and the often vague clinical signs (Sandvik, 2004). Despite such complications, a wide range of studies have estimated the magnitude of economic costs accruing from BVDV (Houe, 2003). In 2001, BVDV disease costs in the Swiss cattle population were estimated to be 8.8 m CHF per year (95% CI: 5.5 - 12.9 m CHF) (personal communication P. Schaller). But the relevant question is not what the disease costs are, but to what extent it is beneficial to control the disease (McInerney, 1996).

In 2008, the Swiss Federal Veterinary Office (FVO) initiated a compulsory national eradication programme for BVDV based on individual identification and elimination of PI animals in the Swiss cattle population. In the initial phase, the whole Swiss cattle herd was tested for virus detection in a short period of time. Tissue samples were taken using special ear tags and tested in certified laboratories using either real-time reverse transcriptase polymerase chain reaction (rtPCR) or enzyme-linked immunosorbent assay (ELISA) to detect virus-positive animals. When required, confirmatory tests were performed using blood samples (Presi and Heim, 2010). Persistently infected animals were slaughtered and new infections avoided by animal movement restrictions. Since October 2008, all newborn calves have been subject to antigen testing to identify and slaughter PIs (calf phase). The eradication programme will cease at the end of 2011 and a surveillance programme will be implemented to document disease freedom and to detect infected animals early enabling rapid response. The projected duration of the mitigation programme is 2008 to 2017. As of November 2010, 3 million cattle have been tested and 19,064 PI animals were found and slaughtered, thereby reducing the PI prevalence of newborn calves from an initial 1.47% (95% CI: 1.44-1.50%) to 0.13% (95% CI: 0.12-0.14%).

The aim of this study was to conduct an economic evaluation of the Swiss mitigation programme for BVDV. The objectives were 1) to calculate the costs and benefits of the eradication programme to determine its break-even point and the residual amount (RA) potentially available for surveillance expenditures, 2) to estimate the cost of putative surveillance strategies to confirm disease freedom following BVDV eradication and 3) to assess the overall economic value of the mitigation programme.

METHODS

General overview

In part 1 of this research, the eradication cost and benefit were calculated to determine the break-even point of the programme and the RA justifiable for surveillance expenditure after BVDV eradication. The total benefit of the eradication programme was calculated as the disease costs avoided when compared with a baseline scenario. Because the results of a BVDV survey conducted ten years ago (Rüfenacht et al., 2000) and the data from the initial phase (Presi and Heim, 2010) showed very similar PI prevalences of 0.64 and 0.80%, respectively, the baseline chosen was a situation of endemic equilibrium.

In part 2, costs of putative alternative surveillance scenarios were calculated to assess their economic acceptability and identify the least-cost option. Four antibody surveillance scenarios were proposed by decision-makers: annual antibody testing in S1) blood of all calves 6 to 18 months old, S2) milk from all first lactating cows, S3) blood of calves 6-18 months old on 50%

of farms and milk from first lactating cows on the other 50% of farms, S4) milk and blood simultaneously in 50% of farms. For practicality, first-lactating beef cows were to be blood instead of milk sampled. For all scenarios, it was defined that the whole herd would be tested for antigen if > 40% of all samples per farm were seropositive.

A stochastic spreadsheet model for the economic analyses was developed using @Risk software for Excel version 5.0 (Palisade Corporation, Newfield, NY, USA). All uncertain data values were integrated as distributions and the model was run with 10,000 iterations over 10 years. Sensitivity runs were conducted to assess the impact of all uncertain parameters with respect to the outputs using the in-built sensitivity analysis tool. All monetary values were expressed in Swiss Francs, CHF (1 CHF = 0.73 € at the time of analysis). All future costs and benefits needed to be translated into present values by multiplying the costs or benefits by the discount factor $1/(1 + r)^t$, where r = 3.5% is the selected discount rate and t the time in years.

Epidemiological model and study population

In an independent project, a stochastic compartmental model was developed to study BVDV spread between farms at country level (Presi et al., 2009). In this model, animals on a farm are allocated to compartments according to their age and health status, the model unit being a combination of age and health status. After each time step the number of animals in a specific compartment is updated according to demographic and infection parameters. The model has a time step of 14 days, the minimal time period to follow the presence of transiently infected (TI) animals. The model's outputs regarding the health situation are the number of PIs, TIs and the number of seropositive animals in each age group at farm level. The effect of mitigation strategies can be evaluated observing the evolution of those numbers.

The study population included 43,267 farms that kept cattle as recorded in the 2008 national agricultural census. Animal categories used were based on the epidemiological model and included calves (CV), heifers (H) and cows (C). The number of PI calves (PIcv), PI heifers (PIh), PI cows (PIc), TI calves (TIcv), TI heifers (TIh) and TI cows (TIc) was either derived from data gathered during the eradication phase of the programme or from epidemiological modelling predictions when data were unavailable.

Part 1: Estimation of the break-even point of the eradication programme and residual amount available for surveillance

The disease costs for the baseline and the eradication programme included production losses and expenditures for palliative treatment of sick animals. Subtraction of the cumulative cost from the cumulative benefit (difference between the eradication programme and the baseline) over a ten year time period yielded the RA potentially available as the maximum expenditure justifiable for surveillance.

Monetary values for production losses were estimated by multiplying the number of TIs or PIs affected by physical loss and price coefficients. Monetary eradication cost comprised labour, operations and expenses. Labour costs were calculated by multiplying the number of working hours per job position spent on the programme by the corresponding wage rate. Costs for operations and expenses were calculated by multiplying the price of a process (e.g. blood sampling, ELISA) by the number of units used. The input data for labour, operations and expenses were either requested from the respective institutions or businesses that delivered the service or indicated by FVO staff involved in the mitigation programme.

Whenever available, Swiss-specific data were used. The number of laboratory tests performed, animals tested, and PIs detected until October 2010 were actual figures from the ongoing programme. For the time after October 2010, epidemiological modelling output was used to estimate costs and benefits. Where no or insufficient data were available, data from the scientific literature were used and complemented by expert opinion and information received from veterinary practitioners in Switzerland.

Monetary eradication benefit: The monetary benefit of eradication comprised avoided production losses and expenditures for palliative treatment (E_{PT}) and laboratory testing (E_{LT}). The production losses included losses due to mortality (L_M), premature culling (L_{PMC}), abortion (L_A), and reduced milk yield (L_{RMY}), and were calculated as outlined below. Input data used are listed in Table 1.

$$L_M = \sum N_{X1} \cdot Mt_{X1} \cdot [(AV]_{X1} + RC_{X1}) \quad (1)$$

Where *N* stands for the number of animals affected, *X1* for PIcv, PIh, PIc, TIcv, TIh, TIc, *Mt* for mortality rate, *AV* for animal value and *RC* for rendering costs.

$$L_{PMC} = \sum N_{X2} \cdot PMC_{X2} \cdot (AV_{X2} - SV_{X2}) \quad (2)$$

Where X2 stands for TIh and TIc, *PMC* for the premature culling rate and *SV* for slaughter value.

$$L_A = \sum N_{X3} \cdot AR_{X3} \cdot CA \quad (3)$$

Where *X3* stands for TIh and TIc, *AR* for abortion rate, and *CA* for costs per abortion.

$$L_{RMY} = [(N]_{PIc} \cdot RMY_{PIc} + N_{TIc} \cdot PropML_{TIc} \cdot RMY_{TIc}) \cdot MY \cdot PM \quad (4)$$

Where *RMY* stands for the rate of reduced milk yield, $PropML_{TIc}$ for the proportion of TIc showing milk loss, *MY* for milk yield and *PM* for the production price per kg milk.

The E_{PT} for PIs (E_{PT1}), TIcv (E_{PT2}), and for TIh and TIc (E_{PT3}) were calculated as follows:

$$E_{PT1} = \sum N_{X4} \cdot PropRVT \cdot [Mt_{X4} \cdot PVT_{X4D} + (1 - Mt_{X4}) \cdot PVT_{X4S}] \quad (5)$$

$$E_{PT2} = \sum N_{TIcv} \cdot PropRVT \cdot [Mt_{TIcv} \cdot PVT_{TIcvD} + (1 - Mt_{TIcv}) \cdot Mb_{TIcv} \cdot PVT_{TIcvS}] \quad (6)$$

$$E_{PT3} = \sum N_{X5} \cdot PropRVT \cdot Mt_{X5} \cdot PVT_{X5D} \quad (7)$$

Where *X4* stands for PIcv, PIh, and PIc, *X5* for TIh and TIc, *PropRVT* for the proportion of morbid animals receiving veterinary treatment, PVT_{XD} for the price of veterinary treatment for animals that die despite treatment, PVT_{XS} for the price of veterinary treatment for animals that survive and *Mb* for morbidity rate.

The E_{LT} for the baseline scenario were:

$$E_{LT} = \sum N_{X6} \cdot PT_{SP} \quad (8)$$

Where *X6* stands for the number of suspect PI animals tested (=RiskUniform(337,404)) and PT_{SP} the price for testing a suspect PI animal.

Table 1. Input data used to estimate Bovine Viral Diarrhoea related disease costs in Switzerland. AC=adult cattle, CV=calves, PI=persistently infected, PIcv=persistently infected calves, PIh=persistently infected heifers, PIc=persistently infected cows, TI=transiently infected, TIcv=transiently infected calves, TIh= transiently infected heifers, TIc=transiently infected cows. Input units in brackets (CHF=Swiss Francs)

Input	Value or risk distribution	Description/source
Mortality rate PI animals (year^{-1})	Pert(0.45,0.5,0.55)	Derived from Viet et al. (2004) and observed data. The half-life of PI animals was set to 1 year and the mortality rate calculated accordingly. This value was taken as the most likely (ML) value. Minimum and maximum values: ML value -/+ 10%.
Mortality rate TI animals (year^{-1})	Pert(0,0.0025,0.006)	Expert estimate based on Houe et al. (1993) and Valle et al. (2005).
Rendering costs AC (CHF)	263	Price list of waste disposal company 'TMF Bazenheid'[a]
Rendering costs CV (CHF)	75	
Premature culling rate TIh and TIc (year^{-1})	Pert(0,0.025,0.057)	Expert estimate based on Valle et al. (2005)
Abortion rate TIh and TIc (year^{-1})	Uniform(0.00011,0.00018)	Calculated based on Rüfenacht et al. (2001)
Costs per abortion (CHF)	Normal(869.7, 497.7)	Derived from Häsler et al. (2006)
Rate of reduced milk yield in PIc (year^{-1})	Pert(0.43,0.48,0.53)	Derived from Voges et al. (2006) who reported a reduction in milk production in PI animals of 48% when compared with non-PI cows. ML value =0.48, minimum and maximum values: ML value -/+ 10%.
Proportion of TIc showing milk loss	Pert(0.30,0.30,0.35)	Derived from R. Bennett (accessed November 2010) who reported that 30% of affected dairy cows suffer a significant drop in milk yield of 20% over a three-week period.
Rate of reduced milk yield in affected TIc (year^{-1})	Pert(0.013,0.014,0.015)	
Morbidity rate TIcv (year^{-1})	Pert(0.03,0.05,0.08)	Assumption based on information from Swiss veterinary practitioners
Proportion of morbid animals receiving veterinary treatment	Pert(0.79,0.84,0.89)	
Price of veterinary treatment (CHF) for		Information collected from Swiss veterinary practitioners. Includes average number of veterinary visits needed for a PI or TI clinical episode, farm visit, administrative costs and the veterinary medical treatment according to the severity of the case.
PIcv and TIcv that die	100	
PIh and TIh that die	170	
PIc and TIc that die	180	
PIcv and TIcv that survive	70	
PIh that survive	110	
PIc that survive	120	
Price for testing a suspect PI animal (CHF)	112.5	Information collected from Swiss veterinary practitioners. Includes farm visit, blood sampling and laboratory cost.

[a] http://www.tmf.ch Price for slaughter waste: 210 CHF/ton for deliveries between 0-4999kg

The number of PIs and TIs, animals tested, laboratory tests performed, animal values (e.g. slaughter value), production data (e.g. milk yield) and market prices (e.g. milk price) are available from the corresponding author on request.

Monetary eradication cost: The monetary eradication cost comprised variable expenditures for labour, operations and expenses for epidemiological modelling, establishment and maintenance of an electronic registration system, sampling, laboratory testing, implementation of movement bans, data analysis, and communication.

In the initial phase, 1,553,526 tests were performed on 1,520,859 cattle on 43,267 farms. Testing costs were the number of tests multiplied by the price of the antigen test (Ptest1=8 CHF). The sampling cost included the price of the farm visit (Pvisit=30 CHF), the sample taking per animal (Psample=5 CHF), the price per ear tag (Ptag1=1.19 CHF) and postage per sample (Ppost=0.4 CHF). All farms were visited once for the initial sampling. An additional visit by the veterinarian became necessary when farmers required a confirmatory test (n=8,267 animals). Further, an additional visit was accounted for each ear tag tissue sample that arrived empty at the laboratory, which happened 13,681 times. For each of the 21,948 animals that needed to be re-sampled, the price of a farm visit, blood sampling (Psampleblood=15 CHF), postage and confirmatory antigen testing (Ptest2=27 CHF) were accounted for. Further, for each of the 12,125 PIs, movement restrictions on the farm applied that were implemented by the cantonal veterinary services as stipulated by the Swiss Animal Health Ordinance (SR 916.401). To implement the movement ban, 0.25 h of cantonal veterinary service time was accounted for. All 12,125 PIs were slaughtered and the value loss calculated by multiplying the number of PIs by the difference in animal value and slaughter value. Of the 12,125 PIs, 7,829 (65%) were calves, 2,374 (20%) were heifers and 1,870 (15%) were cows.

Until 31st October 2010, 1,481,836 tests were performed on 1,465,078 new born calves. Testing costs were the number of tests multiplied by the price of the antigen test. For each calf tested, the price for the calf ear tag including envelope and postage (Ptag2=2.70 CHF) was accounted for. In total, 6,933 calves tested positive and needed to be removed from the population, which was included as the difference in animal value and slaughter value. In total, 832 farms were re-visited to re-test the mothers of the PI calves detected. The farm visit, price for blood sampling, postage and antigen testing were accounted for. Further, 46,660 calf ear tag tissue samples arrived empty in the laboratory. These calves needed to be re-sampled, which resulted in an additional farm visit, cost for postage, blood sample taking and laboratory testing. For each PI animal the loss in value was added to the eradication cost. Further, for each farm with a PI calf, the cost for implementing and lifting the movement ban was calculated. Based on the data available, it was estimated that 832,558 tests on 823,143 newborn calves would be performed from 1st November 2010 to 31st December 2011. According to epidemiological modelling predictions, in these 14 months 50 PI calves would be born and slaughtered.

All farms and cantonal veterinary services received special ear tag pliers that cost 14 CHF/piece. Further, lump sums for epidemiological modelling and data analysis (=0.78 m CHF), establishment and maintenance of an electronic registration system (=0.55 m CHF), communication efforts (=0.17 m CHF), and reference laboratory (=0.32 m CHF) were added to the eradication cost.

Part 2: Estimation of surveillance cost to document disease freedom after eradication

Surveillance cost per scenario for the period 2012-17 comprised expenses for labour, operations and materials. The cost for primary testing comprised the farm visit, sample taking (including material), postage, and laboratory analysis. The cost for the farm visit was the number of farms to be visited multiplied by the price of a farm visit. The price of a farm visit was 30 CHF for a veterinarian. The price of a farm visit by a milk quality consultant was zero, because the samples would be taken during regular farm visits for milk quality control. The cost for sample taking was the number of blood and/or milk samples to be taken multiplied by the price for blood sampling (=7.5 CHF) or milk sampling (=RiskPert(0.5,1.25, 2) CHF). The postage cost was the number of samples taken multiplied by the postage per sample (=0.4 CHF). The cost for the laboratory analysis was the number of samples taken multiplied by the price of the ELISA test (=RiskUniform(2.8,3.2) CHF). If > 40% of the samples per farm tested positive, all cattle were to be blood sampled by a veterinarian and tested for antigen in the laboratory. For this follow-up testing of the whole herd, cost accrued from the farm visit, sample taking, postage and laboratory testing as described above. All prices were the same, apart from the price for antigen testing, which was RiskPert(10,15,20) CHF per sample. Further, annual lump sums for information technology (=20,000 CHF/y), communication (=20,000 CHF/y) and reference laboratory (=60,000 CHF/y) efforts were added to the surveillance cost.

The number of farms visited, number of samples taken as well as the number of farms that needed to be re-visited and the number of animals sampled and tested in the follow-up were derived from epidemiological modelling predictions and are available from the corresponding author on request. Because there will not be any PI animals left in the population after successful eradication, response costs (e.g. epidemiological investigation, removal of positive animals) were not included.

RESULTS

Baseline disease costs

The mean baseline disease costs were 16.04 m CHF (90% CI: 14.71-17.39 m CHF) in 2008 and 14.89 m CHF (90% CI: 13.72-16.08 m CHF) in 2009. The total disease costs in 2008 and 2009 mainly accrued from losses due to mortality (62 and 65%, respectively) and to a lesser extent from losses due to reduced milk yield (23 and 20%, respectively), losses due to premature culling (9 and 8%), expenditures for palliative treatment (7%), expenditures for laboratory testing (0.3%) and abortion losses (0.05%). Sensitivity analyses showed that in 2008 and 2009, the premature culling rates of TIh and TIc had the largest impact on disease costs (regression coefficients 0.73 and 0.66, respectively). The mortality rates for TIs and PIs had regression coefficients between 0.42 and 0.57. The regression coefficients for the rate of reduced milk yield in 2008 and 2009 were 0.15 and 0.14, respectively. All other regression coefficients were <0.1.

Eradication cost, benefit and residual amount for surveillance

In aggregate, the mean estimated total eradication cost was 68.35 m CHF (90% CI: 67.93 – 68.78 m CHF), of which 50% were attributable to the initial phase, 34% to the calf phase until 31 October 2010 and 16% to the calf phase from November 2010 to December 2011. Table 2 lists the detailed eradication cost per eradication phase.

Table 2. Detailed eradication cost for Bovine Viral Diarrhoea estimated for Switzerland in million Swiss Francs (mean and 90% confidence interval where applicable)

	Initial phase	Calf phase until 31 October 2010	Calf phase November 2010 to December 2011
Cost primary sampling	11.93	3.96	2.22
Cost laboratory analysis	12.43	11.85	6.66
Cost re-sampling and re-testing and movement ban	2.07	3.95	1.89
Loss in animal value	6.98 (6.76; 7.21)	2.86 (2.67; 3.06)	0.02 (0.02; 0.02)
Lump sums	0.64	0.92	0.27
Total	*34.05 (33.82; 34.28)*	*23.55 (23.35; 23.74)*	*11.07 (11.07; 11.07)*

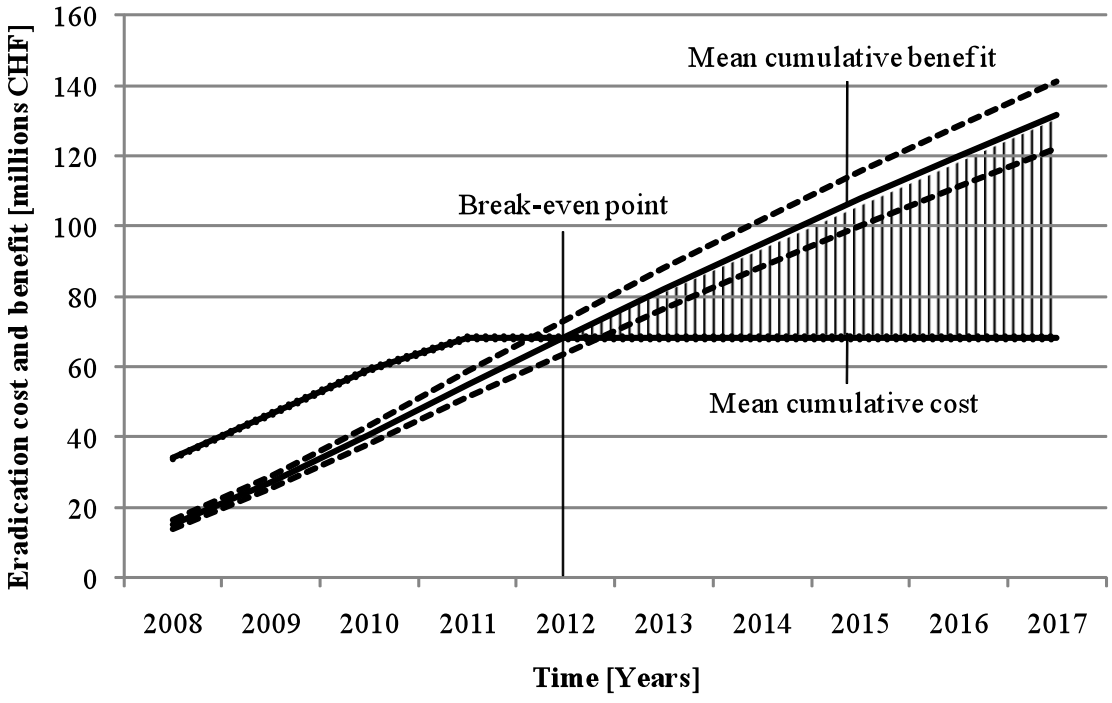

Fig. 1 Mean cumulative eradication cost and benefit with 90% confidence intervals (dotted lines) and break-even point of the Bovine Viral Diarrhoea eradication programme implemented in Switzerland. The hatched area marks the mean residual amount representing the maximum expenditure justifiable for surveillance to document disease freedom after eradication.

In aggregate, the mean total discounted benefit over 10 years was estimated to be 131 m CHF (90% CI: 124-138 m CHF). The break-even point of the programme is reached in 2012 (Fig. 1). The RA resulting from eradication representing the maximum expenditure justifiable for surveillance to document disease freedom in the years 2012-17 was calculated to be 63.15 m CHF (90% CI: 53.72-72.82 m CHF).

Surveillance cost to document disease freedom and net benefit

The cost for the four surveillance scenarios to document freedom from BVDV are summarised in Table 3. The mean discounted surveillance costs for scenarios 1 to 4 were calculated to be between 17.97 and 23.95 m CHF. In S1 and S3, primary sampling cost contributed most to the surveillance cost (77% and 57%, respectively). In S2 and S4, follow-up sampling and testing contributed most to surveillance cost (80% and 44%, respectively).

Table 3. Discounted surveillance cost to demonstrate freedom from Bovine Viral Diarrhoea calculated for Switzerland for the years 2012-17 in million Swiss Francs (mean and 90% confidence interval where applicable). IT= information technology

Cost	Scenario 1	Scenario 2	Scenario 3	Scenario 4
Sampling	16.79	2.15	10.16	9.25
			(10.07; 10.26)	(9.11; 9.38)
Laboratory analysis	3.83	2.03	0.53	2.93
	(3.60; 4.05)	(1.92; 2.13)	(0.50; 0.55)	(2.79; 3.06)
Follow-up sampling and testing	0.57 (0.50; 0.64)	19.26 (16.75; 21.77)	6.76 (5.88; 7.64)	9.80 (8.52; 11.07)
Communication, IT, reference laboratory	0.52	0.52	0.52	0.52
Total	*21.70 (21.46; 21.95)*	*23.95 (21.44; 26.46)*	*17.97 (17.09; 18.87)*	*22.49 (21.21; 23.78)*

The discounted median net benefit of the mitigation programme was found to be 43.40 m CHF using S1 (90% CI: 35.22-47.91 m CHF), 40.93 m CHF using S2 (90% CI: 32.86-45.88 m CHF), 47.08 m CHF using S3 (90% CI: 38.97-51.63 m CHF), and 42.51 m CHF using S4 (90% CI: 34.45-47.17 m CHF).

DISCUSSION

The aim of this study was to investigate the technical and economic relationships of elements of disease mitigation by conducting an economic evaluation of the mitigation programme for BVDV in Switzerland. Results showed that the eradication programme will reach the break-even point in 2012, leaving a RA of 63.15 m CHF (90% CI: 53.72-72.82 m CHF) justifiable for surveillance expenditures to document disease freedom in the years 2012-2017. The estimation of surveillance cost of four putative surveillance strategies to document freedom from BVDV showed that surveillance cost of all scenarios were smaller than this RA. Hence, the mitigation programme is expected to produce a median net economic benefit of at least 40.93 m CHF (90% CI: 32.86-45.88 m CHF).

Surveillance to document disease freedom after eradication is expected to operate at a high level of alert in the first years of the programme. This will allow detecting any BDVD recurrence early and enable rapid response to avoid disease spread. The surveillance strategies proposed comprise testing of all farms in the population every year (scenarios 1-3) or 50% of the farms every other year until the end of the mitigation programme. In aggregate, the mean

discounted surveillance costs were between 18-24 m CHF for all scenarios. Scenarios 2 and 4 were the most costly, because of high cost of follow-up sampling and testing, which contributed 80% and 44%, respectively, to surveillance cost. A decrease in the number of animals and/or farms to be sampled and tested would reduce aggregate surveillance costs. After the first few years of surveillance to document disease freedom, policy makers might want to reduce the number of farms and/or animals to be sampled. Risk assessment methods were shown to be useful if repeated surveys are conducted to document disease freedom (Hadorn et al., 2002; Knopf et al., 2007) and may be considered in that process. When the mitigation programme comes to its end in December 2017, the question will be raised about whether to continue with active surveillance or not. Future surveillance activities will be economically justifiable if the benefit (avoidable disease costs) resulting from the comparison of a situation with surveillance and without surveillance will at least cover surveillance costs. Such calculations will need to take into account the risk of possible introductions of BVDV, the magnitude of an outbreak and related disease costs with and without surveillance.

Currently, EU legislation does not envisage additional guarantees for BVDV for intra-Community trade. This would support national bio-security measures to prevent re-introduction of the disease. However, with an increasing number of BVDV eradication programmes in the EU, in future there may be an increased demand for BVDV free animals, thus producing further benefits from the mitigation programme. Additional benefits or costs may stem from animal welfare, impact on national and international reputation, consumer and industry confidence and trust. Such benefits and costs have real economic value and attempts could be made to measure them using contingent valuation approaches.

It is important to highlight the indispensability of measures of mitigation outcomes. Epidemiological models are not constructed to take into account the economic implications of resource allocation decisions (Howe, 1988), but they capture the biological dynamics and complexity of disease in animal populations and are therefore an important source of data for economic analyses (Perry and Randolph, 2004). The epidemiological model allowed the impact of mitigation activities on the disease dynamics in the population to be assessed. But it had not been specifically designed to underpin economic analyses. Therefore, certain epidemiological outputs could not be directly integrated into economic models, but needed to be amended first to make them fully compatible. By planning epidemiological and economic analyses together from the start, they can be developed in an interdisciplinary, fully compatible way that provides decision-makers with the comprehensive technical and economic information they require.

The mean baseline disease costs were estimated at 16.04 m CHF in 2008 and 14.89 m CHF in 2009, respectively. The availability of the number of TIs in the population retrieved from the epidemiological model allowed including disease costs directly related to this group of animals. Because of the inclusion of TIs, the total disease costs were considerably higher than those estimated by P. Schaller in 2001 that only included PIs (unpublished data). The exact quantification of disease costs in the national cattle herd is difficult, because only very few Swiss-specific data were available. Therefore, a range of assumptions was made regarding coefficients of production losses based on data from the scientific literature. Sensitivity analyses were conducted to assess the impact of uncertain inputs on the total disease costs. Ideally, national data would be used to assess the impact of the eradication programme on performance parameters, as for example done in Norway by Valle et al. (2005). Hässig et al. (2010) recently conducted a study in Switzerland to compare production variables and veterinary costs between dairy herds enrolled in an integrated herd health programme and those without such a programme. Similarly, the comparison of performance indicators such as calving interval, milk

production per lactation and veterinary costs before and after eradication of BVDV would be useful in estimating Swiss-specific disease costs.

Half of the total eradication cost accrued in the first months of the eradication programme when the whole national cattle herd was antigen-tested. Because the samples needed to be taken by a veterinarian in the initial phase, the sampling cost contributed 35% to total cost in that phase. In the calf phase, the sampling cost was reduced considerably, because the samples were taken by the farmer when placing the ear tag in newborn calves. However, large additional costs accrued, because many of the samples arrived empty at the laboratory, mainly due to setting the ear tags incorrectly. In such cases, a farm visit by a veterinarian was necessary to take a blood sample for laboratory analysis. Avoidance of empty samples would reduce the costs for re-sampling and thus overall costs.

By investigating the technical and economic relationships between surveillance, intervention and mitigation, the approach presented provides a structure that helps analyse and understand the use of mitigation resources. It thereby provides a useful framework for decision-makers to understand key concepts that impinge on the economic evaluation of mitigation to inform their decisions about the allocation of resources.

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EVALUATION OF CONTROL METHODS

A SLIGHT SIDE EFFECT ON FERTILITY ASSOCIATED WITH VACCINATION AGAINST BLUETONGUE VIRUS SEROTYPE 8 IN DAIRY COWS

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SUMMARY

Inactivated virus vaccines have been widely used to control bluetongue after introduction of serotype 8 of the bluetongue virus (BTV) in northern Europe in 2006. To evaluate BTV vaccination programs, an appraisal of both protection provided and possible side effects is needed. The objective of this study was to quantify a possible side effect of vaccination against BTV-8 using inactivated vaccines on the fertility of dairy cows in field conditions. The study was performed on dairy herds that were not exposed to BTV. Fertility was assessed by return-to-service following artificial insemination (AI). Statistical multivariate models were performed to quantify a possible side effect of vaccination on conception failure, early and late embryonic death. This study showed that cows receiving a second vaccine injection between 2 and 7 days after AI had a significantly higher risk of 3-week-return-to-service (RR = 1.19 [1.07 – 1.33]). This small side effect might be due to an increase of early embryonic death.

INTRODUCTION

Bluetongue (BT) is a non-contagious, insect-transmitted disease of domestic and wild ruminants caused by the bluetongue virus (BTV). BT has a heavy economic impact, mainly due to the disease effects on animals and to the disruption of international animal trade (Saegerman et al., 2008). In continental France, two BTV serotypes (serotypes 1 and 8) have been spreading concomitantly since 2007. In order to control the progression of the disease in cattle and sheep herds, annual vaccination campaigns, voluntary in 2008 against BTV-8 and compulsory in 2009 and 2010 against BTV-8 and BTV-1, were implemented. To evaluate BTV vaccination programs, an appraisal of both protection provided and possible side effects is needed. Because live attenuated vaccines are known to provoke side effects (Veronesi et al., 2005; Saegerman et al., 2007), inactivated virus vaccines have been used widely in Europe since 2005.

Based on clinical and virological data as well as on immunogenicity, inactivated virus vaccines are considered highly efficacious (Di Emidio et al., 2004; Savini et al., 2008; Schwartz-Cornil et al., 2008). Experimental studies have demonstrated that inactivated vaccines against BTV-2 and BTV-4 are very well tolerated in both sheep (Hamers et al., 2009) and cattle (Di Emidio et al., 2004; Savini et al., 2009). However, very few studies have been conducted to assess vaccination safety in field conditions. A field study conducted in Germany on sheep and

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cattle using three different BTV-8 vaccines demonstrated a high level of safety of the vaccination against BTV-8. Following the second vaccine injection, several sheep developed distinct local reactions and a temporary rise in body temperature (Gethmann et al., 2009). Based on adverse events reports, a review of the safety data of the field use of inactivated BTV-8 vaccines during the mass 2008 vaccination campaign carried out in 12 European countries was performed (European Medicines Agency, 2009). These pharmacovigilance data were consistent with the overall good safety record for all vaccines used. A very low frequency of adverse effects, including abortions, spontaneous death, some effects on milk production and injection site reactions were reported.

Vaccination could also impair fertility through a systemic reaction due to the immunisation (inducing hyperthermia) or stress caused by the manipulation of animals. Thermal stress can affect many reproductive parameters in cows including oocyte quality, embryo development and increased embryo mortality (Wolfenson et al., 2000; Hansen et al., 2001; Bridges et al., 2005). Before becoming implanted in the uterus, embryos are fragile and particularly sensitive to factors such as hyperthermia or stress (Hansen et al., 2001). This could result in an increase in the return-to-oestrus rate in cows vaccinated around the time of AI or before the implantation of the embryo.

If such an adverse effect does exist, it may not have been reported in pharmacovigilance data for several reasons: (i) the time-lag between the vaccination and the return-to-oestrus may be long, (ii) such an effect might be limited and not systematic and (iii) return-to-oestrus is very frequent and highly multifactorial. The objective of this study was to quantify a possible side effect of vaccination against BTV-8 using inactivated vaccines on the fertility of dairy cows in field conditions. This study focused on the possible effect of vaccination before the implantation of the embryo.

MATERIALS AND METHODS

General study design, data selection and assessment of reproductive performances

The general design of this study involved the comparison of the reproductive performance of vaccinated and unvaccinated dairy cows, none of which were exposed to BTV-1 or BTV-8 during 2008. Cows were selected from herds located in Brittany (western France). Three vaccines were used in Brittany during the 2008 voluntary vaccination campaign against BTV-8: Bovilis® BTV8 (Intervet, The Netherlands), BTVPUR® Alsap 8 (Merial, France); and Zulvac® 8 Bovis (Fort Dodge, The Netherlands). Vaccination data were collected for each cow by a regional farmers' organization (UBGDS). The performance data were obtained from herds enrolled in the official Milk Recording Scheme that used artificial insemination in 2008. Information about BTV-1 and BTV-8 exposure of herds during 2008 was obtained from the official French surveillance system.

The first step was the selection of herds that were not exposed to BTV. Because most BT infections in cattle are subclinical (Elbers et al., 2008), the French BTV surveillance system probably led to under-reporting of infected herds. A strategy based on the spatial distribution of reported herds consequently was applied to define unexposed cantons (French districts) in Brittany during 2008. A herd was considered unexposed if it was located in a canton where no BTV-1 or BTV-8 bovine or ovine case was reported during 2008. Overall 150,891 cows in 3,360 herds were selected from the 15,000 herds for which performance data were available.

Reproductive performance was assessed by the occurrence of a repeat AI (return-to-service) after a first or a second AI. There are three causes of return-to-service before the implantation of an embryo: conception failure, early embryonic death (before 16 days) and late embryonic death (after 16 days). Both conception failure and early embryonic death lead to a new heat 3 weeks after AI, and, if the heat is detected, a 3-week-return-to-service. A "3-week-return-to-service" was defined as a return-to-service occurring between 18 and 26 days after AI. A late embryonic death leads to a longer oestrus cycle and thus is followed by a late return-to-service, defined here by a return-to-service occurring after 26 days post AI.

Criteria were applied in order to exclude non-relevant data with regards to the objective. Cows with non-plausible or extreme performance data and herds with unusual management (unusual demographic structure, delayed first service) or suspected of using a bull were excluded. Furthermore, only data from Holstein cows inseminated with semen from Holstein bulls were selected because there is a strong effect of breed on reproductive performances. The study population was composed of 74,238 cows in 2,573 herds in 2008.

Comparison of reproductive performances between vaccinated and unvaccinated cows and selection of unvaccinated cows

It was assumed in this study that if vaccination has a side effect on cattle fertility, the effect would occur within a few days. In order to test an effect of vaccination on conception failure or early embryonic death, a comparison of 3-week-return-to-service rates was performed between cows vaccinated between 3 days before and 16 days after the AI and unvaccinated cows (3-week-return-to-service model). The vaccinated cows were divided into three categories according to the time interval between when they underwent AI and vaccination. Categories were constituted as follows: from 3 days before to 1 day after AI (assuming an effect on conception failure), from 2 to 7 days after AI (assuming an effect on the embryo viability in its first week) and from 8 to 16 days after AI (assuming an effect on the embryo viability in its second week). Two analyses were conducted separately for the first and the second vaccine injection.

A more general model was considered in order to take into account a possible effect of vaccination on conception failure and early and late embryonic death. In this second model, vaccination was considered as a single process including two injections. The analysis involved the comparison of the whole return-to-service rate occurring between 5 days after the second injection and 90 days after AI (90-day-return-to-service model). Doing so, this model also made it possible to take into account the return-to-service occurring more than 26 days after AI in case of undetected heat after a conception failure or an early embryonic death. Only animals vaccinated between 3 days before and 42 days after the AI were selected. Three categories were considered in order to take into account the time interval between AI and vaccination. In the first category (category a), the second vaccine injection was administered between 3 days before and 17 days after AI (corresponding to a first vaccine injection more than 3 days before AI). In this case, the assumption tested was an increase in conception failure or early embryonic death associated with the second vaccine injection. In the second category (category b), the second vaccine injection was administered between 18 and 36 days after AI (first vaccine injection between 3 days before and 15 days after AI). In this case, the assumptions tested were an increase in conception failure or early embryonic death leading to an undetected oestrus associated with the first vaccine injection or an increase in late embryonic death associated with the second vaccine injection. For the last category (category c), the second vaccine injection was administered between 37 and 42 days after AI (first vaccine injection between 16 and 21 days

after AI). In this case, the assumption tested was an increase in late embryonic death associated with the first or second vaccine injection.

Unvaccinated cows were selected according to the date of AI so that both vaccinated and unvaccinated cows underwent AI during the same period in 2008. Moreover, unvaccinated cows were selected so that vaccinated and unvaccinated cows had the same the length of return-to-service observation periods to allow an unbiased comparison.

Statistical model

The relationship between vaccination and occurrence of a possible return-to-service was assessed using multivariate statistical models. To account for factors likely to influence the probability of return-to-service, the association between vaccination status and occurrence of return-to-service was adjusted for several independent variables already described as risk factors for fertility traits in the literature (Hillers et al., 1984; Malher et al., 2006; Marce et al., 2009) as described by Eq. (1):.

$$Y_{ijklmnop} = \alpha + VAC_i + SR_j + LN_k + MY_l + PF_m + CAII_n + MO_o + EN_p + RAND_q + \varepsilon_{ijklmnop} \quad (1)$$

where outcome Y is the 3-week or 90-day-return-to-service following a first or a second AI; α is the intercept; VAC_i is the vaccination status (2 or 4 categories according to the model considered); SR_j is the service rank (two levels); LN_k is the lactation number (four levels); MY_l is the peak milk yield expressed as the maximum at the three first milk records in the lactation (quantitative); PF_m is the minimum of Protein:Fat Contents ratio out of the first three milk records (three levels); $CAII_n$ is the calving-to-AI interval (six levels); MO_o is the month of AI (between three and five categories according to the model considered); EN_p is the proportion of exposed neighbour canton (three levels), $RAND_q$ is a composite variable (random) combining herd number and inseminator number and $\varepsilon_{ijklmnop}$ is the residual error term.

The proportion of exposed neighbouring cantons was taken into account in the analysis as an indirect measure of the possible under-reporting of infected herds. To assess the risk of 3-week-return-to-service, a mixed-logistic regression model was used. For the 90-day-return-to-service model, survival analyses were performed using Cox model. Data analyses were performed using R® software (R Development Core Team, 2007).

RESULTS

Distribution of vaccination and return-to-service rates

In the study population and during the 2008 vaccination campaign, 62.2% of cows were vaccinated (N=46,166 cows; 63,658 AI). Vaccinations were performed from 21 July 2008 to 15 December 2008. Overall return-to-service rates at 3 weeks, 90 days and 200 days after AI were 21.9%, 51.3% and 56.7%, respectively, for the 74,238 cows in herds that were not exposed to BTV in 2008.

Effect associated with a vaccine injection administered between 3 days before and 16 days after AI on 3-week-return-to-service rate

After a first vaccine injection: Selection of AI from cows receiving a first vaccine injection between 3 days before and 16 days after that AI resulted in 3,435 selected AI. These AI were

compared to 10,298 AI from unvaccinated cows in the time period from 2 August 2008 to 27 November 2008. After adjustment, the 3-week-return-to-service rates of vaccinated cows did not differ significantly from unvaccinated cows whatever the time interval between AI and vaccination (Table 1).

After a second vaccine injection: Selection of AI from cows receiving a second vaccine injection between 3 days before and 16 days after that AI resulted in 3,780 selected AI. These AI were compared to 10,594 AI from unvaccinated cows in the time period from 1 September 2008 to 17 December 2008. After adjustment, the 3-week-return-to-service rate was significantly higher for cows vaccinated for the second time between 2 days and 7 days after AI compared to unvaccinated cows (Table 1). This difference corresponded to an increase of 4.2 percentage points of 3-week-return-to-service rate (when adjusting for other factors). Among adjustment variables, rank of service, milk yield, Protein:Fat Contents ratio and calving-to-AI interval were significantly associated with a risk of three-week-return-to-service. Risk was higher for shorter calving-to-AI intervals and after a second AI. The risk was also higher for cows with a higher milk yield and an intermediate Protein:Fat Content ratio (results not shown).

Table 1. Relative risk of 3-week-return-to-service according to the interval between AI and Bluetongue virus serotype 8 vaccination, and the rank of vaccine injection in Holstein dairy herds unexposed to BTV in 2008, Brittany, France

Vaccination status	Vaccination timing (days to AI)	3-week-return-to-service rates (%)	Number of AI	RR [95% CI]	P-value
Unvaccinated	*NA*	22.1	10,298	1	
Receiving a first vaccine injection[a]	- 3 to + 1	21.1	973	0.98 [0.90–1.05]	0.70
	+ 2 to + 7	21.1	1,022	0.97 [0.90–1.04]	0.67
	+ 8 to + 16	21.8	1,440	1.00 [0.94–1.05]	0.98
Unvaccinated	*NA*	22.1	10,594	1	
Receiving a second vaccine injection[b]	- 3 to + 1	23.1	996	1.08 [0.94–1.21]	0.25
	+ 2 to + 7	25.8	1,112	1.19 [1.07–1.33]	0.002
	+ 8 to + 16	22.2	1,672	1.03 [0.93–1.14]	0.55

[a] 12,364 cows in 2,089 herds

[b] 11,786 cows in 2,030 herds

NA : not applicable

Effect associated with two vaccine injections administered between 3 days before and 42 days after AI on 90-day-return-to-service rate

Selection of AI from cows receiving a second vaccine injection between 3 days before and 42 days after that AI resulted in 14,310 selected AI. These AI were compared to 14,310 AI from unvaccinated cows in the time period from 2 August 2008 to 17 December 2008. After adjustment the overall 90-day-return-to-service rate of vaccinated cows did not differ significantly from unvaccinated cows. Whatever the date of vaccination, 90-day-return-to-service was not associated with the vaccination status (Table 2).

Table 2. Hazard ratios of 90-day-return-to-service for the overall model and for the three separate models according to the interval between AI and Bluetongue virus serotype 8 vaccination in Holstein dairy herds unexposed to BTV in 2008, Brittany, France

Models	Vaccination timing (time interval between AI and the 2nd vaccine injection in days)	90-day-return-to-service rates (%)		Number of AI (for both vaccinated and unvaccinated cows)	HR [95% CI]	P-value
		Vac cows[a]	Unvac cows[b]			
Receiving a second vaccine injection (category a)	- 3 to + 17	50.8	49.3	7,862	1.07 [0.99;1.14]	0.07
Receiving a second vaccine injection (category b)	+ 18 to + 37	35.2	35.9	5,081	0.98 [0.88;1.09]	0.74
Receiving a second vaccine injection (category c)	+ 38 to + 42	26.5	28.0	1,333	0.90 [0.72;1.13]	0.37

[a] Vaccinated cows
[b] Unvaccinated cows

DISCUSSION

A slight side effect of vaccination against BTV-8 with inactivated vaccines on dairy cow fertility was found for cows receiving a second vaccine injection from 2 to 7 days after insemination. The only detectable effect was an increase in 3-week-return-to-service rate. This result indicates that the side effect of vaccination is likely to be due to early embryonic death. This study did not highlight any effect of vaccination on conception failure or late embryonic death. For late embryonic death, the non-significant HR was below 1, whereas for conception failure, a non-significant RR above 1 was found. In the latter case, it could either be an absence of effect or a very small effect not detectable with the statistical power of our study.

Very few studies have evaluated the safety of BTV-8 vaccination with inactivated vaccines in field conditions. A Swiss study conducted in 47 cattle herds investigated a possible side effect of BTV-8 vaccination on service success rates (Office Vétérinaire Fédéral, 2009). The overall analysis of services success rate did not show any side effect of vaccination. The authors mention that some models were able to detect a slight decrease in service success rates when second vaccine injections were administered during the service period, but no details were provided on methods or results in this report. A rise of temperature was shown after the second vaccine injection only in sheep (Gethmann et al., 2009). Acute clinical signs were detected 2 to 8 days only after the second injection of both BTV-1 and BTV-8 vaccines (Zulvac® 1 and Zulvac® 8 Bovis, Fort Dodge, The Netherlands) in 22 out of 100 vaccinated sheep flocks (Gonzalez et al., 2010).

Based on the results of this study and review of the literature it can be speculated that two factors could be involved in fertility decrease following vaccination: hyperthermia and stress. A short-term hyperthermia very often is observed after vaccination (Martinod, 1995). An increase of 1°C or more in body temperature can compromise reproductive functions (Hansen et al., 2001). An experimental study showed that an application of heat stress on days 1-7 after insemination can lead to reduced embryonic development (Putney et al., 1988). Animal restraint during vaccination is also a stressful procedure. Stress during vaccination, including fear and anxiety, could have a direct negative impact on fertility. It is impossible here to distinguish effect of immunisation and a possible effect of stress due to manipulation. Nevertheless, it would be possible that both were associated with the decrease in fertility of vaccinated cows.

The virus was spreading widely in France when the vaccination program was implemented. BTV has a negative impact on cattle fertility, including an increase in 56-day-return-to-service rates after a first insemination and an increase in the number of inseminations needed for an assumed pregnancy (Santman-Berends et al., 2010). The strategy based on spatial distribution of reported herds that was applied in this study allowed for studying of the effect of vaccination on fertility independent of exposure. For all the models considered, there was no significant variation of fertility between regions according to the percentage of exposed neighbouring cantons. Accordingly, it is reasonable to assume that the proportion of BTV-infected undetected herds was probably very low or null in our study population.

In this study, only Holstein cows from one French region were selected. However, if hyperthermia and stress are involved in the decreased fertility, the side effect of vaccination is likely to be experienced in other contexts. Similar consequences are likely to be expected in case of a first immunisation with BTV-8 inactivated vaccines including the same adjuvants. However, it is not possible to extrapolate results of a primo-vaccination as shown here to annual booster vaccinations.

This study under field conditions allowed us to quantify a significant but small adverse side effect on fertility after the second BTV-8 vaccine injection given one week after AI in herds that were not exposed to BTV. The side effect is thus likely to pass undetected on a farm. Compared to the negative effect of BTV exposure on fertility, this side effect is small and therefore should not be an obstacle to vaccination. Nevertheless, it can be recommended not to vaccinate using current BTV-8 inactivated vaccines within one week after AI.

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FULL HERD DEPOPULATION AND LOCAL BADGER REMOVAL DURING 2003-2005 LEAD TO REDUCED RISK OF BOVINE TUBERCULOSIS IN IRISH HERDS

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SUMMARY

Herd depopulation is used to combat residual infection with bovine tuberculosis (bTB) in cattle. In previous studies, depopulation was ineffective in reducing future bTB risk in herds, consistent with localised persistence. In 2000, Irish national policy was modified to include a local badger cull, where necessary, following bTB herd depopulation. This policy change was evaluated by comparing future bTB risk in restocked herds following depopulation for either bTB or BSE during 2003-2005. Herds were assigned a 'previous bTB risk', based on bTB history 5 years prior to depopulation. Future bTB risk varied significantly by reason for depopulation and previous bTB risk. Herds depopulated for bTB had the same future risk as BSE herds with no/low previous bTB risk. BSE herds with a high previous bTB risk had a significantly greater future bTB risk. These results suggest that local badger removal is useful in limiting local bTB persistence in depopulated herds.

INTRODUCTION

Persistence of *M. bovis* infection in a herd or locality, for a number of reasons, is a key issue. As for *M. tuberculosis* infection in humans (Manabe & Bishai, 2000), there is increasing recognition of the importance of residual *M. bovis* infection in cattle despite ongoing testing, for example, from Australia (Radunz, 2006) and Ireland (Olea-Popelka et al., 2004, 2008; Kelly & More, in press). Attention has focused on improved diagnostics to identify residual, but previously non-detected, infection in herds (for example, Gormley et al., 2006; Schiller et al., 2010). Further, in Ireland (Griffin et al., 2005) and in GB (Donnelly et al., 2007, Donnelly & Hone, 2010) badgers in particular are important wildlife reservoirs for *M. bovis* infection. Badger removal is associated with a significant reduction in bTB incidence in local cattle (Ó Máirtín et al., 1998; Griffin et al., 2005; Donnelly et al., 2007). Bought-in cattle (Green et al., 2008, Clegg et al., 2008) and environmental contamination (Young et al., 2005) may also play a role in local persistence of infection.

The control of bovine tuberculosis (bTB), due to infection with *Mycobacterium bovis*, in Ireland is well documented (Griffin et al., 2005, Good, 2006). The eradication programme, operating on test and cull principles, was established in 1954. Surveillance comprises testing animals, based on the single intradermal comparative tuberculin test (SICTT) and slaughterhouse monitoring. Each herd has at least one annual full herd test, and

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advanced/clinical cases are rarely encountered. Since the mid 1960s, the bTB animal incidence has remained relatively stable, at approximately 0.4% annually (Good, 2006).

Herd depopulation, including disinfection and a period without bovines, together with a contiguous herd test programme, seeks to address some of the risk factors for persistent local infection, namely infected bovines and environmental contamination from the bovine herd. The intention is to re-establish herds capable of remaining free of bTB. Herd depopulation in the Irish programme has been assessed on several previous occasions. In an early descriptive study, Hahesy et al., (1992) noted a bTB breakdown rate of 36.1% in bTB depopulated herds (bTB-dhs) within 3 years following restocking. Later, Hahesy et al., (1996) observed a similar bTB breakdown rate among bTB-dhs (12%, 20% and 26%, within 1, 2 and 3 years of depopulation, respectively) and herds depopulated for other reasons (10%, 14%, 25%), consistent with local bTB persistence being the consequence of sources other than residual cattle infection. The Irish bTB depopulation policy was modified in 2000 (Good et al., 2003) with the addition of a badger cull programme for 18-months post-depopulation in the environs of the bTB-dhs, to limit spread from an infected badger population (Griffin, 1992, Hahesy et al., 1996, Ó Máirtín et al., 1998).

The aim of this paper is to evaluate the impact of the Irish depopulation policy on the local persistence of bTB infection by comparing the future bTB risk in restocked herds following depopulation for either bTB or BSE during 2003-2005.

MATERIALS AND METHODS

Study population

All Irish herds fully depopulated of cattle in response to a bTB breakdown during 2003 to 2005, and which subsequently restocked, were included in the study. Following depopulation, the premises/holding was cleansed and disinfected and the land left without bovines for at least 4-months. Contiguous herds were tested a minimum of 60-days after the last infected bovine left the bTB-dhs. Also, a badger cull programme was operated in the environs of the bTB-dhs, for 18-months post-depopulation, except where the breakdown was attributed to reason(s) other than badgers.

In Ireland prior to 2005, a confirmed BSE case resulted in herd depopulation. Herds depopulated in response to a BSE case between 2003 and 2005, and which subsequently restocked, were included in the study. These herds (BSE-dhs) were cleansed, disinfected and remained without bovines for not less than one-month. No badger cull was undertaken.

Analysis

Preliminary analysis: For each enrolled herd, data were gathered on the destocking and restocking dates, location, size, composition, type and bTB history (SICTT results and slaughter surveillance) prior to and following depopulation. The BSE-dhs were assigned a bTB risk attribution based on past herd bTB history during the 5 years prior to depopulation, as follows:

Higher risk (H) - at least one bTB episode with ≥2 infected animals [skin reactor, lesion at slaughter];

Lower risk (L) – at least one bTB episode, but without evidence of within-herd transmission (i.e. a single infected animal, >1 infected animal but exclusively introduced animals); and

Default risk (D) - no bTB episode.

All restocked bTB-dhs were categorized as H.

bTB status to the first full-herd test following restocking: The bTB status of each herd was considered positive at the first full herd test following restocking if any animal was deemed bTB-positive, during SICTT or slaughterhouse surveillance, from restocking up to and including this test. For each bTB positive herd, the infection source was considered either introduced or not determined, after considering the bTB-status of the source herd. Using a chi-square test, the bTB-status at the first herd test following restocking for bTB-dhs and BSE-dhs herds was compared.

bTB status after the first full herd test following restocking: The subsequent bTB status was examined from the first herd-level test post-restocking (or time from status restoration, if the herd was bTB-positive at the first test) up until the first of either (i) a bTB-positive designation (following either field or slaughterhouse surveillance) or (ii) the last recorded herd test prior to 31 December 2009. A Cox proportional-hazard model was developed using STCOX in STATA version 11 (StataCorp LP, College Station, TX, USA), based on the outcome variable of time to bTB-positive designation following restocking.

The following risk factors were considered in the survival analysis:

i) Reason for depopulation and bTB risk prior to depopulation *[Reason_risk]:*
   For all bTB destocked herds:
   Reason_risk = 0 *(higher [H] bTB risk)*
   For BSE depopulated herds:
   Reason_risk = 1 *(default [D] bTB risk)*
   Reason_risk = 2 *(lower [L] bTB risk)*
   Reason_risk = 3 *(higher [H] bTB risk).*
ii) Proportion of cows, heifers or females within a herd.
iii) Herd size (treated as a time varying predictor). Part herd tests were deleted except if the herd was bTB-positive at a part test, in which case the herd size of the previous 'whole' herd-level test was used.
iv) Herd type: Dairy, beef, suckler, other
v) Location: Average and maximum herd prevalence rate (the number of bTB test-positive herds divided by the number of herds tested during the year) between 2004 and 2008 within the District Electoral Division (DED). The average and maximum across the 5-years was calculated for each DED *[Variable name: Meanrate]*

A backward selection procedure was used to eliminate terms from the model based on a likelihood ratio test ($p>0.05$). Herd size was treated as a time-varying covariate, changing each time the herd had a full-herd test. Continuous variables were also categorised into 4 groups based on the corresponding quartiles. Whether to treat variables as continuous, categorical or to transform the variable was tested by comparing univariate models using the AIC (Akaike information criteria). To examine the appropriate functional form of a variable, a plot of the lowess smooth of martingale residuals against transformations of the covariate were used. Additionally, the choice of whether to use the proportion of females, heifers or cows within the model was determined by comparing the AIC from the univariate models. Likewise, the choice of using maximum or average herd prevalence rate within the area (DED) was determined from

the AIC of the univariate model. The need for a time-varying-covariate (tvc) was determined by including a tvc in the univariate model and comparing to a model without a tvc using a likelihood ratio test. The proportional-hazard assumption was also checked visually using a plot of -log(-log) survival lines to examine whether the different covariate groups were parallel. In addition, the chi-squared Schoenfield residuals were tested ($p<0.05$) to examine if the hazard ratio varied over time, if significant, the risk factor was included as a time-varying covariate. The model was checked by examining the martingale, influence and schoenfeld residuals.

This study has been reported in accordance with the STROBE statement (von Elm et al., 2007).

RESULTS

There were 68 bTB-dhs and 347 BSE-dhs included in the study. The average herd size at the first herd-level SICTT post-restocking was 46.7 (range 1-148) and 82 (1-833) cattle, for bTB-dhs and BSE-dhs, respectively. Thirty-seven bTB-dhs contained at least one cow (average 29, range 1-85) cows and 272 BSE-dhs, contained at least one cow (average 42, range 1-487). One of the bTB-dhs and two BSE-dhs ceased to exist, (not for bTB reasons) immediately following the first test post-restocking, these herds were therefore censored and not included in the survival analysis.

The numbers (%) of herd bTB breakdowns post-restocking at or prior to first herd-level test, and throughout the study period, are shown in Table 1. There was no significant ($p=0.509$) difference between the breakdowns at the first herd-level test in bTB-dhs compared to BSE-dhs herds.

There was a significant difference ($p <0.001$) in survival times between the depopulation groups (Table 2, Fig. 1). In the final Cox proportional hazards model (Table 3), the risk of future restriction increased with increasing bTB risk among BSE-dhs. There was no significant difference in the risk of a future restriction among bTB-dhs (Reason-risk =0) and BSE-dhs that had a previous bTB default risk (Reason-risk = 1). None of the covariates varied over time.

Table 1. Number (%) of herd bTB breakdowns post-restocking at or prior to first herd level test, and throughout the study period, by reason for depopulation

Breakdowns post-restocking	bTB-dhs (n=68)	BSE-dhs (n=347)
At or prior to 1st herd-level test	5(7.4%)	18 (5.2%)
Attributed to bought-in	1 (20%)	7 (38.9%)
Throughout the study period		
0 – 12 months	5 (7.4%)	30 (8.6%)
13 – 24 months	7 (10.3%)	45 (13.0%)
25 – 36 months	6 (8.8%)	49 (14.1%)
Total 0 – 36 months	15 (22.1%)	93 (26.8%)

Table 2. Kaplan-Meier probability of surviving without a bTB restriction subsequent to the first herd-level test post-restocking to the end of 2009, by reason for depopulation and bTB risk prior to depopulation

Reason for depopulation, bTB risk prior to depopulation	No. at risk	Survival probability	Std. Error
bTB depopulated herds (n=67)			
Reason-risk 0	67	0.708	0.069
BSE depopulated herds (n=345)			
Reason-risk 1	206	0.687	0.036
Reason-risk 2	53	0.489	0.075
Reason-risk 3	86	0.349	0.056

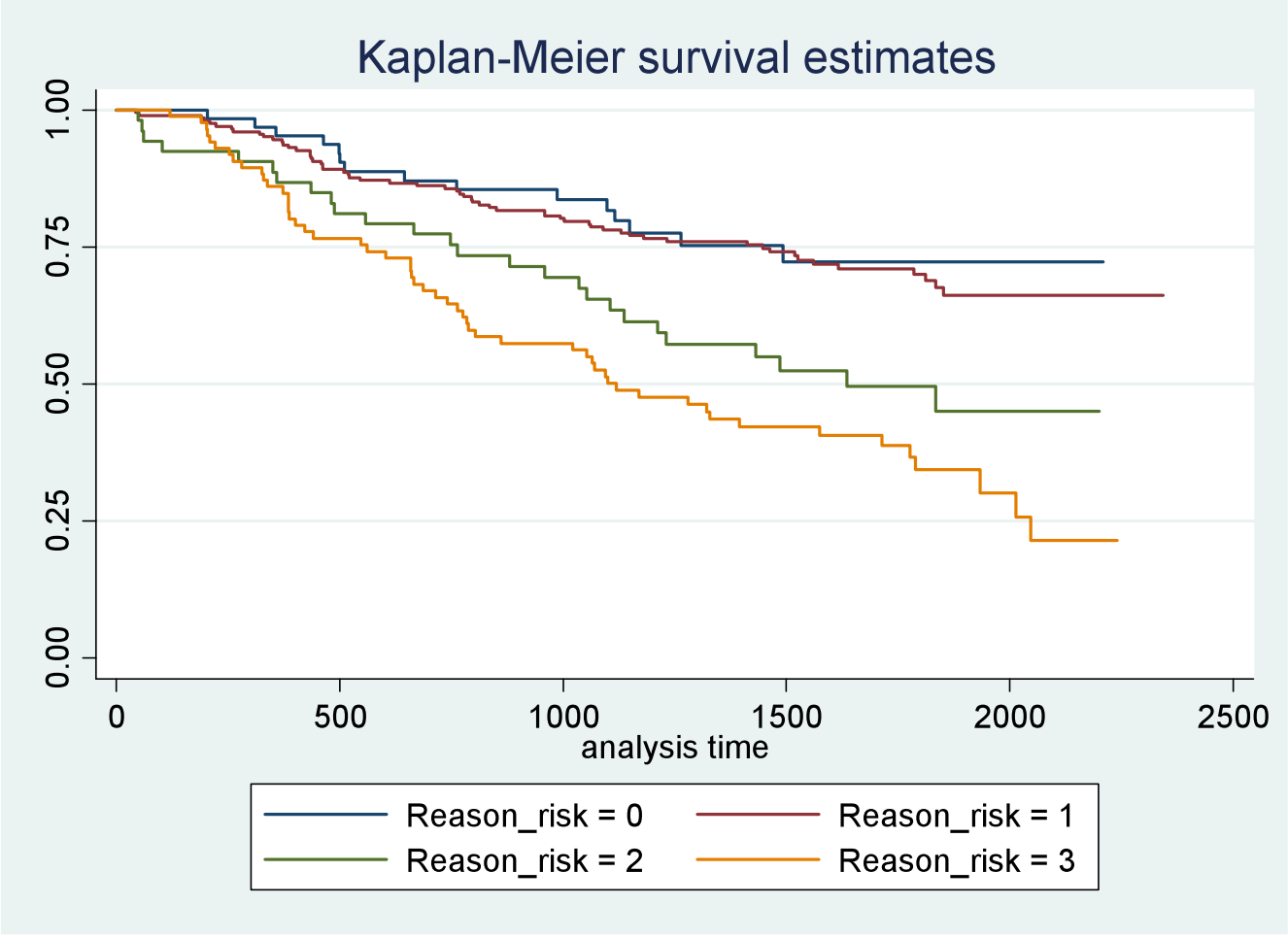

Fig. 1 Kaplan-Meier survival estimates for time to restriction following depopulation by reason-risk for depopulation and prior risk of bTB.

Table 3. Final Cox proportional hazards model of time to a bTB herd restriction following restocking after depopulation.

	Hazard Ratio	Std. Err.	P-value	95% Confidence interval	
				Low	High
Herd size	1.003	0.00	<0.001	1.001	1.004
Proportion of cows	16.05	6.05	<0.001	7.67	33.59
Log meanrate	2.22	0.32	<0.001	1.68	2.94
Reason for depopulation, bTB risk prior to depopulation:					
bTB depopulation					
Reason_risk = 0	referent				
BSE depopulation					
Reason_risk = 1	0.94	0.27	0.827	0.53	1.66
Reason_risk = 2	1.57	0.51	0.169	0.83	2.97
Reason_risk = 3	2.08	0.63	0.015	1.15	3.76

DISCUSSION

The Irish bTB depopulation policy focuses on a number of key drivers for local bTB persistence, namely infection in and environmental contamination from cattle as well as a wildlife reservoir. The results from this study have shown that this policy, as currently applied in Ireland (removal of all bovines, local badger removal if necessary and disinfection), is effective in reducing future bTB risk. These results are in contrast to earlier studies on Ireland, from the 1980/90s (Hahesy et al., 1992, 1996). Prior to 2000 in Ireland, bTB depopulation involved removal of all bovines and associated disinfection. However, a badger cull would not routinely have been applied in depopulated herds, even if badgers had been implicated as a possible source. The current results in respect of depopulation for BSE are similar to recent work from Carrique-Mas et al., (2008) in Great Britain, where depopulation in response to FMD of herds with previous problems with bTB was not associated with reduced future bTB risk.

The study provides insights into the relative importance of the various bTB risks causing persistence. Herd bTB persistence is a reflection of residual infection in cattle and/or re-infection from other sources (including neighbouring or introduced cattle, wildlife or elsewhere). Residual cattle infection is a consequence of imperfect test sensitivity (de la Rua-Domenech et al., 2006), with some infected animals not responding to the routinely used SICTT (Lamont, 1947, Paterson & Ritchie, 1959, de la Rua-Domenech et al., 2006). In the current study, and elsewhere (Hahesy et al., 1996, Carrique-Mas et al., 2008), bTB persistence is for reasons unrelated to the depopulated herd because, by definition, herd depopulation leads to the removal of all residually infected cattle from the herd of concern. Although cattle movements are associated with infection spread, both in Ireland (Griffin, 1993, Flanagan et al., 1998) and GB (Johnston et al., 2005, Gopal et al., 2006), this is probably not a major source of infection in previously non-infected herds (Green et al., 2008, Clegg et al., 2008). In the current study, buying in of new cattle following depopulation cannot explain bTB persistence in the restocked herds, noting that these events occurred in all herds, not just BSE-dhs with a prior high-risk history of bTB. Farm location is recognised as a critical risk factor for bTB in Ireland

(Hammond, 1999, O'Keeffe 2006, Kelly & More in press), suggesting that bTB persistence is associated with the environs of infected herds. In GB, Green et al., (2008) also attributed 75% of bTB infection to local effects within specific high-risk areas. Endemic bTB in badgers is recognized as a critical constraint to bTB eradication (Gormley & Collins, 2000), and badger removal in both Ireland (Ó Máirtín et al., 1998, Griffin et al., 2005, Kelly et al., 2008) and GB (Donnelly et al., 2007) is associated with improved bTB control in cattle herds. Further, targeted badger removal is associated with reduced future bTB risk in Ireland (Olea-Popelka et al., 2009).

Limitations inherent in this study would advocate a degree of caution. National control programmes evolve over time; therefore, within-study comparison (bTB-dhs versus BSE-dhs) is likely to be more robust than a comparison of the current results with those from the earlier work described by Hahesy et al., (1992, 1996). Local badger removal was the main policy difference between each of these two comparison (bTB-dhs [with local badger removal] versus BSE-dhs [without], bTB depopulation during 2003/05 [with local badger removal] versus 1980/90s [without]). In the latter comparison, however, several other differences are likely. In the latter comparison, there are competing biases, both for and against improved resolution of local bTB persistence. On the one hand, the national programme has been modified over time, particularly with the use of the interferon-γ assay and/or anamnestic ELISA to remove potentially infected animals from infected groups (Good et al., 2003). On the other, the criteria for herd depopulation have become increasingly stringent, consistent with more-problematic herds (increasing risk of future bTB risk) in later years. While bTB policy applies to the whole of Ireland, there may be slight variations in its application in different local DAFF offices within Ireland.

As previously (Olea-Popelka et al., 2004, 2008), herd size was confirmed as a risk for bTB in this study, with the proportion of cows being of particular significance (Table 3). BSE-dhs were on average larger than the bTB-dhs. Disinfection during and following a bTB breakdown will reduce, but not eliminate, the risk from environmental contamination. Further, badger cull operations in the environs of the bTB-dhs will have reduced but not eliminated badgers in the area (O'Keeffe, 2006), and thus some risk from this source will remain. Other causes of bTB persistence also undoubtedly exist, e.g. other mammalian sources.

In conclusion, herd depopulation, as currently applied in Ireland, was effective in limiting future bTB risk in problem herds. This finding cannot be attributable solely to removal of residually infected cattle, noting in this study that we found a significantly lower future bTB risk among bTB-dhs compared with BSE-dhs, each with equivalent past bTB history. Rather, targeted badger removal was also important, given that it was conducted in association with bTB-dhs (when evidence in support of a wildlife source was available), but not BSE-dhs. Therefore, consistent with a range of studies, it can be concluded that residually infected cattle (Olea-Popelka et al., 2004, 2008; Kelly & More, in press) and local wildlife (Ó Máirtín et al., 1998; Griffin et al., 2005; Kelly et al., 2008; Olea-Popelka et al., 2008) each contribute to local bTB persistence in Ireland. The strategy of targeted badger removal will be reviewed pending the availability of an effective bTB vaccine for badgers.

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Society for Veterinary Epidemiology and Preventive Medicine

PAST VENUES AND ORGANISERS OF ANNUAL MEETINGS

Year	Venue	Organiser(s)
1983	Southampton	Davies & Thrusfield
1984	Edinburgh	Thrusfield
1985	Reading	Thrusfield
1986	Edinburgh	Thrusfield
1987	Solihull	Thrusfield
1988	Edinburgh	Thrusfield
1989	Exeter	Howe
1990	Belfast	McIlroy
1991	London	Jones
1992	Edinburgh	Thrusfield
1993	Exeter	Howe
1994	Belfast	Menzies
1995	Reading	Paterson
1996	Glasgow	Reid
1997	Chester	Clarkson
1998	Ennis, Eire	Collins
1999	Bristol	Green
2000	Edinburgh	Thrusfield & Mellor
2001	Noordwijkerhout, The Netherlands	van Klink
2002	Cambridge	Wood & Newton
2003	Warwick	Green
2004	Martigny, Switzerland	Stärk
2005	Nairn	Gunn
2006	Exeter	Peeler
2007	Dipoli, Finland	Virtala & Alban
2008	Liverpool	Pinchbeck & Robinson
2009	London	Verheyen & Pfeiffer
2010	Nantes, France	Fourichon & Hoch
2011	Leipzig, Germany	Thulke & Lange

PAST PRESIDENTS

1982-'83	G. Davies
1983-'84	P.R. Ellis
1984-'85	G. Gettinby
1985-'86	R.W.J. Plenderleith
1986-'87	M.V. Thrusfield
1987-'88	K.S. Howe
1988-'89	A.M. Russell
1989-'90	S.G. McIlroy
1990-'91	J.E.T. Jones
1991-'92	J.M. Booth
1992-'93	E.A. Goodall
1993-'94	R.G. Eddy
1994-'95	J.T. Done
1995-'96	G.J. Gunn
1996-'97	M.S. Richards
1997-'98	J.D. Collins
1998-'99	F.D. Menzies
1999-'00	K.L. Morgan
2000-'01	S.W.J. Reid
2001-'02	A.D. Paterson
2002-'03	L.E. Green
2003-'04	J.L.N. Wood
2004-'05	E.G.M. van Klink
2005-'06	D.J. Mellor
2006-'07	E. J. Peeler
2007-'08	J. R Newton
2008-'09	L. Alban
2009-'10	D.U. Pfeiffer

EXECUTIVE COMMITTEE 2010-2011

L. A. Kelly (President), D. U. Pfeiffer (Senior Vice- President), C. Fourichon (Junior Vice-President), T.D.H. Parkin (Honorary Secretary), K. Verheyen (Honorary Treasurer), A. Lindberg, T. Martinez, K. Mintiens, L. Rosenbaum Nielsen, H-H Thulke

Honorary Auditors: Dominic Mellor & Fraser Menzies

LIFE MEMBERS

J.M. Booth, M.J. Clarkson, J.D Collins, G. Davies, J.T. Done, R.G. Eddy, P.R. Ellis, E.A. Goodall, G. Gettinby, K.S. Howe, M.E. Hugh-Jones, W. Martin, F. Menzies, A.M. Russell, M.V. Thrusfield, J. Wilesmith

PLENARY TALKS

Year	Gareth Davies Lecture	Conference Opening Plenary
2011	Karin Schwabenbauer s 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, legistlation 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 vritical 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 (£20 sterling) 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 web site, http://www.svepm.org.uk/, or from the Secretary or Treasurer.

Please send this form to the Society's Treasurer:

Dr Kristien Verheyen
The Royal Veterinary College
Hawkshead Lane
North Mymms
Hatfield
Herts, AL9 7TA
UK

TEL +44 (0) 1707 666 625
FAX +44 (0) 1707 666 574
Email: kverheyen@rvc.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 Secretary of a completed application form and subscription equivalent to the rate for one calendar year. Subsequent 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 four 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. He 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 the Honorary Secretary.
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
Corrected January 1997; April 2002