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ANTIMICROBIAL USE IN LIVESTOCK

THE ASSOCIATION BETWEEN MANAGEMENT AND ANTIMICROBIAL USE IN YOUNG CALVES

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SUMMARY

The relatively high mean antimicrobial usage (AMU) in calves <56 days on dairy farms in the Netherlands is influenced by a small group of farmers with a high AMU. A case-control study was conducted to identify factors that characterise Dutch dairy and suckler herds with structurally (for a period of 2 years) high or low AMU in young calves. Multivariable logistic regression models showed a diverse set of factors to be associated with case herds. These factors were both management related, such as the type of housing used for young calves, and mindset related, such as the measure of agreement with the statement "youngstock need specific management". These results indicate that a change in mindset and subsequent behaviour is necessary in order to reduce the AMU in structurally high-using herds.

INTRODUCTION

In 2008, the Dutch government requested the livestock industry to reduce their antimicrobial usage (AMU). The ultimate goal was for AMU to be reduced by 70% in 2015, relative to the use in 2009. The Dutch Veterinary Medicine Authority SDa monitors AMU in the Netherlands and reports annually on the national trends in AMU, expressed as animal daily defined dose at farm ($DDDA_F$) level (first report published in 2011). The $DDDA_F$ is calculated by dividing the amount of total treated weight by the total weight of cattle present per herd annually (Gonggrijp et al., 2016). Between 2009 and 2014, the overall veterinary use of antimicrobials decreased substantially (SDa, 2015).

The AMU in cattle is reported for each cattle herd type such as suckler, dairy, veal and youngstock. For dairy herds, AMU is currently also presented for different groups within herds, i.e. young calves 0-56 days old, for dry-off therapy and intramammary treatments (cows >2 years old). In young dairy calves, the average use of orally administered antimicrobials is monitored and the mean $DDDA_F$ was 10.9, 4.8 and 3.8 in 2012, 2013 and 2014, respectively (SDa 2013, 2014, 2015). The high $DDDA_F$ in young calves might be a reason for the relatively high prevalence of resistant organisms in younger animals (Khachatryan et al., 2004). However, more than half of the farmers had a $DDDA_F$ of zero in this specific group. This implies that the relatively high mean originates from a relatively small number of farmers with a high $DDDA_F$ in young calves. In suckler herds, specific

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AMU in young calves is not reported, and the existence or extent of suckler herds with a high AMU in calves is unknown.

Factors associated with AMU need to be identified to aid farmers in reducing their usage by improving (young) calf management. Management characteristics that are currently being applied to reduce health problems, and with that AMU, include separation of sick and healthy animals and the quick and sufficient administration of colostrum to young calves (LeBlanc et al., 2006: Morin et al., 2010). The role of the farmers' mindset, which has already proved to be important in calf rearing and calf mortality (Santman-Berends et al., 2014), has not yet been described with regard to young calf health and associated AMU.

The aim of this study was to gain insight into the management and mindset associated with a structurally high AMU in young calves in Dutch dairy and suckler herds.

MATERIALS AND METHODS

Study population

A case-control design was applied based on high or low AMU. The study was restricted to conventional (non-organic) herds, which included 98% of Dutch cattle farms in 2014 (CBS, 2016). Because the suckler sector is rather small, with approximately 3,200 herds in total, with an average low AMU, it was decided that fewer suckler herds than dairy herds would be included in this study. A sample size of 200 dairy herds was chosen to be able to detect risk factors for a high AMU with an odds ratio of at least 2, with 95% confidence and 80% power, considering a risk factor prevalence of 50% in the control group. For suckler herds, a sample size of 100 herds was selected, which enabled detection of risk factors with an odds ratio of approximately 3 (all other parameters were the same). Two groups of herds were selected for inclusion in the study based on the national database in which all supplies of antimicrobials in cattle are registered (MediRund). Herds with a structurally (for a period of 2 years) low $DDDA_F$ in young calves (defined throughout as <56 days old; controls) and herds with a structurally high $DDDA_F$ in young calves (cases) in 2012 and 2013 were included. For dairy herds this meant that structurally low users had a $DDDA_F$ in young calves below 0.5 and structurally high users had a $DDDA_F$ in young calves >28. In suckler herds, structurally low users were defined as herds with a $DDDA_F$ in young calves <0.5 and structurally high users had a $DDDA_F$ in young calves >2. Thereafter, case (high AMU) and control herds (low AMU) were randomly selected from each group, such that a 1:1 case:control ratio per herd type was achieved.

Data collection

A questionnaire was conducted by phone by two employees of GD Animal Health in the period between July and October 2014. The cases and controls were randomly assigned to the interviewers, who were blind to the herd status (case or control). The questionnaire included questions on topics such as the postnatal care/calving management (e.g. availability of a calving pen), type of housing and type and administration of milk and feed. In addition, questions were asked about the general animal health (e.g. health problems in young calves), the mindset concerning youngstock, treatment of animals and specifically young calves (e.g. duration of treatment), knowledge of their own AMU, breed, and having worked off the farm. Besides the information that was obtained from the questionnaire, routinely collected herd data were available, e.g. herd size, growth in herd size, replacement rate, import numbers and

mortality of calves <1 year old. These data were provided by the Netherlands Enterprise Agency (RVO; the Hague, the Netherlands), Rendac (Son, the Netherlands) and GD Animal Health (Deventer, the Netherlands).

Statistical analysis

The data from the questionnaire were combined with the routinely collected data and the case/control status. Descriptive statistics, such as frequency tables and summarising tables were performed to describe the study population.

For the analysis, logistic regression models in Stata 14.0 (StataCorp, 2015) were used, in which a high (case) or low (control) $DDDA_F$ in young calves was included as the dependent variable. The parameters that were available from the questionnaire and routine data were included as explanatory variables. Firstly, the linearity of continuous explanatory variables was checked and variables were categorised in quantiles (terciles or quartiles) when needed. Explanatory variables with less than five observations in any one category were only presented descriptively. All other explanatory variables were pre-screened in a univariable logistic regression model. Explanatory variables that were associated with a P-value <0.2 were considered to be candidates for the multivariable logistic regression analysis. Collinearity between the variables was investigated by means of the bivariate correlation coefficients. When a correlation ≥0.5 was observed, one of the correlated variables was either dropped or a new variable was created that incorporated both variables.

A manual forward model selection method was used to create the final multivariable model. Backward model selection was performed when possible to check for robustness of the results. In both selection methods, a P-value ≤0.05 was considered significant. Only significantly associated variables were retained in the final model. During model selection confounding factors (>25% change in a coefficient) were taken into account and after model selection, all biologically relevant two-way interaction terms were evaluated. The explained variability of the model was presented using the pseudo R^2.

RESULTS

In total, 311 dairy and 199 suckler herds were contacted, of which 200 (64%) and 99 herds (50%) were willing to participate, respectively. Results of one dairy farm were excluded from the analysis because the farm did not meet the inclusion criteria (youngstock raising was outsourced). On one suckler farm, two questionnaires were conducted because the farm had two different breeds with different management practices. Only the questionnaire that involved the main breed was retained in the analysis. Four dairy farms and two suckler farms were excluded because the farmers did not provide the written consent to use their data. Eventually, data from 195 dairy herds (98 cases and 97 controls) and 97 suckler herds (47 cases and 50 controls) were available for analysis.

There was a large variation in farm characteristics, both in case and control herds. Dairy herds classified as a case had a median herd size of 45 calves (<1 year old) and 103 adult cows (means of 59 and 133, respectively). In the dairy herds that were classified as controls, a median of 30 calves and 74 adult cows were present (means of 34 and 85, respectively). The median percentage of growth in herd size during the analysed period in case and control herds was 3.5% (mean=4.2%) and 3.3% (mean=17.5%) respectively. The replacement rate in dairy herds was comparable between cases and controls (mean of 25.0% versus 24.0%).

In suckler herds, case herds had a median herd size of 31 calves (<1 year old) and 45 adult cows present on the farm during the analysed period (means of 48 and 73, respectively). The control suckler herds had a median herd size of 18 calves and 29 adult cows (means of 20 and 34, respectively). The median growth in herd size during the analysed period was 0.8% (mean=1.6%) in case herds and 0.7% (mean=2.3%) in control herds.

Multivariable model for dairy herds

In total, 50 parameters (out of 186 factors that were evaluated) were associated with case herds in the univariable analysis (P-value ≤0.2). The final multivariable model contained six variables and two confounders (Table 1).

Table 1. Results of the final multivariable logistic regression model for high Antimicrobial Usage (AMU; case) versus low AMU (control) in young calves in dairy herds in the Netherlands (N=183: 87 cases and 96 controls)[a]

Factor	Category	N	Odds Ratio	95% Confidence interval	P-value
Type of housing (group housing of calves 0-56 days old)	Non-slatted floor with bedding	116	1		
	Partially slatted floor with bedding	48	4.6	1.5-13.4	<0.01
	Different[b]	19	0.7	0.1-3.3	0.65
Usually start treatment of a sick calf with supportive measures (electrolytes, preparations without antimicrobials, anti-inflammatories)	Yes	127	1		
	No	56	11.4	4.1-31.7	<0.01
Percentage of animals in the herd with respiratory problems	0%	118	1		
	0-10%	33	0.3	0.1-1.2	0.08
	>10%	32	2.3	0.6-8.5	0.21
"Youngstock need specific management" (agree with the statement)	Yes	141	1		
	No	42	4.1	1.4-12.1	0.01
Unfavourable salmonella bulk milk results from the last five tests?	No	145	1		
	Yes, at least 1	38	3.3	1.1-10.2	0.04
Do you know the overall $DDDA_F$ of your farm in 2013?	No	81	1		
	Yes	102	2.5	1.0-6.0	0.05

[a]Confounders: Average number of calves (0-1 year old) present on the farm during the study period and the use of group treatment in calves

[b]'Different' = slatted floor with cubicles (N=12), fully slatted floor (N=4), sand and straw (N=2), rubber with sawdust (N=1)

Herds of farmers who indicated they did not start treatment with supportive measures such as electrolytes, or NSAIDs, more often belonged to the case group. Farmers who initiated treatment with antimicrobials instead of only supportive measures had 11.4 times higher odds of being a case herd. Herds in which young calves were housed on partially slatted floors

combined with bedding instead of non-slatted floors with bedding (complete straw or dust bedding) had 4.6 times higher odds of being a case herd. Farmers who disagreed with the statement "youngstock need specific management" belonged to the case group more often than farmers who agreed with this statement. Farmers with a higher (>10%) percentage of respiratory problems in their calves (versus 0-10%), farmers with an indication for a salmonella infection based on bulk milk testing and/or farmers that knew their $DDDA_F$ of the previous year also had higher odds of being a case herd. The pseudo R^2 of the final model was 45.9%. A check for robustness of the model based on backward model selection was not possible because the full model would not converge.

Other relevant factors in dairy herds

Eighteen factors could not be taken into account in the regression analyses because of a low number of observations in one or more of their categories. This was often the case for the mindset-related questions. Some examples of questions for which the number of observations in one of the categories was too low are shown in Table 2. For example, case herds determined the length of an antimicrobial treatment based on the herd health plan they developed together with the veterinarian less often than control herds did.

Table 2. Factors in dairy herds that could not be analysed due to a lack of observations (<5) but that were different in dairy herds with a high Antimicrobial Usage (AMU; case) versus low AMU (control) in young calves (N=194: 97 cases and 97 controls)

Statement	Percentage of case herds that agreed with the statement (N=97)	Percentage of control herds that agreed with the statement (N=97)
"I apply antimicrobials as little as possible due to the public health risk"	24.7 (N=24)	2.1 (N=2)
"I determine the length of an antimicrobial treatment based on the herd health plan"	48.5 (N=47)	100.0 (N=97)
"Antimicrobials are necessary to keep problems in the herd under control"	25.8 (N=25)	2.1 (N=2)

Multivariable model for suckler herds

In total, 37 parameters (out of 163 factors that were evaluated) were associated with case herds in the univariable analysis ($P \leq 0.2$). The final multivariable model consisted of four variables and three confounders (Table 3). Large suckler herds with an average of 29-152 calves <1 year old had 16.4 times higher odds of being classified as a case herd compared to small suckler herds with on average 1-18 calves. Case herds more often housed cattle of the Belgian Blue breed and experienced significantly more health problems in calves <56 days than control herds. Case herds also agreed with the statement "antimicrobials are necessary to help sick animals" more often than control herds. The pseudo R^2 of this model was 42.9%. The final model was relatively robust to forward or backward construction.

Table 3. Results of the final multivariable logistic regression model for high Antimicrobial Usage (AMU; case) versus low AMU (control) in young calves in suckler herds in the Netherlands (N=87: 39 cases and 48 controls)[a]

Factor	Category	N	Odds Ratio	95% Confidence interval	P-value
Average number of calves <1 year old	1-18	32	1		
	18-29	29	14.3	2.3-89.9	<0.01
	29-152	26	16.4	2.4-114.7	<0.01
Presence of health problems in suckling calves from 0-56 days old	No problems	47	1		
	Diarrhoea and sometimes additional problems	24	4.8	0.9-24.1	0.06
	Other problems (no diarrhoea)[b]	16	7.1	1.3-38.7	0.02
Presence of the Belgian Blue breed	No	56	1		
	Yes	31	13.4	2.9-63.0	<0.01
"Antimicrobials are necessary to help sick animals" (agree with the statement)	No	23	1		
	Yes	64	8.3	1.3-54.6	0.03

[a]Confounders: presence of sick or lame cows in the calving pen, general compliance of the farmer to the advice of the veterinarian, cluster variable: in the first group the calf always stays with the cow and the farmer always agrees with the statement "I only do the most necessary work with regard to my youngstock"; in the second group the calf partly stays with the cow, and the farmer almost never agrees with the statement "I only do the most necessary work with regard to my youngstock"

[b]Other problems = respiratory problems (N=8), omphalitis (N=9)

Other relevant factors in suckler herds

Seventy-three factors could not be included in the regression analyses due to a low number of observations in one or more of the categories. This was often due to questions related to individual housing (which is only applied sporadically in suckler herds) and the case for mindset-related questions. Some examples of relevant differences are shown in Table 4.

Table 4. Factors in suckler herds that could not be analysed due to a lack of observations (<5) that were different in suckler herds with a high Antimicrobial Usage (AMU; case) versus low AMU (control) in young calves (N=97: 47 cases and 50 controls)

Statement	Percentage of case herds that agreed with the statement (N=47)	Percentage of control herds that agreed with the statement (N=50)
"I am not satisfied with my current youngstock rearing and the number of diseased animals"	23.4 (N=11)	2.0 (N=1)
"I use antimicrobials as little as possible due to related costs"	38.3 (N=18)	2.0 (N=1)
"I determine the length of an antimicrobial treatment based on the herd health plan"	100.0 (N=47)	76.0 (N=38)

For example, farmers who belonged to the control group almost never agreed with the following statements: "I am not satisfied with my current youngstock rearing and the number of diseased animals" and "I use antimicrobials as little as possible due to related costs", whereas roughly one-third of the case farmers agreed with these statements (23% and 38% respectively).

DISCUSSION

Antimicrobial usage in young calves is a potential source for antimicrobial resistance, which makes it important to provide tools to enhance prudent use of antimicrobials in this age group. To enable a decrease in AMU, this study attempted to identify factors that were associated with being a structurally high (case) or low (control) user of antimicrobials in young calves in the Netherlands. Both management factors and the mindset of the farmer were observed to be significantly associated with structurally high (cases) or low AMU (controls) in dairy and suckler herds.

Dairy herds that were classified as cases housed calves on partially slatted floors combined with bedding more often than control herds. Previous research has indicated that a slatted floor was a risk factor in the development of diarrhoea in calves housed in groups (Gulliksen, 2009). In addition, dairy case herds more often had unfavourable salmonella bulk milk results in the last 1.5 years, which is also known to be associated with calf diarrhoea (Reynolds, 1986). Diarrhoea problems partly result in antimicrobial treatments being administered, as Constable (2004) showed that 30% of calves with diarrhoea had bacteraemia. Respiratory problems were also associated with dairy case herds. Interestingly, lower odds of being a case herd were found for herds with >0-10% respiratory problems than herds with 0% respiratory problems.

In suckler herds, important risk factors were herd size and the presence of the Belgian Blue breed. In previous research, herd size was also found to be a risk factor for the presence of infections such as salmonellosis (Vaessen, 1998). A larger herd size might increase the infection pressure. Herd size is, however, also a known indicator for underlying management factors that can also influence calf health. The association between the presence of Belgian Blue cattle and being a case herd can possibly be explained by the fact that Belgian Blue calves are more susceptible to respiratory infections than other breeds (Gustin et al., 1985). In addition, double-muscled cattle such as Belgian Blue calves are often born via a caesarean section, as the calf's weight and size are relatively high (De Smet, 2014). Being born via caesarean section instead of natural birth can adversely affect the nonspecific immunity of the newborn calf, leading to a greater susceptibility to disease (Probo et al., 2012). The final multivariable model for suckler herds, which comprised of four significant factors, also included three non-significant confounders, which might indicate the multifactorial nature of a high AMU.

A strong association was found between "usually starting the treatment of a sick calf with supportive measures (electrolytes, anti-inflammatory drugs or other preparations without antimicrobials)" and control dairy herds. Alternative treatments therefore seem to result in a lower AMU. The decisions that farmers make with regard to treatment are affected by their mindset. Mindset already proved to be important in calf rearing and associated mortality (Santman-Berends et al. 2014), mastitis (Jansen & Lam, 2012) and other diseases. Multiple statements about farmers' mindsets appeared to be associated with the herd status regarding AMU in young calves. Farmers of dairy case herds disagreed with the statement "youngstock

need specific management" more often than control dairy farmers. This reflects their attitude towards youngstock, and might be a sign that youngstock are not their highest priority. According to the health belief model, a cue for action towards preventive behaviour (e.g. prevention of disease and therefore AMU in young calves) needs both a belief in a personal threat that needs to be prevented as well as in the effectiveness of the preventive behaviour (Janz & Becker, 1984; Koelen & van den Ban, 2004). The belief in a personal threat is based on the perceived susceptibility of calves to infections (risk) and the subsequent severity of disease (impact). In both control and case herds, a belief in the effectiveness of preventive behaviour might be present, but when a belief in personal threat is absent, no action is taken.

In both dairy and suckler herds, the combination of management/behaviour and mindset appeared to be important. Santman-Berends et al. (2014) indicated three types of farmers with respect to calf management and related mortality: farmers with a high calf mortality 1) who were unaware of the problems, 2) who felt powerless and 3) who were reluctant to change the management in order to improve calf health. This indicates that technical knowledge alone is not enough to make adjustments in calf management. Sufficient attention should be paid to the mindset of the farmer as well. Jansen et al. (2016) described a diverse set of theories (e.g. the previously mentioned health belief theory) affecting people's behaviour in relation to health decisions, which can be applied to mastitis issues, and potentially to health problems and AMU in young calves. Jansen et al. (2016) used the R.E.S.E.T model, which was adapted from van Woerkum et al. (1999) and Leeuwis (2004). This model notes five cues to actions (rules, education, social pressure, economics and tools) which might serve as a general basis to change the mindset of farmers with regard to AMU in young calves. Only if adequate and farmer-specific communication strategies are adopted, can structural changes in technical management be achieved. Earlier research by Speksnijder et al. (2015) showed that veterinarians differed in their attitudes with regard to AMU. Therefore, it is crucial to pay specific attention to the role of the veterinarian in this challenge. Lam et al. (2011) indicated key aspects of communication between the veterinarian and the farmer, in order to stimulate changes in udder health management. These lessons might also be useful with regard to AMU in young calves.

Antimicrobial treatments are a sensitive topic in the Netherlands. This might have resulted in farmers giving socially desirable answers, which often leads to underestimations of true effects. Another factor possibly influencing the study results is the design used. The case-control design of this study enabled identification of factors associated with a structurally high AMU in young calves instead of factors associated with general AMU in young calves. It should be noted that herds were selected as cases or controls based on their AMU in 2012 and 2013, while the interview was conducted in 2014. Although in theory it might be possible that the AMU and management changed within this period, the $DDDA_F$ in 2014 was still high in the majority of case herds (results not presented).

Optimisation of calf management leading to improved calf health and a reduction in the AMU in calves remains important, as the mean $DDDA_F$ applied orally in young calves in dairy herds was still 3.7 with a median of 0 in 2015 (SDa, 2016). This indicates that there is still a minority of herds with a high AMU in young calves. The study provided insight into factors that could encourage more prudent use of antimicrobials in dairy and suckler herds with high a $DDDA_F$ in young calves, and may subsequently support a further reduction of antimicrobials in general.

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WHAT DRIVES POULTRY FARMERS TO USE ANTIMICROBIALS?

A CASE-CONTROL STUDY ON FRENCH TRADITIONAL FREE-RANGE BROILERS

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SUMMARY

A case-control study was carried out in 2016 to identify the risk factors for antimicrobial use (AMU) in French traditional free-range broiler farms. This production type has been engaged in a voluntary process to reduce AMU to the minimum possible. Cases (52) and controls (208) were randomly selected. Two factors were associated with a decreased risk of AMU: using chicken paper topped with starter feed (OR= 0.21; 95% CI = [0.06; 0.71]), and herbal drugs as a prophylaxis (OR= 0.09; 95% CI = [0.01; 0.58]). During the broilers' first 10 days of life, the farmer reporting a problem (OR=20.4, 95% CI = [4.1; 101.4]) and the farmer's perception that the mortality rate in the flock was not low (OR= 10.6; 95% CI = [1.6; 68.3]) were associated with increased odds of treatment. Understanding AMU involves technical factors, as well as how farmers perceive mortality rates in their flock.

INTRODUCTION

Antimicrobial resistance is now a major global public health issue (Laxminarayan et al., 2013) that needs to be rapidly addressed with improved antimicrobial stewardship. Antimicrobial use in farm animals is particularly important, given that the global consumption of antimicrobials is expected to rise by 67% between 2010 and 2030 (Van Boeckel et al., 2015). In France, species bred in intensive production systems were among the most exposed to antimicrobials in 2015 (Méheust et al., 2016). It is therefore necessary to identify levers for change in the way antimicrobials are used. Epidemiological analysis carried out in pig farms showed not only the importance of technical factors for AMU (Hybschmann et al., 2011; van Rennings et al., 2015; Arnold et al., 2016), but also the impact of socio-economic factors on AMU (Casal et al., 2007; van der Fels-Klerx et al., 2011). A cross-sectional survey performed on UK broiler farms highlighted the importance of politics conducted by farmer organisations (FO) as well as farm management for the use of antimicrobials (Hughes et al., 2008). Adherence with biosecurity rules was associated with a decrease in the risk of treatment in French turkey broiler farming (Chauvin et al., 2005). More and more farmers, FO and veterinary surgeons are using alternatives to antimicrobials

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such as herbal drugs, vaccines or probiotics. Nevertheless, the impact of this recent evolution on AMU remains poorly studied in epidemiological research.

Concerning the management of disease situations, Alarcon et al. (2014) stressed the importance of considering farmers' perceptions, in order to better understand their decisions. Wauters and Rojo-Gimeno (2014) have advocated socio-psychological veterinary epidemiology. However, few epidemiological studies focus on the possible discrepancy between the perceptions of farmers and veterinary surgeons.

Based on the analysis that alternative techniques, daily farm management practices and the farmers' perception of their flock situation have not been studied in detail, the case-control study described here aimed to evaluate the impact of these factors in the use of antimicrobials. This study was carried out in French traditional free-range broiler farms, a production engaged in antimicrobial stewardship actions.

MATERIALS AND METHODS

Period of interest in the breeding cycle and geographical area involved

Before having access to an open-air range, traditional French free-range broilers are raised indoors until day 42. Risk factors for AMU are not the same in indoor and outdoor breeding, and most of the antimicrobial treatments in this production are administered before day 42. The study therefore focused on the indoor period. Nine farmer organisations from the two main basins of production (North West and South West France) were contacted, along with one in the centre of the country.

Definition of cases and controls and sample size

A case was defined as a flock of broilers having received at least one antimicrobial treatment between days 1 and 42. The cases were recruited via the practice management software of the veterinary practice(s) that worked with the farmer organisation, or directly via the FO. The controls – untreated flocks of broilers in the same period – were randomly selected among the list of all flock placements. Frequency matching was used; in each FO there were four times more controls than cases. Controls were also selected with a +/-10-day window around the case placement date. The 1:4 case-control ratio aimed to increase the precision of the odds ratio given the limited number of cases (Dohoo et al., 2009). The study focused on the flocks placed between December 2015 and April 2016. The sample size aimed to detect an odds ratio (OR) of 2.5 for a risk factor present in 20% of exposed controls and with a 5% error and a power of 80%.

Data collection and questionnaire

Three previously trained animal health professionals undertook the on-farm administration of the questionnaires between February and June 2016. The questionnaire included 11 sections, of which the first two aimed to gather general data on the farmer and his or her farm. The next sections concerned the flock: biosecurity, facilities, hygiene, animal husbandry practices, health issues, treatments and prophylaxis. The farmer was also asked his or her perception of health issues and mortality. Farming documents (farm register, feed delivery orders, chick delivery orders and prescriptions) were analysed and photographed.

Statistical analysis

The data were entered in a database (Access) and analysed with R software (R Core Team, 2016). AMU status (case versus control) was the binary outcome, and the flock was the unit of analysis. Univariate logistic regression was performed to select the variables ($P<0.25$) included in a multivariate logistic regression model. All potentially connected variables were screened for correlation using a chi-square test. When different variables were correlated ($P<0.05$), variables with smaller P-values in univariate analysis and higher biological interest were conserved. The final model was built using a stepwise regression based on Akaike criteria (Dohoo et al., 2009). Non-significant variables remaining after selection were removed from the model ($P>0.05$), after checking that the AIC was not increasing. Matching was accounted for by forcing FO as a fixed effect in the final logistic regression model (Dohoo et al., 2009; Marsot et al., 2016).

RESULTS

One FO did not report any antimicrobial treatment, so the cases and controls were sampled among the remaining seven FO. The final sample comprised 52 cases and 208 controls. Out of 319 farmers contacted, 23 (7.2%) refused to participate, and 16 (5%) were unreachable. The refusals, mostly motivated by lack of time (17/23) or lack of motivation to answer an extra questionnaire (2/23), mainly concerned control flocks (21/23), and all unreachable farmers were control flocks.

In total, 58% of the farmers in the sample were aged between 40 and 55, and 72% were male. For half of the sample, free-range broiler farming was a secondary production in terms of income: 90% of the farms visited had crop productions, 50% had another type of breeding production (livestock or pigs), while 70% of the sample had between 1 and 4 poultry houses. In total, 53% of the farmers only bred traditional free-range broilers, and 9% also had industrial broiler production.

One hundred variables were screened in a univariate logistic regression, of which 48 had a P-value <0.25 and were considered for multivariate analysis. After removing the main correlations, 26 variables remained for evaluation in the logistic regression model. Eight variables were found to be statistically significant (Table 1) in the final multivariate model. None of the seven categories of FO variable that were forced into the final model were significant. The Hosmer Lemeshow showed that the model had no evidence of poor fit (P-value = 0.91).

Three variables were associated with a decreased odds of antimicrobial treatment: (i) the use of chicken paper topped with starter feed (OR= 0.21; 95% CI = [0.06; 0.71]), (ii) a litter thickness of 10 cm or less (OR= 0.21; 95% CI = [0.05; 0.91]) compared to litter more than 15 cm thick, and (iii) the use of herbal drugs as prophylaxis (OR= 0.09; 95% CI = [0.01; 0.58]). The herbal drugs mostly consisted of essential oils. Among the five variables significantly associated with increased odds of treatment, one concerned the cleaning process: cleaning and disinfection of the concrete perimeter of the poultry house at the previous downtime (OR=3.8, 95% CI = [1.3; 11.3]). Two variables concerned the first days of the chicks: the declaration of a sanitary issue by the farmer during the first 10 days of life (OR=20.4, 95% CI = [4.1; 101.4]) and the farmer's perception of the mortality rate between 1 and 10 days of age being considered "normal" (OR= 10.6; 95% CI = [1.6; 68.3]) or "high" (OR=8.5, 95% CI = [0.95; 75.5]) in comparison with "low". The last two variables associated

with increased odds of AMU concerned the next time period: the declaration of an issue by the farmer between 11 and 42 days (OR=20.1, 95% CI = [6.1; 66.0]) and a phone call between the farmer and the production technician between 11 and 42 days (OR= 6.3; 95% CI = [2.2; 17.7]). This latter variable included all phone calls, regardless of the reason for the call.

Table 1. Results of the multivariate analysis modelling the risk for a flock to have received an antimicrobial treatment (cases, n=52) compared to not having received any antimicrobial treatment (controls, n=208), adjusted for the farmer organisations

Variable	Number of cases (n=52)	Number of controls (n=208)	Adjusted odds ratio	95 % confidence interval	P-value
Use of chicken paper topped with starter feed					
Yes	37	176	0.2	0.06-0.7	0.01
No	15	32	Reference		
Use of herbal drugs in prevention					
Yes	2	35	0.09	0.01-0.6	0.01
No	50	173	Reference		
Thickness of the litter					
11-15 cm	17	64	1.2	0.42-3.6	0.69
10 cm and less	5	57	0.2	0.05-0.9	0.04
15 cm and more	30	87	Reference		
Cleaning and disinfection of the concrete perimeter of the poultry house at the previous downtime					
Yes	37	117	3.8	1.3-11.3	0.01
No	15	91	Reference		
Farmer reported a problem between 1 and 10 days					
Yes	26	24	20.4	4.1-101.4	<0.01
No	26	184	Reference		
Perception of the mortality rate at 10 days					
High	30	53	8.5	0.95-75.5	0.06
Normal	20	83	10.6	1.6-68.3	0.01
Low	2	72	Reference		
Farmer reported a problem between 11 and 42 days					
Yes	26	23	20.1	6.1-66.0	<0.01
No	26	185	Reference		
Phone call between farmer and production technician between 11 and 42 days					
Yes	40	69	6.3	2.2-17.7	<0.01
No	12	139	Reference		

DISCUSSION

Study limitations

Despite a case-recruitment protocol adapted to each FO, the efficiency of case recruitment was very variable in each FO. Exhaustive reporting of AMU was hard to achieve in FO without any systematic tracking of antimicrobial treatments. Systematic checking of the effective status of case or control was done on-farm: false cases if farmers decided not to give the prescribed antimicrobials when broilers recovered by themselves or false controls if farmers gave antimicrobials remaining from a previous treatment. In addition to the information obtained from farmer interviews, on-farm cross-examination of farm register, prescriptions, invoices or drug packaging leftovers helped to determine whether or not the broilers received antimicrobials.

Risk factors for the use of antimicrobials

Based on a large number of free-range broiler farms, this study highlighted the effect of factors (use of herbal drugs, chicken paper topped with starter feed, and farmer perception of the flock health status) that have been poorly investigated so far in terms of AMU. Besides the presented new findings, it was also possible to refine current understanding of the effect of factors previously studied.

Firstly, the use of herbal drugs in prevention was found to be associated with decreased odds of AMU. Prophylaxis plans include vaccines, modern chemotherapy and alternative drugs to modern chemotherapy, such as herbal drugs. Among the prophylactic alternatives to antimicrobials, similar studies showed that probiotics could decrease AMU in poultry production (Chauvin et al., 2005; Hughes et al., 2008). The use of homeopathic agents was also found to be associated with a decreased risk of antimicrobial treatment in pigs (Arnold et al., 2016). Good on-farm practices could be confounders, but several variables were taken into account for the associations reported. Therefore, even if the presence of a confounder cannot be totally excluded, results from this study merit further evaluation (including via experimental approaches) to better understand the effect of herbal drugs (and more accurately of essential oils) in prevention.

Secondly, the use of chicken paper topped with starter feed was found to be associated with decreased odds of AMU. This technical tool aims to optimise the management of the first 10 days of chickens' lives. Successful management during this period is crucial for the remainder of the flock's life, and determines its future technical performance (Heier et al., 2002; Yassin et al., 2009). Chicken paper is covered with feed and is set under the drinking systems and the heating systems, thus gathering all that is necessary for the young chickens. The noise produced by the chickens walking on the paper attracts the rest of the flock. The early satisfaction of their physiological needs ensures the development of their immune system (Panda et al., 2015), making them less vulnerable to disease, and less susceptible to receiving an antimicrobial treatment. Heier et al. (2002) did not find the same results and showed that chicken paper used in industrial Norwegian chicken farms was associated with a higher mortality. They theorised that it was due to the mix of feed and droppings raising the infection pressure. These divergent results could be related to differences in the two breeding systems, such as the lower bird density in free range broilers, and chicken strains that are less productive.

Thirdly, the declaration of a problem in the flock during the whole period studied was found to be associated with increased odds of AMU. This finding is in accordance with the observation that antimicrobial treatments are mostly administered in the context of a sanitary event on the farm: more than 90% of the antimicrobials administrated were given as a treatment (and not a prophylaxis). Additionally, farmer perception and judgement about the level of mortality at 10 days was associated with increased AMU. Some farmers judged a mortality rate considered to be low in this type of production (less than 1%) as "high". Lupo and Prou (2016) studied mortality detection and mortality notification by mussel farmers, and assumed the farmers compared their observations to a previous situation to decide whether or not to notify. Similar hypotheses can be made here: the way farmers perceive the health status of their flock is partly connected to the recent history of health on the farm, and their judgement is partly out of phase with how veterinary surgeons and technicians evaluate the health situation. More studies should be conducted to test this hypothesis.

Among the other significant variables, cleaning and disinfection of the concrete perimeter of the poultry house at the previous downtime was found to be associated with an increased risk of AMU. Hypotheses can be made that this finding is a case of reverse causality, with farmers having recurrent health problems trying to get rid of this issue by re-enforcing the cleaning and disinfecting operations. Surprisingly, this study also shows that the thinner the litter, the lower the odds of treatment with antimicrobials. This variable aimed to test the hypothesis that when the litter was thick enough, the broilers were more comfortable (better absorption, better isolation, etc.), and as a consequence less susceptible to diseases. Bilgili et al. (2009) showed that lower bird density associated with higher quantity of bedding and duration of lighting were associated with a decreased risk of footpad dermatitis. The difference between the results of Bilgili et al. (2009) and the results observed here could be explained firstly by the fact that different outcomes were observed (footpad dermatitis lesions versus use of antimicrobials) and secondly because different strains of broilers were studied. Two assumptions can be made for better explaining the results from the present study: the thickness of litter could be linked to a set of management practices, or it could have an indirect impact on broiler health, as shown in a study of the risk of intestinal lesions due to coccidiosis with higher amounts of litter (Henken et al., 1992). A more detailed analysis is necessary to investigate the role of litter on AMU, taking into account (among other things) the amount and the type of litter. Lastly, phone calls between the farmer and his or her technician were also found to be associated with increased odds of AMU. This result is consistent with the role of technicians in FO, who work at the interface between the farmer and the veterinarian.

In conclusion, this study highlights the effect of protective practices, which may contribute to reduce AMU in poultry. Moreover, it stresses that socio-psychological factors may be as important as technical factors in explaining the use of antimicrobials in traditional free-range broilers. It is necessary to work ahead of actual problems by improving prevention, because once a problem is declared, antimicrobials are often used. The discrepancy between the perception of veterinary surgeons, technicians and farmers of the health flock situation has to be taken into account. To decrease the risk of AMU once a problem is identified, work should focus on how technicians and veterinary surgeons can modify farmers' perception of the situation, and help them to find alternative solutions to AMU.

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ENVIRONMENTAL AND VECTOR-BORNE DISEASES

SOUP-*E.COLI*-FIELD LOGISTICS-EPI-AND-GENOMICS: A SAMPLING STRATEGY TO CAPTURE BACTERIAL DIVERSITY IN A CHANGING URBAN ENVIRONMENT

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SUMMARY

Urbanisation has been linked to a risk of emerging infectious diseases. Understanding transmission processes for known pathogens may shed light on mechanisms of disease emergence and is essential for informing public health and planning policy. The Urban Zoo Project examines the "flow" of micro-organisms in Nairobi, using the landscape genetics of *E. coli* to model this diverse and highly fragmented urban environment. A total of 2,351 samples were collected, yielding more than 1,800 whole genome sequences from 99 households across the city, stratified by socio-economic and livestock-keeping status. This is a uniquely structured dataset, which may answer questions about the influences of livestock keeping and poverty on bacterial diversity in urban settings. However, care is required in analysis and interpretation, as some households and sample types yielded only a small number of isolates, which reduces the power to detect diversity and assess the phylogenetic relatedness of bacteria in some settings.

INTRODUCTION

Zoonotic diseases have traditionally been perceived as a rural problem, but the challenges posed by several neglected tropical diseases have now shifted to urban settings (Neiderud, 2015). More than half of the world's population now live in urban areas, and this proportion is expected to continue rising, particularly in Africa and Asia (United Nations, 2014). Cities present many opportunities for infectious pathogens to flourish: dense human populations, highly interconnected through globalised travel networks; areas of overcrowding and poor sanitation; conditions ideal for insect vectors, or rodent hosts to proliferate; highly fragmented environments, promoting closer contacts with wildlife or livestock may all contribute to the rapid emergence and spread of pathogens in urbanised areas. Yet despite the recognition that urban centres present an increased risk of pathogen emergence (Institute of Medicine (US), 2003), the transmission processes involved are largely unstudied, and addressing this gap is essential for informing policies both for public health and urban planning.

Approximately 80% of novel pathogens have zoonotic origins (Woolhouse and Gowtage-Sequeria, 2005), and processes that lead to their emergence are thought to be similar to those promoting the spread of known zoonoses. *Escherichia coli* is a well-characterised bacterium

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with a diverse range of host species, existing both as a commensal in the gut, and as an opportunistic pathogen, as well as being able to survive for extended periods in a range of external environments. The Urban Zoo Project is using this bacterium as a model to understand the flow of bacterial strains in an urban environment, taking a landscape genetics approach (Manel et al., 2003) to characterise isolates collected from around the city of Nairobi, Kenya.

Nairobi typifies the rapid urbanisation common in sub-Saharan Africa, having expanded from a population of 350,000 in 1962 to almost 10 times that number in less than 50 years (APHRC, 2014). Between 60 and 70% of the population are estimated to live in informal settlements, or slums (APHRC, 2014), where people are socially and economically marginalised, infrastructure and basic social service provision are largely absent, and environmental conditions are poor. However, these areas are frequently juxtaposed with very different types of neighbourhoods – wealthy areas delineated by sharp boundaries, such as security walls, enforcing a high level of social segregation. Complex value chains within and outside the city supply livestock products to residents (Alarcon et al., 2017), and over 116,000 ruminants and 28,000 pigs are estimated to be kept in the city (KNBS, 2011). Human and animal waste is disposed of poorly, often alongside food producers, and a diverse array of wildlife species co-exist with the human and domestic animal population. Whereas many studies of genetic diversity make use of convenience samples to construct phylogenies, this study aimed to combine the powerful tools of genomics with structured epidemiological sampling and analysis methods, to assess risk factors for sharing bacterial strains across these diverse ecological niches, and hence identify potential transmission pathways presenting an increased risk for disease emergence. This paper describes the complex sampling strategy developed by the Urban Zoo Project, in an attempt to represent the diversity of *E. coli* across Nairobi municipality.

MATERIALS AND METHODS

Map layer data

Three main priorities guided household selection: socio-economic diversity, population distribution and livestock keeping. Publicly available data from the censuses had only a coarse spatial resolution. However, disaggregated socio-economic data at a much finer scale was produced by a previous project on water availability and distribution in Nairobi, implemented by the Institut Français de Recherche en Afrique (IFRA). The technical report (Ledant, 2011) describes how different land-use patterns (for example residential, industrial, institutional) were identified from pan-sharpened satellite imagery. Physical characteristics (eg. tree cover, plot size, amount of gated space, roofing type, presence of agriculture) were then used to define and discriminate between 17 different classes of residential neighbourhoods, the assumption being that neighbourhoods with similar physical characteristics are also similar in terms of other qualities, such as socio-economic status and service provision. The classification was validated with 817 household questionnaires. The map layers from the IFRA project were kindly made available to the Urban Zoo study.

Selection of neighbourhoods

The 17 physical neighbourhood classes defined by the IFRA project were ranked by average income and merged into seven wealth groups (Table 1).

Table 1. Adaptation of 17 neighbourhood classes identified by IFRA study (Ledant 2011) to seven wealth groups by the Urban Zoo Project, and the number of sub-locations with a dominant wealth group identified and selected in the Nairobi municipality

Characteristics of physical neighbourhood classes identified by IFRA study (adapted from Ledant, 2011)					Urban Zoo Project re-classification		
Code	Tree cover	Defining characteristics	Neighbourhood description [a]	Average monthly income per capita [b]	Wealth group	Possible sub-locations	Targeted sub-locations
A	>13.5%	Detached housing with intense tree cover	Detached housing on very large plots *(>3000 m²)*	39890	1	8	3
B			Detached housing on large plots *(400 - 3000 m²)*	22462	2		
C	>13.5%	Attached and semi-detached housing	Attached housing on medium plots *(<400 m²)* with important tree cover	22084	2	8	4
D	3 - 13.5%	Apartment building	Apartment buildings *with gated space*	22084	2		
E	3 - 13.5%	Attached and semi-detached housing	Higher standing row houses *(plot size >190 m²)*	13352	3	5	3
F			Lower standing row houses *(plot size <190 m²)*	6153	4	3	3
G	<3%	Roof cover >50% tiles	Lower standing *apartment buildings*	6153	4		
H			New areas of dense *single housing* development	3855	5		
J	<3%	Roof cover >40% concrete	High density multi-storey buildings	3855	5	9	5
K	3 - 13.5%	Apartment building	Apartment buildings *with open access*	3855	5		
L	3 - 13.5%	Peripheral areas	Peripheral areas *(mainly residential)*	3855	5		
M			Peripheral areas with rural component *(presence of agriculture)*	2165	6		
N	3 - 13.5%	Collective housing	Community housing *with gated space*	2165	6		
P			Community housing *with open access*	2165	6	24	11
Q			New areas of low-quality housing *(built-up area <37%)*	2165	6		
R	<3%	Roof cover >85% corrugated iron sheets	High density planned low-quality housing *(built-up area <37% AND public space >20%)*	2165	6		
S			High density unplanned low quality housing (slums) *(built-up area <37% AND public space <20%)*	1301	7	13	4

[a] Words in italics give further sufficient and necessary physical characteristics that are used to allocate residential neighbourhoods to each class

[b] Kenyan Shillings (KES). In 2011, USD 1 was worth approximately KES 80

Local sub-location (administrative) boundaries were then overlaid on maps of each wealth group, and areas of similar neighbourhood types were identified, with the aim of maximising the geographical spread across the municipality.

This process identified 70 possible sub-locations containing at least two or three contiguous polygons of the same residential neighbourhood class. The dominant wealth group was determined for each sub-location by extracting the proportion of population belonging to each neighbourhood class within the sub-location boundaries. The number of sub-locations to visit in each wealth group was weighted by population and the number of neighbourhood classes that went into the formation of each wealth group. For example, 11 sub-locations dominated by wealth group 6 were selected, due to both the population density and the variety of neighbourhood classes that make up this grouping (Table 1).

Purposive selection of 33 sub-locations for the study was undertaken in three rounds: a) selection of slum and peripheral rural areas with high livestock densities, in which project activities were already being carried out (8 sub-locations); b) selection of one sub-location to represent each remaining neighbourhood class (15 sub-locations) with the highest population proportion for that class; c) selection of 10 further sub-locations to make up the target number for each wealth group, attempting to maximise geographical spread, distribution between neighbourhood classes and the proportion of population belonging to the dominant class. Three random geographical points were selected within the dominant housing types for each sub-location, and sub-locations were fully randomised to determine the visiting order.

Selection of households

Local chiefs and elders for each sub-location assisted in recruitment, which was normally carried out a few days before the visit to the households. In the presence of these local officials, random geographical points were identified on the ground, and the nearest households that met the inclusion criteria (Table 2) were identified, to obtain a triplet of two livestock-keeping and one non-livestock-keeping household. Sub-locations were randomised to one of two categories (large ruminant/small monogastric and large monogastric/small ruminant) to try and obtain a roughly equal proportion of households that would definitely keep cattle or pigs.

Human members of a household were defined as those who either slept on the premises, or (in order to include staff) spent at least 8 hours a day on the plot most days, and interacted regularly with the core household in a way likely to facilitate sharing of pathogens, such as sharing food prepared on the premises, regularly handling food, animals, animal manure or contact with human excrement (for example nannies looking after young children). Other families who lived on the plot (such as tenants), but who had separate cooking facilities and did not contact or share livestock or livestock products belonging to the core household were excluded.

Once households had been identified and consented to participate, appointments were made for visits by the study teams. Normal procedure was for the house team (consisting of two clinical officers, and one to three veterinarians/animal health workers) to visit one household per day on 3 consecutive weekdays – usually Monday to Wednesday. The wildlife team, consisting of two to three veterinarians overseen by a doctoral student, would visit each household in the sub-location for 3 consecutive days, with one long early morning and (if appropriate) one evening visit in order to set mist nets for trapping wild birds and bats,

respectively. All field staff were Kenyan nationals, fluent in both Kiswahili and English, and participants could opt to complete the questionnaires in either of these languages.

Table 2. Criteria defining livestock keepers

	Large ruminant	Large monogastric	Small ruminant	Small monogastric
Necessary and sufficient	Cattle	Pigs	Goats/sheep	Poultry or rabbits
Optional	Any other species	Any other species	Pigs, poultry or rabbits	Cattle, sheep or goats
Exclusion	None	None	Cattle	Pigs

Ethics statement

Ethical approval for human and animal sampling was obtained through the Institutional Research Ethics Committee and the Institutional Animal Care and Use Committee at the International Livestock Research Institute (ILRI), Nairobi. Permits for wildlife sampling were obtained through the National Museums of Kenya and the Kenya Wildlife Service.

Data collection

Each household was asked to nominate people to provide answers to the following questionnaires (different members within the household could answer each questionnaire): a) household composition, socio-economic data and wildlife interaction; b) livestock ownership, management, sourcing, sales and antimicrobial use; c) food purchasing, preparation and consumption of own livestock products. Every human member of the household was invited to contribute a faecal sample and answer questionnaires on their occupation, exposure to livestock, food consumption and medical history. Environmental samples were taken from the kitchen and external household environment, including water samples from tanks, ponds or effluent. Any animal-source foods were sampled, and up to 20 rectal swabs were taken from all livestock species present. Wild birds were caught using mist nets and cloacal swabs collected, rodents were captured using live and kill traps, and bats, primates and carnivores trapped and sampled if householders reported frequent sightings. Questionnaires and data associated with samples were recorded using Open Data Kit (ODK) Collect software (Hartung et al., 2010) on electronic tablets, and uploaded to databases held on servers at ILRI.

Laboratory procedures

All samples were enriched in buffered peptone water for 24 hours before plating onto eosin methylene blue agar (EMBA), a selective and differential media for coliform bacteria. After 24 hours incubation at 37°C, five colonies were selected and sub-cultured for a further 24 hours on a second round of EMBA. Each of the five purified isolates, plus the original sample were sub-cultured on Müller-Hinton (MH) agar and stocked in cryovials at -20°C. One purified isolate per original sample grown was selected at random, and confirmed as *E. coli* by biochemical testing, using triple sugar iron agar (TSI), Simmons citrate agar, and

motility-indole-lysine media. If an isolate was deemed to be something other than *E. coli*, then a second isolate was selected at random from the archived vials. All isolates identified as *E. coli* by biochemical testing were grown-up on MH for 24 hours before DNA was extracted using commercial kits (Purelink® Genomic DNA Mini Kit, Invitrogen, Life Technologies, Carlsbad, California). DNA was transported under licence to The Wellcome Trust Centre for Human Genetics, Oxford, UK, for sequencing on the Illumina HiSeq 2500 platform.

RESULTS

Recruitment by socio-economic status and livestock type

Participants from all 17 types of neighbourhood class, with the exception of class G (lower-standard apartment buildings), were recruited to the study. However, it was not always possible to recruit houses in the targeted physical neighbourhood class, or even in other classes within the same wealth group, for every sub-location. Aggregation of wealth groups 1 and 2, and groups 3 and 4 occurred naturally in the field (Table 3), mainly due to the difficulty in finding livestock keepers (especially large livestock keepers) in the wealthier and middle-class sub-locations. For example, in three of the four sub-locations targeting wealth group 2 neighbourhood types, at least one recruited household actually fell under the wealth group 1 neighbourhood class (detached houses on plots greater than 3,000 m^2). For wealth groups 5, 6 and 7, all households recruited were of a neighbourhood class belonging to the correct wealth group.

Table 3. Households by neighbourhood class for the top four wealth groups

Wealth Group	Neighbourhood class	Target Wealth Group 1	2	3	4
1	Detached housing on very large plots	7	6[a]		
2	Detached housing on large plots	2[a]	4		
	Attached housing on medium plots		1		
	Apartment buildings with gated space		1		
3	Higher standing row houses			9	3[a]
4	Lower standing row houses				6
	Lower standing apartment buildings				0

[a] Households where the neighbourhood class fell outside of the target classes for the sub-location

Twelve different species of livestock (cattle, pigs, sheep, goats, rabbits, guinea pigs, chickens, ducks, geese, turkeys, guinea fowl and pigeons) were sampled over the course of the study. The distribution of livestock between neighbourhood classes varied according to species (Fig. 1). Chickens were the most common species encountered, kept by 83% of the 66 livestock-keeping households, and these along with goats, rabbits and other poultry types were distributed relatively evenly across all neighbourhood classes. However, cattle and sheep were found almost exclusively in either the very wealthy areas, the very poor areas, or the areas on the eastern and western periphery of the city. The distribution of pigs was similar, except that they were not found in the higher wealth groups, although one pig-keeper in a dense new-build area (wealth group 5) was recruited.

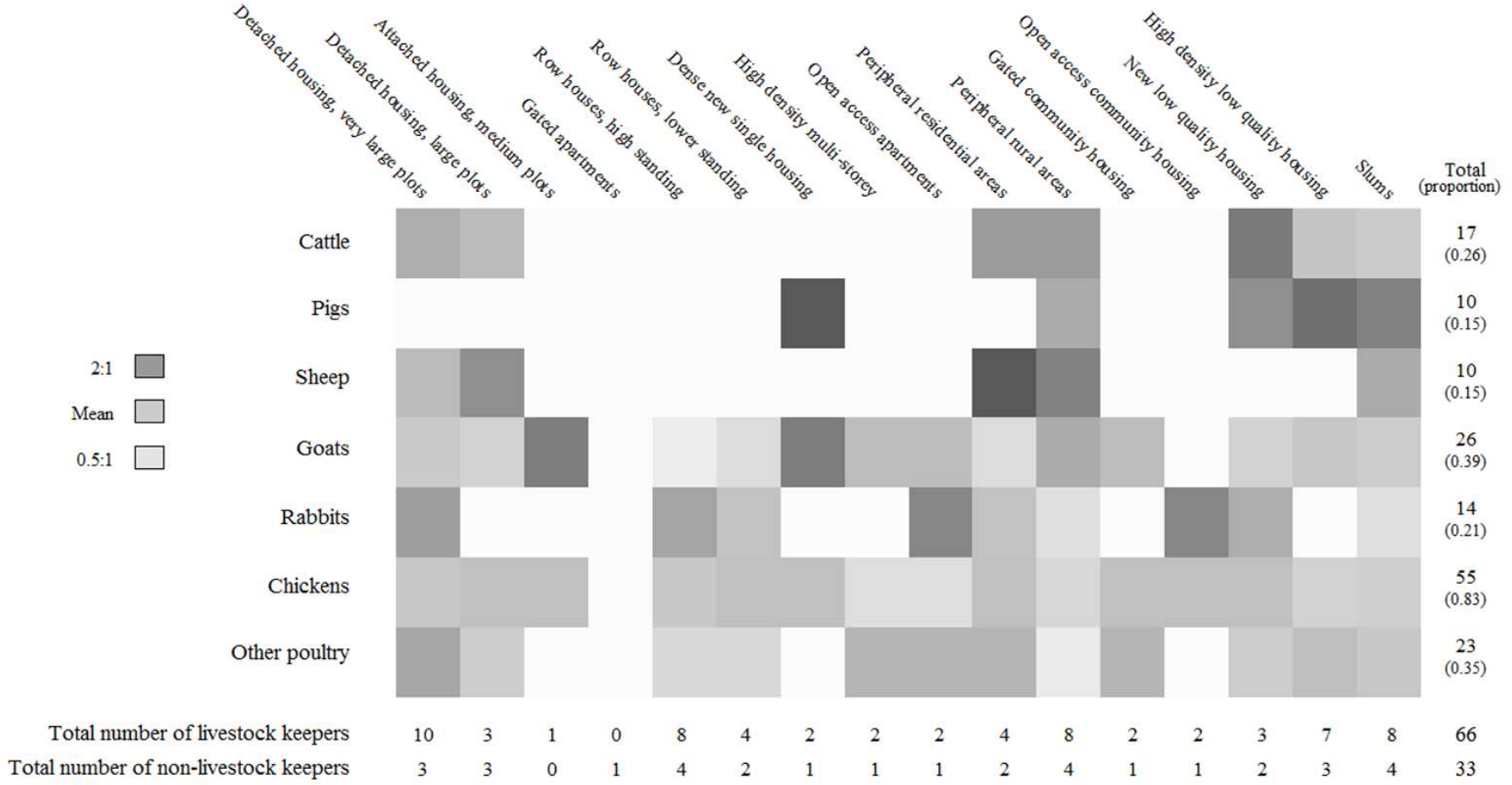

Fig. 1 Heatmap showing different types of livestock found in recruited households by neighbourhood class. For each livestock species, cells are shaded relative to the mean proportion of all livestock-keeping households owning that species. The evenness of the shading for each species gives an idea of how well the recruitment protocol worked in terms of achieving an even distribution of that species between wealth groups (shown by dotted lines)

Participation rates and sample sizes

The number of members per recruited household ranged from one to 19, including staff members and unrelated household residents. However, full participation by every member was only achieved in 20 of the 99 households. Composition of the household varied by wealth group (Fig. 2), with households at the lower end of the wealth-scale having more children (median = 2, compared to median 1 child in wealth groups 1 and 4, and median 0 children in wealth groups 2 and 3).

Households in the top two wealth categories had more staff (median eight and two staff, for wealth groups 1 and 2, respectively), whereas the median number of staff members for all other wealth categories was 0. The number of other samples collected also varied considerably between households, depending on the number and number of types of livestock kept, the availability of wildlife (which varied geographically) and animal-source food types in the household.

A total of 2,351 samples were collected, but recovery rates of *E. coli* varied by sample type: fewer than 80% of samples from rabbits, guinea pigs, rodents, bats, eggs and dairy products showed bacterial growth on EMBA. Wild birds, meat, tank water and kitchen surface samples also had growth rates of less than 80%, but in addition, more than 5% of the isolates that did grow from these samples were deemed not to be *E. coli* by biochemical testing. In contrast, of the samples collected from humans, other livestock and the outdoor environment, 95% of samples collected yielded *E. coli* isolates that were confirmed with biochemical testing and sent for sequencing. Although not all sequence data is available at the time of writing, from the first 961 isolates sent, 936 (97.4%) have been mapped to a known *E. coli* genome, whilst sequence data from the remaining 25 isolates has classified them as coliforms belonging to other Enterobacteriaceae, mixed samples or unknown species. Due to a combination of these factors, the number of *E coli* sequences per household varies from two to 38.

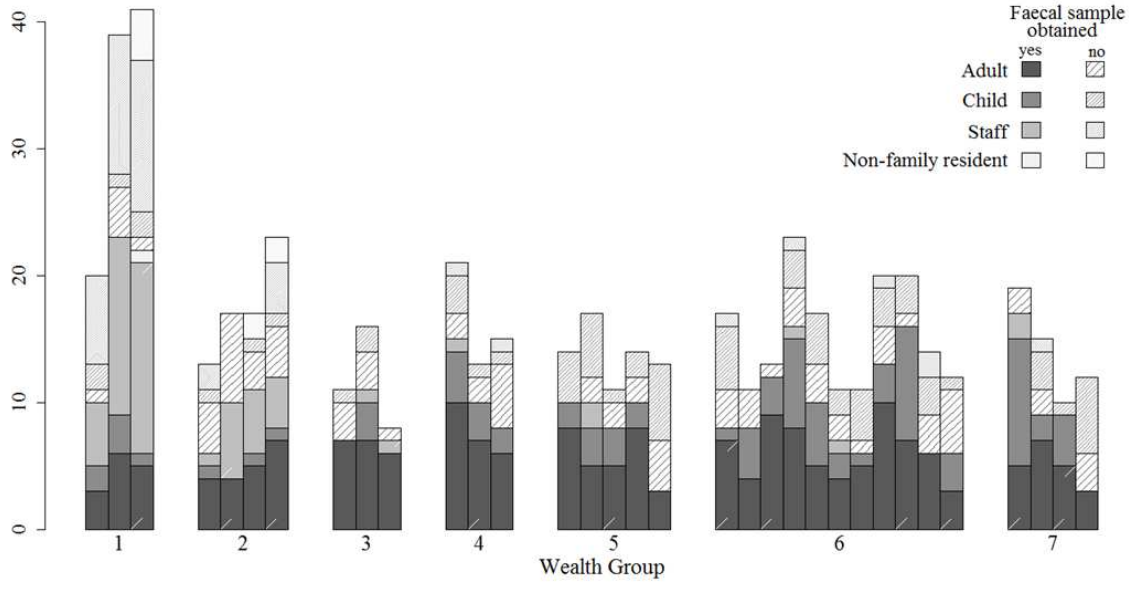

Fig. 2 Barplot of humans in recruited households in the 33 sub-locations, showing number of participants providing faecal samples for the study

DISCUSSION

This is believed to be one of the few studies so far to combine epidemiological sampling with phylogenetic analyses based on whole genome sequencing of microbes. Landscape genetics studies often rely on convenience samples, and are frequently exploratory in nature (Storfer et al., 2010); by contrast this study has generated a unique, carefully stratified data-set to test hypotheses around urban livestock-keeping and socio-economic status as risk factors for exposure to zoonoses. Purposive sampling was required in order to ensure inclusion of a diversity of livestock and socio-economic status in the households, the number of which was necessarily limited by resources. However, random sampling techniques (spatial point selection, random selection of animals within households and random selection of bacterial isolates) were introduced wherever possible. Together with the wealth of data on potential risk factors collected from each household, this should reduce the risks of selection bias, uncontrolled confounding, clustering, and failure to identify effect modifiers for stratified data – common problems in molecular epidemiology studies (Field et al., 2014).

It was found during the course of the study that it was not possible to recruit the desired triplet of large livestock keeper, small livestock keeper and non-livestock keeper in every sub-location. Certain neighbourhood types (particularly the new-build and middle class neighbourhoods) had local byelaws in place, prohibiting the keeping of large livestock. Whilst using local officials to recruit households was essential to secure the confidence of participants and maintain good relations in many sub-locations, it was believed to have also deterred a small number of potential participants, who may have been keeping livestock illegally. Pigs, in particular, were restricted to the low-income housing types and, like cattle and sheep, highly clustered within certain neighbourhood types. Goats, poultry and small stock were more widely distributed, yet even here, there were observable differences that may influence pathogen transmission. For example, scavenging indigenous chickens were predominant, but some households kept large flocks of exotic layer or broiler types – also restricted to both the wealthiest and the poorest sub-locations. This co-variance of livestock type and wealth group categories must be considered during analyses. The use of an alternative measure, such as a wealth index (Rutstein and Johnson, 2004) that could be derived from the household data collected, correlates with wealth group and may be a useful alternative. This could also remove the problem of how to define the households that fell outside the target neighbourhood typology for the wealthier sub-locations, where housing types were generally less uniform and identifying livestock keepers was more challenging.

Although faecal samples were collected from people not present in the household during the visit (such as school-age children), there were other unanticipated issues with sample collection. People in poor areas tend only to buy meat late in the day, just prior to cooking, as they have no cold storage, hence most meat samples came from wealthier households. Unusual livestock species, such as guinea pigs and pigeons, were limited to only two or three households. The laboratory protocols, using two rounds of selective media plus biochemical testing, yielded a very high proportion of usable sequences for the target organism; however, the above factors together with variation in isolation rates has meant that some sources are under-represented in the final dataset. Phylogenetic analyses are necessarily carried out on data that lack independence due to their relatedness, but while control for genetic relatedness is standard, statistical problems such as intra-cluster variability and small sample sizes are often ignored (Garamszegi and Møller, 2010). Despite efforts to balance the number of samples per household, the very small number of data points obtained from some households must be taken into account during the analysis as a potential source of bias, and will reduce

the power to detect diversity and phylogenetic relatedness of bacteria in some settings. Representing the city has proved complex, as without knowing the genetic diversity in advance, an effective sample size calculation is impossible (Bartoszek, 2016), and therefore much care will be required when interpreting comparisons between the genetic data.

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DEVELOPMENT OF STANDARDISED METHODOLOGIES TO INFORM THE DESIGN AND EVALUATION OF CONTROL PROGRAMMES FOR ANIMAL AFRICAN TRYPANOSOMIASIS

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SUMMARY

The work demonstrates methodologies to measure the impact of Animal African Trypanosomiasis (AAT). The net value of cattle production of two study areas in Cameroon and Zambia were modelled for scenarios 'with' and 'without' AAT, and monetary differences were calculated over a 10-year period. The outputs from these models can be used as inputs for economic evaluations to ensure public resources for AAT control are being utilised effectively, aid the design of future control programmes and prioritise areas for resource allocation. This toolset is desperately needed for AAT, which is one of the biggest constraints to livestock production in sub-Saharan Africa.

INTRODUCTION

Animal African Trypanosomiasis (AAT) is one of the most important disease constraints to livestock production on the African continent (Swallow, 2000). The impact (estimated at billions of USD annually) are such that AAT influences where people settle and the intensity and diversity of both crop and livestock industries (Ilemobade, 2009). The disease is vector-borne, transmitted primarily by tsetse flies and caused by a parasite protozoan (*Trypanosoma* spp.). Cases are usually chronic with intermittent fever, anaemia, lymphadenopathy and lethargy, leading to weight loss and reductions in fertility and milk production; acute disease and fatalities can also occur (CFSPH, 2009). The epidemiology of AAT is complex with several species of host, *Trypanosoma* and tsetse flies potentially involved in the transmission cycle. The impact of disease can be reduced by trypanocide application and the introduction of trypanotolerant cattle breeds. However, there is increasing resistance to trypanocides and trypanotolerant breeds are considered less productive by some communities (Sinyangwe et al., 2004; Mamoudou, 2007). Therefore, reduction in exposure is largely reliant on control of the tsetse vector by methods such as insecticide treatment of cattle (ITC), traps or targets, ground or aerial insecticide spraying or changes in livestock management. The Pan-African tsetse and trypanosome eradication campaign (PATTEC) was established in the year 2000 and has set tsetse eradication as its goal. Although this goal presents a considerable challenge that would require extensive resources and there is debate as to its feasibility, it has renewed interest in AAT research and tsetse control.

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Decision-making regarding AAT control should be informed by several components considering the disease epidemiology, ecology, economics, infrastructure and socio-political context (Shaw, 2009). The numerous control programmes for the disease, covering different ecological areas, are a wealth of information for the design of future studies. However, in a systematic review of control operations in five sub-Saharan African countries it was concluded that the control operations are rarely submitted to full evaluation nor their details made available to those outside the implementing institution (Meyer et al., 2016). In addition, only one of the studies identified included a full economic evaluation (Putt et al., 1988). Assessments predicting the efficiency of resource allocation under different control scenarios using techniques such as cost-benefit analyses would be highly valuable in the planning of future programmes. In addition, these analyses can be used to prioritise areas where control of the disease is likely to yield the greatest benefits.

In order to perform economic analyses, an estimate of the baseline impact of the disease and the likely impact of proposed control strategies is needed. The disease and its control are highly complex and contentious, with numerous variables influencing the processes and outcomes from control programmes. For example, estimates of the impact of AAT on different parameters vary significantly in the scientific literature, depending on the type of study conducted, livestock management, species of tsetse and trypanosome (T&T) present and breeds kept (CFSPH, 2009). The aim of this study was to develop a framework that can be used to compare the net value of cattle production in different settings and under different AAT control scenarios. This framework was then applied to Mambwe district in the Eastern Province of Zambia and Faro et Déo district in the Adamaoua Region of Cameroon to estimate the current net value of cattle in the study area and to predict the potential changes under AAT suppression. This is the first stage of this assessment and these results will then be used as inputs into a cost-benefit analysis to predict the benefits arising from different possible control strategies for the disease.

MATERIALS AND METHODS

Study areas

Mambwe district is located in a game management area in the Eastern Province of Zambia and around three-quarters of this district is tsetse infested. Although the savannah fly *Glossina morsistans morsistans* is the main tsetse species present in the area (Van den Bossche & Staak, 1997), *G. pallidipes* and *G. brevipalpis* are also present. The livestock system is mixed agro-pastoral, all cattle are trypanosensitive zebus and cattle density is low (Table 1). The Faro et Déo district of Cameroon is part of the most important cattle-producing region in the country (Adamaoua region), supplying both local and international markets (Mamoudou et al., 2009). Here, trypanosensitive cattle are reared extensively, with a system of communal herding. *G. m. submortisans*, *G. fuscipes fuscipes* and *G. tachinoides* are the main tsetse species present.

Table 1. Cattle numbers in the study areas

Cattle type	Faro et Déo	Mambwe
Cows	42,059	4,328
Heifers	23,253	1,787
Calves	21,806	2,261
Oxen	17,466	816
Bulls	26,146	209
Young males	36,273	1,411
Total	168,003	10,812
Cattle density	15.2 cattle/km^2	2.4 head/km^2

Over the years, the Cameroonian government has directed significant resources to the control of the disease in this region, and the district is now divided into three zones from south to north. Namely, the plateau (where tsetse populations have been supressed), the buffer zone (used as a barrier to tsetse invasion from the valley to the plateau) and the tsetse-infested valley (Mamoudou et al., 2009). Expansion of T&T control is being considered by the Department of Veterinary Services for both these study areas.

Herd model

An animal-level herd model was developed in order to estimate the baseline impact of AAT in the study area (summarised in Fig. 1 and described in detail below). Six age-sex groups of cattle (calf, heifer, young male, cow, adult ox and adult bull) were defined and the cattle population, production outputs, control costs and AAT incidence and sequelae were simulated on an annual basis. The model was stochastic in order to incorporate variability in cattle type and productivity, as well as uncertainty and variability surrounding estimates of AAT frequency and impact. Model parameters were sourced from longitudinal studies of sedentary herds, experimental infection models, entomological surveys and field-based studies of cattle. In addition, data from official sources (Mission Spéciale d'Eradication des Glossines, MSEG, Cameroon and Department of Veterinary Services, Zambia), the opinion of local experts and a field study comprising interviews with cattle owners in the area were utilised where appropriate (Fauron et al., 2016; Holt et al., 2016a). The model was developed in R version 3.3.0 (R Core Team, 2014) using the packages mc2d, psychometric and msm.

Impact of AAT on baseline productivity: The production of each animal during each year is calculated according to a combination of its age-sex-breed group, its health status regarding AAT and its survival that year. Input values for baseline mortality and offtake rates for each cattle group, calving rate and age at first calving were sourced from the literature. In Mambwe district, the main breed kept was Angoni, whereas in Faro et Déo farmers predominantly kept Fulani and Gudali cattle (Fauron et al., 2016). Therefore, for Faro et Déo, breed was modelled as a Bernoulli distribution with Fulani representing around 85% of the cattle population. Relevant literature was used to estimate the range of values for different production parameters and local experts were consulted to ensure these values were appropriate for the current situation. The estimates were combined in probability distributions based upon the information available. The percentage reduction in milk and meat production, draft power, and fertility in animals affected by AAT were sourced from the literature and

ranges were decided in consultation with local experts. These values were then multiplied by values from the baseline productivity distributions to estimate losses due to AAT. The number of deaths, births and sales in each age-sex category were collected each year to calculate the population size for the following year.

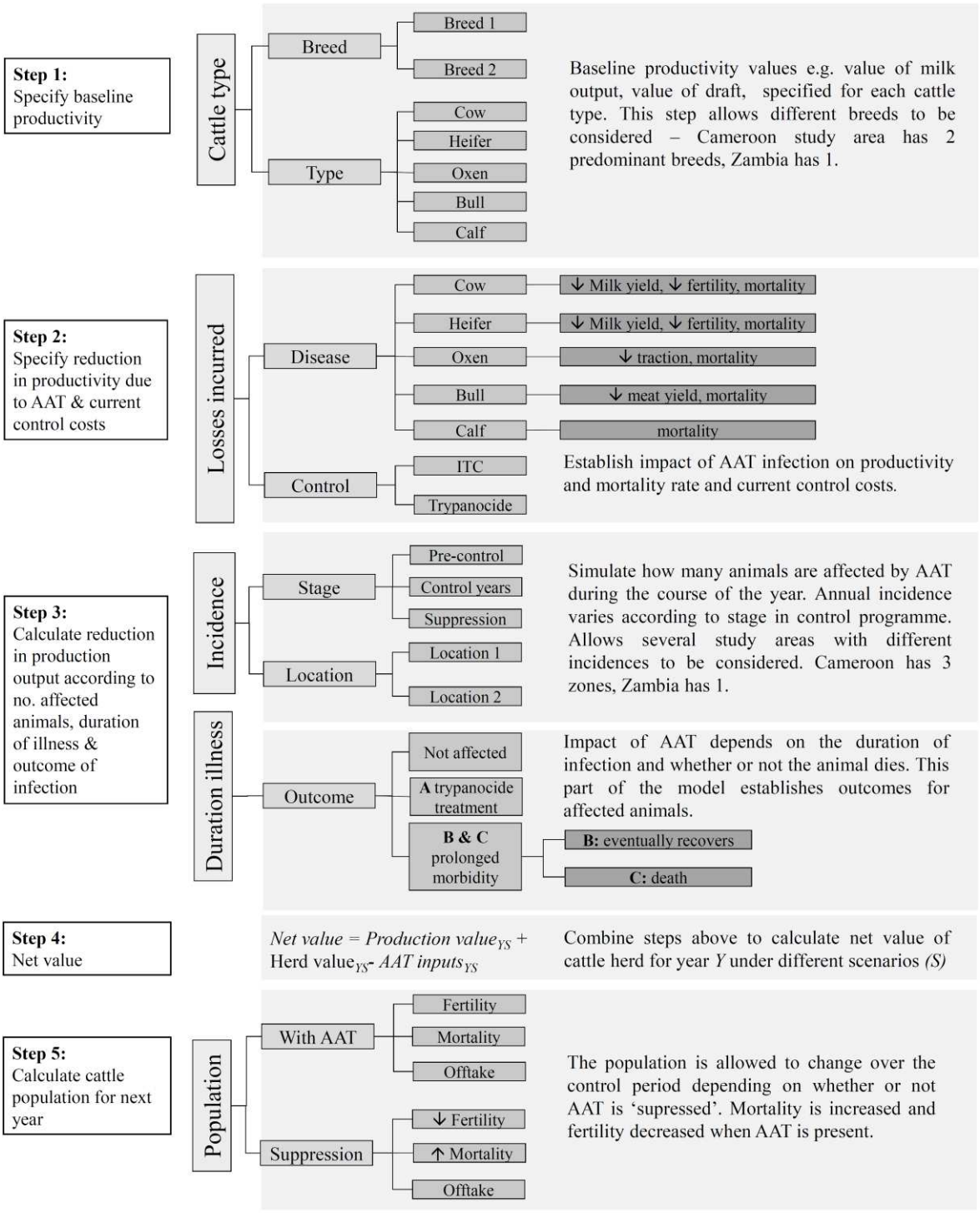


Fig. 1 Overview of the model structure to estimate net value of cattle production in the study areas

Annual incidence and outcomes following infection at district level: For the 'with' AAT scenario (scenario 1), estimates of the annual incidence for Faro et Déo were taken from a study on sentinel and transhumant herds in the study area (Mamoudou, 2007). In Faro et Déo, >90% of cattle herds are transhumant and move from plateau to valley for grazing during the dry season (*personal communication* – F. Omourou), while 2.5% and 7.5% are sedentary in the valley and plateau, respectively. Mamoudou (2007) found that 12 out of 78 seronegative calves seroconverted during their first transhumance, therefore the annual incidence for transhumant cattle was modelled as Beta(s + 1, n – s + 1), where s = 12 and n = 78. Annual incidence for the plateau and the valley were taken from sedentary sentinel herds in this study. During a longitudinal study in the Eastern Province of Zambia, 155 new infections were detected in 85 sentinel cattle over a period of 19 months (Simukoko et al. 2011). The monthly incidence of AAT in cattle was estimated at 6 and 10% in two studies in the area (Van den Bossche, Doran, and Connor 2000; Simukoko et al. 2011). Therefore, annual incidence of AAT in Mambwe district under scenario 1 was modelled using a Uniform distribution, as between 72% and 100%. For scenario 2: 'without' AAT, the annual incidence was considered to be between 1 and 5%, as both study areas have possible tsetse reinvasion sources.

Outcomes following infection in terms of duration of illness and mortality were established to estimate losses due to AAT. Cattle treated with trypanocides typically recover quickly to a state of productivity similar to unaffected cattle. However, treatment failure was reported in both study areas due to poor compliance with dosage, resistance and the purchase of fake or sub-standard drugs (Fauron et al., 2016). Therefore, two outcomes were possible following infection: i) animal given successful treatment and quickly recovers (Group A) or ii) animal considered 'untreated' either because left untreated or due to treatment failure. Murray et al. (1983) showed that on average, an untreated zebu will physically deteriorate for 12 weeks and then slowly start to recover (Group B) or die from a further drop in its packed cell volume (Group C). Mortality was estimated by a distribution based on an experimental field study in a group of untreated calves and adult zebus residing in an endemic area (Dolan, 1998). The probability of treatment success, mortality rate in untreated animals and the duration of reduced productivity for each group were modelled using BetaPert distributions. Whether or not an animal belonged to group A or died (group C) if considered untreated were modelled using Bernoulli distributions. The costs of prophylactic trypanocide drugs was estimated based on four prophylactic doses per year per animal in scenario 1, and zero doses in the absence of AAT. The number of curative doses used for an animal in groups A and B/C was one and two, respectively.

Estimating total net value of cattle in the study areas: The models described were run for 2,000 simulations to estimate the net value of cattle production in both districts for scenarios 'with' (1) and 'without' (2) AAT. For each year (y) of the simulation of a scenario (S), the total value of the herd production P_{YS} (defined by Eq. (1)), inputs related to AAT I_{YS} (defined by Eq. (2)) and the herd value at the end of the year H_{YS} (defined by Eq. (3)) were extracted from the model.

$$P_{YS} = milk_{YS} + meat_{YS} + draft_{YS} \quad (1)$$

$$I_{YS} = pre\_tryp_{YS} + cur\_tryp_{YS} + itc_{YS} \quad (2)$$

$$H_{YS} = \sum_y cattle_i * price_i \; I \quad (3)$$

$$\text{Net value} = P_{YS} - I_{YS} + H_{YS} \quad (4)$$

These values were combined additively to estimate the net value of cattle for the study area in that year (defined by Eq. (4)). Finally, the total net value of cattle was compared with a scenario where AAT is eliminated.

RESULTS

The current annual net value of cattle in the study areas was estimated to be 110.3 and 8.2 mil USD or 661.9 and 758.4 USD per head of cattle in Faro et Déo and Mambwe, respectively (Table 2). The majority of production revenue was estimated to come from draft power in Faro et Déo, while the contribution of draft and milk outputs to the total revenue were similar in Mambwe. The Faro et Déo study area had a much higher production output due to a larger population size. However, the market prices for livestock products are currently higher in Zambia. Annual total spending on AAT control is currently estimated at 813,298 USD in Faro et Déo and 145,376 USD in Mambwe. The majority of this amount is linked to the purchase of curative trypanocides. Based on our assumptions, it was estimated that around 3 and 6% of the total herd are lost to AAT annually in Faro et Déo and Mambwe, respectively.

Table 2. Median production outputs (*P*: milk, milk, meat & draft), inputs (*I*: trypanocides & ITC) and herd value (*H*) from cattle production in the study areas for year 1, scenario1: 'with' AAT. Median values are given with 5th and 95th percentiles

	Faro et Déo x10^6	Mambwe x10^6
Milk (kg)	16.2 [16.0;16.5]	2.2 [2.1;2.3]
Milk (USD)	4.9 [3.6;6.2]	2.8 [2.4;1.8]
Meat (kg)	9.4 [8.2;10.7]	2.1 [1.7;2.5]
Meat (USD)	11.3 [8.1;15.2]	0.55 [0.45;0.65]
Draft (hrs)	14.7 [14.2;15.2]	0.63 [0.52;0.75]
Draft (USD)	44.3 [31.7;53.4]	3.1 [2.0;4.4]
Total $P_{1,1}$ (USD)	60.0 [47.6;72.6]	6.4 [5.3;7.8]
Trypanocides (USD)	-0.71 [0.69;0.73]	-0.13 [-0.15;-0.10]
ITC (USD)	-0.26 [0.22;0.30]	-0.02 [-0.02;-0.01]
Total $I_{1,1}$ (USD)	-0.81 [0.69;0.93]	-0.15 [-0.17;-0.11]
AAT deaths	4964 [4136;5957][a]	640 [272;1,218][a]
Total $H_{1,1}$ (USD)	51.3 [41.8;60.5]	1.9 [1.8;2.1]
Net value (USD)	110.3 [94.9;126.3]	8.2 [7.0;9.5]

[a]All costs are in millions of USD except AAT deaths, which are absolute values

Potential benefits arising from AAT elimination

According to the analysis, elimination of AAT in Mambwe and Faro et Déo districts could generate additional annual benefits of up to 5.6 (90% credible interval (CI): 5.5- 5.6) and 44.0 (90% CI: 35.6-58) million USD per year by the end of year 10 (Table 3). This figure accounts for increased milk and meat production, calving rate and draught power, as well as reduced mortality, morbidity and veterinary drug expenses. The value of differences in meat production is much lower than other parameters, as the model assumes that around a third of the cattle with AAT have some salvage value. Over the 10-year period, the median

cumulative additional production generated by the control of AAT was valued at 20.5 (90% CI: 18.7-22.3) mil USD in Mambwe which is 1,893 (90% CI: 1,734-2,062) USD per initial head of cattle. For Faro et Déo, this was estimated to reach 80.3 (90% CI: 69.6-89.1) mil USD or 418 (90% CI: 415-530) per initial head of cattle; these values are undiscounted.

Table 3. Possible additional benefits on cattle production arising from AAT elimination in Faro et Déo and Mambwe estimated over 10 years. Median values are given with 5th and 95th percentiles, all costs are in millions of USD

	Faro et Déo		Mambwe	
Parameter	Difference (percentile)	Value of difference ($x10^6$)	Difference (percentile)	Value of difference ($x10^6$)
Milk (T)	3,021 [2,940;3,413]	10.7 [10.5;10.7]	1,754 [1502;1990]	2.2 [2.1;2.2])
Meat (T)	1,044 [710;1,203]	1.4 [1.3;2.0]	40 [14;53]	0.25 [0.24;0.27]
Draft (hrs)	4.3 [3.9;20.2]	14.5 [8.8;15.9]	3.5 [3.0;3.6] $x10^5$	1.8 [1.1;2.4]
Total $P_{1,1}$		18.2 [12.5;21.1]		4.4 [4.0;4.6]
Total $I_{1,1}$		-1.2 [-1.4;-1.0]		-0.29 [-0.22;-0.35]
Total $H_{1,1}$		26.1 [14.6;37.5]		0.95 [0.77;1.1]
Difference in net-value		44.0 [35.6;58.3]		5.6 [5.5;5.6]

DISCUSSION

This study estimated that suppression of AAT (defined as annual incidence between 1 and 5%) could generate an additional production over a 10-year period valued at 80.3 and 20.5 mil USD in Faro et Déo and Mambwe, respectively. In a survey of cattle-owning households across 17 study areas in five AAT-affected countries, which is linked to this project, communities in Cameroon were estimated to have the highest vulnerability to AAT (Holt et al., 2016b). This was due to the high importance of cattle in this setting, the large trypanosensitive herds practising transhumance, the reportedly high occurrence of treatment failure, constant AAT challenge and a lack of tsetse control in some areas (Holt et al., 2016b). Despite this, estimated cattle density here is 15.2 trypanosensitive cattle/km^2, which is closer to average cattle-densities in tsetse-free sub-Saharan African regions (Kristjanson et al., 1999). Therefore, increases in production following suppression of AAT were expected to be high. The game management areas of Mambwe contain an abundance of wildlife hosts and vegetation that support tsetse populations, therefore the high burden of AAT has led to a very low density of cattle in the area. The model estimates that a growth in cattle population following AAT removal due to reduced mortality and increased fertility could increase herd value by up to 953,000 (90% CI: 231,000-1,606,000) USD per year and increase production by up to 5.3 million USD per year. However, the benefits may be higher if restocking from outside the district occurred; this is not included in the current model.

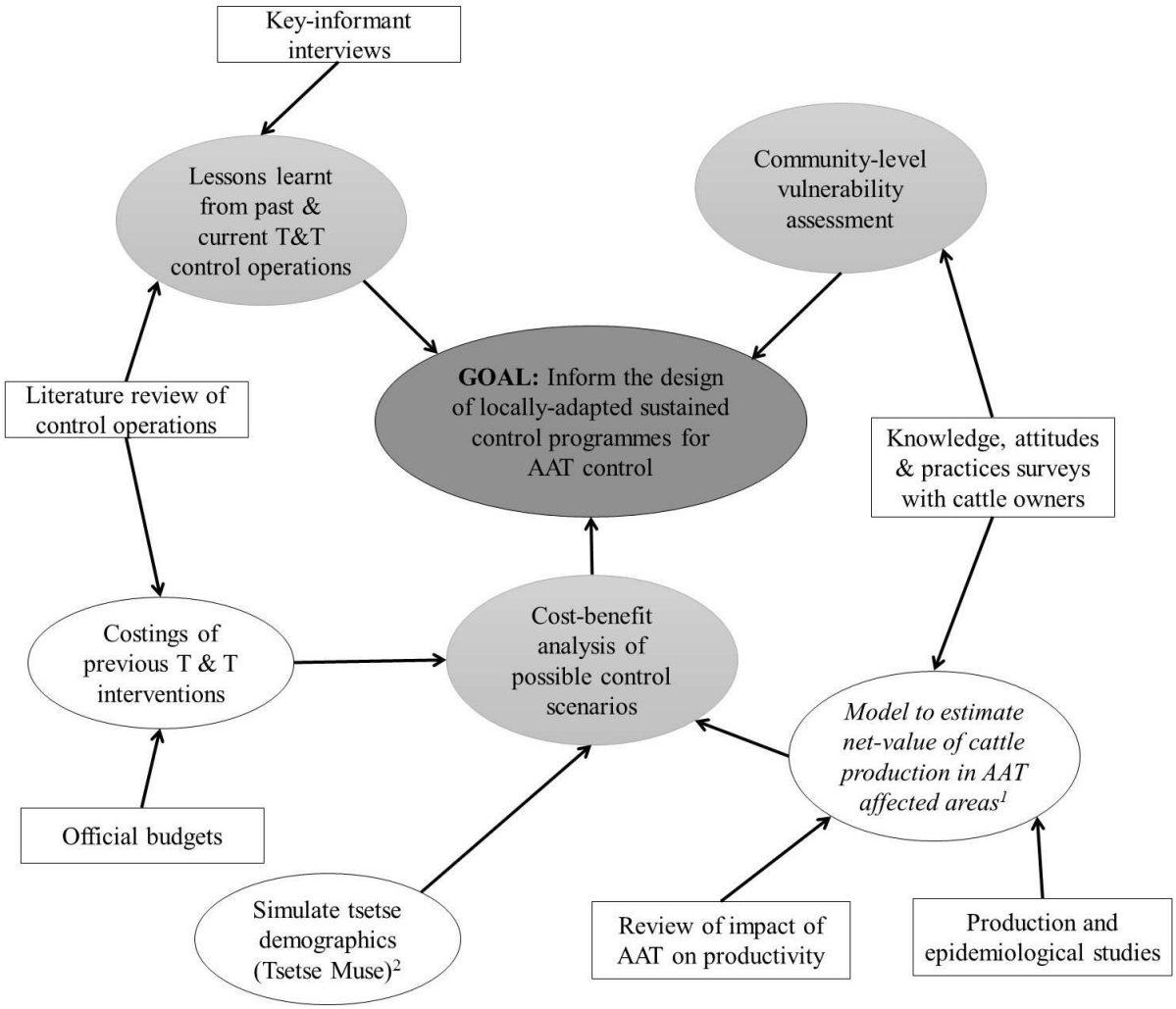
In 2001, it was estimated that only 1.3% of tsetse infested areas had active T&T control programmes (Allsopp, 2001). Therefore, decision makers need information to target locations where control programmes are most likely to yield the most benefits. It is envisioned that this model can be adapted to different geographic areas and used as a component of priority-setting advice. They can also be used to justify public spending on AAT over other diseases. The impact that AAT has on a community is a result of complex interactions between

environmental, political, socio-cultural, entomological and livestock management factors (Shaw, 2003). Although not all impacts could be included, this model was parameterised from a comprehensive review of existing literature, interviews with farmers in the study area, expert elicitation and reports and budgets from official sources. Therefore, in order to reduce model assumptions and uncertainty, parameters were triangulated from several sources where possible. Both countries have reported human African trypanosomiasis (Simarro et al., 2011), therefore including the impact on the public health sector might add value to future work.

Farmers are heavily reliant on chemotherapy and through field surveys conducted as part of this project, trypanocide use was found to be ubiquitous and treatment failure was increasingly reported (Fauron et al., 2016). In both study areas, draft power provision was greatly reduced due to AAT, indicating the disease threatens both the sustainability of cattle and crop production in the area. If control programmes are not expanded, then alternative management strategies are needed to reduce the risk of AAT. Farmers in Faro et Déo already manage the disease by only entering the valley region during the dry season where AAT risk is at its lowest. Some farmers in West Africa manage the disease by using trypanotolerant breeds such as N'Dama or Baoule (Agyemang, 2005); similarly, the introduction of trypanotolerant breeds may be of benefit in these areas. However, communities in Faro et Déo have a strong cultural preference for the traditional Fulani cattle, and trypanotolerant breeds are considered to have reduced traction, which is one of the main uses of cattle in these areas. However, the latter may be offset in areas with high morbidity and mortalities in trypanosensitive draft animals (Van der Waaij et al., 2003), or by the use of crossbreeds with some trypanotolerance.

Despite the vast potential benefits, these estimates do not account for the costs of suppressing AAT, and further data are needed to inform the selection of control methods to maximise the benefits obtained from resources invested. In addition, the estimates do not consider whether suppression is feasible or how long it would take to achieve. A systematic review of control operations in Burkina Faso, Cameroon, Ethiopia, Uganda and Zambia has been conducted in parallel to this study. The results indicate that past AAT control operations were often successful in the short term. However, sustainability was a central issue for most control operations that targeted non-isolated tsetse populations, leading to a permanent reinvasion pressure. This has been attributed to a loss of motivation to maintain control efforts once AAT levels were low and livestock owners perceived the disease as less important. Furthermore, the limitation of community engagement in the development of AAT control polices and the reliance on external funding seem to prevent the appropriation of control by the beneficiaries. Although this analysis did not assume tsetse elimination would be achieved, sustained tsetse suppression was modelled, and successful examples of this are rare, particularly in non-isolated populations (Meyer et al., 2016).

Indeed, previous control campaigns in Cameroon appeared to successfully clear areas that subsequently suffered from re-invasion or resurgence of tsetse following cessation of control activities (Mamoudou et al., 2009b). Therefore, a systems approach to AAT is needed to understand how communities are affected by the disease and inform the design of locally-adapted sustainable control programmes. Figure 2 shows how this study fits into the larger project, which aims to tackle some of these issues.



[1]Current study is presented italicised,
[2]Tsetse Muse is an existing model available from http://www.tsetse.org/

Fig. 2 Overview of the model structure to estimate net value of cattle production in the study areas

In the final stages of this project, simulations of the net value of cattle production under different control scenarios are being combined with a model to estimate the spatial and temporal distribution of tsetse under different control scenarios (implemented in Tsetse Muse: http://www.tsetse.org/) and data on costings of possible control scenarios to perform cost-benefit analysis (CBA) of T&T control in the different study areas. This is being undertaken in collaboration with district officials in order to inform AAT control in the study areas. It is envisioned that the results from this project will be useful for the evaluation of existing – and design of future – control programmes, ensuring that they have tangible benefits in the communities they are targeting.

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AN APPROACH TO ASSESS HABITAT SUITABILITY FOR VECTOR-BORNE DISEASES: A CASE STUDY OF *ANGIOSTRONGYLUS VASORUM* IN THE UK

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SUMMARY

The potential use of boosted regression tree modelling in predicting habitat suitability for vector-borne diseases is illustrated using *Angiostrongylus vasorum* in the UK as a case study. A predictive risk map was developed by utilising a combination of environmental, host and climatic factors associated with disease presence and survival. This map can be used to communicate the relative risk of the disease in different areas and to target regions for disease control.

INTRODUCTION

There is concern that changes in climatic conditions in recent years have resulted in concurrent changes in habitat suitability for the vectors responsible for disease transmission. Ultimately, this has led to variation in patterns of disease distribution and has increased the risk of diseases in areas where infection was previously absent or at low risk (Balogun et al., 2016).

Remote sensing and satellite imaging data are now relatively easy to obtain, and can be used to develop individual spatial layers describing a range of environmental and climatic factors which may impact vector survival.

Boosted regression tree (BRT) modelling is a regression method that has been used extensively in ecology to identify geographical suitability for species survival, taking into account environmental and climatic factors. BRT combines two algorithms: regression trees (models that relate a response to their predictors by recursive binary splits) and boosting (an adaptive method for combining many simple models to improve predictive performance). It includes a stochastic component that allows the variance in the final model to be reduced by using only a random subset of data to fit each new tree (De'Ath & Fabricius, 2000; Elith et al., 2008). It is particularly suited to modelling spatial data as it does not require observations to be independent and is unaffected by variations in data coverage that may arise as a result of sampling bias.

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Using remote sensing data as input variables, BRT modelling can be used to predict the habitat suitability of vector-borne diseases and to estimate the relative contribution of each variable to the final estimate. A case study of *Angiostrongylus vasorum* in the UK is presented in order to illustrate the methodology. *A. vasorum* is a parasitic nematode that can result in cardiopulmonary disease, neurological signs and/or coagulopathies in dogs following the ingestion of infected slugs or snails. It was first reported in southern England in 1980 (Simpson & Neal, 1982) and recent studies have confirmed its spread beyond historical UK endemic foci (Kirk et al., 2014; Helm et al., 2015).

MATERIALS AND METHODS

A comprehensive literature review was conducted to identify potential factors associated with the presence, development and survival of *A. vasorum*. Factors identified were grouped into three categories: environmental factors, climatic factors and definitive hosts (dogs and foxes). Potential sources for UK data describing these factors were identified.

Environmental factors

Three environmental factors were considered: land cover, the normalised difference vegetation index (NDVI) and the enhanced vegetation index (EVI).

Land cover: The Land Cover Map (LCM2007) raster layer was used. LCM2007 is a parcel-based thematic classification of satellite image data covering the entire UK, and is derived from a computer classification of satellite scenes obtained mainly from Landsat, IRS and SPOT sensors. It contains 26 land subclasses, of which 23 were available describing the UK (Table 1).

The normalized difference vegetation index (NDVI) and enhanced vegetation index (EVI) are the two most frequently used vegetation indices. These are calculated using different algorithms that assess whether or not the target being observed contains green vegetation. Data were processed using ERDAS Imagine 9.2 software to extract the NDVI and EVI values for the UK. Seasonal means were estimated using values from each season from 2000 to 2010, inclusive.

Climatic factors

Two indexes were considered: an Index of soil wetness (Mt index) and a Water budget-based forecast index (Wb-bs forecast index). Algorithms previously developed for similar helminthic diseases were used to estimate climatic indexes, using remote sensing data as inputs.

The Mt index was calculated using Eq. (1) proposed by Ollerenshaw and Rowlands (1959) and data were obtained from the Met office and CGIAR-CSI (Table 2).

$$Mt\ index = \frac{n(R-P+125)}{25} \quad (1)$$

Within Eq. (1), n is the number of rain days, R is rainfall in mm and P is the potential evapotranspiration in mm.

Table 1. Land cover subclasses, percentage of each land subclass in the UK and code used for BRT analysis

Land subclass	Code used for BRT	Percentage in study area
Inland bare ground	C0	0.91
Sea / Estuary	C1	5.37
Water (inland)	C2	0.59
Littoral rock [a]	-	-
Littoral sediment	C4	0.11
Saltmarsh	C5	0.05
Supra-littoral rock [a]	-	-
Supra littoral sediment [a]	-	-
Bog (deep peat)	C8	2.41
Dense dwarf shrub heath	C9	2.75
Open dwarf shrub heath	C10	7.96
Montane habitats	C11	2.27
Broad-leaved / mixed woodland	C12	3.32
Coniferous woodland	C13	5.82
Improved grassland	C14	25.18
Neutral grassland	C15	3.91
Set-aside grassland	C16	0.13
Bracken	C17	0.37
Calcareous grassland	C18	1.91
Acid grassland	C19	5.59
Fen, marsh, swamp	C20	0.04
Arable cereals	C21	9.71
Arable horticulture	C22	15.57
Arable non-rotational	C23	0.13
Suburban / rural developed	C24	4.45
Continuous urban	C25	1.38

[a]Not present in the study area

The Wb-bs forecast index was based on the growing degree day (GDD) concept and surplus water. Wb-bs was calculated using Eq. (2) proposed by Malone and colleagues (1987), and data were obtained from the Met office and CGIAR-CSI (Table 2).

$$Wb - bs = (GDD\ x\ days\ in\ month), if[R - P\ x\ 0.8] > 0, +(GDD\ x\ n) * (R - P), if\ (RP) > 0 \quad (2)$$

Within Eq. (2), R is rainfall in mm, P is the potential evapotranspiration in mm and GDD is the average mean temperature minus the base development temperature for the cycle of most helminths (Malone et al., 1987).

Table 2. Variables, units of each variable and remote data source from which data were obtained

Abbreviation	Variable name	Units	Source	Time period considered
N	Number of rainy days	Days with at least 1 mm rain Raster map 5km grid cell	Met Office	Between 1981 and 2010
R	Rainfall	mm – monthly average Raster map 5km grid cell	Met Office	Between 1981 and 2010
P	Evapo-transpiration	Mm – monthly average Raster map 5km grid cell	CGIAR-CSI	Between 2000 and 2010
Tmin	Minimum Temperature	°C - Raster map 5km grid cell	Met Office	Between 1981 and 2010
Tmax	Maximum Temperature	°C - Raster map 5km grid cell	Met Office	Between 1981 and 2010

Definitive hosts

Canids are the definitive hosts of *A. vasorum* and population densities of dogs and foxes were therefore considered within the analysis. There was no census data available for either population in the UK, so densities were estimated as follows:

- The dog population density per km^2 was estimated using previously reported patterns of dog ownership in urban and rural settings in the UK (Murray et al., 2010) and the census data for the human population (CENSIN 2000). Estimates per km^2 were converted to $5km^2$ using the mean value.

- The fox population density (per $5km^2$) was estimated based on the fox density per geographical land class previously described by Webbon and colleagues (2004). However, land cover from 2007 was used instead of land cover from 1982, and the land class description was used to determine the equivalent category in each dataset.

Individual spatial data layers were developed for each predictor variable. Environmental and climatic factors were grouped by season (spring, summer, autumn and winter) between 2000 and 2010.

Outcome

The outcome measure for our models was obtained from the results of a previous study (Kirk et al., 2014) recording the proportion of veterinary practices that reported seeing at least one confirmed case of *A. vasorum*. The geographic location of practices that returned a completed questionnaire (n=1,419) was transformed into continuous surface data using kriging in order to determine the presence or absence of disease per 5 km^2.

ArcGIS 10.2.2 was used for the generation and manipulation of all spatial data layers.

Risk factors modelling

A BRT modelling method (Elith et al., 2008) was used to (i) identify the variables that most closely described *A. vasorum* distribution and (ii) generate a predictive risk map of potential habitat suitability for the parasite in the UK.

The presence or absence of disease per $5km^2$ was used as the outcome variable, and the environmental factors, climatic factors and definitive hosts were included in the model as predictor variables. Ten-year composites stratified by season were used separately for EVI, NDVI, Wb-bs forecast index and the Mt index. All predictor variables (n=17) were included in the initial BRT model and a backward elimination process was followed. Each time a variable was removed, the cross-validation (CV) deviance was compared with the CV deviance of the initial model until a change >0.04 was observed, at which time the previous model was accepted as the final model. A learning rate (lr) of 0.01 and a tree complexity (tc) of 5 were used.

The relative contribution of each variable in predicting habitat suitability for *A. vasorum* was determined, together with the general trends displayed by each variable and any interactions between the main effects.

Model fitting was implemented using the gbm package (version 1.6-3) in R 2.6.1, together with the cross-validation stage-wise function presented by Elith et al. (2008).

RESULTS

The median number of dogs and foxes per 5 km^2 was 14 and 4 animals, respectively (Table 3). The range of NDVI and EVI values were similar across seasons, with median values higher during spring. In contrast, Wb-bs forecast index values for spring were smaller compared to the other seasons, while the highest median values for Mt index were during summer (Table 3).

Six variables were retained in the final BRT model (Fig. 1). Land cover accounted for 27.5% of the variation in *A. vasorum* suitability, with abandoned or unmaintained grassland having the highest risk of occurrence. Dog density accounted for 18.3% of the variation with risk of infection rising rapidly with increasing dog density, before plateauing above 400 dogs/$5km^2$. Winter NDVI accounted for 15.3% of the variation with the highest probability of occurrence between an NDVI of 4,000 and 8,000.

Table 3. Median, first and third quartiles of predictor variables

Variable[a]	Median (1st quartile – 3rd quartile)
Dogs per 5km^2	14 (4 – 32)
Foxes per 5km^2	4 (3 – 5)
NDVI	
Spring	7162 (6390 – 7591)
Summer	7034 (6254 – 7556)
Autumn	6234 (5573 – 6800)
Winter	5652 (5050 – 6242)
EVI	
Spring	5116 (4058 – 5732)
Summer	4770 (4021 – 5544)
Autumn	3556 (3013 – 4194)
Winter	3221 (2770 – 3761)
Mt index	
Spring	9 (0 – 12)
Summer	40.5 (25 – 59)
Autumn	33 (25 – 43)
Wb-bs index	
Spring	0 (0 – 0)
Summer	60 (0 – 240)
Autumn	387 (260 – 607)

[a] Land cover is not included in this table (see Table 1)

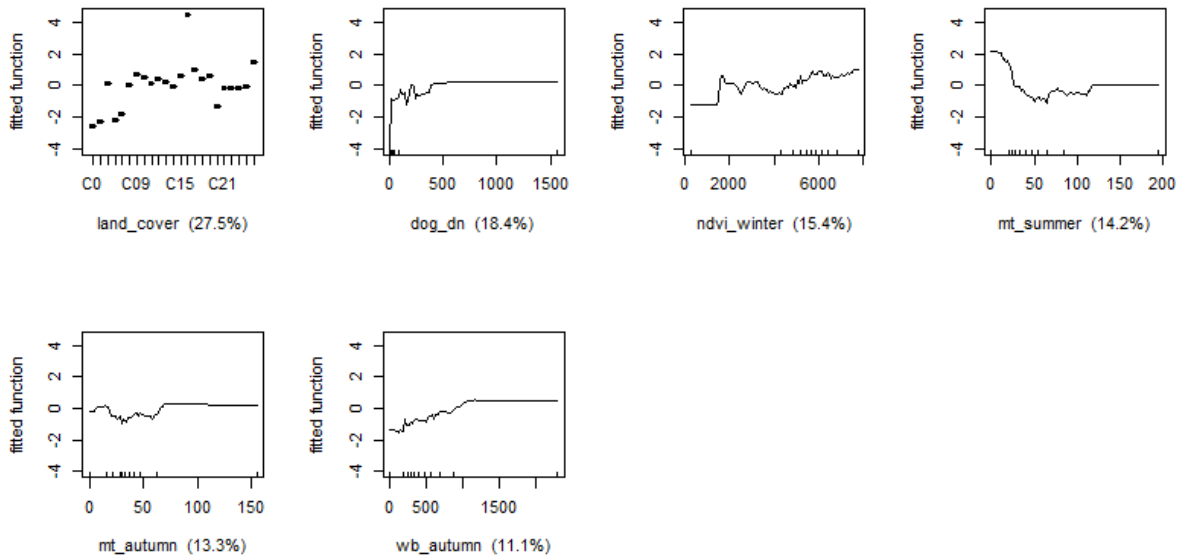

Fig. 1 Response curves for the six variables retained in the final BRT model. Panels (top left to bottom right include: land cover (see Table 1 for codes used in each subclass), dog density (dog_dn), mean NDVI in winter (ndvi_wi), mean Mt index in summer (mt_summer), mean Mt index in autumn (Mt_autumn) and mean Wb-bs in autumn (wb_autumn). Y-axes are on the logit scale. Dashes at inside bottom of plots show distribution of observations, in deciles, across the variables

The predictive risk map for *A. vasorum* distribution shows that a large proportion of the UK is suitable for *A. vasorum*, with a higher suitability in southern and central England (Fig. 2).

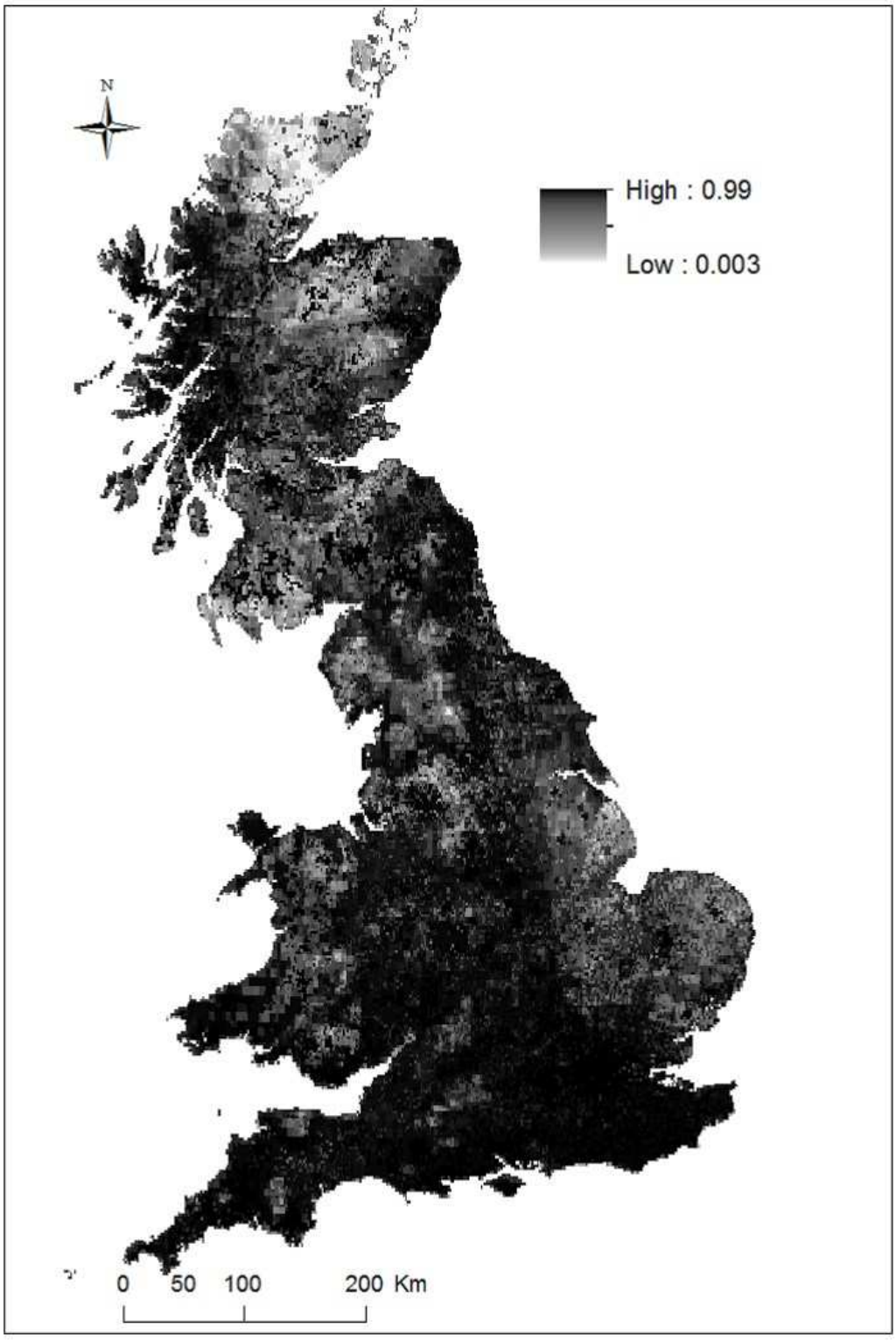

Fig. 2 Predictive risk map of *A. vasorum* distribution in the UK using the six predictor variables retained in the final BRT model

DISCUSSION

In recent years, a number of vector-borne diseases have been reported in areas that were previously assumed to be unsuitable for the survival of the vector (Altizer et al., 2013; Majekodunmi et al., 2013; Yactayo et al., 2016). It is therefore important to develop tools that can be used to provide information for policy makers and clinicians on the relative risk of vector-borne diseases in different areas, so preventive measures can be put in place and resources can be prioritised.

Climate plays a key role in parasite transmission and population diversity and dynamics. The activity of terrestrial gastropods (intermediate hosts) is highly susceptible to temperature, moisture and vegetation, and development of metastrongyloid larvae in the intermediate host is also temperature-dependent (Jenkins et al., 2006). Therefore, changes in climate might influence the spatial distribution of some parasites in certain geographic areas.

In the case of *A. vasorum*, the increasing number of publications since its first description in domestic dogs (in 1854) clearly mirrors the growing interest of canine angiostrongylosis worldwide. Nonetheless, the recent literature mainly focuses on case reports in dogs in areas where infection has not previously been reported (Hurníkova et al., 2013; Jolly et al., 2015; Pantchev et al., 2015; Alho et al., 2016,). The first attempts to understand the habitat suitability for canine angiostrongylosis were performed by Morgan and colleagues (2009) in a study showing the influence of climate as well as vegetation on the disease and predicting a global geographic distribution of *A. vasorum*. More recently, a nationwide distribution of *A. vasorum* in Great Britain confirmed that the lungworm had spread beyond historical endemic foci (Kirk et al., 2014). This study confirms that habitat suitability for *A. vasorum* is widespread in the UK, and identifies high risk areas as well as the main factors accounting for the majority (61%) of the variation in habitat suitability: land cover, dog density and vegetation index for winter.

The maps developed in this study can be used to alert local practitioners to the relative risk of *A. vasorum* infection in their area. The methodological approach presented here has wider applicability beyond this specific case study.

ACKNOWLEDGEMENTS

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METHODS AND MODELLING

MODELLING BOVINE VIRAL DIARRHOEA VIRUS (BVDV) SPREAD BETWEEN DAIRY CATTLE FARMS ON A REGIONAL SCALE: RELATIVE CONTRIBUTION OF TWO BETWEEN-HERD TRANSMISSION PATHWAYS

L. QI*, E. VERGU, B.L. DUTTA AND P. EZANNO

SUMMARY

Between-herd transmission routes such as trade movements (MV) and neighbour contacts (NB) are responsible for the spread of Bovine Viral Diarrhoea Virus (BVDV). The objective of this study was to propose a procedure including modelling and data analyses to evaluate the relative contributions of these transmission routes to virus spread. The model, consisting of within- and between-herd modules, was developed via a novel data-driven mechanistic approach to simulate the spread of BVDV between dairy cattle herds within a region using a comprehensive dataset of cattle life trajectory. Analyses of simulated data showed that NB caused over 75% of all infection events. The average duration of within-herd infections caused by NB tended to be shorter than those caused by MV. The model was sensitive to parameters concerning neighbourhood radius and between-neighbour transmission rate. Both routes should be considered when implementing control strategies to decrease the spread of BVDV on a large scale.

INTRODUCTION

Bovine Viral Diarrhoea Virus (BVDV) is widespread among industrial cattle herds. Infected animals are either transient (TI) or persistent (PI). PI individuals are produced strictly through vertical transmission, and are the main contributors to virus shedding throughout their short lifetimes. TI individuals are occur through horizontal transmission and shed fewer viruses during a limited infectious period (normally less than 2 weeks; Brownlie et al., 1987, Houe, 1999, Lindberg, 2003). An increase in reproductive disorders and calf mortality (Baker, 1987, Carlsson et al., 1989, Lindberg, 2003, Ersbøll et al., 2003, Svensson et al., 2006) and a reduction in milk production incurring significant financial losses (House, 2002, Fourichon et al., 2005, Heuer et al., 2007) were observed in infected herds. It has been shown that BVDV infection within a herd fades out after a few years in a large proportion of the cases after a single virus is introduced (Damman et al., 2015, Viet et al., 2007, Ezanno et al., 2008). However, all infected herds can spread BVDV to other herds, as long as a between-herd "connection" exists. Therefore, on a regional scale, a herd can be infected through several transmission routes and at several time points, which is expected to enhance

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virus persistence at both herd and regional levels. Thus, a better understanding of BVDV spread within a region is crucial when targeting control and eradication.

On a regional scale, infectious pathogens are transported between herds through different pathways, such as displacement of live agents (human, other animals and insects), environmental routes (air, water and soil; Keeling et al., 2008), and trade movements (Heinrichs, 1993, Groenendaal et al., 2004, Gates & Woolhouse, 2014). It is necessary to account for multiple interactions among herds when investigating BVDV spread and persistence in a metapopulation. Some understanding of the main routes of between-herd transmission at regional level has been achieved. Animal trade movements have been recognised as the main route of virus transportation, thereby influencing the persistence and prevalence in infected herds and the size of epidemics (Courcoul et al., 2010). Studies on neighbour contacts showed that the presence of a PI-herd (herd with PI animals) in the neighbourhood increased the risk of other herds in the same neighbourhood becoming PI-herds (Ersbøll et al., 2010). However, in the previous studies, the interactions among herds were overly simplified (Courcoul et al., 2010), or only a single transmission pathway was considered (Ersbøll et al., 2010, Tinsley et al., 2012). In order to fully represent BVDV propagation mechanisms within a region, a more comprehensive study including multiple transmission pathways along with all available resources such as field observations, expert knowledge and theoretical analyses is required.

Additionally, as it has been shown that both BVDV prevalence and persistence at herd level vary with herd-specific characteristics such as herd size (Ezanno et al., 2007), it is also important to account for demographic features for each herd when considering between-herd transmissions.

To study such a complex biological system combining several scales, modelling appears to be a suitable approach. In particular, modelling is complementary to observations when either biological information about the system is not sufficient or observational studies are difficult to perform. In general, modelling interactions on multiple scales and their coupling could be either a top-down or a bottom-up approach, depending on the system involved and objectives in question (Meier-Schellersheim et al., 2009, Qu et al., 2011). Considering the herd-level dynamics as building blocks for a bottom-up approach, the spread of BVDV on a regional scale can be modelled.

The objective of this study was to propose a modelling-based approach to investigate BVDV spread within a heterogeneous bovine metapopulation and to interpret the contributions of different between-herd transmission pathways. A novel stochastic multiscale epidemiological model in discrete time was developed by combining data-driven and mechanistic approaches. Two between-herd transmissions – trade movements (MV) and neighbour contacts (NB) – were considered. The Brittany region located in France was chosen as a case study for simulations and data analyses.

DATA AND METHODS

This section introduces the proposed regional model, the data used, and the corresponding data-mining process.

Description of datasets and data-mining process

Two kinds of data were used in this study. The first were extracted from the French cattle identification database that contains information on individual cattle life trajectories from birth to death in France. In particular, information on cattle movement (date, source and destination herds) was used to connect herds. Since the full life trajectory of cattle is recorded, it was possible to calculate some of the herd-specific parameters such as annual birth and age-based outgoing rates, in order to establish demography for each herd. The Brittany region located in North-Western France, densely populated with dairy cattle herds, was chosen as a case study. Data on cattle life trajectories over 9 years (1st Jan 2015 – 31st Dec 2013) were used. Herds existing over the 9 years of observation and with more than ten adults were included in simulations. A metapopulation consisting of 12,750 herds and trade information on female animals over 9 years was established. The values of two parameters needed for establishing herd-specific demography (yearly birth and outgoing rates) were calculated when creating the metapopulation. The outgoing rate was calculated for each age group as the ratio between the total number of animals leaving the herd by means other than trade and the number of days all animals were present (Beaunée et al., 2015).

The second dataset used in this study concerns the geo-location of French town borders, available on internet, with which random geo-coordinates can be assigned to each farm using R language (package: maptools). Since real coordinates of herds are not available for confidentiality reasons, this random geo-location was used to define the neighbourhood of each herd.

Description of regional model framework

Since a regional farming system includes many interacting heterogeneous herds, within-herd dynamics and between-herd interactions should be specified for all simulated herds in the region. The proposed regional model framework includes data, and within and between-herd simulators, describing the individual herd demographic and infectious dynamics and their interactions (Fig. 1).

Three factors – herd-specific demography, infection process and vertical transmission – were developed as a within-herd simulator representing BVDV spread within a dairy herd (Ezanno et al., 2007). The between-herd simulator includes two routes (MV and NB). The herd-specific demography process and MV are driven by observed data. NB is integrated into the within-herd infectious process, while the quantification of within-herd vertical transmission requires information on animal trade movement to account for movements of R (immune) dams and to calculate the probability of them carrying a PI foetus.

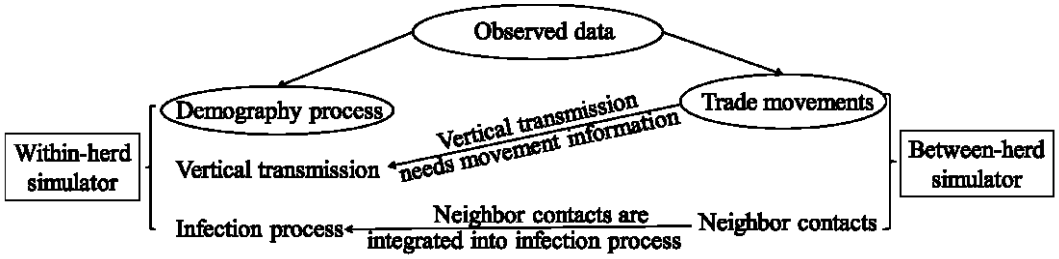

Fig. 1 Conceptual model framework of the spread of BVDV on a regional scale, coupling data, within and between-herd demographic and infection dynamics

Within-herd simulator

The within-herd model describes BVDV spread in a structured dairy cattle herd. It is a discrete-time stochastic compartmental model with a time-step of 2 weeks (Fig. 2).

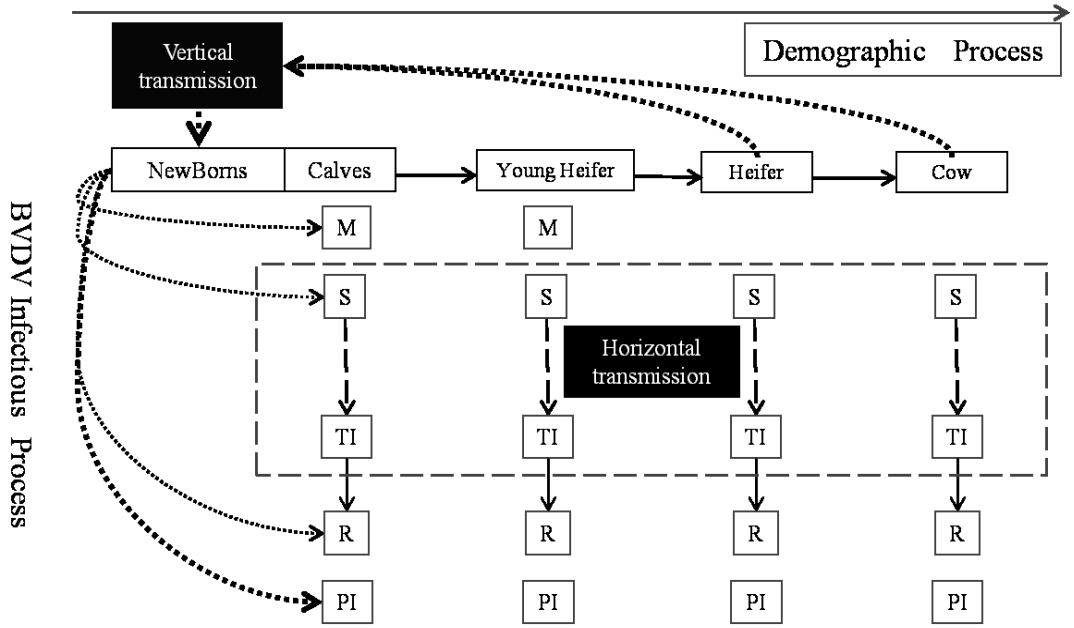

Fig. 2 Simplified view of the within-herd simulator of BVDV spread (see Ezanno et al., 2007 for the full description). Each herd comprises four age groups (calves, young heifers, heifers and cows). The five health states are: M – protected by maternal antibodies; S – susceptible; PI – permanently infected; TI – transiently infected; R – immune. M state only exists in calves and young heifers, and becomes S state before entering the Heifers group. S, PI, TI and R states exist in all age groups

At each time-step, the number of animals (N_g^X) in age group g and health state X, is updated from that in the previous time-step following a Binominal distribution (Eq. (1)),

$$N_g^X(t+1) \sim Bin\left(N_g^X(t), r_g\right) \quad (1)$$

where r_g is the herd-specific outgoing rate of age group g calibrated from observed data.

The infection process consists of horizontal and vertical transmissions. The horizontal transmission makes S become TI, and was modelled by a Binomial distribution with an infection probability encompassing the frequency-dependent transmission (Ezanno et al., 2007). The health status of newborns (M, S, PI, or R) is defined at birth, based on the occurrence of vertical transmission. Newborns from S and PI dams have the same status as their mothers. TI dams give birth to R calves. The challenge was to assign a health status to newborns from R dams, which could be PI, R, or M, depending the gestation period during which the dam was exposed to BVDV. A calibration procedure, described in the following sub-section, was proposed and used in the model to complete the missing information from the database.

Vertical transmission modelling: Vertical transmission is an important factor in BVDV spread as it is the only path to produce PI animals. One method to define vertical transmission is to explicitly model the gestation process based on a probability of

fertilisation, of abortion due to BVDV infections, and of vertical transmission if the dam is infected mid-gestation (Ezanno et al., 2007). As it was not possible to quantify herd-specific infertility probabilities from currently available datasets, a new vertical transmission procedure was proposed, allowing more information to be incorporated into these parameters compared to an assumption of uniform distribution.

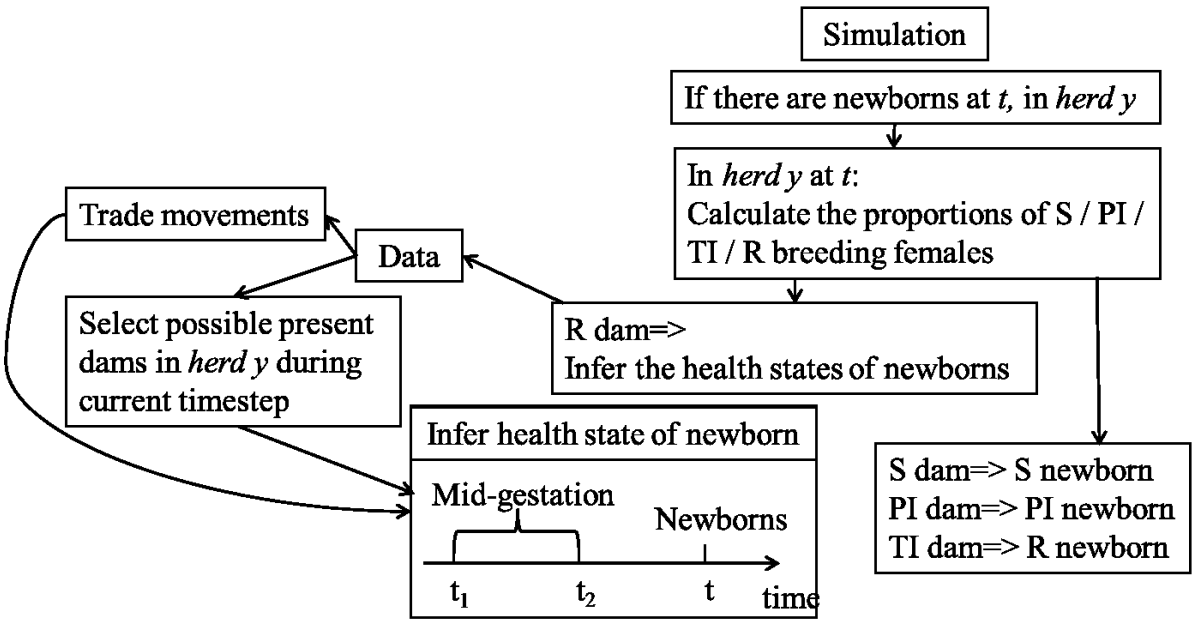

Fig. 3 Proposed estimation procedure for vertical transmission. t is the current time, and t_1 and t_2 show the range of mid-gestation during which vertical transmission might occur. The procedure starts from the "simulation" box. At each time-step, the health status of the dams giving birth in herd y are deduced from the simulated proportions of breeding females per health state. In case of S, PI, and TI, newborns will be S, PI, and R, respectively, whereas for R dams, the health status of newborns should be inferred

As shown in Fig. 3, the number of newborns in *herd y* was calculated at each time step according to the birth rate and the number of dams present in the herd. Then the number of dams giving birth was set equivalent to the number of calculated newborns. The health status of dams was estimated according to the distribution of dams among health states in *herd y*. In case of an R dam giving birth, a random R dam is chosen from the database. The health status of the newborn is then estimated by integrating trade movement information. If the selected R dam was R or TI before mid-gestation (t_1), the newborn is M. If the R dam was S before t_1, the exposure to BVDV should be inferred, accounting for possible trade movements of the dam (i.e. the exact location). If the R dam was exposed after t_2, the newborn is R. If the R dam was exposed between t_1 and t_2, it produces a PI newborn with a fixed probability as previously assumed at within-herd level (Ezanno et al., 2007).

Between-herd simulator

The between-herd simulator extends the model from farm level to a regional metapopulation scale. Two between-herd transmission routes – MV (animal trade movements) and NB (seasonal neighbour contacts) – were included in the model.

MV was used to model purchasing and selling transactions between farms. Such trade behaviour could happen between farms separated by either short or long distances, and it could result in the direct introduction of animals with different BVDV status into the destination herds (Heinrichs, 1993, Gates et al., 2014). Details such as age at movement, date of movement, source and destination herds were included in the model. The missing information concerned the health status of moving animals, which were imputed by using the simulated distributions among health statuses of animals from the same age-groups in the source herds.

In contrast, NB represents direct and indirect interactions between farms within a short distance. Precise modelling of NB would require data on the geo-location of pastures and their use by animals, which are currently not available at the level represented in this model. Therefore, a simple approximation was preferred for representing NB, using only the geo-location of French town borders. Herds from a given town were distributed randomly over space within their town. As shown in Fig. 4, the neighbourhood of one herd was defined within an assumed circle of fixed radius R_{nb}. All herds in the same neighbourhood could have an impact on others.

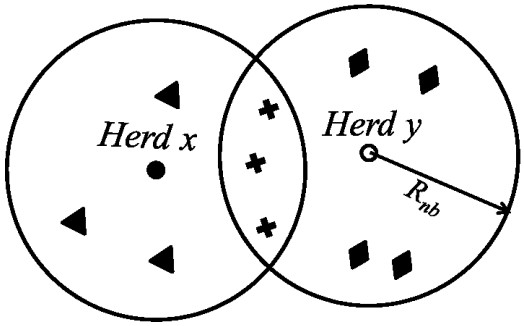

Fig. 4 Definition of neighbourhood. Each symbol (▲, ×, ●, ○ and ♦) represents one herd. The neighbourhood of each herd is assumed to be a circular area with a fixed radius R_{nb}. For instance, the neighbourhood of *Herd x* (●) includes herds ●, ▲ and ×. The neighbourhood of *Herd y* (○) includes herds ○, ♦ and ×

During modelling, NB was integrated in the horizontal transmission process of the within-herd simulator. All simulated herds were assumed to use a similar pasturing season, from mid-March to mid-November. During pasturing season, the probability of infection for age-group g of *herd y* during time interval t to t+1, $prob_{inf}^{herd\,y}(g,t)$, was updated by adding a neighbour impact as follows:

$$prob_{inf}^{herd\,y}(g,t) = 1 - e^{-\left(p_{inf}^{herd\,y}(g,t) + p_{inf}^{nb}(g,t)\right)} \quad (2)$$

where $p_{inf}^{herd\,y}(g,t)$ is the within-herd force of infection for age-group g during time interval t to t+1; $p_{inf}^{nb}(g,t)$ is the force of infection due to NB on age-group g during time interval t to t+1, due to all PI animals pasturing in the neighbourhood of *herd y*. By assuming the animals at pasture mix equally, $p_{inf}^{nb}(g,t)$ was modelled by a scaled mass action (Ögren et al., 2002).

Initial conditions and parameters used in simulations

The initial herd size was defined for each simulated herd according to observed data. On 1st January 2005, one PI cow was introduced into each of the 10% uniformly randomly chosen herds. Details on the parameter values used are given in Table 1. The two parameters related to NB are assumed based on expert opinions.

Table 1. Parameters adopted in simulation

Parameter and definition	Value	Reference
r_g: age-based outgoing rates per 2 weeks	[0,1]	Calculated from data
r_b: yearly birth rate	[0,0.36]	Calculated from data
s_r: sex-ratio	0.5	(Ezanno et al., 2007)
m_P: rate of mortality of PIs per 2 weeks	0.026	(Ezanno et al., 2007)
m_{P0}: probability of mortality of PI calves at birth	0.01	(Ezanno et al., 2007)
β_w^P: within-group transmission rate for PIs per day	0.5	(Moerman et al., 1993)
β_w^T: within-group transmission rate for TIs per day	0.03	(Baker, 1987)
β_b^P: between-group transmission rate for PIs per day	0.1	(Niskanen et al., 2003)
β_b^{nbP}: between-neighbour transmission rate for PIs per day	0.03	Expert opinion
R_{nb}: neighbourhood circle radius (in km)	2	Expert opinion

Simulation scheme

The analyses of simulated data did address BVDV spread on a regional scale and the contribution of the two between-herd transmission routes. For each simulation scenario, results were obtained based on ten runs (which were assumed sufficient to illustrate the stochastic variability within the scenario) over 9 years. Data analyses were carried out at both within- and between-herd level, neglecting initially infected herds.

Firstly, simulations were carried out by varying the inclusion of different between-herd transmission routes (none, each single and both between-herd transmission routes together). The evolution of the regional prevalence (number of infected herds in the metapopulation) over 9 years was calculated for each of the four scenarios, and the contribution of each transmission route was shown through the comparison of obtained results.

Secondly, for the scenario with two between-herd transmission routes, the evolution of the regional incidence (number of newly infected herds in the metapopulation) over 9 years and the cumulated regional incidence were calculated. The contribution of each transmission route was investigated by identifying and counting the number of newly infected herds caused by each route in both bi-weekly and cumulated regional incidences.

Furthermore, to investigate the details of virus spread, all within-herd infection events (where BVDV-free herds were infected) recorded were classified into two groups according to their causal infection route. Five characteristics were selected to compare both groups of infections: (1) the proportion of R animals in the infected herd at the beginning of the infection; (2) the duration of the within-herd infection (time between an infection starting in one herd and the following local fade out); (3) the average number of animals in the infected herd over the duration of the within-herd infection; (4) the cumulative number of new PIs

over the duration of the within-herd infection; (5) the cumulative number of new TIs over the duration of the within-herd infection. The distributions of these five characteristics were calculated to characterise and distinguish the infections of the two groups.

Finally, a parsimonious sensitivity analysis was performed with respect to β_b^{nbP} and R_{nb}. Three values presenting low, mild and high neighbour impact were chosen for each parameter: $\beta_b^{nbP} = 0.01$, 0.03 and 0.1 per day, $R_{nb} = 0.5$ km, 2 km and 6 km. (1) To examine the model sensitivity to β_b^{nbP}, mild R_{nb} (2 km) was adopted and three scenarios were designed, one for each of the three β_b^{nbP} values. (2) To analyse the model sensitivity to R_{nb}, two β_b^{nbP} values (0.03 and 0.1) were chosen and six scenarios were designed with the three R_{nb} values considered. The impact of these parameters on regional prevalence and incidence was then assessed.

RESULTS

Analyses of BVDV spread and the contribution of between-herd transmission routes

Regional prevalence: Both NB and MV increased the regional prevalence. NB led to significant periodic variations of the regional prevalence (Fig. 5).

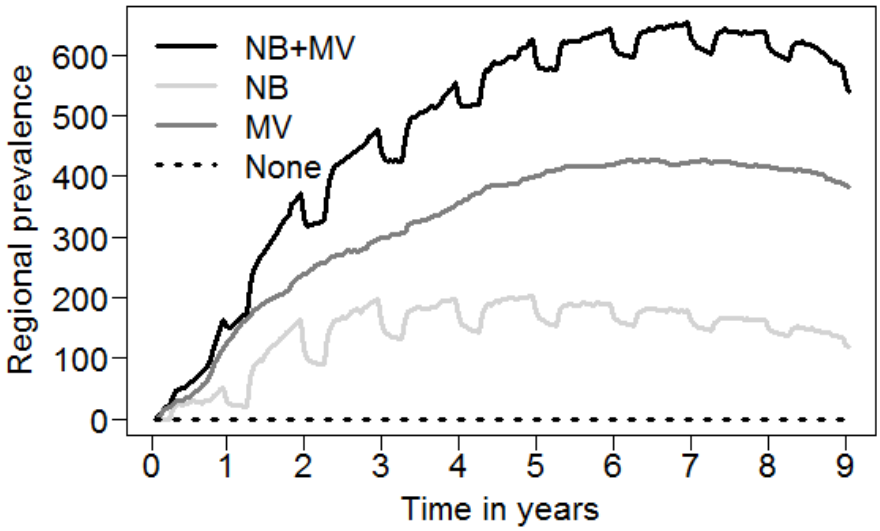

Fig. 5 Average number of infected herds at regional level. Between-herd transmission routes are: trade movements (MV) and neighbour contacts (NB)

Regional incidence: The regional incidence also showed significant periodic variations because of NB. For the cumulative incidence, NB played the major role by producing more than 75% of on average 6,357 new within-herd infections (Fig. 6).

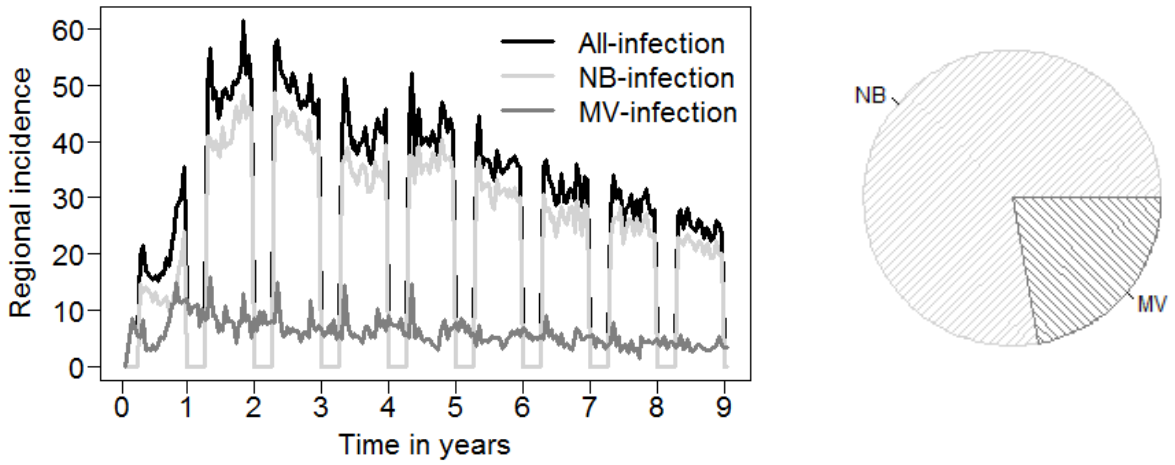

Fig. 6 Lines show the average regional incidence. The pie graph shows the distribution of cumulative incidence over 9 years among the causes of infections

Distributions of five characteristics of infection events: The distributions of five characteristics defined for identifying within-herd infection events due to each of the two transmission routes are shown in Fig. 7. The infections introduced by NB tended to die out faster, led to lower cumulative numbers of PIs and TIs, and started with fewer R animals (Fig. 7).

Sensitivities of regional prevalence and incidence to variations of β_b^{nbP} and R_{nb}

The model was highly sensitive to β_b^{nbP}. Larger β_b^{nbP} led to higher regional prevalence and incidence, and a stronger seasonal periodic effect (Fig. 8). The sensitivity of regional prevalence to R_{nb} is influenced by β_b^{nbP}, and is higher for larger between-neighbour transmission rates (Fig. 9).

DISCUSSION

The study shows that on average, NB contributes to most of the within-herd infection events, while MV induces longer persisting infections. Considering the interactions between the two between-herd transmission routes, herds in the metapopulation are likely to be infected several times, leading to continued BVDV spread at a regional level. Simulation results from the proposed model agree with field observations. In the Brittany region, BVDV infection is assumed to be endemic and even dairy herds acquiring very few animals may be infected (Joly, pers. Comm., 2007). The work from Courcoul et al. (2010) also indicated that BVDV persisted over time with both MV and NB.

The findings provide insights about the possible control strategies that could be applied on a regional scale, aiming to eradicate BVDV. Although testing animals or guaranteeing their virus-free status before trading could contribute to reducing the regional prevalence, it might not be enough to eradicate BVDV in densely populated areas with many contacts at pasture and a long-lasting pasturing season. The efficiency of control strategies needs to be further assessed within the proposed modelling framework.

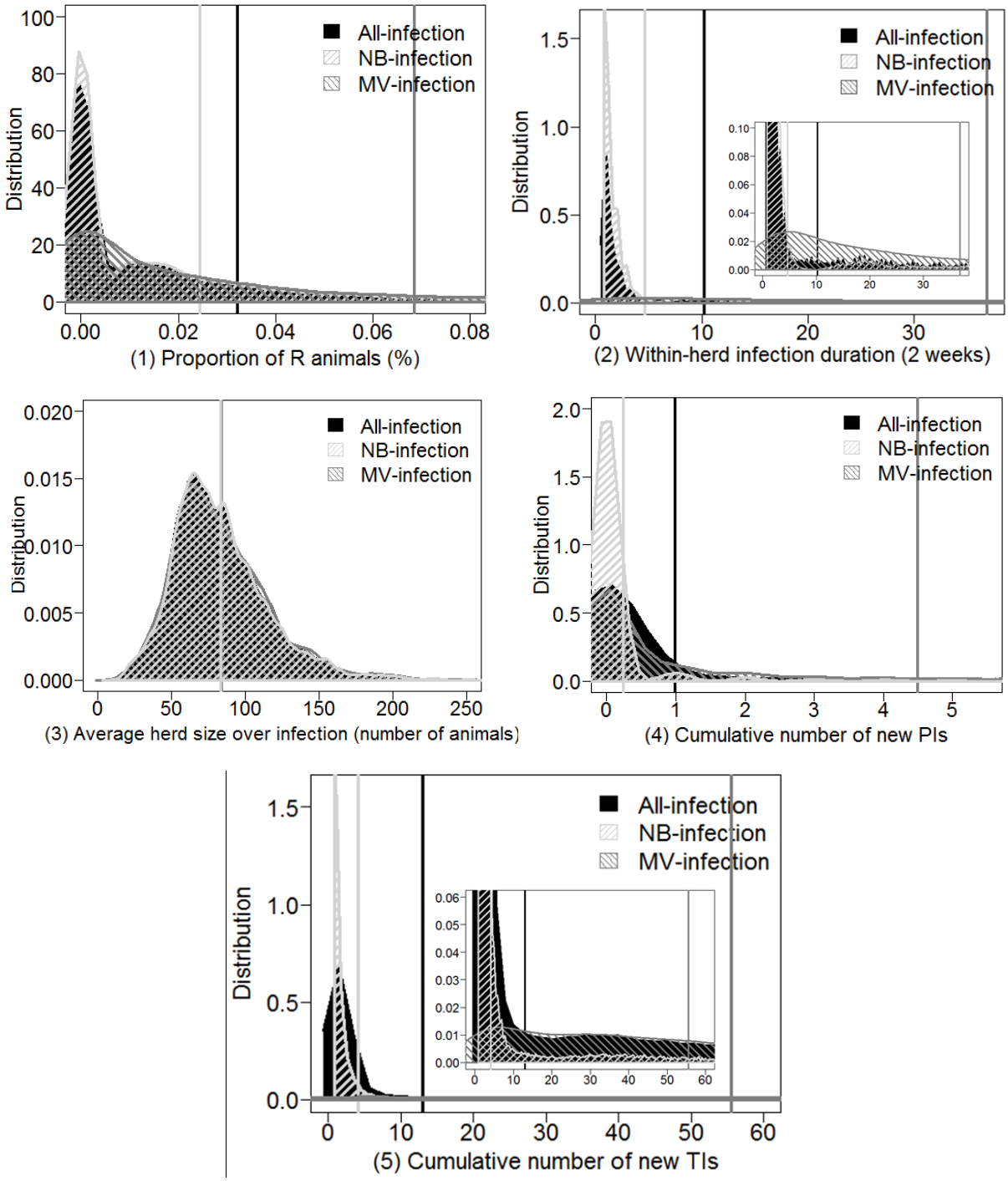

Fig. 7 Distributions of five characteristics of within-herd infection events recorded during simulations: (1) proportion of R animal in the infected herd at the beginning of the infection; (2) duration of the within-herd infection; (3) average number of animals in the infected herd over the duration of the within-herd infection; (4) cumulative number of new PIs over the duration of the within-herd infection; (5) cumulative number of new TIs over the duration of the within-herd infection. The vertical lines are the mean values of the variable

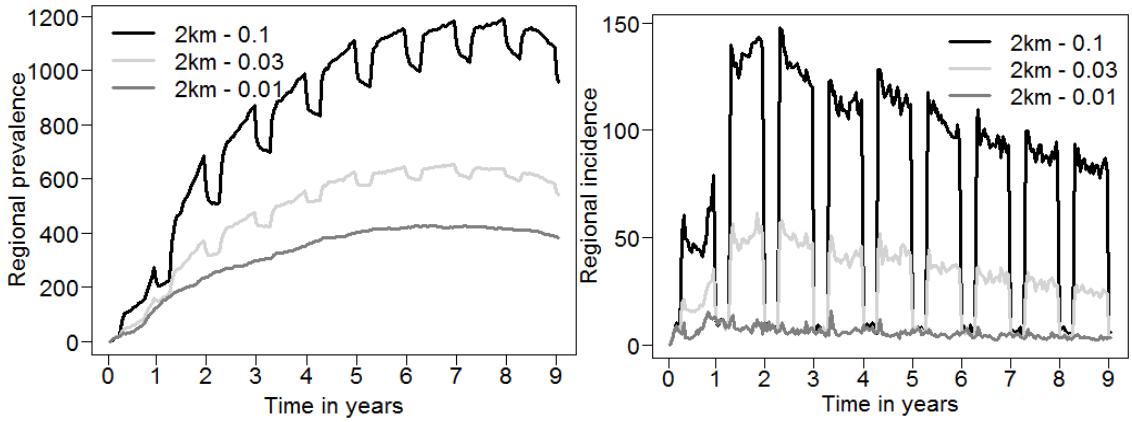

Fig. 8 Regional prevalence and incidence with R_{nb}= 2 km and β_b^{nbP}=0.01, 0.03 and 0.1

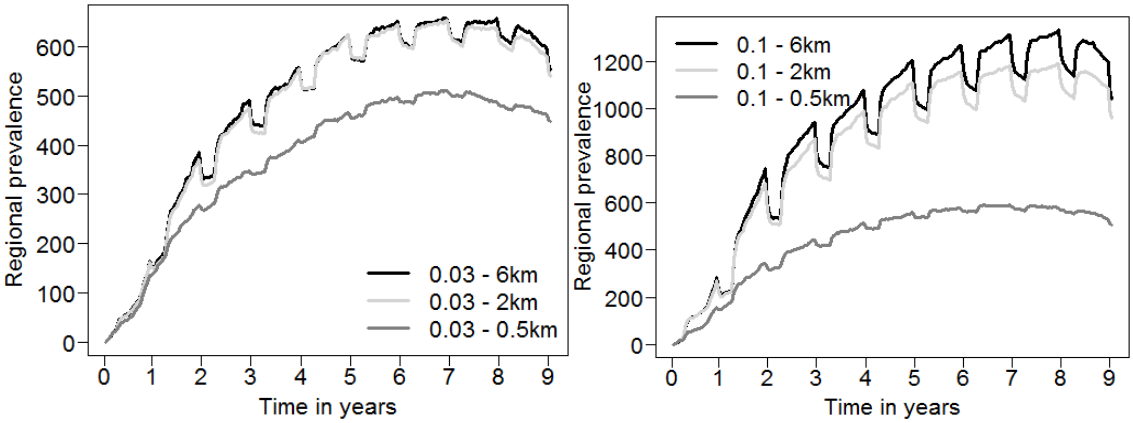

Fig. 9 Regional prevalence with R_{nb} = 0.5 km, 2 km and 6 km, and β_b^{nbP} = 0.03 and 0.1

Two main assumptions were made when modelling NB. Firstly, that the pasture season and neighbourhood radius were the same for all herds. Secondly, that a scaled mass action is adopted to model the impacts of neighbours on infection transmission. This first assumption is a plausible approximation as a first attempt, but data on pasture would allow refining the contact structure between herds at pasture. The latter assumption is appropriate if the animals are equally mixed on pastures, for example, the animals can use a common resource (such as water or food) between neighbouring herds. However, this assumption does not apply when neighbouring relations become heterogeneous. In future, the assumptions made about NB could be updated if relevant epidemiological data become available. For example, the parameters concerning NB could be calibrated according to longitudinal and regional epidemiological data, leading to a more precise model. However, as mentioned above, a precise description of NB requires a considerable amount of data, the trade-off between efficiency and accuracy should be well considered.

In the metapopulation framework, infectious disease propagation can be viewed as a complex system where structured and managed host populations localised in space interact through contacts at various levels: between individuals within age-groups, between age-groups within a herd, and between herds on a regional scale. The proposed model framework is convenient to model such a system. Firstly, this is a general framework that can be directly used to study BVDV spread in other contexts, as long as data on individual animal life trajectory records are available. The object-oriented design of the computer implementation ensures that the proposed model framework is extendable. For instance, beef herds could be

added to the model to establish a full picture of cattle herds within a region. Approaches to control the infection spread could be evaluated within the proposed framework. Thus, this model could become a convenient tool for animal health managers, such as animal health services, to evaluate the control options accounting for territorial specificities (animal herd densities, farming systems, etc.).

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SAMPLING STRATEGIES TO CONTROL MISCLASSIFICATION BIAS IN LONGITUDINAL UDDER HEALTH STUDIES

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SUMMARY

Milk bacteriological culture in udder health cohort studies is likely to provide biased estimates of measures of disease frequency or association, due to the absence of a gold standard to diagnose intramammary infections. In cohort studies, diagnostic errors can lead to selection and misclassification biases of these estimates. These biases were estimated through Bayesian simulations, and sampling strategies to improve the diagnostic procedure are proposed.

INTRODUCTION

A cohort study is the standard method to estimate the incidence of diseases and identify their natural history, by analysing the association between a baseline exposure and risk of disease over the follow-up period. Subjects with the outcome at baseline are excluded from the follow-up, while new incident cases of exposure are identified. It is assumed that prevalent and non-prevalent cases can be differentiated with no error so that only susceptible cases are included in the follow-up. Incident cases are likewise assumed to be correctly identified.

Using imperfect tests may lead to biased estimates of disease frequency and of association with exposure. For instance, in longitudinal udder health studies in which bacteriological culture is commonly used for diagnosis, both quarters at risk of becoming infected and subsequent incident intramammary infections (IMI) can be wrongly identified. In the former, the bias resulting from IMI misclassification could be considered a selection bias, while in the latter it would be commonly defined as misclassification bias. Different methods can be used to limit or address these biases, such as study design, improving the diagnostic procedures by using duplicate or triplicate samples for identifying quarters at risk and incident IMI, or addressing the biases analytically (McInturff et al., 2004; Dufour et al., 2012). Little is known about the relative impact in cohort studies of the selection bias resulting from misidentification of quarters at risk of becoming infected compared to the more traditional misclassification bias. Furthermore, the impact on measures of association of any reduction of this selection bias using more accurate diagnostic procedures is unknown. With the often limited resources available for milk sample analyses, a better understanding of the relative impact of the selection and misclassification biases would allow a more appropriate distribution of the resources for sample collection and diagnostic procedures in a manner that would optimise the balance between the cost and precision of a study.

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The objectives of this study were to evaluate the relative impact of selection and misclassification biases resulting from IMI misclassification on measures of disease frequency (incidence) and of association with hypothetical exposures. The effect of improving the sampling strategy by collecting duplicate or triplicate samples at first or second sampling was also assessed.

MATERIALS AND METHODS

Datasets from a hypothetical cohort study were simulated and analysed based on a separate scenario for two common mastitis pathogens representing two distinct prevailing patterns. The two scenarios investigated were *S. aureus* and coagulase negative staphylococci (CNS). *Staphylococcus aureus*, a relatively uncommon pathogen (prevalence <5%) with a low incidence (0.01 NIMI/quarter-month), can be identified with excellent sensitivity (Se; ~90%) and almost perfect specificity (Sp; >99%, at 100 CFU/ml) by bacteriological culture. CNS are more prevalent (10-30%), with a high incidence (~0.3 NIMI/quarter-month), and with milk bacteriological culture having a fair Se (~60%) but an excellent Sp (95%, at 200 CFU/ml; Dohoo et al., 2011; Dufour et al., 2012).

The generated datasets for each scenario were emulating a longitudinal cohort study with two milk samples collected 1 month apart, from each quarter of a random sample of 30 cows per herd from 100 herds, with a herd-level exposure having a known strength of association. The first milk sample (S_1) was used to identify quarters at risk of IMI at the beginning of the cohort, while the second (S_2) was used to identify the outcome (acquisition of a new IMI). A hypothetical exposure with known strength of association (OR~3.0) was generated. To make the scenario more realistic, exposure was equally associated with odds of a prevalent IMI on the first milk sample as with odds of IMI acquisition on the second sample (as observed in Dufour et al., 2012). Exposure was randomly associated with the odds of eliminating an existing IMI. Correlation of the two specific types of IMI by cow and by herd was obtained from Dufour et al. (2012) to produce realistic datasets. For each scenario, 100 datasets were generated.

Sensitivity and Sp to diagnose *S. aureus* and CNS were represented as Beta distributions with the following shape parameters: (0.90, 0.85) and (0.60, 0.55) for Se; (1, i.e. uniform distribution) and (0.95, 0.90) for Sp, for *S. aureus* and CNS, respectively. Analyses for each of the two scenarios were conducted separately. In each dataset, new S_1' and S_2' variables were generated by applying the scenario misclassification parameters to the S_1 and S_2 samples. Incidence and measures of association with the hypothetical exposure were computed using first the S_1' and S_2' variables (total bias), then S_1' and S_2 (selection bias only), and finally the S_1 and S_2' variables (misclassification bias only). If the selection and misclassification biases were deemed important, the effect of improving Se and Sp on the first and/or second sampling(s) was assessed by applying different scenarios: duplicate samples - parallel on S_1 and S_2; duplicate samples - parallel on S_1 and single on S_2; duplicate samples - parallel on S_2, single on S_1; duplicate samples - series on S_1 and S_2; duplicate samples - series on S_1, single on S_2; duplicate samples - series on S_2, single on S_1; duplicate samples - parallel on S_1 and series on S_2; duplicate samples - series on S_1 and parallel on S_2, and triplicate samples with a two out of three interpretation (Table 1).

Table 1. Hypothetical gain or loss in sensitivity (Se) and specificity (Sp) using different hypothetical sampling strategies

Sample	Duplicate series		Duplicate parallel		Triplicate	
	Se	Sp	Se	Sp	Se	Sp
S. aureus	-0.1	0	+0.1	0	0	0
CNS	-0.25	+0.05	+0.15	-0.05	0	+0.1

Incidence of IMI and measure of association with exposure (odds ratio; OR) were estimated using Markov Chain Monte Carlo (MCMC), implemented using the Stan modelling language (Carpenter et al., in press) through the rstan (Stan Development Team, 2016) interface to R (R Core Team, 2015). Each MCMC sample used four sampling chains with 100 burn-in samples followed by 500 monitored samples. Models were run under the Amazon EC2 cloud-computing environment.

RESULTS

Incidence

For *S. aureus*, biases were small with an observed incidence of 0.29 versus a true incidence of 0.25 IMI/100 quarter-months (Table 2).

Table 2. Existence of bias for measure of intramammary infection incidence (IMI/100 quarter-month; median and 95% credible interval)

	S. aureus	CNS
True incidence	0.25 [0.09-0.53]	28.94 [25.48-32.72]
Single sample, total bias	0.29 [0.12-0.63]	28.57 [24.38-32.71]
Single sample, selection bias only	0.33 [0.13-0.66]	40.69 [36.43-45.07]
Single sample, misclassification bias only	0.22 [0.08-0.49]	22.59 [18.68-26.99]

In the CNS scenario, diagnostic errors in the two samples led to important selection (40 IMI/100 quarter-month) and misclassification (23 IMI/100 quarter-months) biases for estimation of IMI incidence. These biases were in opposite directions and therefore the incidence measure obtained using single sampling on both the first and second test (29 IMI/100 quarter-months) was exactly the true value and no specific sampling strategies had to be considered (Table 3).

Table 3. Effect of sampling strategy on estimated measure of intramammary infection incidence (IMI/100 quarter-month; median and 95% credible interval. S_1: first sample; S_2: second sample)

	S. aureus	CNS
Duplicate samples, parallel on S_1 and S_2	0.26 [0.10-0.56]	35.93 [32.50-39.62]
Duplicate samples, parallel on S_1 and single on S_2	0.24 [0.09-0.53]	27.05 [23.35-30.38]
Duplicate samples, parallel on S_2, single on S_1	0.32 [0.13-0.66]	37.81 [33.97-42.13]
Duplicate samples, series on S_1 and S_2	0.33 [0.13-0.66]	16.61 [11.62-21.81]
Duplicate samples, series on S_1, single on S_2	0.36 [0.16-0.71]	30.72 [25.78-35.43]
Duplicate samples, series on S_2, single on S_1	0.27 [0.10-0.60]	15.25 [10.94-19.96]
Duplicate samples, parallel on S_1 and series on S_2	0.22 [0.07-0.51]	14.34 [10.36-18.05]
Duplicate samples, series on S_1 and parallel on S_2	0.39 [0.17-0.76]	40.27 [35.75-45.25]
Triplicate samples	0.29 [0.12-0.60]	23.58 [19.51-27.73]

Association

In the *S. aureus* scenario the OR for exposure showed little bias (Table 4, observed OR of 3.1 versus true OR of 3.2). Therefore, no particular sampling strategy was considered. The CNS scenario, however, revealed the presence of a large misclassification bias moving the association towards the null value (Table 4, OR of 1.7 versus true OR of 2.6). Little improvement could be brought about using different sampling strategies aiming to improve Se and/or Sp on first and/or second sampling and using a two out of three interpretation for IMI definition. This latter strategy only corrected the measure of association to an OR of 2.0 (Table 5).

Table 4. Existence of bias for measure of association between exposure and probability of incident intramammary infection (Odds ratio; median and 95% credible interval)

	S. aureus	CNS
True association	3.18 [1.31-8.55]	2.58 [1.89-3.55]
Single sample, total bias	3.11 [1.40-7.75]	1.72 [1.44-2.10]
Single sample, selection bias only	3.16 [1.44-7.61]	2.66 [2.10-3.41]
Single sample, misclassification bias only	3.14 [1.26-8.73]	1.70 [1.34-2.14]

DISCUSSION

With the scenarios studied, our results indicated that selection and misclassification biases of a low prevalent and incident disease diagnosed with high Se and Sp, are minimal and do not require specific sampling strategies to improve the unit at risk or case identification. However, when investigating a highly prevalent and incident disease diagnosed with an average Se and high Sp, a bias toward the null would be observed for the measure of association with exposure, and this bias could not be controlled by modulating the sampling strategy.

Table 5. Effect of sampling strategy on measure of association between exposure and probability of incident intramammary infection (Odds ratio; median and 95% credible interval. S_1: first sample; S_2: second sample)

	CNS
Duplicate samples, parallel on S_1 and S_2	1.75 [1.46-2.19]
Duplicate samples, parallel on S_1 and single on S_2	1.73 [1.42-2.15]
Duplicate samples, parallel on S_2, single on S_1	1.76 [1.47-2.16]
Duplicate samples, series on S_1 and S_2	1.61 [1.30-1.96]
Duplicate samples, series on S_1, single on S_2	1.69 [1.42-2.05]
Duplicate samples, series on S_2, single on S_1	1.68 [1.34-2.08]
Duplicate samples, parallel on S_1 and series on S_2	1.68 [1.32-2.12]
Duplicate samples, series on S_1 and parallel on S_2	1.74 [1.46-2.15]
Triplicate samples	2.04 [1.66-2.54]

Many studies have looked into the effect of misclassification on statistical inferences, including biased prevalence and incidence rate estimates (Rogan & Gladen, 1978; Quade et al., 1980) and biased relative risk estimates (Barron, 1977; Greenland, 1980). Nondifferential misclassification of disease generally leads to bias towards null in the estimated associations as well as reduced statistical efficiency (Bross, 1954; Barron, 1977; Copeland et al., 1977). This bias depends mainly on the Sp of the test used for rare diseases (Copeland et al., 1977). If the Sp of the test is perfect, then bias is absent (Poole, 1985). The effect of misclassification of disease at baseline to calculate incidence has been considered less frequently. In longitudinal studies, the nondifferential misclassification of disease at baseline, especially imperfect sensitivity, can lead to over- or under-estimation of the observed cumulative incidence risk ratios (Pekkanen et al., 2006). This bias can be significant for disease with a low true incidence, a high true prevalence, a substantial disease duration (i.e. as long as the interval between first and second test), and a poor test Se. To minimise bias, disease subjects at baseline should be excluded from the cohort based on a highly sensitive test (Pekkanen et al., 2008). Case identification during the follow-up period should use a highly specific test having a high positive predictive value (Brenner & Gefeller, 1993). Our results show that a longitudinal study for a low prevalent and incident disease, identified with a highly sensitive and specific test, do not suffer from bias and using a single sample strategy with this test will give satisfactory estimates of incidence and of the association with exposure. However, a more prevalent and incident disease diagnosed with an imperfect sensitivity will give a biased measure of association, despite tentative efforts to improve its diagnosis with various sampling strategies. It is therefore necessary to correct the bias at the analytic stage, for instance by incorporating the Se and Sp of the test in the modelling strategy (Magder & Hugues, 1997; McInturff et al., 2004).

Increasing the number of samples or tests can prevent bias in some situations, but in others, efforts can be spared by maintaining a single sampling approach. When designing longitudinal studies, evaluating potential biases and best sampling strategy is as critical as the choice of test. An R package was developed for such appraisal. Correcting remaining biases using analytical methods can complement the choice of a good sampling strategy.

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MAXIMUM ENTROPY ECOLOGICAL NICHE MODELLING FOR SURVEILLANCE OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) IN THE UNITED STATES

M.A. ALKHAMIS*, C. VILALTA, A. ARRUDA, A.M. PEREZ AND R.B. MORRISON

SUMMARY

Risk maps for porcine reproductive and respiratory virus (PRRSv) outbreaks were developed for the Midwest (MW) and Southeast (SE) regions of the US to compare the ecological dynamics of the disease in the two most pig-dense regions of the country. Maps were developed by modelling the ecological niche of the virus using surveillance and relevant demographic and environmental data collected between 2009 and 2016. Data fit the model relatively well in both regions (cAUC=0.6 in the MW and 0.7 in the SE). The contribution of pig density to PRRSv risk was higher for the MW (87%) compared to the SE (58%), whereas land cover and environmental variables were more important in the SE (42%) than in the MW (13%). Results suggest that the ecological dynamics of PRRSv are different in both regions, which may have important implications in the design of effective preventive strategies in the country.

INTRODUCTION

Infection with the porcine reproductive and respiratory syndrome virus (PRRSv) causes significant financial losses to the swine industry of North America, where the pathogen is endemic (Perez, 2015; Neumann, 2005). The disease is characterised by reproductive and respiratory disorders in sows and growing pigs, respectively (Quaife, 1989; Elazhary et al., 1991). PRRSv strains are classified into two types referred to as type I or European strain and type II, or North American strain, with the latter causing most of the outbreaks in the US (Ropp et al., 2004; Murtaugh, 2009). Infected pigs may transmit the virus directly and indirectly through all bodily secretions (Rossow, 1998). Movements of infected animals and contaminated fomites, as well as air, have been heavily implicated in maintaining PRRSv circulation and spread across the US (Cutler et al., 2011; Perez et al., 2015).

PRRSv maintains an endemic state in North America, with annual seasonal increases in the number of outbreaks. However, the disease also causes occasional seasonal epidemics through new emerging viral strains (Arruda et al., 2015). Those epidemics are usually the cause of heavy losses to the industry as they result in far-reaching direct losses, and require prompt mobilisation of diagnostics, control and prevention resources. Many studies have

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identified strong relationships between season and number of outbreaks as well as emergence of new PRRSv strains (Holtkamp et al., 2010; Tousignant et al., 2015). Similarly, other studies were able to establish associations between herd size and frequency of PPRSv outbreaks (Holtkamp et al., 2012; Kwong et al., 2013; Truong & Gummow, 2014). Therefore, demographic and climatic factors could both contribute to the prediction of the probability of outbreak occurrence and definition of geographical ranges of suitable areas (known as the ecological niche) for introduction, maintenance and spread of PRRSv.

To date, no studies have been able to quantify the combined role of season and herd size in defining the spatial range of high-risk areas for PRRSv outbreaks across different swine production systems in the US. This poses a challenge for the early detection, control and prevention of severe PRRSv outbreaks. Mapping the spatial risk of PRRSv outbreaks and quantifying the role demographic and climatic risk factors play on PRRSv risk may help to increase producers' awareness and guide risk-based interventions. The objective of this study was to compare the ecological dynamics, as suggested by the relative contribution of demographic and environmental variables to disease risk, of PRRSv in the two most densely populated regions of the country. This study integrates spatial information regarding PRRSv surveillance with relevant demographic and environmental factors. Results provide useful insights into the epidemiology of PRRSv in the most important swine production areas of the country, and can be useful in guiding risk-based surveillance, including early detection, control, and prevention of PRRSv outbreaks in the US.

MATERIALS AND METHODS

Data Source

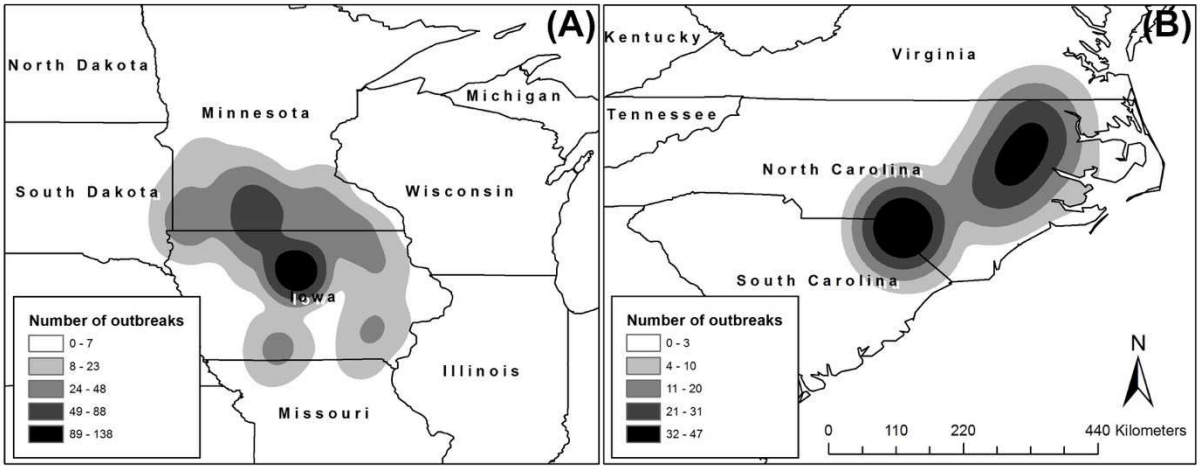

Fig. 1 Geographical locations of PRRSv outbreaks smoothed using a kernel density function, in the Midwest (A) and Southeast (B) regions of the US

This study used a confidential dataset obtained from a voluntary programme referred to as the Swine Health Monitoring Project, which includes data from approximately 40% of the sows in the country. Data were collected from 29th July 2009 to 28th September 2016 from breeding farms in the Midwest and Southeast regions, which account for most of the swine production in the country. The dataset contained information about sow farms that shared weekly PRRSv data at premises level, including geographical location, the production system of the farm and the date at which the outbreak was observed. PRRSv status classification

used in this study followed criteria described elsewhere (Holtkamp et al., 2011). A smoothed kernel density function was used to represent the geographical density of PRRSv outbreaks in both swine production regions to protect data confidentiality (Fig. 1A and B).

The geographical range of PRRSv high-risk areas was predicted using a set of demographic and climate predictors, including: 1) pig density; 2) climate; 3) land cover. A kernel density function with a spatial resolution of 5 km^2 was used to represent the density of pig farms in both regions (referred to as pig density) and was derived from locations of all pig farms across the US in 2012. Locations of all pig farms in the US were retrieved from United States Department of Agriculture Census of Agriculture website (www.agcensus.usda.gov). Climate data were obtained from the WorldClim website (http://www.worldclim.org), which compromises interpolated global climate data commonly used for ecological modelling and GIS (Hijmans et al., 2005). This source consisted of 19 climatic, referred to as Bioclim variables, and derived from global temperature and precipitation data and. Bioclim variables retrieved in the form of raster data, with a 5 km^2 resolution. However, in this study, bioclimatic variable 8-9 and 18-19 were excluded due to known spatial artefacts in those four variables, as suggested elsewhere (Reeves et al., 2015). Thus, only 15 bioclimatic variables were included in the subsequent analysis. Finally, an estimate of the geographical distribution of 16 different land cover features in the US (which included forests, croplands, grasslands, urban, etc.) was obtained by using the high spatial resolution (0.5 km^2) land cover data grid, known as MODIS-based global land cover climatology, from the United States geographical survey (USGS) webpage (http://www.landcover.usgs.gov). Thus, the final raster dataset compromised 17 demographic and climatic predictors.

The Raster package (Hijmans, 2016a) implemented in R statistical software (RDCT, 2015) was used to convert all of the above predictor layers into a common projection and map extent. Each raster was cropped so that the geographical extent of the spatial analyses covered the two swine production regions. Furthermore, scatter-plots were used to visually inspect for collinearity between each pair of predictors. Finally, because the land cover layer was at a different spatial scale (0.5 km^2) from other environmental variables (5 km^2), all predictors' layers were aggregated and resampled to create a uniform raster stack with a final spatial resolution of 5 km^2.

Ecological Niche Modelling

A presence-only maximum entropy ecological niche modelling technique (Maxent; Phillips et al., 2006) was used to predict the geographical range of PRRSv high-risk areas in Midwest and Southeast swine production regions. The Maxent program version 3.3.3 was implemented as a function in the Dismo package in R (Hijmans, 2016b). This method has recently become popular for predicting the spatial distribution of infectious diseases of both public health and veterinary significance (Reeves et al., 2015; Alkhamis, 2016a; Alkhamis & VanderWaal, 2016). The Maxent algorithm extracts associations between presence data (location of PRRSv outbreaks) and predictor variables to build an ecological niche model for each region. These ecological niche models use the extracted associations to characterise the demographic and climatic requirements for the species or disease agent, and subsequently deploy those associations to predict suitable geographical locations in non-sampled areas. The default logistic model was used to ensure that predictions gave estimates between 0 and 1 for the risk per map cell. Furthermore, the default convergence threshold, regularisation, and number of iterations were used in the analysis of both regions. Initially, a purely bioclimatic Maxent model was fitted for both regions (which included only the 15 bioclimatic

variables) to assess their association with PRRSv presence data. Next, the selected bioclimatic variables that had greater than 10% relative contribution to the prediction were selected and included in the subsequent ecological niche models, along with the above non-bioclimatic predictors. A Jackknife test was used to calculate the contribution of each environmental variable to the final model's prediction.

The performance of the candidate Maxent models was evaluated by partitioning the data into training and testing sets and using the threshold independent method. The threshold independent method characterises the performance of the model across the full range of possible probability thresholds for presence/absence predictions (Elith & Leathwick, 2009). A k-fold partitioning scheme was used to create 5 partitions and randomly sample each partition with replacement, where each candidate Maxent model was tested five times (k=5) against 10,000 randomly generated background points (pseudo-absences). Subsequently, the area under the curve (AUC) was calculated through a receiver operator characteristic (ROC) plot of the sensitivity (the proportion of true predicted known presences, known as omission error) vs. 1 - specificity (proportion of false predicted known absences, known as commission error) over the whole range of threshold values between 0 and 1. The training set (training-data AUC) was used for model building, whereas the testing sets (testing-data AUC) were used to evaluate model accuracy using the average value of the AUC calculated for each partitioned set. An AUC value of 0.5 represents an entirely random predictive model, while an AUC value of 1 represents a perfectly discriminating predictive model. Commonly, Maxent models with AUC values >0.75 for both training and testing data were usually considered reliably discriminating models (Soberón, 2005; Elith et al., 2006). Finally, because of the large geographic area analysed in this study, a calibrated AUC (cAUC) was used for the final Maxent model to evaluate the presence of the spatial sorting bias (SSB) as suggested elsewhere (Hijmans, 2012). If the cAUC value was close to 1, then one can conclude the absence of SSB (i.e. a swine production region with high observed outbreaks have small impact on the resultant Maxent model), whereas if the value was close to zero, then SSB is present in the data (i.e. a swine production region with high observed outbreaks have large impact on the resultant Maxent model).

RESULTS

Only three environmental predictors contributed to the geographical range of high-risk areas for PRRSv outbreaks in the Midwest, with AUC values closer to 1 than 0 (Table 1). Pig density was by far the most important environmental predictor, followed by precipitation seasonality and land cover in the Midwest (Table 1). Areas with high pig densities and precipitation variability between 40% and 60% over the course of the year were found mostly suitable for PRRSv outbreaks in the Midwest. For the Southeast region, four environmental predictors contributed to the geographical range of high-risk areas for PRRSv outbreaks, with AUC values higher than those of the Midwest Maxent model (Table 1). Similar to the Midwest, pig density was the most important predictor, followed by precipitation of the wettest month, land cover and temperature seasonality, although the relative contribution of pig density was smaller for the Southeast compared to the Midwest. Specific to the Southeast, areas with high pig density, with precipitation amounts between 120 millimetres and 200 during the wettest months, and near croplands were mostly suitable for PRRSv outbreaks.

Table 1. Estimates of relative contributions of the environmental variables to each Maxent model and their validation AUCs values

Variable	% Contribution	Training Data AUC[a]	Test Data AUC±SD[b]	cAUC[c] ±SD
Midwest region (Minnesota/Iowa)				
Pig Density	87.3	0.83	0.82±0.01	0.60±0.01
Precipitation Seasonality	11.1			
Land Cover	1.6			
Southeast region (North Carolina)				
Pig Density	58.5	0.97	0.96±0.03	0.70±0.02
Precipitation in the Wettest Month	19			
Land Cover	13.3			
Temperature Seasonality	9.1			

[a] Area under the curve
[b] Standard deviation for the test and calibrated AUC
[c] Calibrated AUC for test data

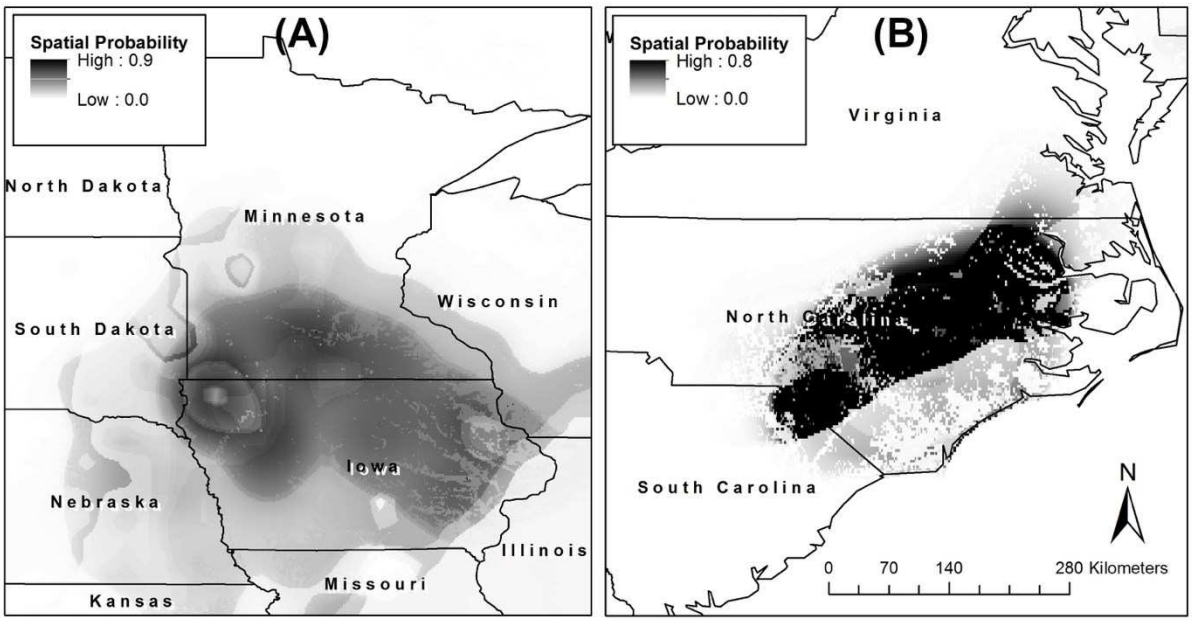

Fig. 2 Predicted spatial probability of PRRSv risk, predicted by the final Maxent models, in two swine production systems between 2009 and 2016. (A) Predicted spatial risk in the Midwest. (B) Predicted spatial risk in the Southeast

Generally, the predicted spatial range of high-risk areas (Probability <0.6) in the Midwest included south Minnesota and north and central Iowa (Fig. 2A). For the Southeast, the predicted spatial range of high-risk areas (Probability <0.8) included east central parts of North Carolina and a small part of northeast South Carolina (Fig. 2B).

DISCUSSION

This study presented the results of a novel approach to map the spatial risk for PRRSv in swine populations of the US. Ecological niche modelling, based on presence-only outbreak data, was used to reveal the spatial range of geographical areas suitable for PRRSv circulation and spread, and the environmental requirements that predict its distribution in the two largest swine production regions in the US. The model was used to compare the relative contribution of environmental predictors (after accounting for pig density) in both regions. Results provide quantitative knowledge on the variability of the ecological dynamics of PRRSv at the regional level in the US.

The highest incidence of PRRSv outbreaks was observed in the Midwest, with 627 outbreaks observed between July 2009 and September 2016 in 14 swine production systems. Therefore, both southern Minnesota and Iowa were predicted to be the highest risk areas for the disease (Fig. 2). The AUC values of the Midwest Maxent model suggest that the selected environmental variables were adequate predictors for PRRSv outbreaks between 2009 and 2016 (Table 1). Unlike the Midwest model, the Southeast model identified only east and east-central North Carolina, and northeast South Carolina as high-risk areas for PRRSv outbreaks, and the predicted geographical range did not extend beyond the areas of the observed outbreaks (Fig. 1 and Fig. 2). However, the AUCs values of the Southeast model suggest a more robust prediction than the Midwest model (Table 1).

As expected and reported elsewhere (Holtkamp et al., 2010; Holtkamp et al., 2012; Perez et al., 2015), pig density accounted for most of the background spatial risk in the Midwest. Although still important, however, the relative contribution of pig density to risk was lower in the Southeast compared to the Midwest (Table 1). Unlike the Midwest model, the Southeast model suggested that seasonality had a relatively larger role in predicting the risk of PRRSv outbreaks in which the combination of rainy seasons and the large temperature variation over the course of the year led to the seasonal increases in the number of outbreaks. That said, breeding farms in the Midwest and Southeast are characterised by high biosecurity measures, where pigs are contained in a closed environment in an attempt to minimise the impact of the weather. Thus, the effect of weather may be due, at least in part, to weather-related anthropogenic activities such management practices, transportation of live animals or semen and nearby farming activities, which occurs in seasonal patterns, as suggested elsewhere (Holtkamp et al., 2012; Perez et al., 2015). Inclusion of such variables related to spatio-temporal patterns of pig movements would substantially improve predictions of the models presented here, but such information was not available.

Reasons for the differences in the relative contribution of factors to PRRSv in each region are yet to be elucidated. One may argue that with pig density being relatively stable in a given cell (farm), then one would expect a more predictable output, in terms of estimates of risk, in the Midwest compared to the Southeast, as also suggested by the relative consistency of spatial clustering and risk in the region reported elsewhere (Tousignant et al., 2015). In turn, a different attitude towards control in sow herds in the Southeast compared to the Midwest, may result in higher persistence of PRRSv in the former, thus resulting in a higher influence of weather in risk, given that pig density is relatively constant.

One limitation of the present study is that it only represents PRRSv occurrence in sow farms, as data on other types of farms were not available to us. Inclusion of such data may have improved the strength of the predictions. For example, it has been found that virulent emerging strains, such as the recent 1-7-4 RFLP-type PRRSV observed between 2014 and

2015, were most likely to originate and spread from sow farms into other production types (Alkhamis et al., 2016b). Furthermore, control of PRRSv outbreaks is focused on sow herds, with the objective of weaning PRRSv-free pigs and transporting them to sites located at varying distances, where they are grown and finished until reaching market weight (Linhares et al., 2014). Thus, the inclusion of further information related to farm demographics will shed further insight into the spatial epidemiology of PRRSv, in terms of identifying the contribution of such demographics to the incidence of outbreaks and their role in shaping the geographical extent of PRRSv high-risk areas.

In summary, results suggest that the ecological dynamics of PRRSv are different in the two largest swine production areas of the US, which may affect the ability to predict risk in both regions and, most importantly, the effectives of prevention and control strategies in the country.

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CONTROLLING PARATUBERCULOSIS

IDENTIFICATION OF PHENOTYPIC TRAITS OF RESISTANCE TO LIMIT MAP SPREAD IN A DAIRY CATTLE HERD USING A MODELLING APPROACH

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SUMMARY

Cattle show variable responses to *Mycobacterium avium* subsp. *paratuberculosis* (MAP) exposure, leading to paratuberculosis, a chronic infectious disease. Current control strategies for this disease are ineffective. However, recent studies highlighted the heritability of resistance to paratuberculosis, paving the way to a potential genetic selection of more resistant animals. Our objective was to identify the phenotypic traits of resistance to paratuberculosis that strongly influence MAP spread within a dairy cattle herd. Sixteen phenotypic traits were investigated and they were assumed to vary alone or in combination. A cluster analysis was performed to identify groups of scenarios. An ANOVA quantified trait contribution to model output variance. Four traits were highlighted: resistance acquisition speed, infectious dose, quantity of MAP shed in faeces by heavy shedders and duration before heavy shedding state. Trait combinations contributed up to 12% of output variance. We advocate that future genetic selection should focus on these four traits simultaneously.

INTRODUCTION

Paratuberculosis is an enzootic bacterial disease of domestic ruminants. This worldwide disease is responsible for economic losses in dairy cattle herds. Susceptible animals are infected in utero by infected dams or by ingestion of *Mycobacterium avium* subsp. *paratuberculosis* (MAP).

The response to MAP exposure varies among animals. Paratuberculosis is present in infected herds with a low prevalence ranging from 2.8 to 27% infected animals (Good et al., 2009; Raizman et al., 2011) Among animals of the same age (whose exposure to MAP can be assumed to be similar), some stay infection free, while others become infected. In experimental studies, challenging calves of the same age with a similar MAP infectious dose led to varying severity in paratuberculosis lesions, serological responses, and quantities of bacteria shed in faeces (Mortier et al., 2011, 2013, 2014, 2015). Evolution in infection course is also highly variable among animals. For example, the latency and incubation periods vary from 4 months to 15 years (Matthews, 1947; Mitchell & Medley, 2012; Nielsen, 2008; Stewart et al., 2007; Van Roermund et al., 2007). These variations in response to MAP exposure are explained by different mechanisms and individual characteristics. The

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combinations of these characteristics, denoted as phenotypic traits, define the phenotypes of animals as resistant or susceptible to paratuberculosis.

Two kinds of measures are available to control paratuberculosis in a herd: hygienic measures that reduce the amount of MAP present in the farm environment and in the feed, and test-and-cull measures that detect and eliminate the infected animals in infected herds. These measures are hard to implement and are often ineffective in controlling paratuberculosis at herd or regional levels (Bastida and Juste, 2011; Beaunée et al., 2015). Commercialised vaccines against paratuberculosis decrease MAP shedding and attenuate clinical signs in infected animals, but do not prevent infection (Bastida and Juste, 2011; Kalis et al., 2001). Moreover, cross reaction with diagnostic tests for *Mycobacterium bovis* is described in most of these vaccines (Behr and Collins, 2010).

The individual variability in responses to MAP exposure could lead to the development of innovative control measures for paratuberculosis if the most resistant animals can be selected. Genetic studies have highlighted that cattle resistance to paratuberculosis is heritable, with a heritability ranging from 0.01 to 0.23 (Kirkpatrick, 2010; Kirkpatrick and Shook, 2011; Küpper et al., 2012; van Hulzen et al., 2011; Zare et al., 2014). The resistance of cattle to paratuberculosis, measured by the ability of animals exposed to MAP to be seronegative, is associated with few genetic markers (Alpay et al., 2014; Kirkpatrick et al., 2011; Purdie et al., 2011; Van Hulzen et al., 2012; Zanella et al., 2011). Potential genetic selection for paratuberculosis resistance in cattle has to focus on the most relevant phenotypic traits. It is therefore crucial to identify the most influential phenotypic traits of resistance to paratuberculosis that limit MAP spread within dairy cattle herds.

As paratuberculosis is a chronic disease, observational and experimental studies are difficult and expensive to implement. Therefore, modelling is the most appropriate approach to study MAP spread within a herd. Several models of MAP-spread dynamics have been developed so far on different scales (as reviewed in Marcé et al., 2010) and more recently Al-Mamun et al., 2016; Beaunée et al., 2015; Koets and Gröhn, 2015; Lu et al., 2010; Marcé et al., 2011; Martcheva et al., 2015; Robins et al., 2015; Smith et al., 2015). One study assessed the effectiveness of a potential genetic selection for resistance to paratuberculosis to achieve eradication (van Hulzen et al, 2014). Three phenotypic traits: the length of the susceptibility period, the length of the latency period, and the susceptibility to infection were investigated individually using a modelling approach. This paper concluded that hundreds of years were needed to eradicate paratuberculosis in a herd using only genetic selection and ranked the investigated traits by effectiveness. However, this effectiveness might have been underestimated as combinations of traits were not considered. In addition, how MAP would spread if introduced in a herd once selection had been achieved was not described. Finally, additional phenotypic traits are of interest but have not yet been investigated.

Our aim was to identify which phenotypic traits have the strongest influence on MAP-spread dynamics in a dairy cattle herd. Several phenotypic traits such as disease susceptibility, infection course, and shedding were investigated both individually and combined.

MATERIALS AND METHODS

To assess the influence of improving phenotypic traits of cattle resistance on paratuberculosis, a scenario assuming current values for these traits has been compared to

scenarios assuming individual and combined improved traits. Traits were assumed to have been improved by genetic selection of animals more resistant than the current ones (denoted as reference animals) in the whole herd.

Choice of a model and study design

A stochastic mechanistic compartmental model has been chosen. This model offers the most up-to-date description of MAP-spread dynamics and accounts for all of the major processes of MAP transmission and infection. It represents both infection and demographic processes, as herd structure and herd dynamics have been shown to highly influence MAP spread (Marcé et al., 2011). As a mechanistic model, each of the processes and mechanisms involved are coded by a parameter, which allowed us to represent variations in traits of resistance through a small number of changes in the model.

Main features of the model: the chosen model and its equations were detailed in Marcé et al. (2011) and in Beaunée et al. (2015) for its optimised version in the C++ language.

Briefly, the infection course is represented by different compartments, each corresponding to a health state: susceptible animals (S) can be infected by ingesting MAP and become transiently infectious (T) and start to shed MAP. Then, after spending an average duration (ν_T) in state T, animals are assumed to stop shedding and become latently infected (L). Infected animals shed the bacteria again after an average latent period (ν_L), becoming moderate shedders (Is) for an average duration ν_{Is}. The disease evolution ends with a state for heavy shedding or clinical symptom occurrence (Ic) that lasts on average for 6 months before culling. Animals not infected before 1 year of age (μ) are assumed to become no more susceptible (R). The susceptibility decreases with age with an exponential decay of parameter h. The demographical dynamics are representative of a typical Western Europe Holstein dairy herd with a high renewal rate (1/3 of the cows per year) and five age groups (unweaned calves, weaned calves, young heifers, bred heifers and adult cows). The herd is assumed to be closed, with no exchange of animals with other herds.

Susceptible animals can be infected in utero if born to infected dams, by a contact with MAP present in the farm environment and shed in faeces by infectious animals (either locally or in the general environment of the farm), or by ingestion of contaminated milk or colostrum.

Investigated phenotypic traits: cattle resistance could be expressed in two ways: resistance and tolerance. Resistance, expressed by susceptible animals, is the ability to prevent infection that can occur before birth (a lower probability to be infected by vertical transmission) and after birth (a faster decrease in susceptibility and a younger age to become no more susceptible). Tolerance is defined as the ability to cope with infection and is expressed by infected animals.

Table 1. Parameters used in the model coding for the investigated phenotypic traits of resistance to paratuberculosis and tested values

Parameter	Corresponding phenotypic trait	Univariate design [Min -ref.[c] -Max]	Multivariate design				References[d]
			V1	V2	V3	V4	
u^a	Susceptibility duration	[1 - 52 - 132]					A
h	Resistance acquisition speed (coefficient)	[ø- 0.1 - 10]	0.2	0.3	0.4	0.5	B
α	Infectious dose required to be infected (x 10^6 bacteria)	[ø - 1 - 10^6]	1.5	2	2.5	3	C
$v_T{}^a$	Duration of transient state	[1- 25 - 132]			-		D
$v_L{}^a$	Duration of latent state	[1 – 52- 208]			-		E
$v_{Is}{}^a$	Duration of moderate shedding state	[1- 104 - 520]	95	86	77	68	F
$v_L+v_{Is}{}^a$	Duration before heavy shedding and clinically affected state	[ø - 156 - 468]	234	312	390	468	G
$v_T{}^a$ *with* $v_T+ v_{L=}Cte^b$	Duration of transient state with constant duration before moderate shedding state	[1 - 25 - 76]			-		H
$v_{Is}{}^a$ *with* $v_T+v_L+ v_{Is=} Cte^b$	Duration of moderate shedding period with constant duration before heavy shedding or clinically affected state	[1 - 104 - 179]			-		I
$\varphi Milk_X$	Factor of decrease in MAP shed in milk for animals in health state X (% of quantity shed by a reference animal)						
$\varphi Milk_{Is}$	moderate shedding state (*Is*)	[0 - 100 - ø]	50%	10%	5%	0%	J
$\varphi Milk_{Ic}$	heavy shedding and clinically affected (Ic)	[0 - 100 - ø]					K
$\varphi Feces_X$	Factor of decrease in MAP shed in faeces for animals in health state X (% of quantity shed by a reference animal)						
$\varphi Feces_T$	transient state (*T*)	[0 - 100 - ø]	50%	10%	5%	0%	L
$\varphi Feces_{Is}$	moderate shedding state (*Is*)	[0 - 100 - ø]					M
$\varphi Feces_{Ic}$	heavy shedding or clinically affected (*Ic*)	[0 - 100 - ø]	66%	50%	40%	33%	N
φP_X	Factor of decrease in probability of in utero transmission for cow in health state X (% of probability in a reference animal)						
φP_{LIs}	latent and moderate shedding states (*LIs*)	[0 - 100 - ø]	50%	10%	5%	0%	O
φP_{Ic}	heavy shedding or clinically affected (*Ic*)	[0 - 100 - ø]					

[a]Durations expressed in weeks; [b]Cte means constant; [c]Value of the parameter for a reference animal, without improvement of resistance

[d]Corresponding references: A (Hagan, 1938; Rankin, 1962; Whitlock & Buergelt, 1996); B (Windsor & Whittington, 2010); C (Begg & Whittington, 2008); D (Mitchell & Medley, 2012; Stewart et al., 2007; Van Roermund et al., 2007); E (Mitchell & Medley, 2012; Nielsen, 2008); F (Matthews, 1947); G (Matthews, 1947; Mitchell & Medley, 2012; Nielsen, 2008); H (Mitchell & Medley, 2012; Nielsen, 2008; Stewart et al., 2007; Van Roermund et al., 2007); I (Matthews, 1947; Mitchell & Medley, 2012; Nielsen, 2008; Stewart et al., 2007; Van Roermund et al., 2007); J (Sweeney et al., 1992); K (Giese, 2000); L (Van Roermund et al., 2007); M (Rossiter & Burhans, 1996); N (Jørgensen, 1982; Whittington et al., 2000); O (Benedictus et al., 2008; Whittington & Windsor, 2009)

Four phenotypic traits are related to the ability to prevent MAP infection: the duration of the susceptible period, the resistance acquisition speed, the dose of MAP required to be infected, and the probability of in utero transmission. The ability to cope with infection is related to: the duration of the latent state, the duration before occurrence of the heavy shedding or clinically affected state, and the quantities of MAP shed through the different transmission routes by animals in each infectious state.

Initial conditions and simulation protocol: the initially naïve herd was contaminated by the introduction of one moderate-shedding cow. MAP spread was monitored for 25 years and 500 runs for each studied scenario. A scenario was defined as a set of values for the 16 studied phenotypic traits. Scenarios assuming that each phenotypic trait varies individually were investigated (denoted hereafter as the univariate study design). A multivariate study design was then performed. Regarding the calculation capacity constraints, the results from the univariate study design were used to reduce the number of traits and defined tested values per trait in the multivariate study design. In this second part of the study, eight phenotypic traits were assessed with five values each in a complete factorial design (total: 380,625 scenarios), as it was of interest to explore all orders of interactions.

Model outputs: Four model outputs describing MAP-spread dynamics in a herd were analysed: (i) the persistence was the proportion of runs where the infection persisted until 25 years after MAP introduction, (ii) the cumulated incidence was the mean cumulated number of newly infected animals over the 25 years, (iii) the prevalence of infected animals was the median prevalence of infected animals at 25 years and (iv) the prevalence of affected animals was the median prevalence of heavy shedders and clinically affected animals at 25 years. The latter two were calculated only when infection persisted over the 25 years.

Output analysis

A cluster analysis was performed on scenarios of the multivariate study design to identify groups of closely related scenarios. The predicted persistence and cumulated incidence were standardised and used to build the clusters. The number of clusters was defined by looking at the within-group sum-of-squares by number of clusters. Clusters were built using the k-means clustering method (kmean function in R package "factomineR"). An ANOVA was performed to investigate the contribution of each phenotypic trait their interactions to the variance of the four model outputs.

RESULTS

The univariate study design highlighted seven phenotypic traits inducing an improvement of at least one of the model outputs and nine non-influential traits if varied alone (Fig. 1). Influential phenotypic traits were: decrease in susceptibility duration, increase in resistance acquisition speed, increase in infectious dose, increase in latent state duration, decrease in quantity of map shed in faeces by heavy shedders and clinically affected animals, and increase in duration before entering the heavy-shedding and clinically affected state. The results from the univariate design study were used to identify phenotypic traits to investigate in the multivariate study. Non-influential phenotypic traits were simplified in order to decrease the number of traits to study: phenotypic traits involving the same mechanisms of resistance were assumed to be unique traits (e.g. decreased shedding by similar routes); when traits were strongly correlated, only one was kept.

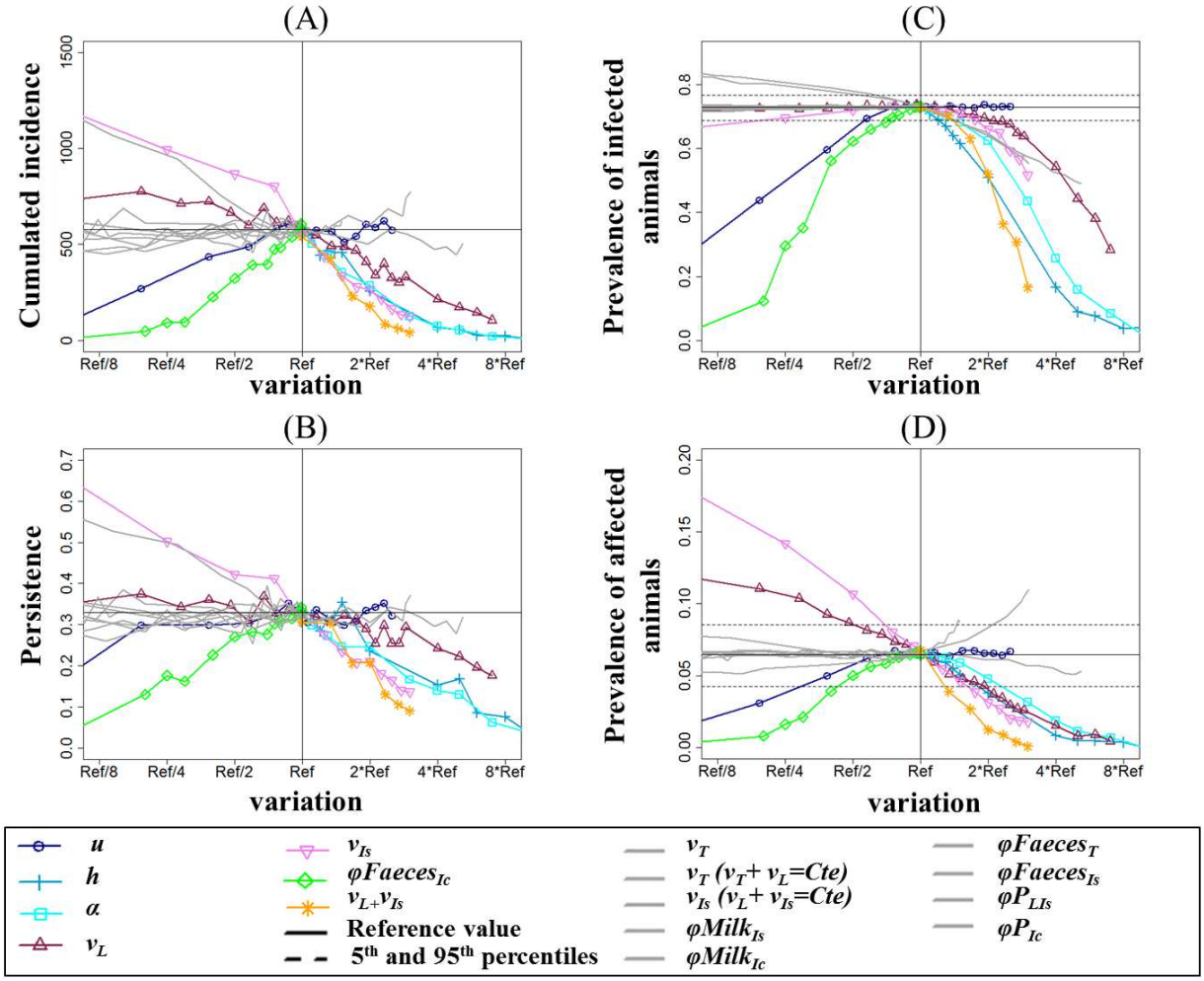

Fig. 1 Effect of variations in phenotypic traits tested in the univariate design study on the four model outputs. The x-axis is defined using relative values for parameters compared to their reference value (Ref; see Tab. 1 for more details on parameters and values)

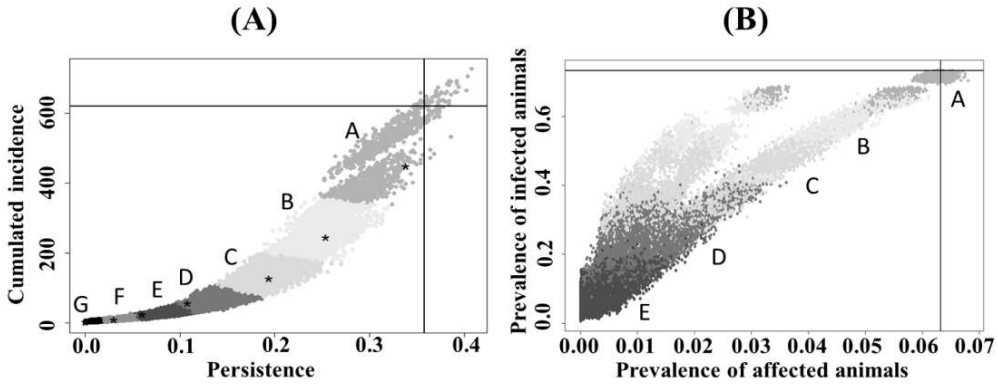

Fig. 2 Distribution among seven clusters (A to G) of the scenarios investigated in the multivariate study design for the four model outputs. Lines represent output values for the reference scenario; asterisks are centroids of clusters

Seven clusters among the multivariate study design were built (Fig. 2). Clusters A and B grouped low control scenarios with a low decrease in persistence and cumulated incidence. Clusters C, D, and E contained moderate control scenarios with a moderate decrease in persistence and cumulated incidence. Clusters F and G grouped complete control scenarios with very low persistence and an almost nil cumulated incidence. More than 80% of the scenarios lay in clusters F and G.

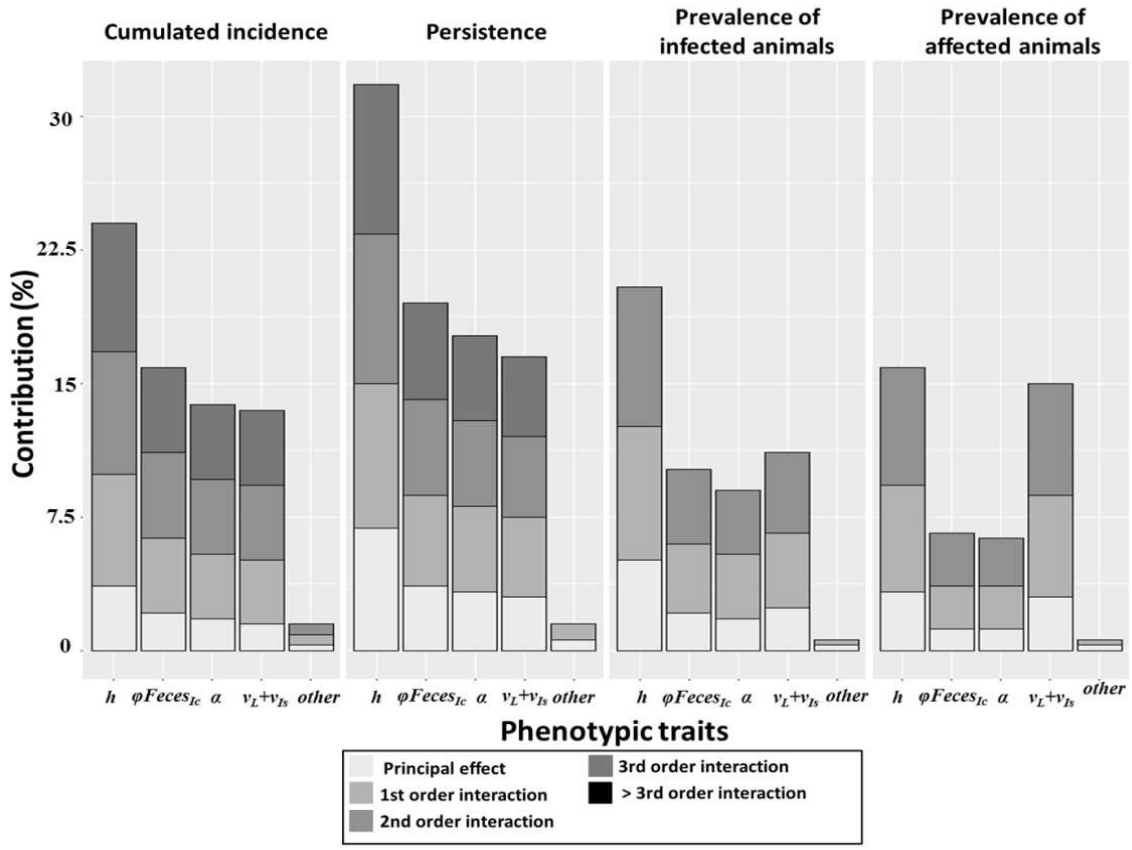

Fig. 3 Contribution of the phenotypic traits to variations of the four model outputs as given by the ANOVA. Definitions of tested parameters are listed in Table 1

The ANOVA performed on scenarios of the multivariate design identified four strongly influential phenotypic traits (listed from most to least influential; Fig. 3): (i) increase in resistance acquisition speed (h), (ii) decrease in quantity of MAP shed in faeces by heavy shedders or clinically affected animals ($\varphi Faeces_{Ic}$), (iii) increase in duration before heavy shedding and clinically affected state (v_L+v_{Is}), and (vi) increase in infectious dose (α). Interactions between these four phenotypic traits contributed to up to 12% of the variance of the model outputs. Among the 625 scenarios investigating the variations of the four most influential traits, 535 allowed a decrease in the cumulated incidence at 25 years from 619 cases (if no trait was improved) to 25 cases. For example, three scenarios allowed the threshold of 25 cases of cumulated incidence to be reached with moderate improvement of the traits (Fig. 4): (i) a 34% decrease in the quantity of MAP shed in faeces by heavy shedders and clinically affected animals and a 50% decrease in the infectious dose (both at V_1) combined with a doubled duration before heavy shedding and clinically affected state (V_2), (ii) all of the four strongly influential phenotypic traits at their V_1 value (see Tab. 1 for tested values), and (iii) a 34% decrease in the quantity of bacteria shed in faeces by heavy shedders and clinically affected animals and a 50% increase in the duration before entering

the heavy shedding and clinically affected state (both at V_1) combined with a tripled resistance acquisition speed (V_2).

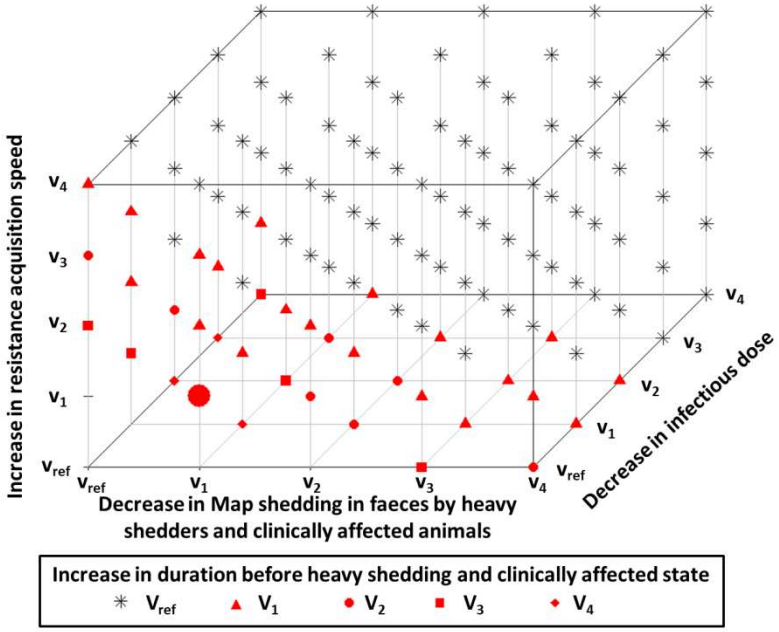

Fig. 4 Minimal increase (among tested values) in the duration before entering the heavy shedding and clinically affected state required per triplet of values (x,y,z axes) for the three other strongly influent phenotypic traits to reach less than 25 cases of cumulated incidence. When there is no symbol in the triplets, it means that none of the tested values for this duration allowed reaching less than 25 cases of cumulated incidence. The large dot (●) represents a combinations of variation in phenotypic traits where x is at V_1, y is at V_{ref} and z is at V_1, the fourth trait (increase in duration before heavy shedding and clinically affected state) should be at V_2 or higher to have less than 25 cases of cumulated incidence (see example (i) in the main text).

DISCUSSION

Variations in four out of the 16 phenotypic traits of resistance to paratuberculosis strongly influenced MAP-spread dynamics in a dairy cattle herd: (i) increase in resistance acquisition speed, (ii) decrease in quantity of MAP shed in faeces by heavy shedders or clinically affected animals, (iii) longer incubation period, and (vi) increase in infectious dose. Combinations of these traits contributed up to 12% of the model output variance, which denoted a real added-value of selecting for several influential phenotypic traits simultaneously.

The increase in resistance acquisition speed was the most influential phenotypic trait, a finding in agreement with Van Hulzen et al. (2014). The increase in latency period was highlighted in this previous study as the second most influential trait. Our study did not show an influence of this trait when varying it without delaying the heavy shedding and clinically affected state, though lengthening the incubation period (i.e. the duration before entering the heavy shedding and clinically affected state) was influential. The decrease in the level of

MAP shedding in faeces by heavy shedders and clinically affected animals was not tested previously, but it was shown here to considerably influence MAP spread. This trait is of double interest: firstly, it can serve as a potential target for genetic selection; secondly, it can be managed using current control measures used in the field, such as hygiene and test-and-cull measures. Finally, the increase in infectious dose was highlighted as being influential, which also was identified by Van Hulzen et al (2014).

This study identified several combinations of the four most influential phenotypic traits of resistance to paratuberculosis in cattle, which allowed for a cumulated incidence of fewer than 25 cases over the 25 years of simulation to be reached. This is close to a situation where the disease can be considered as under control. Such a cumulated incidence could not be reached in our simulations when varying a single trait. This result confirmed that future genetic selection for resistance to paratuberculosis in dairy cattle should focus on combinations of several traits.

In our model, only calves younger than 1 year of age could be infected by MAP, yet a recent study noted that adult cows still are susceptible to paratuberculosis (Espejo et al., 2013) However, adult infections as described in this study correspond to rare events occurring when a naïve cow is introduced into a highly contaminated herd (Pradhan et al., 2011), which is not the case for animals present already in the herd at the start of the infection spread. In addition, our simulated herd was assumed to have no contact with other herds. Taking into account the possible reintroduction of MAP into the herd (e.g. by buying an infectious animal) could increase the disease persistence and influence of MAP spread in the herd. However, it is expected that it would not influence the identification of relevant phenotypic traits as found here.

ACKNOWLEDGEMENTS

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EVALUATION OF ADAPTIVE TEST STRATEGIES FOR CONTROL AND ERADICATION OF PARATUBERCULOSIS WITHIN DAIRY CATTLE HERDS

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SUMMARY

Paratuberculosis is a chronic infection caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP). A long subclinical phase challenges the test strategies and interpretation of diagnostic information. A bio-economic herd simulation model was used to adapt the sampling interval in response to the estimated true prevalence in the herd, so the farmer could reduce the prevalence to a given tolerance level focusing on profit maximisation. When the prevalence was below the tolerance level, the sampling interval was longer, and when the prevalence was above the tolerance level, the sampling interval was shorter. The results showed that the adaptive test strategy could be used to reduce the prevalence in the simulated herd, which depended on both the sampling interval and the tolerance level used. Moreover, the simulations showed a potential for saving costs for testing when combining the adaptive strategy and a reduced risk-based test strategy, while still preserving disease control.

INTRODUCTION

Paratuberculosis is a chronic infection caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP). It is widely spread in dairy cattle in Europe (Nielsen & Toft, 2009). Transmission is mainly via MAP shed in the environment, and infected animals are often infected as calves, because the susceptibility decreases with the age of the animal (Sweeney, 2011). Paratuberculosis has a long subclinical phase during which infected animals can shed MAP (Whitlock et al. 2000). MAP-specific antibody ELISA for milk and serum samples, bacteriological culture and PCR on faecal samples exist for detection of infected and infectious animals (Nielsen, 2014). None of these tests are perfect, particularly for discrimination between infected and non-infected, and infected and infectious stages. However, test-positivity is highly associated with the probability of being infectious, and consequently the timing of testing is essential when constructing test-strategies (Nielsen & Toft, 2006). Subclinical animals can have a lower milk yield and body weight, thus reducing the farmer's income from milk and slaughter values (Ott et al., 1999). In the clinical phase, the animals have severe diarrhoea and will eventually die.

One way forward in a control strategy is to use a strict test scheme to continually screen cows for MAP infection (Whittington & Sergeant 2001, Sergeant et al. 2008). This can help the farmer to identify infectious or affected cows for culling before they negatively impact

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the herd. In Denmark, the ID Screen ELISA test (IDvet, Grabels, France) is used on milk samples in the voluntary national control programme for paratuberculosis. Lactating cows are sampled quarterly and cows are classified into three risk categories: high-, medium- and low-risk cows, based on the repeated ELISA results (Nielsen et al., 2007). The test costs are covered by the farmers, and some farmers may be reluctant to enter the paratuberculosis programme or stay in the programme when the prevalence has been reduced to a level where the infection no longer appears to affect the production performance. Furthermore, farmers that have always had a low prevalence may not want to spend money on quarterly testing when positive cows are rarely found. The farmer may think that the tests (and associated costs) are to no avail, even if the continual testing helps to keep the prevalence low.

This study evaluated a new test strategy that can adapt to the purpose of retaining control of paratuberculosis in a herd, without necessarily eradicating the infection (Kirkeby et al. 2016b). The strategy includes automatic adjustment of the sampling interval using a long sampling interval when the within-herd prevalence is low, and a shorter sampling interval when the prevalence is above a certain threshold. For the first time, a reduced testing approach was also simulated, where only the test-positive cows in the herd followed the full test scheme and cows with only negative previous tests are tested once per lactation. This reduced testing approach is currently used in the Danish control programme for paratuberculosis. The first objective of the present study was to evaluate the epidemiological and economic consequences of the adaptive test strategy. All scenarios were run in a herd with standard hygiene (and mean prevalence of paratuberculosis, following Kirkeby et al. 2016a). The second objective was to evaluate the efficiency of the reduced test scheme for eradication of MAP in the herd.

MATERIALS AND METHODS

Simulations were performed using the iCull model, a mechanistic, stochastic, dynamic bio-economic model that simulates individual cows in a standard Danish dairy cattle herd with daily time steps (Kirkeby et al. 2016a). The model simulates a realistic dairy farm with lactation curves, somatic cell counts and more details based on data (Græsbøll et al. 2016). All simulations in this study were of a herd with 200 dairy cows over 10 years. A 3-year burn-in period and 500 repetitions were used in all simulations. As the infection progresses in the individual animal, the milk ELISA value increases. The milk yield of the individual cow is therefore adjusted according to the ELISA value (Græsbøll et al. 2014, Kirkeby et al. 2016a). All simulated scenarios described a herd with an initial true prevalence of 5.6% (corresponding to a herd with a median prevalence and thus standard level of hygiene, Kirkeby et al. 2016a). All scenarios in this study used closed herds (i.e. without introduction of livestock), which is common in 50% of all dairy herds in Denmark. This means that the probability of eradication is higher than when an open herd is simulated.

All simulated scenarios used the test-and-cull strategy, which may be sufficient for reducing the prevalence within a herd (Lu et al. 2008, Kirkeby et al. 2016a). For comparison, baseline scenarios (for each of the two herd types) were simulated, reflecting the current strategy for Danish herds with a sampling interval fixed to 3 months. In the adaptive strategy, a prevalence cutoff (PC) is set to reflect the prevalence that the farmer will accept. Lowering the prevalence below the PC can be costly, for example due to increased test costs or costs associated with culling of false-positive reactors. Therefore, the farmer may wish to keep the prevalence low, without necessarily aiming for eradication. In the adaptive strategy, the sampling interval is varied according to the estimated true prevalence in the herd. The true

prevalence is continuously estimated with the Rogan-Gladen approach (Rogan & Gladen, 1978). If the estimated true prevalence drops below the chosen PC, the farmer uses a long sampling interval (LSI). Then, if the prevalence exceeds the PC, the farmer switches to a short sampling interval (SSI). In this study, two different PC levels of 1% and 5% were simulated (Table 1). Summary information of the number of simulated days before switching to LSI (when the estimated prevalence was lower than the chosen PC) was saved during the simulations. Likewise, it was recorded if the farmer switched back to SSI (meaning that the prevalence went above the chosen PC again). The probability of switching to LSI, as well as the probability of switching back to SSI, was then calculated from the 500 repetitions of each scenario. Lastly, the probability of eradication of MAP was also calculated from these data.

Table 1. Parameters used in this study

Parameters	Setting	Unit
Long sampling interval (LSI)	365/730[a]	Days
Short sampling interval (SSI)	31/91/182	Days
Initial prevalence	5.6	%
Prevalence cutoff (PC)	1/5	%
Reduced testing	On/Off	

[a]Reduced testing was not combined with LSI = 730 because of the reduced testing approach uses a one-year cycle

Besides the adaptive test scheme, it was also simulated that the farmer could choose to use a reduced testing approach. This reflects a current alternative test strategy in Denmark, where farmers can choose to test cows just once per lactation, but cows that receive a positive test result (which may be true positive or false positive) still have to be tested every third month. In this way, the farmer saves costs for testing because only positive cows will follow the full test scheme while the rest will follow the reduced test scheme. Cows in the reduced test scheme are sampled at least 180 days prior to calving. The reduced testing approach was combined with the adaptive test scheme to evaluate the efficiency of both (Table 1).

The iCull model is a bio-economic model, and is therefore able to retrieve economic information in each scenario, in particular the costs of testing. The economic calculations in the model are further described in Kirkeby et al. (2016a).

RESULTS

Generally, the higher the PC, the higher the prevalence was at the end of the simulations (Fig. 1). Likewise, the longer the LSI and SSI, the more time it took to reduce the prevalence. When the PC was set to 1% in the scenario with SSI/LSI = 31/365, the median number of simulated days before switching to LSI was 849. This was the fastest among all the scenarios, excluding the reduced testing scenarios. The probability of reaching LSI within the 10 simulated years was generally high – between 64% and 100% in all simulations (Table 2). The probability of switching back to SSI (after LSI was reached) was between 48% and 94% in the scenario with PC = 1% and between 46% and 81% when PC = 5% (Table 2).

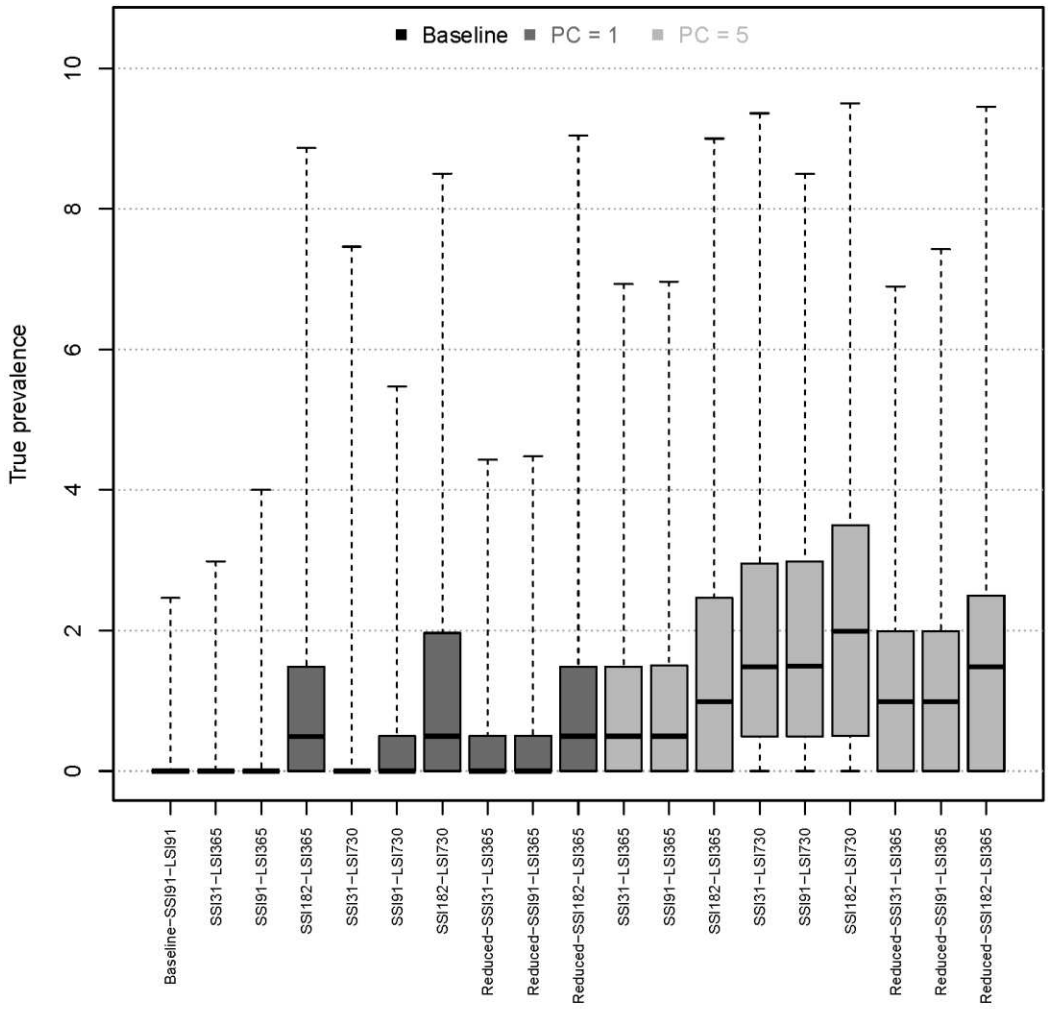

Fig. 1 Boxplot of the true prevalence after 10 simulated years for each scenario. In these scenarios, the farmer chose a prevalence cutoff (PC) as the maximum tolerable MAP prevalence. When the prevalence exceeded this value, a short sampling interval was used. When the prevalence was lower than this value, a long sampling interval was used. X-axis labels show the short sampling interval and long sampling interval and whether the reduced testing strategy was used. SSI = Short sampling interval, LSI = Long sampling interval. So in the second scenario the SSI is 31 days, the LSI is 365 days and reduced testing was not used. The solid bar is the median value, the box represents 25th–75th percentiles and the whiskers show the range of values

Use of the reduced testing strategy decreased the probability of eradication when comparing scenarios with the same PC, SSI and LSI. The reduced testing approach did not have any noticeable impact on the probability of switching to LSI. Furthermore, the reduced testing approach generally increased the probability of switching back to SSI after LSI was reached.

Table 2. Results of the simulations. Parameters used in this study. SSI = Short sampling interval, LSI = Long sampling interval, Reduced = Reduced testing strategy. The probabilities of eradication, switch to LSI and back to SSI again were calculated from 500 simulations

Scenario	Days before LSI: Median (5%:95%)	Prob. of eradication	Prob. of LSI	Prob. of SSI again
Baseline		0.87		
PC = 1%:				
SSI31-LSI365	849 (212:1824)	0.92	1	0.94
SSI91-LSI365	1640 (361:3259)	0.78	0.89	0.82
SSI182-LSI365	1911 (452:3364)	0.43	0.64	0.8
SSI31-LSI730	863 (209:1886)	0.79	1	0.72
SSI91-LSI730	1726 (361:3182)	0.69	0.9	0.5
SSI182-LSI730	1927 (452:3364)	0.39	0.71	0.48
Reduced-SSI31-LSI365	246 (119:398)	0.65	1	1
Reduced-SSI91-LSI365	799 (179:1731)	0.61	1	0.98
Reduced-SSI182-LSI365	1585 (270:3364)	0.41	0.81	0.81
PC = 5%:				
SSI31-LSI365	257 (88:553)	0.4	1	0.79
SSI91-LSI365	520 (88:1362)	0.41	1	0.75
SSI182-LSI365	756 (88:2090)	0.34	1	0.7
SSI31-LSI730	248 (88:491)	0.24	1	0.55
SSI91-LSI730	516 (88:1271)	0.24	1	0.46
SSI182-LSI730	717 (88:1908)	0.17	0.99	0.5
Reduced-SSI31-LSI365	153 (88:243)	0.31	1	0.79
Reduced-SSI91-LSI365	270 (88:634)	0.32	1	0.81
Reduced-SSI182-LSI365	475 (88:1726)	0.29	0.99	0.79

In the baseline scenario, approximately 2750 EUR were spent yearly on ELISA tests. This cost was reduced in all scenarios with PC = 1%, except those with SSI = 31. When PC = 5%, all scenarios reduced the costs for testing compared to the baseline (Fig. 1). All the reduced testing scenarios had lower costs than the equivalent scenarios without reduced testing. Increasing the LSI to 730 days reduced the costs for testing in the normal hygiene herd. This was also true for the level of PC, so that higher PC reduced the costs for testing.

DISCUSSION

This study showed that the adaptive test strategy is a feasible way to save costs for testing while still controlling paratuberculosis within a dairy farm, supporting previous results (Kirkeby et al. 2016b). In the baseline scenario, the costs for testing were about 2,750 EUR per year. In the scenario where PC was set to 1%, SSI = 3 months and LSI = 1 year, the normal hygiene herd could generally save 500 EUR yearly for testing (Fig. 2). If this strategy

was combined with the reduced testing approach, the costs for testing were around 1,200 EUR, saving about 1,550 EUR yearly (Fig. 2).

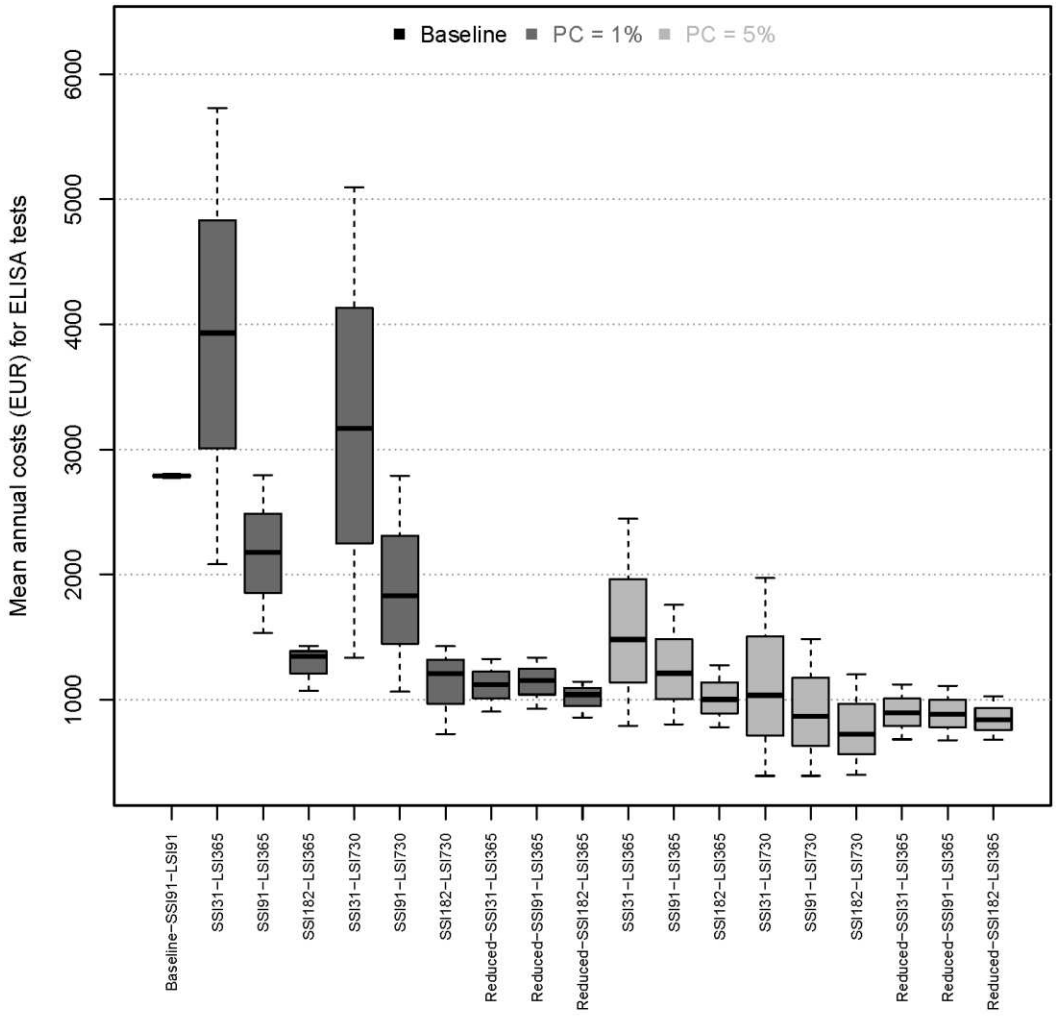

Fig. 2 Boxplots of the mean annual expenses (EUR) for ELISA testing for each scenario. X-axis labels show the short sampling interval, long sampling interval, prevalence cutoff and whether the reduced test scheme is used. SSI = Short sampling interval, LSI = Long sampling interval. So in the second scenario, the SSI is 31 days, the LSI is 365 days and reduced testing was not used. The solid bar is the median value, the box represents 25th–75th percentiles and the whiskers show range of values

When combining the reduced test approach with the adaptive strategy, the costs for ELISA testing were generally reduced (Fig. 2). However, the reduced test strategy was not as efficient in decreasing the prevalence (Fig. 1). The individual farmer must decide how to balance between reducing test costs and prevalence in the herd. This consideration should also include the speed of decrease in prevalence and the likely economic effect of the chosen strategy. This study described some new tools to perform this task, namely PC, SSI, LSI and reduced testing. The likely outcomes of using the different strategies in a typical Danish herd are provided in this paper, and should be used as a tool allowing farmers to prioritise.

Increasing the PC resulted in an increase in the prevalence at the end of the simulations. However, it also dramatically reduced the costs for testing, especially in the scenarios with long test intervals (Fig. 2). If a farmer believes that reducing the prevalence to 5% is achievable and realistic, he may use the results from this study to decide on a higher PC. This can be an important first step towards the control of paratuberculosis within a herd. Farmers have to prioritise their efforts against different diseases, and a more relaxed control scheme could potentially be an affordable method to get more farmers to control paratuberculosis. Another argument for using the adaptive test approach is to keep farmers within a control programme. Once the prevalence is close to zero, the farmer may want to leave the control programme because the prevalence of MAP is low and he must prioritise other diseases, etc. However, if the adaptive test scheme is used, the costs for testing will also be low, increasing the incentive to stay in the programme, but with a purpose that more closely resembles surveillance than control.

Long SSI intervals caused the prevalence to increase, because no tests were performed for long periods. This negates the advantages of repeated testing, which has generally been an asset of the Danish paratuberculosis control programme. When the SSI was decreased from 3 months to 1 month, no noticeable change could be seen (Fig. 1). This is probably because the SSI of 3 months is really good for identifying infected animals. In a herd with lower hygiene (thus higher prevalence of MAP), the results will likely be very different because it is often not possible to prioritise culling of MAP cows only (Kirkeby et al. 2016a).

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ZOONOSES AND ANTIMICROBIAL RESISTANCE

QUANTITATIVE RISK ASSESSMENT OF CAMPYLOBACTER IN BROILER CHICKENS - ASSESSING INTERVENTIONS TO REDUCE THE LEVEL OF CONTAMINATION AT THE END OF THE REARING PERIOD

M. CROTTA*, M. GEORGIEV AND J. GUITIAN

SUMMARY

A standard broiler flock was reproduced and used as a baseline to simulate the effects of different mitigation strategies on the proportion of highly contaminated flocks (%HCFs) entering the slaughterhouse. The baseline model predicted 18.8% of HCFs. A positive effect was attributed to enhanced biosecurity, while adopting the thinning practice had a negative effect. When both effects were tested simultaneously, results were not conclusive with an effect on %HCFs ranging from -20.21% to +77.65%. When mitigation strategies operating on *Campylobacter* concentration in the intestine were tested, a reduction of 100% and 99.6% in %HFCs were estimated following a generic treatment with bacteriocins and bacteriophages, respectively. Reduction in %HCFs as a function of immunisation measures were explored and a reduction of 15% in the rate of transmission led to a %HCFs reduction of almost 50% at slaughter. The main parameters and assumptions underlying the baseline model were discussed by sensitivity analysis.

INTRODUCTION

Chicken meat is a well-known source of *Campylobacter*; in 2010 the European Food Safety Authority (EFSA) estimated that between 20% and 30% of the total cases of campylobacteriosis across the EU could be attributed to chickens and chicken meat (EFSA, 2010). In 2011, the Panel on Biological Hazards issued a scientific opinion on control options and performance objectives and/or targets at different stages of the broiler meat chain. The major conclusions were: (i) there is a linear relationship between the prevalence of *Campylobacter* in broiler flocks and public health risk and (ii) reducing the numbers of *Campylobacter* in the intestines of chickens at slaughter by 3 log CFU/g units would reduce the public health risk by at least 90%. Although the linearity of the relationship should be considered a simplification and interpreted cautiously, a recent review supports the hypothesis that mitigation strategies aimed at reducing the level of contamination of the birds entering the slaughterhouse would result in a significant reduction of the risk for human health (Meunier et al., 2016). Following these considerations, the aim of this study was to quantify the effect of farm-level mitigation strategies on the level of contamination of broiler flocks at depopulation. The proportion of Highly Contaminated Flocks (%HCFs) sent to

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slaughter was used as unit of comparison. The threshold used to define a flock as 'highly' contaminated (5.09 log CFU/g) was previously adopted by Georgiev et al. (2016) and used in their epidemiological study exploring factors associated with the risk of a broiler flock being highly colonised at slaughter in the UK.

Three categories can be distinguished among those explored: (i) management practices aimed at reducing the exposure of chickens to the pathogen (enhancement of biosecurity, avoidance of partial depopulation, early final depopulation), (ii) interventions aimed at increasing the resistance of broiler chickens to colonisation (e.g. through vaccination or use of feed additives) and (iii) mitigation strategies aimed at reducing the pathogen's load in the caecal contents of infected birds (bacteriophage therapy and bacteriocins). The assessment was made by developing a baseline probabilistic model aimed at capturing the dynamics of within-flock transmission of *Campylobacter* in a typical broiler chicken flock and comparing the proportion of HCFs obtained under baseline conditions with that obtained when different strategies were implemented.

MATERIALS AND METHODS

Baseline model

The baseline model aimed to estimate the proportion of flocks with an average contamination level higher than 5.09 log CFU/g as a function of the within-flock prevalence (*WFP*) and the individual level of contamination in colonised birds. The assessment of the mitigation strategies affecting the pathogen's load in the caecal contents of infected birds was made by adopting the overall effects of the interventions already summarised by EFSA (EFSA 2011). The *WFP* can be expressed as the ratio between the number of birds colonised with *Campylobacter* over the total number of birds in the flock. This value is calculated at the day of final depopulation (*dpday*) and assumed to be dependent on (i) the age or day of the cycle at which the flock became colonised ($Cday^{+}$) and (ii) the spread of *Campylobacter* within the flock following colonisation, measured as the rate at which non-colonised birds become colonised.

In the model, $Cday^{+}$ defines the moment at which the spread starts, which is in turn dependent on a number of biological variables such as the total number of birds in the flock (*Nb*) and the number of infected birds at t_0 (It_0).

The age at which the flock became infected: The dynamics describing the broiler becoming colonised by *Campylobacter* and the time at which this occurs in a typical broiler flock are largely unknown. Applying a Bayesian model to several longitudinal datasets on *Campylobacter* infection in UK broiler flocks, Goddard et al. (2014) estimated that the time at which a flock becomes infected ranges between 10 and 45 days, with a most likely value around 30–35 days (Goddard et al. 2014). Accordingly, the day of colonisation ($Cday^{+}$) was modelled as:

$$Cday^{+} = Pert(Min, Max, Most\ likely) \quad (1)$$

Where Min=10, Max=45 and Most likely is a Discrete (30,31,32,33,34,35). In total, 10,000 iterations were run and the cumulative distribution obtained for $Cday^{+}$ was used to estimate the daily probability of a flock becoming infected. Therefore, the chances that each day has to be $Cday^{+}$ were finally modelled as:

$$Cday^{+} = Discrete(10,..,dpday; p_{10}^{+},\ldots,p_{dpday}^{+}) \quad (2)$$

Where *dpday* is the day of final depopulation and p^{+}_{10} … p^{+}_{dpday} are the estimated probabilities according to Eq. (1).

Spread of infection: The spread of *Campylobacter* within the flock following its colonisation on $Cday^{+}$ was assumed to exhibit logistic growth. The results of two experiments (Van Gerwe et al., 2005) were fitted to a logistic growth curve:

$$Ib_t = \frac{K\ Nb\ Ib_0}{Ib_0 + (kNb - Ib_0)e^{-rate*t}} \quad (3)$$

Where Ib_t is the number of colonised birds at time t, Nb is the flock size, K the carrying capacity of the environment (assumed equal to 1) and *rate* is the coefficient representing the growth rate of colonised birds in the total population. The parameterisation of a logistic function was already used in a previous work (Katsma et al., 2007), where *rate* was estimated extrapolating from the original work the actual number of infected birds in the population at each data point. Using the original dataset, the hypergeometric process was used to include the uncertainty surrounding the number of infected birds detected in each sampling time given the sample size. Having obtained the distributions describing the number of colonised birds at each sampling point allowed the simulation of alternative outcomes for each i^{th} sampling event: 10,000 simulated datasets were fitted to the logistic growth function (Eq. (3)) and as many values for *rate* were obtained. To parameterise the distribution describing the uncertainty in *rate* from the obtained values, the Maximum Likelihood Estimation (MLE) method for a Gamma distribution was used.

Within-flock prevalence estimation: In each simulated scenario, the *WFP* was defined as the predicted proportion of infected birds on *dpday*. The probability distribution describing the *WFP* was obtained through the simulation of 500,000 production cycles in which $Cday^{+}$ was randomly sampled according to Eq. (1), and the spread of the infection modelled by fitting a logistic growth model in which the coefficient *rate* was sampled from its uncertainty distribution.

Infected birds in infected flock at slaughter: The actual number of infected birds in the flock *N(Ib)* was estimated after each iteration as:

$$N(Ib)_i = Nb * WFP_I \quad (4)$$

Level of contamination of the flock: The level of contamination of the flock is generally estimated by bacteriological count of a number of pooled caeca (N_c) randomly sampled at the slaughterhouse. Therefore, the final result can be assumed to be a function of: (i) the number of contaminated caeca sampled and (ii) the level of contamination in a positive sample. The hypergeometric process was used to estimate the number of contaminated caecal samples (N_c^{+}) as a function of *Nb*, N_c and $N(Ib)_i$:

$$N_c^{+} = Hypergeometric\ (N_b; N(Ib)_i; N_c) \quad (5)$$

Level of contamination in caeca: The ability of *Campylobacter* to reach high level in caecal contents after infection has been widely reported (Shanker et al., 1990; Nauta et al., 2005; Uyttendaele et al., 2006). The intestinal carriage of *Campylobacter* in contaminated

chicken carcasses entering the slaughterhouse (C_c) was estimated from a previous study (Rosenquist et al., 2006) and assumed to be adequately described by the normal distribution:

$$C_c = Normal(\mu_c; \sigma_c) \quad (6)$$

The final level of contamination of the flock (*Fl*) was inferred from the estimated level of contamination of a standard pooled sample of 10 caeca samples/batch using the central limit theorem.

Baseline settings and risk outputs

In the baseline model, 500,000 infected flocks were simulated. It was assumed that each flock was raised in a broiler house with 20,000 birds (*Nb*) under a standard biosecurity (B-) and without partial depopulation (T-). The simulation was initiated assuming that the infection was due to one initially colonised chicken (Ib_0=1) and according to the industry dataset (Georgiev et al., 2016), day 38 of the cycle was selected as the most likely day of clearance (*dpday*). At the end of the simulation, the cumulative probability distribution obtained for *Fl* was used to estimate the expected %HCFs at slaughter. Once the baseline output was obtained, different management conditions and mitigation strategies were tested and results compared to the baseline scenario. Moreover, in order to assess the relative effects on the output of the inputs described by probability distributions ($Cday^+$; C_c; *rate*), a sensitivity analysis was performed and tornado charts were used to show the inputs ranked by effects on the output mean.

Measures to prevent chickens' exposure: Enhanced biosecurity

The relationship between enhancement of farm biosecurity and risk of flock colonisation has been established, among others, in a recent epidemiological study (Georgiev et al., 2016) where the adjusted Relative Risk (RRa) was obtained. Results of that study indicate that batches raised under standard biosecurity are significantly more likely to be colonised at high level than batches raised under enhanced biosecurity (RRa= 1.30 (95% CI: 1.05 – 1.48). Since the baseline model assumed a standard level of biosecurity (B^-), the effect of enhanced biosecurity on the proportion of highly contaminated flocks at slaughter was obtained using the RRa as multiplicative coefficient as follows:

$$P(B+T-) = P(B-T-)*1/RRa_{(B-)} \quad (7)$$

Where, (B-T-) is the proportion of highly contaminated flocks obtained from the baseline model. In this case, the scenario (B+T-) estimates the proportion of highly contaminated flocks at slaughter if all the infected flocks were grown under enhanced biosecurity management.

Measures to prevent chickens' exposure: Thinning

The estimated RRa for the factor of thinning (T+) resulted 1.55 (CI 1.18-1.87) for the flocks grown under enhanced biosecurity management. In the baseline model, the partial depopulation was not practised, so the effect of thinning on the proportion of highly contaminated flocks was estimated through the scenario (B-T+), in which 100% of the flocks were thinned before the end of the production cycle:

$$P(B-T+) = P(B-T-)*RRa_{(T+)} \quad (8)$$

An additional scenario (B+T+) was also assessed, in which the flocks were all assumed to be partially depopulated and raised under enhanced biosecurity measures.

$$P(B+T+) = P(B-T-)* RRa_{(T+)}*1/RRa_{(B-)} \quad (9)$$

Measures to increase resistance to colonisation

Interventions aimed at increasing resistance to *Campylobacter* colonisation include the use of additives such as organic acids and phytocompounds in drinking water or feed, vaccination, and selective breeding. Those measures are expected to reduce or even prevent colonisation. In either case, the result would be a reduction in the number of birds being colonised and therefore, the *WFP*. Assuming that those strategies would exert their effects on the spread of infection (*rate*, Eq. (3)), the reduction of *WFP* was assessed as a function of the expected increase of the resistance to colonisation. To this end, the increase of resistance was represented as a decrease in *rate,* and results of %HCFs in scenarios with the parameter arbitrarily decreased by 1%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70% were compared.

Measures to reduce the microbial load in colonised animals

The interventions aimed at reducing the bacterial load in infected birds have been recognised as important on-farm mitigation strategies to reduce the average microbial load in contaminated flocks at slaughter (EFSA, 2011) and the available options such as the use of bacteriophage and bacteriocins have been very recently reviewed (EFSA, 2011; Robyn et al., 2015; Meunier et al., 2016). The efficacy of those interventions depends on a number of biological and technical factors, and their effect is still difficult to estimate quantitatively (Hermans et al., 2011). For this reason, a generic modelling approach to evaluate the reduction in %HCFs at slaughter due to a reduction in the pathogen's load in intestines was performed to assess the potential benefit of interventions with this general aim. Adopting the values reported in the EFSA scientific opinion (EFSA 2011), the assumed effects on *Campylobacter* reduction in intestines of colonised birds were fixed to 3 log CFU/g and Uniform(5,1;5,9) log CFU/g for a generic treatment with bacteriophages and bacteriocins respectively. In the model, both mitigation strategies affecting the level of contamination in infected birds are assumed to act on individual μ_c (Eq. (6)). Considering that the reductions in caecal load described above are only rough approximations of the expected effects and that the effect of such mitigation strategies on %HCFs cannot be directly inferred by coefficients, simulations were used to explore the general relation describing the changes in %HCFs as a function of: (i) the expected reduction on the caecal load and (ii) the *WFP*.

Uncertainty in the baseline scenario

The effects of the interventions under investigation on %HCFs were estimated by comparing the outputs of the different scenarios obtained by means of Monte Carlo simulations with that of the baseline. The effects were estimated using a standard broiler flock as a baseline. Therefore, certain flock characteristics were assumed and although the production process of broiler chickens is highly standardised, in reality, some inputs such as *Nb, rate* or *dpday* might be different among the farms. The same applies to It_0 at $Cday^+$. Those inputs are expected to have an impact on the *WFP* and consequently on *Fl* and %HFCs. To quantify those effects, the baseline values were replaced by distributions describing the variability and the uncertainty surrounding the parameters. The output of the model obtained with those inputs was used to perform a sensitivity analysis and tornado

charts were used to represent $Cday^+$, *Nb*, It_0, *Cc, dpday* and *rate* ranked by effect on the output mean. The distributions describing *Nb* were obtained assuming a conservative discrepancy of ±100% from the baseline information, while the effect of the uncertainty surrounding the initial number of shedders was tested assuming that It_0 may range from 0.05% (It_0=1) to 5% (It_0=1,000) of the total population. The day of final depopulation depends on several biological, economical and practical factors; industry data were used to estimate the parameters (Minimum; Most Likely; Maximum) of the Pert distribution describing the uncertainty in *dpday*.

RESULTS

Baseline model

The probability distribution describing $Cday^+$, indicates that the day of infection has 35.67% chance of falling within the range 10-28, 73.06% within the range 10-35 and 98.35% within the range 10-42.

Following the estimation of the parameters obtained by the MLE, the Gamma distribution describing the *rate* was:

$$rate = Gamma(652.2; 0.0010) \quad (10)$$

The distribution shows a mean of 0.698 with a standard deviation of 0.027.

Over 100,000 simulated flocks, the *WFP* at slaughter was 46.35% on average. The *WFP* was below 50% in 72.4% of simulated scenarios and close to 90% at the 90th percentile. The average value recovered for *Fl* was 1.83 log CFU/g, with a standard deviation of 2.7 log CFU/g. The %HCFs was 18.8%.

Result of the sensitivity analysis (Fig. 1) clearly shows that the $Cday^+$ is the input with the greater influence on the output.

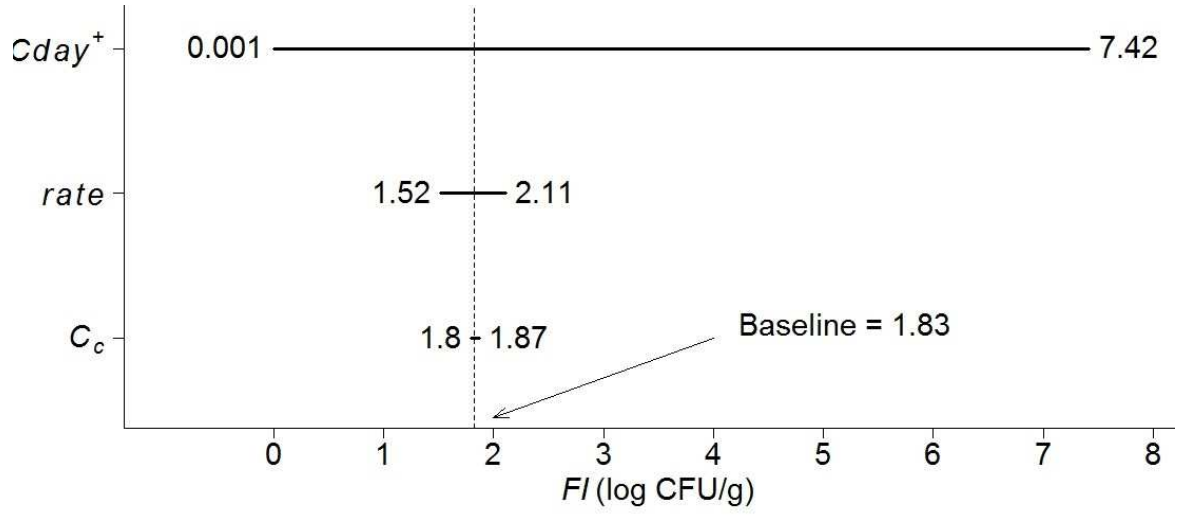

Fig. 1 Tornado chart representing the model inputs ranked by effect on the output (*Fl*) mean

Effects of mitigation strategies

The estimated %HCFs for the scenarios in which enhanced biosecurity (B+T-), partial depopulation (B-T+) or both management options (B+T+) were enabled are reported in Table 1. The confidence limits associated to the RRa of the factors under investigation were used in Eq. (7) – Eq. (9), therefore, the 'best' and the 'worst' scenarios reflecting the uncertainty surrounding the estimates were reported. Interestingly, when both the biosecurity measures and the thinning practice were adopted, the combined effect of the factors was not conclusive. In fact, the uncertainty surrounding the effects led to a reduced and increased proportion of highly contaminated flocks when the best and the worst scenarios, respectively, were assessed.

Table 1. Resulting %HCFs at slaughter when the effect of management conditions affecting the introduction of pathogen and/or the spread of the infection were simulated. Numbers in brackets represent the ±deviation from the baseline output in percentage

Scenario	Output	Best scenario	Worst scenario
Baseline (B-T-)	18.8%	//	//
B+T-	14.4% (-23.4%)	12.7% (-32.44%)	17.9% (-4.78%)
B-T+	29.1% (+54.78%)	22.1% (+17.55%)	35.1% (+86.70%)
B+T+	22.4% (+19.14%)	15.0% (-20.21%)	33.4% (+77.65%)

Under the assumed effects for on-farm mitigation strategies aimed at reducing the microbial load in colonised animals, the %HCFs decreased by 100% and 99.6% when the use of bacteriocins and bacteriophages were simulated.

The effects on the distributions describing *Fl* (mean, 5th and 95th percentile) and %HCFs of different expected resistance (expressed as decreasing *rate*) against *Campylobacter* colonisation are reported in Table 2.

Table 2. Results obtained for *Fl* and %HCF when the effect of interventions aimed at enforcing the individual resistance to *Campylobacter* were tested. Numbers in brackets represent the ±deviation from the baseline output in percentage

Decrease in rate (% baseline)	*Fl*	5th p.ile	95th p.ile	%HCF
Baseline	1.82	0.00	8.65	18.8%
-1%	1.78	0.00	7.57	18.21% (-3.08%)
-5%	1.61	0.00	7.47	15.34% (-18.34%)
-10%	1.38	0.00	7.26	12.55% (-33.20%)
-15%	1.16	0.00	6.85	9.80% (-47.82%)
-20%	0.94	0.00	6.15	7.22% (-61.58%)
-30%	0.54	0.00	3.83	2.92% (-84.43%)
-40%	0.24	0.00	1.59	0.56% (-97.03%)
-50%	0.08	0.00	0.75	0.01% (-99.92%)
-60%	0.02	0.00	0.00	0.00% (-100%)
-70%	0.01	0.00	0.00	0.00% (-100%)

As expected, %HCFs was greatly affected by the transmission rate; a reduction of 15% in the rate of transmission led to a 50% decrease in the probability of %HCFs at slaughter with respect to the baseline. Similarly, the general relationship explaining the reduction in %HCFs as a function of *WFP* and the reduction of the level of contamination in caeca clearly indicated how more drastic effects are needed from mitigation strategies operating on the individual level of contamination in caeca if the *WFP* is high (Fig. 2).

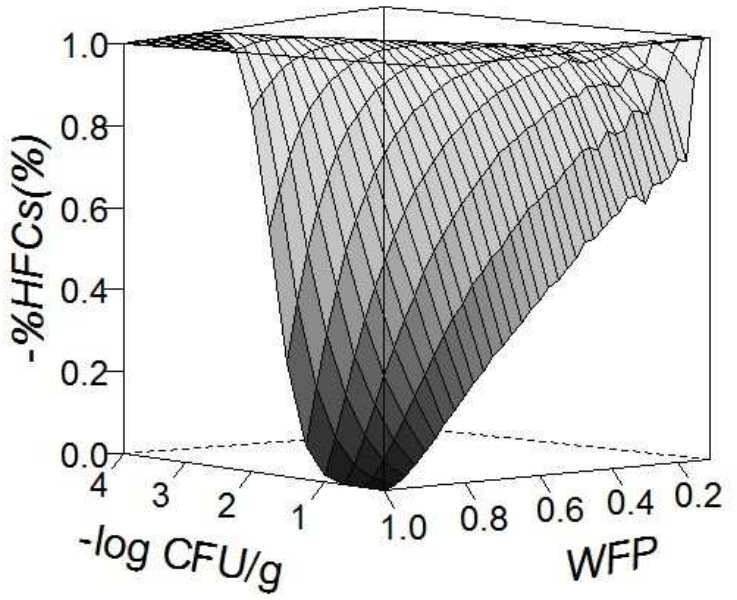

Fig. 2 Graphical reproduction of the change *%HCFs* as a function of: (i) the expected reduction effect on the caecal load (-log CFU/g) and (ii) the within-flock prevalence (*WFP*)

Uncertainty in the baseline scenario

To evaluate the effect that the fixed inputs have on the model output, the baseline values were replaced by the distributions and a sensitivity analysis was performed (Fig. 3). $Cday^{+}$ remained the input with the greatest effect on the output mean.

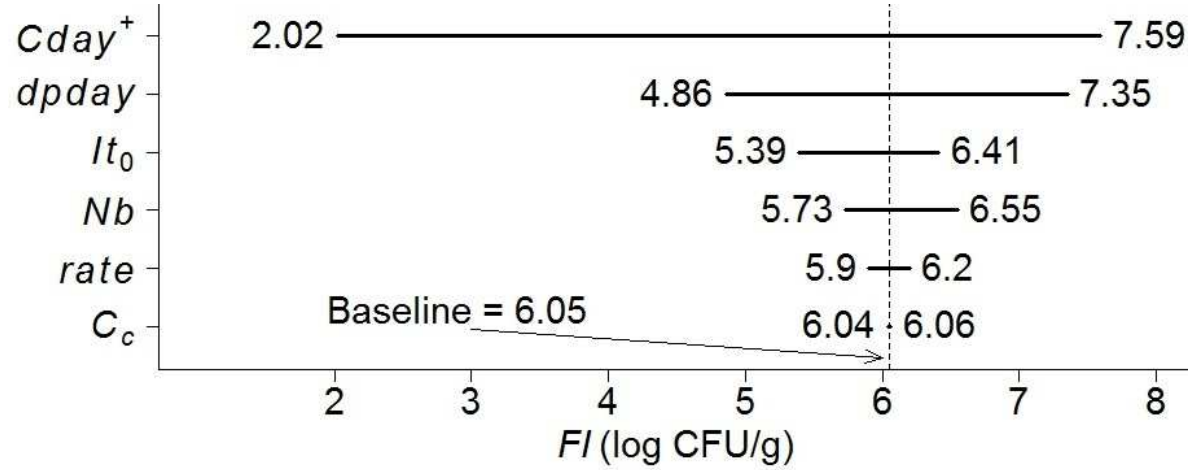

Fig. 3 Tornado chart representing the model inputs ranked by effect on the output (Fl) mean

DISCUSSION

In this work, a stochastic model was implemented that can be used to quantify: (i) the effect of mitigation strategies for which the specific point in time during the cycle and magnitude of the effect are both known and (ii) the effects of factors for which the specific point in time that the effect takes place is unknown but the overall effect on the output at the end of the cycle is known.

When targeted mitigation strategies aimed at reducing the bacterial load were tested, results clearly indicated that under the potential effects assumed by the model, treatment with bacteriocins and bacteriophages were consistently effective in reducing the level of contamination at individual level and thus %HCFs at slaughter. However, great care should be taken when considering these estimations; as previously remarked, the effects of those mitigation strategies were estimated by experimental trials in a controlled environment and might not have captured variability under field conditions. Nevertheless, research efforts on measures to combat the survival of *Campylobacter* in colonised broilers are still ongoing and seem to be promising (Hammerl et al., 2014; Robyn et al., 2015; Gracia et al., 2016; Guyard-Nicodeme et al., 2016), the simple approach proposed to quantify those effects might be easily applied as soon as new evidence becomes available. Similarly, despite the encouraging results of the experimental studies conducted so far, vaccines and other immunisation strategies aimed at enforcing the resistance against colonisation by neutralising and eliminating the pathogen at mucosal level are not yet available (Meunier et al., 2016). Results reported in Table 2 provide a general understanding about the magnitude of the impacts on *WFP* as a function of a generic treatment aimed at decreasing within-flock transmission.

In contrast, the coefficients used to correct the baseline estimation as a function of the adoption of enhanced biosecurity measures or/and the practice of partial depopulation were obtained from an exhaustive epidemiological study conducted in UK in 2014. Therefore, these estimates are likely to represent effects under field conditions. In this respect, it should be noted that the results recovered for the scenarios under investigation (B-T-; B+T-; B-T+ and B+T+) were obtained assuming that all the simulated flocks operated under the same conditions. However, if the actual proportions of flocks operating under different management practices in the population are known, those fractions might be used to weight the results and obtain an estimation of the overall prevalence of HCFs in the whole population.

It can reasonably be assumed that the general effects related to enhanced biosecurity measures are exerted on the parameters governing the *WFP* ($Cday^{+}$; *rate*) rather than *Fl*; this is supported by some recent findings in which chickens kept in an experimental 'bio-secure cube' became infected several days later (or remained *Campylobacter*-negative) than those kept in a standard environment (Battersby et al., 2016). Furthermore, results of a systematic review on on-farm sources of *Campylobacter spp.* concluded that the factors increasing the risk of contamination of a new flock seem to be related to biosecurity aspects such as insufficient cleaning and disinfection, insufficient downtime, and the presence of an adjacent broiler flock (Agunos et al., 2014). Recently, Sommer et al. (2016) conducted a cross-country study to identify on-farm risk factors for the colonisation of broiler flocks with *Campylobacter* and confirmed the on-farm factors associated with the level of biosecurity as significant. On this basis, the general relationship displayed in Fig. 2 can be considered as graphical evidence in support of the benefits that could be obtained when mitigation strategies operating at different levels are applied simultaneously. In fact, if biosecurity

measures or strategies aimed at reducing the *WFP* are in place, less drastic mitigation strategies operating on the level of contamination are required to get a significant effect on the occurrence of HCFs.

The on-farm model, although relatively simple, provided an exhaustive understanding of the dynamics leading to the *WFP* and the *Fl* in infected flocks and the related biological factors involved (i.e. *Nb*, It_0, *rate*, and C_c). The data used by Goddard et al. to parameterise the Bayesian model were collected from epidemiological studies related to commercial broiler chickens in UK. It is therefore believed that the distribution describing $Cday^+$, can be considered as an acceptable approximation to describe the first day of *Campylobacter* infection in the UK broiler chicken flock.

For the transmission of *Campylobacter* within the flock, the logistic growth model proposed by Katsma et al. (2007) was adopted. The main difference is that the Bayesian method allowed us to describe the parameter *rate* as a distribution instead of a fixed value. This gave us the opportunity to formally consider the uncertainty underlying this input and assess its influence on the outcome by means of sensitivity analysis. Furthermore, some baseline information (*Nb*, It_0 and *dpday*) was included in the model as initiative input (Eq. (3), *WFP*) and the potential impact on the outcome as a function of a variation in those values should be taken into account. The sensitivity analysis reported in Fig. 3 clearly showed how variations in those inputs might lead to significant consequences; as a practical example, if *dpday* is brought forward by 2 days or *Nb* decreased by 5,000 units, the baseline proportion of HCFs decreased by 28% and increased by 8.7%, respectively (results not shown).

As in any model aimed at describing the complexity of a real system, some assumptions and limitations are recognised. The first assumption is related to $Cday^+$, where the baseline model assumes that the transmission never starts before the tenth day of the cycle. The sensitivity analysis highlighted the importance of this input, but the threshold assumed by the model finds its justification from epidemiological data and biological characteristics such as passive immunity (Newell & Fearnley, 2003; Lin, 2009). However, if new evidence and data become available, the model can be easily updated operating on Eq. (1). An important limitation highlighted by the sensitivity analysis concerned the effect of the uncertainty related to It_0. The transmission model was initiated assuming one initial infected bird, but in reality the initial number of shedders is likely to be strictly related to the source of contamination.

In this work, all the main aspects involved in *Campylobacter* contamination were explicitly accounted for at flock level and it was shown how expected effects of different mitigation strategies could be included in quantitative risk assessment models. The level of flock contamination at the end of the rearing period is a well-known critical factor with recognised effects on human health. The provided results highlighted how understanding the role and relationships of the individual inputs involved in the occurrence of highly contaminate flocks is crucial. The reported results and the identified relationships together with the structure of the model itself are practical instruments for decision makers.

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SPATIAL DISTRIBUTION OF SMALL RUMINANT AND CATTLE BRUCELLOSIS IN JORDAN AND AN ASSESSMENT OF THE EFFECT OF ENVIRONMENT, DEMOGRAPHICAL FACTORS AND LIVESTOCK OWNERS' PRACTICES

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SUMMARY

Brucellosis remains endemic in many parts of the world including Jordan. In this study, the spatial pattern of ruminant brucellosis and the potential effect of ecological, geographical and demographical factors on the risk of ruminant brucellosis in Jordan using Boosted Regression Trees (BRT) was explored. Ruminant brucellosis is heterogeneously distributed in Jordan. Geographical patterns for cattle and small ruminants showed a higher risk of seropositive status in the northern part of Jordan, which should therefore be prioritised for disease control. The practices of livestock owners were stronger predictors of small ruminant flock seropositivity against *Brucella spp.* than environmental factors or livestock density.

INTRODUCTION

Ruminant brucellosis was first reported in Jordan in 1971 and has remained endemic since then in spite of control efforts (Al-Talafhah et al., 2003; Al-Ani et al., 2004). A recent nationwide cross-sectional study (Musallam et al., 2015a) estimated the seroprevalence in cattle herds and small ruminant flocks at 18.1% (95% CI: 11 - 25.3) and 34.3% (95% CI: 28.4 - 40.4), respectively.

Baseline prevalence estimates are important to decide the best control strategy for brucellosis (FAO, 2009). Moreover, knowledge of demographic and spatial heterogeneities in distribution of the infection and of its potential clustering within certain areas and production systems may allow the targeting of control programs, which is especially important in low-income countries such as Jordan, where resources are limited.

Climatic conditions are important determinants of the proliferation and distribution of vector-borne zoonoses such as leishmaniasis (Samy et al., 2014) and Rift Valley Fever (RVF) (Wilson, 1994). However, climatic conditions are also important for the distribution of non-vector-borne bacterial diseases, as they determine the environmental persistence of the pathogen and therefore transmissibility. For example, *E. coli* O157:H7 adopts different environmental conditions and was proven to be able to survive outside the cattle host (Lim et al., 2010).

The ability of *Brucella* to persist in the environment is determined by the availability of favourable environmental conditions such as pH ≥4, low temperature and high humidity –

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under such conditions the organism remains infective for several months (European Commission, 2001).

The objectives of this study are to: i) assess the potential influence on the risk of seropositive status against *Brucella* spp. of spatially varying risk factors unaccounted for in previous analysis of herd/flock-level risk factors; ii) visualise and explore the spatial patterns of cattle and small ruminant brucellosis in Jordan and iii) assess the relative importance of environmental factors, demographical factors and livestock owners' practices on the serological status of cattle herds and small ruminant flocks against *Brucella* spp. infection.

MATERIALS AND METHODS

Target and study populations and data collection

The target populations were all cattle herds and small ruminant (sheep and goats) flocks in all the Jordanian governorates (n=12). The study population consisted of 333 small ruminant flocks and 204 cattle herds, randomly selected as a part of a nation-wide cross-sectional study that was carried out between March and October 2013 (Musallam et al., 2015a).

For each selected flock/herd, the following data were collected: i) geographical location recorded; ii) data regarding production parameters and health events; iii) data on husbandry and management practices that could influence the risk of introducing or maintaining *Brucella* spp. in the flock/herd, and iv) data on knowledge attitudes and practices (KAP) regarding brucellosis. The serological status of each flock/herd regarding brucellosis was ascertained using indirect ELISA (Brucelisa160M for cow's milk samples and RBT followed by competitive ELISA (COMPELISA) for sheep and goat serum samples.

Influence of spatially varying risk factors on the herd/flock-level serological status against *Brucella* spp.

To assess the potential influence on the risk of seropositive status against *Brucella* spp. of spatially varying risk factors unaccounted for in previous analysis of herd/flock-level risk factors, the residuals from the logistic model used in the multivariate analysis of herd/flock-level risk factors (Musallam et al., 2015a) were obtained, plotted and interpolated using ordinary kriging, implemented in Arc GIS 10.2.2; ESRI, 2014.

Visualisation and exploration of the spatial clustering of cattle and small-ruminant brucellosis in Jordan

To visualise the geographical distribution of the sampled flocks/herds, choropleth maps were created using Arc GIS 10.2.2; ESRI, 2014. The maps represented the distribution of seropositive flock/herd by district. The spatial scan statistic was used to identify significant clusters of seropositive flocks/herds. The analysis was implemented in SaTScan V.8.1.1 (www.satscan.org) using a Bernoulli probability model, a circular window set to contain a maximum of 50% of the population at risk, and Monte Carlo randomisation with 999 permutations. The clusters for these were considered statistically significant where $P<0.05$. The analysis was done for cattle herds and small ruminant flocks separately and then repeated to consider them together.

Assessment of the relationship of environment, demographic factors and livestock owners' practices with serological status against *Brucella* spp.

The explanatory variables that were considered in this study were:

1) Environmental variables:

The available published evidence on the association between ecological and geographical factors and ruminant brucellosis is scarce. Therefore, six different variables based on data availability and the *a priori* plausibility of association with the likelihood of flocks or herds being infected with *Brucella* spp were selected. These variables were:

a) Altitude expressed in meters above sea level (masl).

b) Average annual relative humidity (%)

c) Average annual rainfall (mm)

d) Average annual temperature (^{0}C)

The above data were obtained for the point location of each study herd/flock from the Land-process Distributed Active Archive Centre (LDAAC, 2014; http://eros.usgs.gov/#/Find_Data/Products_and_Data_Available/gtopo30_info).

e) Land cover; there are four main physiographic regions in Jordan: Jordan Rift Valley and Wadi Araba, the Highlands, the Arid Zone (Plains), and Badia (Eastern Desert; Al-Jaloudy, 2006).

f) Climatic zone; there are four bioclimatic subdivisions in Jordan: Mediterranean, Irano-Turanian, Saharo-Arabian, and Sudanian (Al-Jaloudy, 2006).

2) Demographical factors presented as density of the different ruminant species (small ruminants and cattle) at district level, estimated as: number of cattle or sheep and goats divided by the area of the district (km^2). Data regarding the total small ruminants and cattle population in each district were obtained from the annual report of the Ministry of Agriculture of Jordan (MOA, 2013) and the total area of each district was obtained from the Royal Jordanian Geographic Centre (RJGC).

3) Husbandry and health-management practices of livestock owners (including likely actions in the event of abortion in their animals) were obtained from a Knowledge, Attitudes and Practices (KAP) study of livestock owners regarding brucellosis in Jordan (Musallam et al., 2015b). Variables considered were: a) introducing new animals to the flock/herd from other flocks/herds in the previous year, b) whether the newly introduced animals were kept in separate pen, herd or house for a certain period, c) feeding aborted material to dogs d) burning aborted material, e) burying aborted material, f) throwing aborted material in fields or streets, and g) throwing aborted material in water channels. Different variables included in this study are presented in Table 1.

To identify the associations between ecological, geographical factors and livestock owner's practices and the serological status of the studied flocks/herds Boosted Regression Trees (BRT) were used. Boosted Regression Trees were implemented in R 3.2.1 (R

Development Core Team, 2015), package ''dismo'' version 0.8–17 and package ''gbm'' version 2. A bagging factor of 0.5 was used, as suggested by Friedman (2002).

Table 1. Environmental, demographical factors and husbandry and health-management practices that were included in a BRT model for ruminant brucellosis seropositivity in Jordan, 2014

Variable	Description	Data source
Brucella status	Serological status of the flock/herd	Musallam et al., 2015a
Animal density	Density of animals (animal/km^2)	MOA[a], 2013; RJGC[b]
Average temperature	Average annual temperature (°C)	LDAAC[c], 2014
Altitude	Altitude above sea level (m)	LDAAC[c], 2014
Average rainfall	Average annual rainfall (mm)	LDAAC[c], 2014
Relative humidity	Relative humidity (%)	LDAAC[c], 2014
Climatic zone	Climatic zone	Al-Jaloudy, 2006
Land cover	Land cover	Al-Jaloudy, 2006
Animal introduction	Adding new animals to the herd/flock during the last year	Musallam et al., 2015a
Animal separation	Separate newly added animals	Musallam et al., 2015a
Feed dog	Feeding aborted material to dogs	Musallam et al., 2015a
Bury aborted	Burying aborted foetus	Musallam et al., 2015b
Burn aborted	Burning aborted foetus	Musallam et al., 2015b
Throw in streets	Throwing aborted foetus in streets	Musallam et al., 2015b
Throw in water canals	Throwing aborted foetus in water canals	Musallam et al., 2015b

[a]Ministry of Agriculture
[b]Royal Jordanian Geographic Centre
[c]Land-process Distributed Active Archive Centre

In this study, the model was fitted with a Bernoulli distribution because the dependent variable has a binary outcome (seropositive or seronegative). Several combinations of the *lr* (0.001, 0.05, 0.1) and *tc* (3, 4, 5, 6) parameters were tested. The model with the highest cross-validated Receiver Operating Characteristic (ROC) Area Under the Curve (AUC) score was selected. More details and information on the BRT fitting procedure can be found in (Elith et al., 2008).

The analysis was done for both cattle herds and small ruminant flocks separately and then repeated to consdier them together. To assess the collinearity between selected variables, Cramer's phi-prime (Ø) statistic was calculated; variables were considered collinear if Ø >0.7. When a pair of variables was found to be collinear, only the more biologically plausible variable was kept in the analysis.

RESULTS

Residuals of the logistic regression model

The mean value of the residuals of the logistic regression model for cattle herds and small ruminant flocks was -0.05 and -0.02, while the interquartile range was 0.31 and 0.42,

respectively. Figure 1 presents kriging interpolated plots of the residuals for cattle herds (a) and small ruminant flocks (b).

The spatial distribution of the residuals is heterogeneous for cattle herds and less heterogeneous for small ruminant flocks. For cattle herds, there is a variation in the northeastern part of the country, suggesting that the used logistic regression model for cattle did not fit the cattle data very well, and additional factors (rather than those studied in the cross-sectional study) are likely to influence the spatial distribution of the seropositivity of cattle herds against *Brucella* spp.

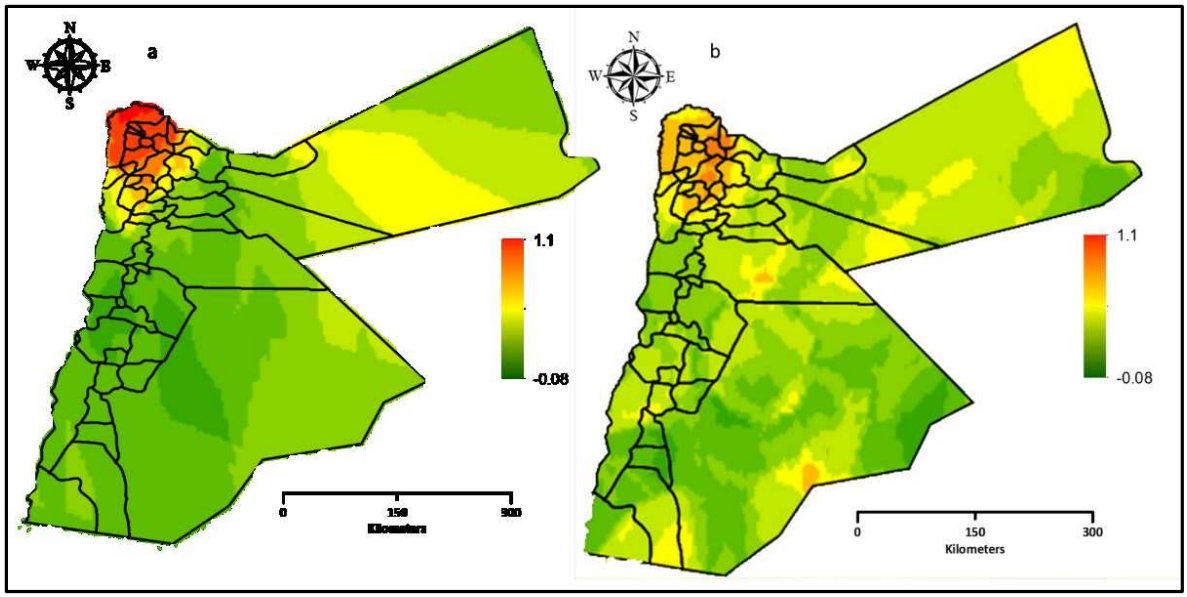

Fig. 1 Kriging interpolated map of the spatial distribution of the residuals from the logistic regression model used for the analysis of risk factors for cattle herds (a) and small ruminant flock (b) seropositivity against *Brucella* spp. in Jordan

Spatial pattern of cattle and small ruminant brucellosis

The point locations of seropositive and seronegative cattle herds and small ruminant flocks are presented in Fig. 2. Results of the spatial scan statistic demonstrate that there were two small-sized significant clusters ($P<0.05$) for seropositive cattle herds, one in the northwest of the country with a radius of 6.59 km ($P=0.005$) and the other in the northeast of the country with a radius of 1.11 km ($P=0.036$). The relative risk for a herd to be positive if located within the area identified as a cluster, relative to the risk of being positive if outside it, was 6.19 and 6.03 for the two clusters, respectively.

For small ruminant flocks, one small-sized significant cluster was detected in the northeast of the country with a radius of 3.78 km. The relative risk for a flock to be positive if located within the area identified as a cluster relative to the risk of being positive if outside it was 4.42 ($P=0.042$). When both cattle herds and small ruminant flocks were considered together in the analysis, one small-sized significant cluster was detected in the northern part of the country with a radius of 3.74 km. The relative risk for a farm to be positive if located within the area identified as a cluster relative to the risk of being positive if outside it was 4.17 ($P=0.03$; Fig. 3).

BRT model

The results of a collinearity analysis showed an association between two of the explanatory variables, namely climatic zone and average rainfall. The BRT model was run considering the two variables together and it was repeated including only one each time; no difference in the outcome was observed, so both were included in the model.

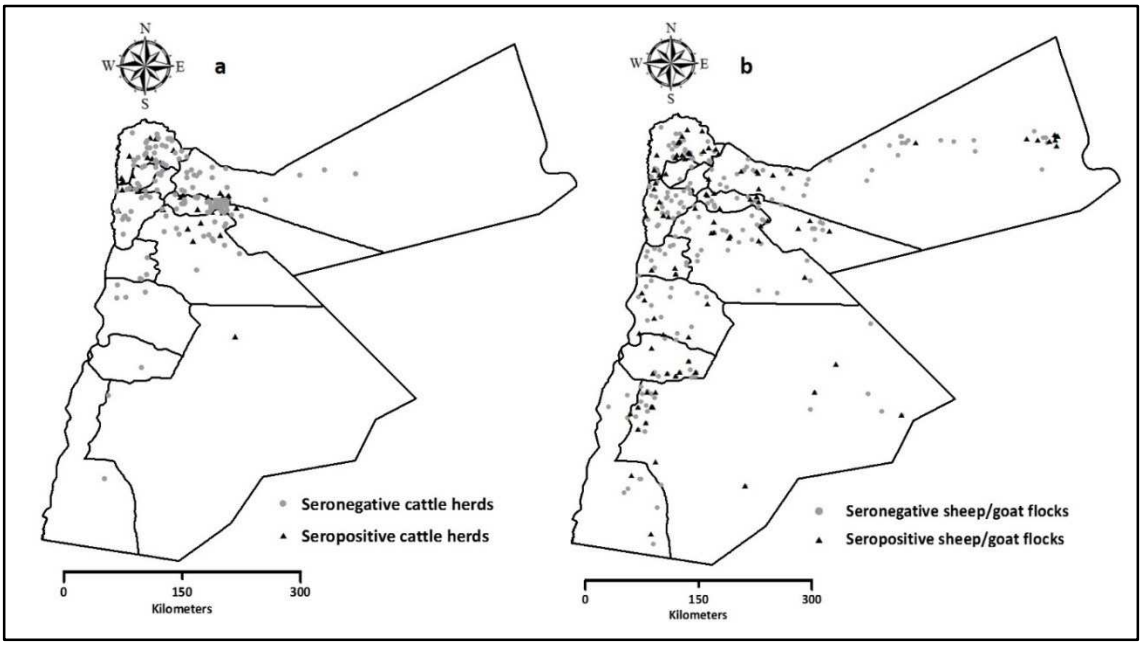

Fig. 2 Point locations of seropositive and seronegative cattle herds (a) and small ruminant flocks (b). One dot on the map may present more than one flock or herd

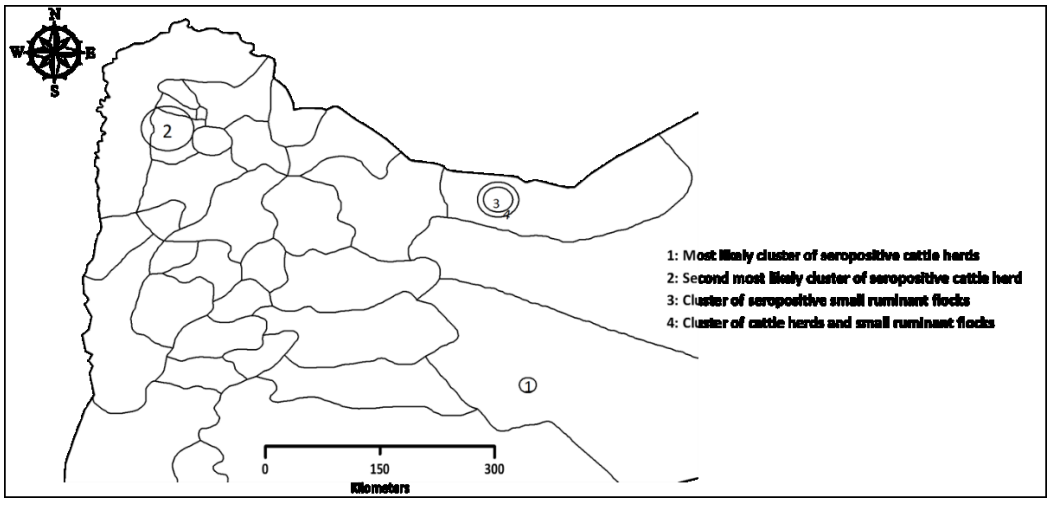

Fig. 3 Location of spatial clusters of seropositive cattle herds, seropositive small ruminant flocks and seropositive farms

When the BRT model was run for cattle herds only (n=204), the number of retrieved regression trees was very small because the number of observations (cattle herds) was low, so the model was run using small ruminant flocks only and then the analysis was repeated

considering cattle herds and small ruminant flocks together. Results of small ruminant flocks only are presented. The selected BRT model was fitted using an *lr* of 0.001, *tc* of 3 and a total of 8,850 decision trees. This combination showed a cross-validated ROC AUC score of 0.952. The relative importance (the % by which each explanatory variable influenced the outcome) of the explanatory variables in the BRT model was identified (Fig. 4). The results show that feeding aborted material to dogs had a relative importance (RI) of 32.7% in increasing the risk of ruminant brucellosis in Jordan, separating newly added animals gained an RI of 30.3% in decreasing the risk of seropositivity against *Brucella* spp. infection and adding new animals from other districts had an RI of 6% in increasing the risk.

DISCUSSION

Brucellosis remains a major public health problem in many countries including Jordan, where some evidence suggests that the number of human cases may be increasing (Abo-Shehada & Abu-Halaweh, 2013) and the estimated seroprevalence in cattle herds and small ruminant flocks is among the highest in the world – 18.1% (95% CI: 11 - 25.3) and 34.3% (95% CI: 28.4 - 40.4), respectively. Control of the disease in humans relies on a reduction in the prevalence and eventual eradication of infection in ruminants, where it is generally accepted that the best strategy will depend on the baseline level of disease and the availability of resources (Benkirane, 2001; McDermott et al., 2013; Okello et al., 2015).

This study represents the first attempt to map and describe the spatial patterns of ruminant brucellosis in Jordan. Previous studies were carried out to describe the spatial patterns of brucellosis for small ruminants in Spain (Mainar-Jaime et al., 2005), Italy (D'Orazi, et al., 2007) and Iran (Haghdoost et al., 2007); for cattle in Brazil (Borba et al., 2013), and for humans in Azerbaijan (Abdullayev et al., 2012) and China (Li et al., 2013). The results of these studies showed that brucellosis tends to have a heterogeneous spatial distribution.

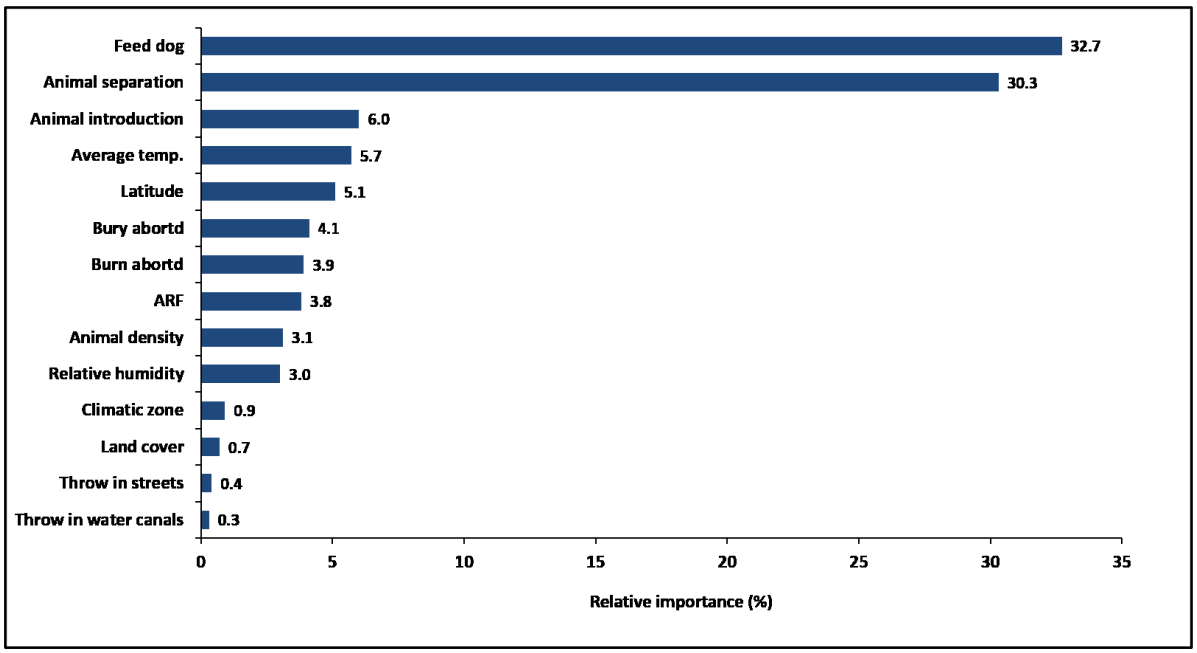

Fig. 4 Relative importance (%) of the explanatory variables included in the Boosted Regression Trees (BRT) model on the serological status of small ruminant flocks against *Brucella* spp. in Jordan. Contributions are presented so that the sum adds to 100

The role of spatially varying risk factors on disease distribution has often been explored for vector-borne diseases such as dengue fever and non-vector-borne diseases such as highly pathogenic avian influenza, the distribution of which is assumed to be driven by environmental determinants (Dhingra et al., 2014). The role of spatially varying environmental risk factors is likely to be less important for a disease such as brucellosis, for which transmission is likely to take place because of relatively close contact between infectious and susceptible domestic ruminants. However, environmental conditions influencing the survivability of the bacteria in the farm environment could be of importance.

The heterogeneous spatial distribution of the residuals of previously developed logistic regression models, described in the cross-sectional study (Musallam et al., 2015a), indicate that the models (particularly the cattle model) did not fit the data very well and suggest the potential effect of additional factors on the outcome (i.e. serological status of the flocks and herds in the northern parts of the country).

Both cattle and small ruminant brucellosis are not homogeneously distributed across Jordan, with a higher proportion of cattle herds and small ruminant flocks seropositive against *Brucella* spp. in the northern part of the country. The high risk of seropositivity within small ruminant flocks in the northwestern districts compared to other districts can be explained by the high density of the cattle population in this area, since most cattle herds are concentrated in the districts within the northern governorates, where the climatic and geographical features are favourable for cattle production (Al-Jaloudy, 2006). In contrast, small ruminant flocks are distributed all over the country, with higher densities in the northeast (Mafraq governorate) and southeast (Ma'an governorate). Although small in size, the detection of significant clusters of seropositive cattle herds and small ruminant flocks by spatial scan statistics in the northern districts was not unexpected, and confirms the localisation of infection in the districts of the northern governorates.

Moreover, the free animal movement between the northern governorates and the illegal trading of small ruminants near the northern border of the country plays a significant role in the persistence of infection in the northern districts. The presence of a significant cluster of seropositive cattle herds in the northeastern districts of the country was expected, because this district (Dolel) has the highest cattle density in Jordan. The other significant cluster in the northwestern districts could result from the presence of communal pastures in this area, where ruminants are allowed to graze together (Al-Talafhah et al., 2003).

The detection of a small-sized significant cluster of seropositive small ruminant flocks in the northeast district of Mafraq governorate can be explained by the presence of many large-sized flocks (>500 animals) and the presence of small ruminant markets where animals are traded.

In this study, BRT models were used because they are more flexible, can model variables of any type and fit interactions between different explanatory variables, making them superior compared with GLM (Elith et al., 2008).

The practices of livestock keepers in the event of abortions in their animals have a high influence in increasing the risk of a small ruminant flock being seropositive (e.g. 33.6% for feeding aborted material to dogs), while biosecurity practices such as separating newly added animals are also highly influential (31%), reducing the risk of small ruminant flock seropositivity. In contrast, the relative importance of environmental and demographic factors on the risk of small ruminant flock seropositivity was low. The results of the BRT analysis

confirmed that ruminant brucellosis in Jordan is highly associated with management and livestock owners' practices, but not geographical and ecological factors which have a smaller relative importance for the distribution of the seropositive flocks.

The low relative importance of small ruminant density (3%) in increasing the risk of flock seropositivity does not contradict the finding of a significant spatial cluster in the northeastern districts because other factors contributed to the presence of this cluster.

The observed spatial patterns suggest that there may be potential for prioritising control or vaccination activities in the northern areas of the country, and the results of the BRT analysis suggest that any control programme will not be efficient unless it is associated with public health education programs that increase the livestock owners' awareness of the high-risk practices.

Although their relative influence in affecting the risk of seropositivity of small ruminant flocks in Jordan was low, environmental factors have been found to be influential in the distribution of natural foci of brucellosis in the European hare (*Lepus europaeus*) in the Czech Republic (Pikula et al., 2005).

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OPTIMISING THE MEASUREMENT OF ANTIMICROBIAL RESISTANCE FOR USE IN EPIDEMIOLOGY

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SUMMARY

Antimicrobial Resistance (AMR) can be measured in different ways. Two classes of measurements (sample-level and isolate-level) from faeces samples of livestock were compared and the agreement of both measurement types was estimated. The sample-level screening provided higher estimates of prevalence than the isolate-level of prevalence. It was proposed that such data offer evidence supporting a reappraisal of measuring AMR for epidemiological purposes. In particular, it was suspected that sample-level screening may, in some epidemiological studies, be more relevant than the commonly used isolate-level screening.

INTRODUCTION

Baseline prevalence estimates are fundamental to most epidemiological questions. Antimicrobial Resistance (AMR) is no different, except that there is no single way of defining AMR at the individual animal level (Davison et al., 2000). Furthermore, some of the possible ways of measuring AMR are fundamentally different in the sense that they are applied to different strata in the host, where "strata" here refers to isolate-level and sample-level screening for resistance to a specific antimicrobial.

Ideally, it would be known how to translate results from one method to another and which measure is most useful for which epidemiological question. An example of the former (how one method of measurement is translated to another - namely from sample-level to isolate level or vice versa) is provided here.

In this study, measures of isolate-level and sample-level AMR for particular antimicrobials are compared in order to demonstrate the difference and the association between the two measures.

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MATERIALS AND METHOD

Sampling

Sub-samples were taken from 41 faecal samples (25 from cattle and 16 from sheep) submitted for routine parasitological (i.e. non-bacterial) screening to the SAC Veterinary Investigation Centre, Inverness between August 2013 and July 2014.

Comparative study

Each sample was 'streak' cultured on three plates: a standard MacConkey plate and two containing antibiotic (ampicillin 16mg/L or nalidixic acid 15 mg/L). The streaking process on the plates involved sequentially streaking sub-samples from one streak to the next, with the result that the concentration of sample decreased with each consecutive streak on a plate (Amyes et al., 1992; Gunn et al., 2008; Humphry & Gunn, 2014).

Where present, one *E. coli* colony from each of the two antibiotic-containing plates was randomly selected resulting in 0-2 "resistant" isolates. From the standard (non-antibiotic) plate, 8-10 colonies were selected to make a total of ten isolates selected per sample. These ten colonies were biochemically confirmed as *E. coli* and tested for the Minimum Inhibitory Concentration (MIC) for ampicillin, and nalidixic acid using concentrations from an appropriate standard with priority given to EUCAST (European Committee on Antimicrobial Susceptibility Testing) breakpoints, or, where these were unavailable (in the case of nalidixic acid), BSAC (British Society for Antimicrobial Chemotherapy) breakpoints (Table 1) were used.

Table 1. The two antibiotics, the concentrations (µg/mL) used in the agar plates for testing the sample using the plate streak method and the MIC breakpoints chosen to determine an isolate's categorisation as sensitive/resistant

Units (µg/mL)	Ampicillin	Nalidixic acid
Concentration used in streak plate	16	15
Sensitive threshold for isolate MIC	MIC [a] ≤8	MIC[b] ≤16
Resistant threshold for isolate MIC	MIC[a] >8	MIC[b] >16

[a]EUCAST, 2015
[b]BSAC, 2012

It is common to assume 100% specificity, that is, when resistance is identified by any method, then it is assumed that the sample contains phenotypically resistant bacteria of the species of interest. Although the assumption of 100% specificity may appear justified, reasons why it may not be so include contamination of the sample, miss-identification of a resistant colony as being of the bacterial species under consideration, incomplete mixing, or denaturation of the antimicrobial agent such that some or all of the substrate does not contain the appropriate concentration. For the purposes of this study, this assumption does not need to be made. Similarly, a 100% test sensitivity was not assumed. That is, not all samples where no resistance was detected were assumed to be truly sensitive to antibiotic.

Comparative data analysis

Only samples from which eight or more validated *E. coli* isolates were identified from the control plate (i.e. without the antimicrobial present) and tested for MIC were included in the

analysis. Where a sample resulted in more than eight isolates being tested (maximum of ten), a sub-sample of eight was randomly selected from these data. Hereafter, these data will be referred to as the comparative study data.

RESULTS

In total, 41 samples were tested. An insufficient number of confirmed *E. coli* isolates were obtained from one sample to be included in the data analysis. Of the remaining 40 samples, three showed resistance by streak plating to nalidixic acid, and fourteen showed resistance by streak plating to ampicillin. The 40 samples all yielded eight or more isolates from the control plate for further testing.

The data demonstrated that there could be a substantial range of sensitivities among isolates picked both within and between samples (ampicillin, Fig. 1). There appear to be two clusters of phenotype when the distribution of MICs is plotted as a histogram (Fig. 1). It was observed that from samples deemed resistant through plate screening, there could be more than one phenotype of bacterium present, but from samples deemed sensitive through plate screening there were no bacteria isolated that had an MIC deemed resistant according to EUCAST thresholds (Fig. 1). A similar pattern but with fewer samples resistant and fewer isolates resistant was observed for nalidixic acid.

Table 2 gives two-by-two tables of samples measured as resistant (to ampicillin or nalidixic acid) or sensitive according to the streak plate method and according to the testing of eight isolates for their MIC (EUCAST/BSAC method). One or more isolates from one of these samples was interpreted as defining the sample as resistant using EUCAST/BSAC thresholds. It is apparent from the 2-by-2 classification table that, compared to a sample of eight isolates tested for MIC and classified under EUCAST or BSAC guidelines, the streak plating method appears more likely to categorise a sample as resistant. This is either because it is more sensitive or less specific (or a combination of both) than the measured MIC-based isolate method for both ampicillin and nalidixic acid.

Table 2. A cross classification of resistance to ampicillin or nalidixic acid for samples tested using the measured EUCAST or BSAC isolate MIC-based method and the sample level streak plating method from samples taken in the calibration study

			Streak plating method	
Measured			Sensitive	Resistant
EUCAST/BSAC	Ampicillin	Sensitive	26	8
MIC method		Resistant	0	6
with eight isolates	Nalidixic acid	Sensitive	37	2
per sample		Resistant	0	1

A McNemar chi-squared test confirmed that the apparent difference in "marginal proportions" (i.e. prevalence using each method) was statistically significant different at the 5% level in the case of ampicillin (P=0.01), but not for nalidixic acid (P=0.45). Note that no isolates were classified as resistant using the EUCAST method from samples that were classified as sensitive using the streak plate method.

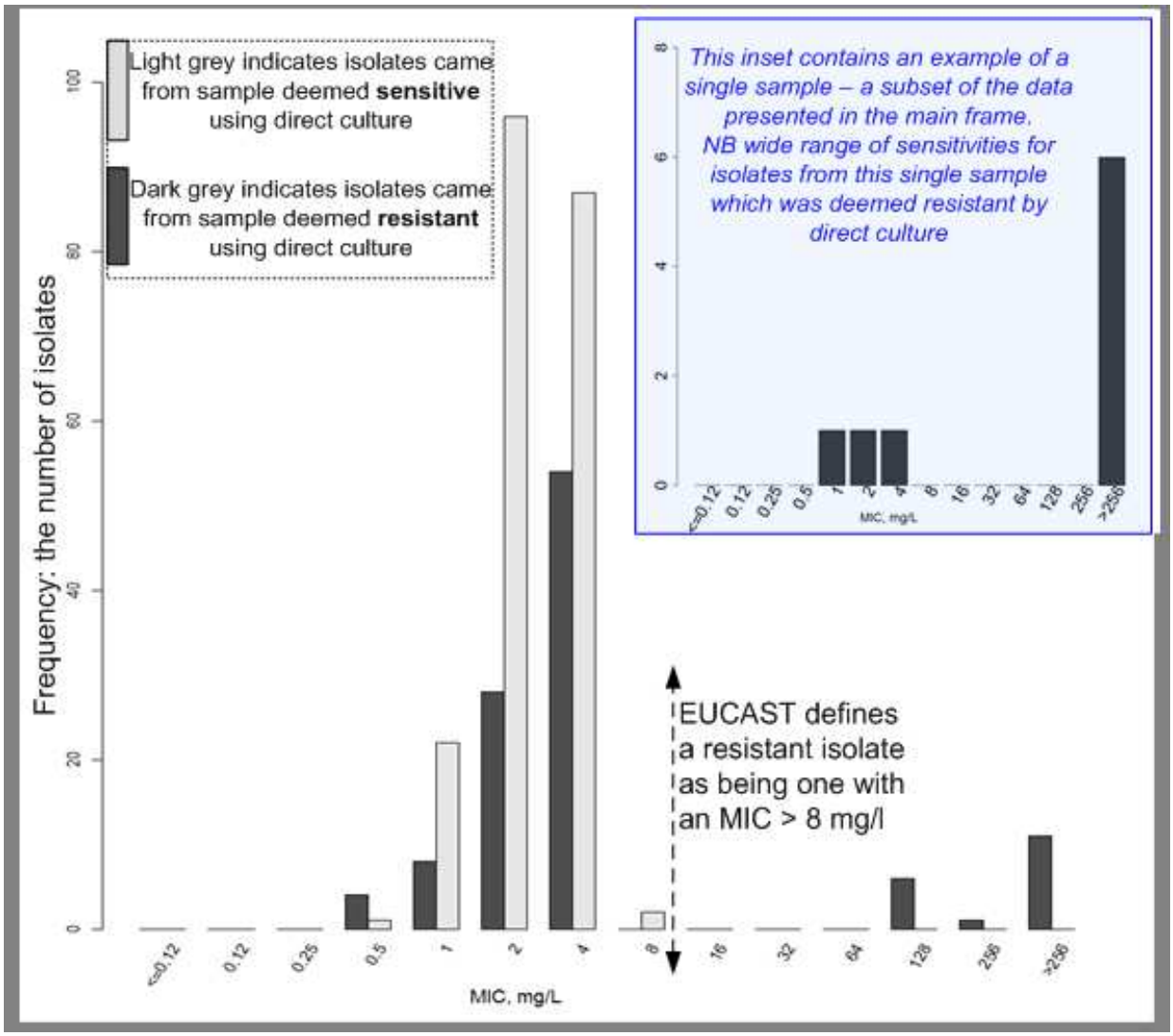

Fig. 1 Frequency of MIC to ampicillin for each of the eight *E. coli* isolates from each of the forty samples. The frequency of MICs of isolates from a selected sample (deemed positive by direct culture) is presented in the inset

DISCUSSION

It was demonstrated here that a sample-level test (streak plating) gave a much higher probability (and hence higher prevalence estimate) of categorising a sample as resistant than testing one or eight *E. coli* isolates by measuring their MIC and comparing with agreed breakpoints. This result is easily understood when taking into account the high density of *E. coli* in the sample medium (faeces; Smith & Crabb, 1961). The difference is due to the screening of a large number of isolates that occurs when using a sample-level test.

It is also noted that by taking both measures on the same samples it should be able to back-predict the prevalence from one measure of resistance to the other measure based on historic data in which only one of the measures had been taken. To do this, the same pattern of agreement as measured in the current samples needs to be assumed, had this also applied in the historic samples.

It is the nature of resistant organisms to be at a significant evolutionary advantage in comparison to sensitive organisms when in the presence of the antibiotic to which they are resistant. Therefore, even when such organisms are present in very low density it seems likely that their presence is very important for the amplification through selection and transfer of resistance to other parts of the wider system (e.g. as described by systems maps; Department of Health, 2014). When this argument is correct, then it is very likely that measurements that are inherently designed to test a whole sample will, for some epidemiological purposes, be more relevant than measurements that are designed around the individual isolate or bacterium.

It is also important to recognise the existence of other important methods not used here, which are also used for defining resistance. For example, disk diffusion at both the sample level and the isolate level, PCR identification of known genes at both the sample level and the isolate level and rapid whole gene sequencing at the isolate level. In an ideal epidemiological world, studies would exist that allowed translation of results from any one of these methods into estimates of what those results would have been if using a different method. In reality, where these translations are not always possible, it is important to specify which method is used and therefore precisely what prevalence measure has been estimated. Also in an ideal epidemiological world, knowledge of which of these methods are most useful for important epidemiological questions (such as the cost/benefit of reduced antimicrobial usage in livestock production) would exist (Woolhouse et al., 2015). The study presented here relates two of those measures with one another, and is a small contribution to that ideal epidemiological world.

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ESTIMATING DISEASE OCCURRENCE

CHRONIC KIDNEY DISEASE IN CATS IN THE UK: PREVALENCE, RISK FACTORS AND SURVIVAL

M.C. CONROY*, D.C. BRODBELT, D.G. O'NEILL, Y.M. CHANG AND J. ELLIOTT

SUMMARY

Chronic kidney disease (CKD) is a common disease of older cats. There is limited previous research on CKD in cats presenting to primary-care practice in the UK. Cats diagnosed with CKD were identified from electronic clinical records from the VetCompass database. Prevalence was estimated and risk factors for diagnosis of CKD and survival following diagnosis were investigated. CKD was found to be a common disease of older cats attending VetCompass primary-care practices. Risk factors and prognostic indicators were found to be consistent with previous research carried out in referral and geriatric populations, with breed, hyperthyroidism and low body weight associated with diagnosis, and serum phosphate, urine protein: creatinine ratio and feeding of renal diets associated with survival. The results of this study can aid the diagnosis of CKD, provide evidence for prognostic estimates and highlight the need for improvement in veterinary education for earlier detection and management of CKD.

INTRODUCTION

Chronic kidney disease (CKD) is a commonly recognised disease process in cats. It is a progressive disease process that has been defined as kidney damage that has been present for at least 3 months (Polzin, 2010; Bartges, 2012). CKD does not become clinically apparent until around 75% of the kidney is damaged (Brown et al., 1997). CKD causes many clinical signs, the most commonly reported being anorexia, weight loss, depression, polydipsia/ polyuria and vomiting (Lulich et al. 1992; Elliott & Barber 1998; Caney 2016). CKD causes considerable morbidity in cats, with most having more than one of these clinical signs (Elliott & Barber, 1998). The International Renal Interest Society (IRIS) has published guidelines on staging the severity of CKD based on serum creatinine concentrations, with sub-staging using urine protein: creatinine ratio (UPC) and systolic blood pressure (IRIS, 2015).

Previous studies have estimated the prevalence of CKD in cats attending primary-care practice in the UK as between 1.7% - 3.6% (O'Neill et al., 2014; Sanchez-Vizcaino et al., 2015). Prevalence increases with age, with up to 80% of cats >15 years estimated to have CKD in some studies (Marino et al., 2014). Previous estimates are difficult to compare due to the highly variable case definitions and the populations sampled. CKD is likely to be underdiagnosed, with up to 15% of apparently healthy cats being diagnosed with CKD following a routine health check (Paepe et al., 2013).

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Risk factors reported for CKD diagnosis include age (DiBartola et al., 1987; Lulich et al., 1992; Elliott & Barber, 1998), sex (White et al., 2006; Greene et al., 2014), breed (Lulich et al., 1992; Greene et al., 2014), hyperthyroidism (Williams et al., 2010; Markovich et al., 2014), Feline Leukaemia virus (Glick et al., 1978; Beatty, 2014) and Feline Immunodeficiency virus (Baxter et al., 2012; Poli et al., 2012). Vaccination (Finch et al., 2016) and incomplete recovery from acute kidney injury (Reynolds & Lefebvre, 2013) are also hypothesised to predispose to CKD. Previous research into risk factors for CKD diagnosis has been limited mainly to referral populations and questionnaire-based studies, which may reduce the generalisability of the results. A lack of information on the risk factors for diagnosis of CKD in cats limits clinicians' ability to implement targeted effective screening programmes, with cats usually only being identified once clinical disease is apparent (Lees, 2004).

Kidney disease has been identified as the most common cause of death in cats >5 years of age in the UK (O'Neill et al., 2015). Previous median survival estimates have ranged from 21 days – 1,151 days depending on disease severity at diagnosis (Elliott & Barber, 1998; Elliott et al., 2000; Boyd et al., 2008). Proteinuria (Syme et al., 2006; King et al., 2007), anaemia (Kuwahara et al., 2006; King et al., 2007), serum phosphate levels (Kuwahara et al., 2006; King et al., 2007; Chakrabarti et al., 2012) and feeding a low protein, low phosphate diet (Elliott et al., 2000) have been associated with survival following CKD diagnosis. No single variable reliably predicts survival following diagnosis of CKD.

The use of primary-care veterinary data to investigate disorder prevalence, risk factors and survival can provide results that are relevant to UK primary-care practice, allow estimation of disease burden and may aid development of screening protocols and prognostic estimates for cats diagnosed with CKD. The aims of this study were to estimate the prevalence of CKD in cats, to identify risk factors for diagnosis of CKD and to evaluate the survival of cats following CKD diagnosis in cats attending primary-care practice in the UK. It was hypothesised that purebred cats were at greater risk of CKD diagnosis than crossbred cats.

MATERIALS AND METHODS

Anonymised electronic patient records (EPR) derived from the VetCompass database (VetCompass, 2016) were used to identify cats for study inclusion. The retrospective cohort of cats attending VetCompass practices during the study period was used to identify CKD cats and estimate prevalence. A case control study nested within this cohort was undertaken to evaluate risk factors for diagnosis. Incident cases and frequency age-matched controls were used. Survival analysis following CKD diagnosis was undertaken. The Royal Veterinary College Clinical Research Ethical review Board granted ethical approval (URN M2015 0050).

All cats registered at 244 primary-care practices participating in VetCompass from 1st Jan 2012 – 31st Dec 2013 were included. In order to test the hypotheses, sample size calculations indicated that 290 cases and 290 controls were required if purebred cats had twice the odds of CKD compared to crossbred cats, assuming 80% power and 95% confidence (Epi-Info7, Centres for Disease Control and Prevention, Atlanta, GA). Potential cases were identified from these cats by searching the EPR for VeNom diagnosis codes (*renal failure, renal disorder, renal disease, chronic renal failure, renal insufficiency*), treatment (*KD, RCW feline renal, renal food, renalzin, ipakitine, alucap, semintra, tumil K, renal profile, UPC*) and free text terms (*CKD, CRD, CRF, chronic renal/kidney failure/disease/insufficiency*) appropriate

to CKD diagnosis. The results from all searches were merged and duplicates removed. A random sample of 20% of the potential cases was selected for detailed review. The case definition required a diagnosis of CKD (or synonym) recorded by the vet within the EPR, or a diagnosis of kidney disease (or synonym) within the EPR with evidence of disease chronicity, which was defined as the presence of clinical signs for at least 3 months. Controls were selected from the cats that were not identified in the searches, were frequency matched by age (<9 years or ≥9 years) and were excluded if CKD was suspected by the vet in the clinical notes but either not confirmed or ruled out. Hyperthyroid cats were required to be non-azotaemic when the total T4 levels were <40nmol/l. Demographic data were extracted automatically and further data required to answer the research questions were extracted manually from the EPR (date of diagnosis, method of diagnosis, treatment, survival and medical history). Data were exported to Microsoft Excel 13 for checking and cleaning, and exported to Stata 11 (StataCorp LP, College Station, TX) for statistical analysis.

Statistical analysis

Prevalence estimates: As only 20% of the potential cases were reviewed in detail, a stratified analysis was undertaken to estimate the prevalence of CKD, using the Stata survey commands. Two strata, the cats that had their EPR read in detail and the cats that were not identified in the searches, were used. Sampling weights were proportional to the inverse of the probability of being sampled (Dohoo, 2010).

Descriptive statistics: Medians and interquartile ranges (IQR) were calculated for continuous variables. Age was categorised as <9 years or ≥9 years. Bodyweight was categorised into quintiles. Breed was categorised into crossbred or purebred, with a purebred being a cat recorded as a breed recognised by International Cat Care (International Cat Care, 2015). Serum creatinine at diagnosis was categorised based on IRIS guidelines (IRIS, 2015). Vaccination was categorised as annual vaccination, kitten/non-annual vaccination or no vaccination recorded. Non-steroidal anti-inflammatory drug (NSAID) use was categorised as one-off treatment, recurring one-off treatment or long-term treatment. Days since last anaesthetic was categorised into 0 – 179 days, 180 – 364 days and no anaesthetic recorded within the last 365 days. The variables heart murmur, arrhythmia and gallop rhythm recorded were highly collinear, so were combined into one variable: heart auscultation abnormality. Co-morbidities, such as hyperthyroidism, were categorised by when they were diagnosed relative to CKD diagnosis.

Risk factor analysis: Binary logistic regression was used to evaluate risk factors for CKD diagnosis. Variables associated with diagnosis of CKD ($P<0.2$) in the univariable analysis were carried forward to the multivariable analysis. A manual backwards stepwise model-building approach was used for the multivariable model. Potential confounders were investigated by identifying any marked change in the odds ratio after removing the variable from the model. Age was forced into the model. Biologically plausible interactions were investigated and tested with the likelihood ratio test (LRT). Collinearity was investigated by examining the variance inflation factor and tolerance (Hair et al., 2014). Outliers and leverage were assessed by examining the standardised Pearson residuals, delta deviance and delta beta residuals, model fit was assessed by the Hosmer-Lemeshow test, and predictive ability by examining the area under the ROC curve (Hosmer & Lemeshow, 2000; Dohoo, 2010). Statistical significance was set at the 5% level.

Survival analysis: A Cox proportional hazards model was constructed to investigate variables associated with survival following CKD diagnosis. Variables associated with

survival at the univariable level ($p<0.2$) were taken into the multivariable model. A manual backwards stepwise model building approach was used. Confounders and interactions were assessed as in the risk factor analysis. Visual inspection of the log cumulative hazard plot and examination of the Schoenfeld residuals were used to assess the proportional hazard assumptions. Model fit was assessed by examining the Cox-Snell residuals and outliers were checked using the deviance residuals (Dohoo, 2010). Statistical significance was set at the 5% level.

RESULTS

The denominator population included 353,448 cats attending 244 VetCompass clinics. Searches of the EPRs identified 11,836 potential cases of which 2,368 were reviewed in detail. This identified 625 newly diagnosed incident and 338 pre-existing cases, with 625 age-matched controls also identified.

Prevalence estimates

Overall, period prevalence was estimated at 1.2% (95% confidence interval (CI) 1.1% - 1.3%). Prevalence was found to increase with age, with the prevalence in cats <9 years 0.1% (95% CI 0.08% – 0.2%) and 3.6% (95% CI 3.3% - 3.9%) in cats ≥9 years. Prevalence was found to be highest in Burmese cats (Table 1).

Table 1. Period prevalence of chronic kidney disease in cats attending primary-care practice in the UK between 1st Jan 2012 and 31st Dec 2013

		Denominator	Prevalent cases	Period prevalence[a]	95% Confidence interval
Overall		353448	961	1.2%	1.1% - 1.3%
Age (years)	0 to <4.5	118136	17	0.05%	0.02% - 0.07%
	4.5 to <9	67842	58	0.4%	0.3% - 0.5%
	9 to <13.5	45597	212	1.9%	1.6% - 2.1%
	13.5 to <18	30586	478	4.7%	4.3% - 5.1%
	18 to <22.5	7672	186	6.5%	5.6% - 7.4%
	22.5 +	305	1	1.1%	0% - 3.2%
Breed	Crossbreed	341791	800	1.1%	1.1% - 1.2%
	Burmese	3173	44	5.0%	3.5% - 6.4%
	British Shorthair	7057	14	0.9%	0.4% - 1.3%
	Bengal	4167	3	0.3%	0.0% - 0.7%
	Persian	4202	25	2.3%	1.4% - 3.2%
	Siamese	2990	22	2.9%	1.7% - 4.1%
	Main Coon	2428	4	0.8%	0.02% - 1.5%
	Other purebred	11531	38	1.5%	1.0% - 1.9%

[a] Calculations carried out based on the stratified sampling strategy with proportional weighting

Descriptive statistics

Demographics: The following analyses were performed on incident cases only. Median age and weight at diagnosis of CKD were 14.8 years (IQR 12.1 – 16.7) and 3.54 kg (IQR

2.97 – 4), respectively. Weight was recorded in 43.8% of cats and was more frequently recorded in cases (51.9%) than controls (36.8%). Sex was recorded in 99.4% of cats, with females accounting for 46.7% (292) of cases and 44% (275) of controls. Neuter status was recorded in 72% of cats, and 59.5% (372) of cases and 55.6% (346) of controls were neutered. Breed was recorded in 98.3% of cats and most cases (84.4%, 530) and controls (88.5%, 553) were crossbred.

Risk factor analysis

Univariable analysis: The following variables were broadly associated (P<0.2) with CKD diagnosis and carried into the multivariable analysis: weight at diagnosis, breed, having a heart auscultation abnormality or goitre, vaccination status, NSAID treatment, days since previous anaesthetic, hyperthyroidism, arthritis, cystitis, and hepatopathy (all prior to CKD).

Multivariable analysis: The strongest association was identified between cats with pre-existing hyperthyroidism and CKD diagnosis, with these cats having 5.7 times the odds of CKD diagnosis than cats without hyperthyroidism. Purebred cats were at increased odds of CKD diagnosis in comparison to crossbred cats (see Table 2). Model fit was found to be adequate (P=0.2) and the area under the ROC curve indicated good predictive ability (0.78).

Survival analysis

Incident cases had their clinical notes examined up until the 31st Dec 2015. In total, 380 (60.8%) cats died during the follow-up period and 224 (35.8%) were lost to follow-up. Most deaths (90.3%, 343) involved euthanasia. CKD was the most common reason for death (37.9%, 144), with poor quality of life recorded for just over a fifth (22.6%, 86) of deaths. During the study period, 92 cats were not seen again by a vet following diagnosis of CKD. Most of these cats (82.6%, 76) were euthanised at diagnosis. These cats were excluded from further analysis. A total of 526 cats were included in the survival analysis.

Univariable survival analysis: Median survival time was 388 days from CKD diagnosis (IQR 88-1,042 days; Fig. 1). The estimated all-cause mortality rate following diagnosis of CKD was 6.5 deaths per 10 CKD cat years at risk (95% CI 5.8-7.3).

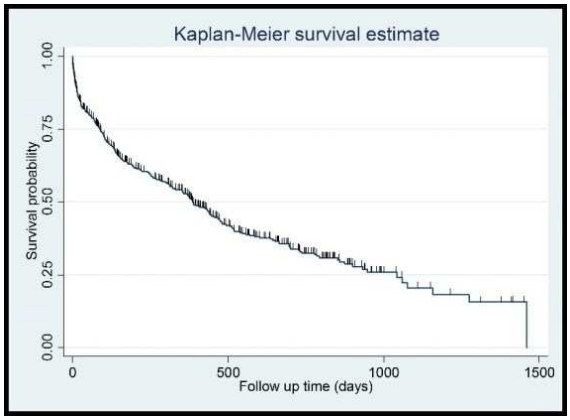

Fig. 1 Kaplan-Meier curve for all cats following CKD diagnosis. Dashes indicate censored cats. N=526

Table 2. Multivariable analysis of risk factors for diagnosis of chronic kidney disease in cats attending primary-care practice in the UK between 1st Jan 2012 and 31st Dec 2013

Variable		Case	Control	Odds Ratio	95% Confidence Interval	*P*-value (LRT)
Age	<9 years	58	58	Reference		0.2
	≥9 years	559	559	0.75	0.48 - 1.15	
Breed	Crossbred	522	545	Reference		0.03
	Purebred	87	59	1.72	1.15 - 2.58	
	Unknown	8	13	1.07	0.38 - 2.93	
Heart auscultation abnormal	No	304	263	Reference		0.08
	Yes	113	57	1.64	1.06 - 2.54	
	Not Recorded	200	297	1.12	0.83 - 1.52	
NSAID treatment	Not recorded	383	345	Reference		0.02
	One-off treatment	140	179	0.81	0.60 - 1.11	
	Repeated one-off treatment	51	79	0.7	0.44 - 1.10	
	Long-term treatment	43	14	2.34	1.12 - 4.88	
Goitre	No	188	32	Reference		0.04
	Yes	61	17	0.42	0.21 - 0.85	
	Not recorded	368	568	0.13	0.08 - 0.19	
Hyperthyroidism	No	547	607	Reference		<0.0001
	Yes	70	10	5.7	2.78 - 11.68	
Cardiomyopathy	No	593	598	Reference		0.003
	Yes	19	24	0.26	0.10 - 0.65	
Days since last anaesthetic	0 - 179 days	39	68	Reference		0.005
	180 - 364 days	20	32	1.21	0.55 - 2.70	
	No anaesthetic recorded within 1 year	558	517	2.07	1.28 - 3.36	
Weight	0 - 3 kg	64	48	Reference		<0.0001
	3.01 - 3.5 kg	56	50	1.03	0.57 - 1.85	
	3.51 - 4.05 kg	53	51	0.84	0.46 - 1.54	
	4.07 - 4.78 kg	31	82	0.36	0.19 - 0.67	
	4.85 - 9.6 kg	21	81	0.26	0.13 - 0.51	
	No weight recorded	392	305	0.7	0.40 - 1.3	

The following variables were found to be broadly associated (P<0.2) with increased survival following CKD diagnosis at the univariable level: neutered cats, purebred cats, heavier cats, cats presenting for geriatric health check or pre-anaesthetic bloods, the use of biochemistry and urinalysis at diagnosis, low grade of heart murmur, presence of hypertension, IRIS stage 1 at diagnosis, serum phosphate <1.5mmol/l at diagnosis, UPC <0.2 at diagnosis, use of renal diet treatment, use of phosphate binder treatment, use of potassium supplementation, use of amlodipine treatment, no anabolic steroid treatment, no IVFT

treatment, presence of periodontal disease, presence of cystitis, no cardiomyopathy, presence of hepatopathy and no constipation.

Multivariable analysis: Serum phosphate at diagnosis was found have the largest association with survival, with cats with a reported serum phosphate of ≥1.5mmol/l at diagnosis to be at 5.78 times the hazard of death in comparison to those cats with a serum phosphate of <1.5mmol/l. There was an interaction between time and serum phosphate, with no association found over 400 days after diagnosis. UPC was also found to be a negative prognostic indicator, with cats with a reported UPC of >0.4 having 4.96 times the hazard of death in comparison to those with a UPC of ≤0.2. Table 3 shows the multivariable Cox regression results. Proportional hazard assumption was satisfactory (P=0.22), and examination of the Cox-Snell residuals indicated a good fit.

DISCUSSION

CKD was found to be a common disease in geriatric cats, with just under 1 in 28 cats ≥9 years being diagnosed during the study period. Risk factors for diagnosis were found to be similar to those previously identified, and serum phosphate concentrations and control of serum phosphate through treatment was found to be associated with survival following diagnosis.

The prevalence of CKD calculated in the present study was lower than previous estimates. O'Neill et al. (2014) estimated a prevalence twice as high from a similar population, though differences in the case definition, the study population and the study duration may have partially accounted for the difference in estimates. The estimate is closer to two estimates for the prevalence of renal disease in primary-care practices, one based in the UK (Sanchez-Vizcaino et al., 2015) and the other in the US (Lund et al., 1999). These combined all renal presentations, so the prevalence of CKD may be similar to the estimate in the present study.

Body weight was found to be associated with both diagnosis and survival. Cats with lower body weight at diagnosis are likely to have more advanced CKD, explaining the increased risk of diagnosis and decreased survival identified. Increasing progression of weight loss may also influence owners and veterinarians in opting for euthanasia. Previous studies have identified genetic conditions that may predispose some breeds to CKD, such as polycystic kidney disease in Persians and familial amyloidosis in Abyssinian cats (Boyce et al., 1984; Lyons et al., 2004). This may explain the increased odds of diagnosis seen in purebred cats as a group. Interestingly, purebred cats also had improved survival in comparison to crossbred cats. Breed has not previously been identified as associated with survival in cats diagnosed with CKD (Kuwahara et al., 2006; Syme et al., 2006; Boyd et al., 2008), and purebred cats have not been found to live longer than crossbred cats (O'Neill et al., 2015). It is possible that purebred cats are more likely to be insured (Egenvall & Nødtvedt, 2009) and insurance status may be a confounder in the association between purebred status and survival. The long-term use of NSAID therapy was identified as a risk factor for CKD diagnosis. This could be due to a low level nephrotoxicity causing kidney injury (Reynolds & Lefebvre, 2013; Khan & Khan, 2015).

Table 3. Multivariable Cox regression analysis for survival following chronic kidney disease diagnosis in cats attending primary-care practice in the UK between 1st Jan 2012 and 31st Dec 2013

Variable			N	Deaths	Hazard Ratio	95% Confidence Interval	*P*-value (LRT[a])
Breed		Crossbred	446	261	Reference		0.001
		Purebred	80	40	0.57	0.40 - 0.81	
		Unknown	7	3	0.3	0.09 - 0.96	
Serum phosphate at diagnosis	0-400 days after diagnosis	<1.5mmol/l	20	4	Reference		<0.0001
		1.5mmol/l +	68	47	5.78	1.91 - 17.32	
		Not recorded	445	190	4.35	1.49 - 12.71	
	>400 days after diagnosis	<1.5mmol/l	12	7	Reference		
		1.5mmol/l +	11	6	0.47	0.15 - 1.44	
		Not recorded	159	50	0.45	0.20 - 1.01	
UPC[b]		0-0.2	28	6	Reference		0.01
		0.21-0.4	19	8	3	1.02 - 8.34	
		>0.4	15	11	4.96	1.78 - 13.81	
		Not measured	471	279	2.77	1.21 - 6.35	
Renal diet		No	143	94	Reference		0.0009
		Yes	390	210	0.58	0.45 - 0.75	
Phosphate Binder		No	442	257	Reference		0.02
		Yes	91	47	0.68	0.48 - 0.95	
IVFT[c]		No	419	222	Reference		<0.0001
		Yes	114	82	1.97	1.48 - 2.62	
Cystitis		No	440	262	Reference		0.02
		Yes	93	42	0.69	0.49 - 0.97	
Constipation		Before CKD diagnosis	8	8	Reference		0.01
		At CKD diagnosis	3	2	0.35	0.07 - 1.78	
		After CKD diagnosis	10	5	0.15	0.05 - 0.50	
		No constipation	512	289	0.23	0.11 - 0.51	
Weight		1.65 - 2.78	39	31	Reference		0.0003
		2.8 - 3.23	37	25	0.87	0.50 - 1.51	
		3.25 - 3.65	45	30	0.54	0.32 - 0.92	
		3.67 - 4.3	43	29	0.47	0.28 - 0.80	
		4.3 - 7.5	44	25	0.48	0.27 - 0.83	
		No weight recorded	325	164	1.4	0.82 - 2.41	

[a]LRT: Likelihood ratio test
[b]UPC: Urine protein: creatinine ratio
[c]IVFT: Intravenous fluid therapy

The reduced odds associated with recent anaesthesia and CKD diagnosis found in the present study conflicts with previous studies (Greene et al., 2014). It may be that vets do not perform anaesthesia on cats they suspect of having CKD, or provide better support and monitoring during anaesthesia to these cats to reduce the risk of renal hypoperfusion. Residual confounding by age may partly explain this apparent association, as older cats may

be less likely to undergo anaesthesia whilst also being more likely to be diagnosed with CKD. Further research into anaesthetic practices to explore this association is merited. No association was identified between vaccination and CKD diagnosis in the current study, unlike earlier studies (Finch et al., 2016). Previous studies relied on owner reports, and it is possible that social desirability bias influenced the results. Misclassification is also a possibility if vaccination was carried out at a different clinic. Hyperthyroidism was identified as a risk factor for CKD diagnosis in the current study, as has previously been reported (Langston & Reine, 2006; Williams et al., 2010; Markovich et al., 2014). However, the presence of a goitre was found to be protective against diagnosis of CKD. This may be due to undiagnosed hyperthyroid cats having any CKD masked due to the increase in GFR from the hyperthyroidism. Recording of thyroid palpation within the clinical notes was variable, with it being more common in cases than controls. This may have biased the results seen here. Other conditions that have previously been identified as associated with CKD are periodontal disease and cystitis (Greene et al., 2014; Finch et al., 2016). Neither of these were identified as a risk factor in the present study. It was not possible to record periodontal disease severity in this study, unlike in previous studies. This may explain the lack of association found, as severity may be associated with CKD diagnosis. The previous study identifying an association between cystitis and CKD diagnosis was performed in primary-care practices in the USA, so population differences or differing underlying causes of cystitis may be the reason no association was seen in the present study. In contrast, co-presence of cystitis was associated with increased survival after diagnosis. More frequent veterinary visits associated with cystitis may explain the improved survival associated with cystitis diagnosis in the current study.

Serum phosphate levels at diagnosis and proteinuria were associated with survival following diagnosis, as previously identified (Syme et al., 2006; King et al., 2007; Boyd et al., 2008; Chakrabarti et al., 2012). An interaction with time was identified between serum phosphate and survival, with an association observed in the first 400 days following diagnosis, and no association detected over 400 days after diagnosis. As the main treatment for CKD is phosphate restriction, the loss of association between serum phosphate at diagnosis and survival over time is logical. In agreement with previous reports, low phosphate low protein diets were also associated with improved survival in cats in the current study (Elliott et al., 2000). This supports the potential benefit from these diets even though compliance may be lower than in a research setting. The use of IVFT was found to be a poor prognostic indicator. It is likely that cats receiving IVFT are at a more advanced stage of CKD (Elliott & Barber, 1998). Constipation secondary to dehydration has been identified in cats with CKD (Korman & White, 2013). The association seen in the present study between constipation and survival may be related to the severity of CKD at the time of diagnosis, with cats previously diagnosed with constipation being at a more advanced stage of CKD.

There are limitations to this study. Data were not collected primarily for research, so recording quality may not have been consistent across all EPRs. The case definition required veterinary diagnosis alone and did not need the veterinarian to perform biochemical or urine analytical support, so misclassification of cases may have occurred. Misclassification of variables may have occurred if the history available was limited or inaccurate. Insurance data were not available for this study. Insurance status has been identified as a risk factor for diagnosis of a number of other conditions in similar populations (O'Neill et al., 2016) and may also have been a confounder for some of the other variables in the final models. It is also possible that residual confounding by age was present in the risk factor analysis.

CKD is a common disease of older cats presenting to primary-care practice in the UK. Identification of higher risk cats may allow targeted screening to identify cats with CKD before clinical signs become apparent. Improved knowledge of positive and negative survival indicators provides veterinarians with evidence for prognostic estimates, monitoring and treatment protocols. The present study highlights the requirement for improved education of owners and vets concerning the diagnosis, treatment and monitoring of CKD, and these findings may aid in improving the health and welfare of cats attending primary-care practices in the UK.

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MIGHTY MODELS FROM LITTLE DATA GROW: ESTIMATING ANIMAL DISEASE PREVALENCE

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SUMMARY

Global datasets relating to prevalence of animal pathogens are a useful input for risk assessments. However, missing data, and the resulting uncertainty, could potentially bias model outputs. This paper reviews the challenges associated with using freely available datasets and explores methods of estimating data, where necessary. By filling these data gaps, the final models can achieve more robust predictions with reduced uncertainty.

INTRODUCTION

Globalisation, with its associated increase in the frequency of movements of animals, humans and trade products around the world, has been acknowledged as a risk factor for the spread of pathogens between countries and is a motivating factor behind the implementation of many pathogen incursion risk assessments (Simons et al., 2016). Risk assessments are therefore being conducted by both international organisations (ECDC, 2016) and individual countries, specifically those wishing to understand the potential threats to their livestock populations and to avoid negative implications for the trade of both live animals and animal products (Roberts et al., 2016).

One of the aims of the Animal Health and Welfare ERA-NET consortium (ANIHWA)-funded project SPARE ('Spatial risk assessment framework for assessing exotic disease incursion and spread through Europe') is to develop a generic risk assessment framework for the entry of exotic animal pathogens into the European Union (EU) (SPARE, 2016). The first step in a risk assessment is termed the release or entry assessment. In general terms, this is defined as the 'evaluation of the probability of introduction of an agent from its origin until the point of entry into a country or area' (USDA, 2016). This necessitates the derivation of estimates for the animal disease situation in each 'origin' country of the world requiring data on animal disease prevalence (e.g. the number of recorded outbreaks per year), and demographic livestock data (e.g. the total number of animals and farms per species). In order to ensure a correct interpretation of these data, it is important that they are comparable between countries. Furthermore, they should ideally arise from robust animal health surveillance, which ensures that confidence in the health status of animals resident in the country of origin and those moving between countries is maintained and trade barriers are justified (Hoinville et al., 2013).

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One such data source is the World Animal Health Information Database (WAHIS) and its predecessor Handistatus II (OIE 2016a; OIE 2016b), which are maintained by the Office International des Epizooties (OIE). The OIE operates a global surveillance system that provides valuable data sources for animal diseases by collating information that all member countries (MCs) are obliged to report. The OIE is recognised as a reference organisation by the World Trade Organization, and had a total of 180 MCs in 2016. Each MC is expected to report the animal diseases that it detects within its territory. Therefore, the data available from the OIE database could be considered as one of the most globally comprehensive. Nevertheless, all global datasets dealing with such data are inherently subject to differences in the quality of individual reporting and the monitoring systems for disease on which reports are based. From the point of view of utilising the OIE data in a quantitative risk assessment, one of the most important issues is 'missing data', i.e. MCs where the data are only reported as presence of a disease, without a numerical estimate of scale, or for which no information is reported at all. Absence of reported disease could be considered to imply the disease-free status of a country, when in reality this may not be the case.

To assist in obtaining objective estimates for risk assessments, it is therefore expedient to develop reliable methods that can utilise the data that are available to estimate values for MCs where data are missing. Previous research has used a number of different methods to deal with missing data in human health disease incidence datasets, including grouping countries based on predictive associations (McDonald et al., 2015). The development of a method to obtain objective estimates for MCs with missing data is presented here. This method is illustrated with a case study for number of outbreaks of classical rabies in MCs, based on data on classical rabies outbreaks from the OIE databases (i.e. those outbreaks recorded as rabies). Results are presented to compare the effectiveness of the different grouping methods across multiple species by conducting statistical analysis of the results for MCs with available data.

MATERIALS AND METHODS

Overview

For the purposes of the analysis presented here, and for future integration within a larger risk assessment model, it was decided that the method employed to fill the data gaps should be automated as far as possible, with generic rules implemented when certain data are absent. An imputation method, sometimes termed 'farcasting' by analogy with forecasting, was used, whereby empirical models were fit to existing data and predictions were generated for the missing values from the fitted model (McDonald et al., 2015). To achieve this, countries were grouped together according to a predefined set of rules, all the historical data from the countries in each group were then combined and a probability distribution fit to these grouped data.

Historical input data

For the analysis presented here, the main inputs were the number of reported outbreaks and animal demographic data (e.g. number of animals in a country). Data for the number of reported outbreaks by country, k, species, s, and year, y, $N_{ob}(k,s,y)$, were obtained from all 180 MCs in the OIE databases over the years 1996-2014. The following species were considered for this analysis: pigs, cattle, sheep, goats, cats, dogs. For the period 1996-2005, data were obtained from Handistatus II (OIE, 2016b). For the period 2005-2014, data were

obtained from the country annual reports, located in the OIE reporting history section of the WAHIS interface (OIE, 2016a). Where no reports were available for a country, the data were considered to be missing. A breakdown of the number of outbreaks by species was not readily available from the WAHIS data, so were estimated based on the average proportion of cases attributed to each species from both the WAHIS and Handistatus data (e.g. if there were 100 outbreaks of rabies reported in the data and the ratio of reported cases from the available data was 40% pigs and 60% cattle, then it was assumed that 40 outbreaks were attributed to pigs and 60 to cattle).

There were considerable differences in the animal demographics between countries that could influence the number of recorded outbreaks. Therefore, to obtain a statistic that was comparable between countries, the number of outbreaks was weighted by an animal demographic metric; the number of 'animal establishments' by country and species, $N_{est}(k,s)$. For pigs, cattle, sheep and goats this was the number of animal establishments as defined in the WAHIS database for 2014; essentially equivalent to farms. In the absence of reliable data, it was assumed that it would be acceptable to treat cats and dogs as single entities (i.e. the number of establishments was equal to the number of animals). The average number of animals per farm was considered as an alternative statistic, but as it was not directly reported in the OIE database, it was decided that the additional level of uncertainty in deriving this estimate was too great; for example it was not clear for some countries whether the farms were made up of lots of farms of average size, a few very large farms, or lots of small 'back yard' farms. While the reporting of the data for number of establishments was sporadic and appeared to be subject to uncertainty (e.g. the same numbers of livestock/farms in a country being reported for numerous consecutive years, when one would expect some variation as animal production systems are dynamic and vulnerable to external factors), the value was directly reported in the OIE data and so considered to have less uncertainty than the number of animals per farm. However, due to the high level of uncertainty, only one point estimate of number of animal establishments was used, rather than an attempt to estimate values for each year. If these data were not recorded in the 2014 annual report, then the most recent historical observations were used. Data on the numbers of cats and dogs per country were estimated based on a collection of previous studies/literature reviews (OIE 2016a; WSPA 2008; FEDIAF 2014).

The number of outbreaks, $N_{ob}(k,s,y)$ was divided by the number of establishments, $N_{est}(k,s)$, to obtain the 'establishment prevalence' of disease by country, k, species, s and year, y, $P_{est}(k,s,y)$, see Eq. (1):

$$P_{est}(k,s,y) = N_{ob}(k,s,y) / N_{est}(k,s,y) \quad (1)$$

Groupings of countries

It was essential that the country grouping method was relevant to the subject matter and provided robust predictions. A number of different measures, G, for grouping countries were investigated, with six different groupings considered in this analysis. Two groupings were the official United Nations (UN) geographical regions and sub regions, *UNregion*, *UNsubRegion*, respectively (UN, 2016). A further grouping, *AltUNsubregion*, developed in a previous analysis was a modified version of the *UNsubRegion* accounting for some specific epidemiological considerations (Adkin et al., 2004). Two further groupings used k-means cluster analysis to group countries into 5 and 10 groups based on the gross domestic product (GDP) per person, *Gdp5* and *Gdp10* respectively, under the hypothesis that a country's

economic activity might be proportionate to its disease prevalence and/or livestock demographics (Jacobsen & Koopman, 2005). Estimates using the whole world as the sixth group were used for comparison.

Fitting a probability distribution

For each grouping method, a similar calculation was performed: for each subgroup, *g,* all non-zero entries of the establishment prevalence, $P_{est(k,s,y)}$, relating to MCs, *k*, in the subgroup *g* were extracted. A gamma distribution was fitted to these data as the shape of the distribution is very flexible depending on the choice of parameters, so it was considered likely to provide a reasonable fit for a large number of datasets. In addition, it is restricted to positive values, as was the case with the data. The gamma distribution was fitted using the *fitdistr*[†] function in R, which uses maximum likelihood methods to determine the best fitting values for the gamma distribution (Eq. (2)):

$$P_{fit}(g,s) = fitdistr(P_{est}(k,s,y) > 0,' gamma'), where\ k \in g. \quad (2)$$

Predictive accuracy

For all the countries that had OIE reported outbreaks of rabies in the species considered in this analysis, the accuracy of the model predictions was evaluated by two methods. The first method was a comparison of the overall fit using a Kolmogorov-Smirnov (KS) test in R[††] to compare the raw data of rabies outbreaks over all years for country *k* and species *s*, against the fitted distribution from the group, $K_{s(k,s)}$, see Eq. (3):

$$K_s(k,s) = ks.test\big(P_{est}(k,s,y) > 0,' pgamma', P_{fit}(g,s)\big), where\ k \in g. \quad (3)$$

A statistically significant result for the KS test implied confidence that the two sets of data (raw and fitted) came from the same underlying distribution and thus the fitted distribution could be considered a reasonable estimate for the observed data. The different grouping scenarios were compared by evaluating the proportion of MCs where this was not the case at the 1% level, i.e. had a KS test *P*-value >0.01.

The second method assessed the grouping fit at country level, by comparing the observed median number of outbreaks per country from the OIE data, $N_{ob(k,s)}$, against the median, 25th and 75th percentiles of the predicted number of outbreaks per country using the grouping fit, $N_{obFit(k,s)}$. The predicted number of outbreaks was obtained by multiplying the observed number of establishments, $N_{est(k,s,y)}$, by the 50th, 25th and 75th percentiles of the fitted distribution, $P_{fit(g,s)}$. If the median of the raw OIE data fell between the 25th and 75th percentiles of the fitted distribution then it was considered that the fitted distribution was a reasonable estimate for that country. Additionally, the squared difference between the observed and predicted country medians were calculated for different grouping methods, *G* (Eq. (4)):

$$S_{diff}(k,s,G) = (N_{ob}(k,s) - N_{obFit}(k,s,G))^2 \quad (4)$$

[†] https://stat.ethz.ch/R-manual/R-devel/library/MASS/html/fitdistr.html
[††] https://stat.ethz.ch/R-manual/R-devel/library/stats/html/ks.test.html

Statistics were generated for the total squared difference over all countries (Eq. (5)):

$$S_{diffTot}(s, G) = \sum_k S_{diff}(k, s, G) \quad (5)$$

and a comparison between two grouping methods, G_1 and G_2, for the number of countries where the squared difference was smaller for G_1 than for G_2, $N_{diff}(s,G_1)$ (Eq. (6)):

$$N_{diff}(s, G_1) = \sum_k (S_{diff}(k, s, G_1) < S_{diff}(k, s, G_2)) \quad (6)$$

RESULTS

Approximately 60% of observations in the rabies dataset were a non-zero value. This varied between species; while 44% of countries reported at least one value for rabies in dogs, <1% of countries reported at least one value for deer (Table 1).

Table 1. Percentage of countries with at least one reported outbreak of classical rabies by UN sub region and species

UN Sub Region	Pig	Deer	Cattle	Sheep	Goat	Cat	Dog	Buffalo
South America	29%	0%	64%	29%	21%	29%	29%	14%
Western Africa	0%	0%	38%	6%	6%	6%	75%	0%
Central America	38%	0%	100%	38%	38%	50%	63%	25%
Eastern Africa	11%	0%	58%	26%	26%	32%	58%	0%
Northern Africa	0%	0%	43%	43%	43%	43%	57%	0%
Middle Africa	0%	0%	11%	0%	0%	0%	33%	0%
Southern Africa	40%	0%	100%	60%	80%	100%	100%	0%
Northern America	25%	0%	0%	25%	25%	0%	25%	25%
Caribbean	9%	0%	13%	9%	17%	9%	13%	0%
Eastern Asia	0%	0%	29%	14%	14%	14%	29%	0%
Southern Asia	22%	0%	44%	11%	22%	11%	56%	33%
South-Eastern Asia	0%	0%	45%	0%	0%	36%	45%	0%
Southern Europe	23%	0%	31%	23%	23%	31%	38%	0%
Australia & New Zealand	0%	0%	0%	0%	0%	0%	0%	0%
Melanesia	0%	0%	0%	0%	0%	0%	0%	0%
Micronesia	0%	0%	0%	0%	0%	0%	0%	0%
Polynesia	0%	0%	0%	0%	0%	0%	0%	0%
Central Asia	20%	0%	100%	80%	0%	80%	100%	0%
Western Asia	6%	0%	33%	33%	28%	33%	50%	6%
Eastern Europe	70%	20%	100%	90%	60%	100%	100%	0%
Northern Europe	7%	0%	21%	21%	14%	21%	21%	0%
Western Europe	0%	0%	38%	13%	0%	50%	50%	0%
Overall	**13.0%**	**0.9%**	**39.5%**	**22.4%**	**19.3%**	**28.7%**	**44.4%**	**4.5%**

This value for deer was thought to be largely due to the low country-level prevalence of rabies in deer. Due to the lack of data, results for deer and buffalo were considered highly uncertain and were not considered further. Table 1 also shows that while Southern Africa tends to have a lot of data, Australasia has no reported outbreaks. This highlights an important consideration in the choice of the most appropriate grouping method; a method that provides a very good distribution fit for one region, but has no data for the other regions may not be as good as one that has reasonable data for all groups but has a poorer distibution fit.

Figure 1 shows the results of the overall fit for the different grouping measures for rabies. There was a maximum increase of 13.8% in the number of countries with a KS test *P*-value >0.01, between treating the whole dataset as one epidemiological unit and the best grouping measure. The *AltUNsubregion* was on average the best across all species, although results varied by species, with the *Gdp* groupings performing better for goats.

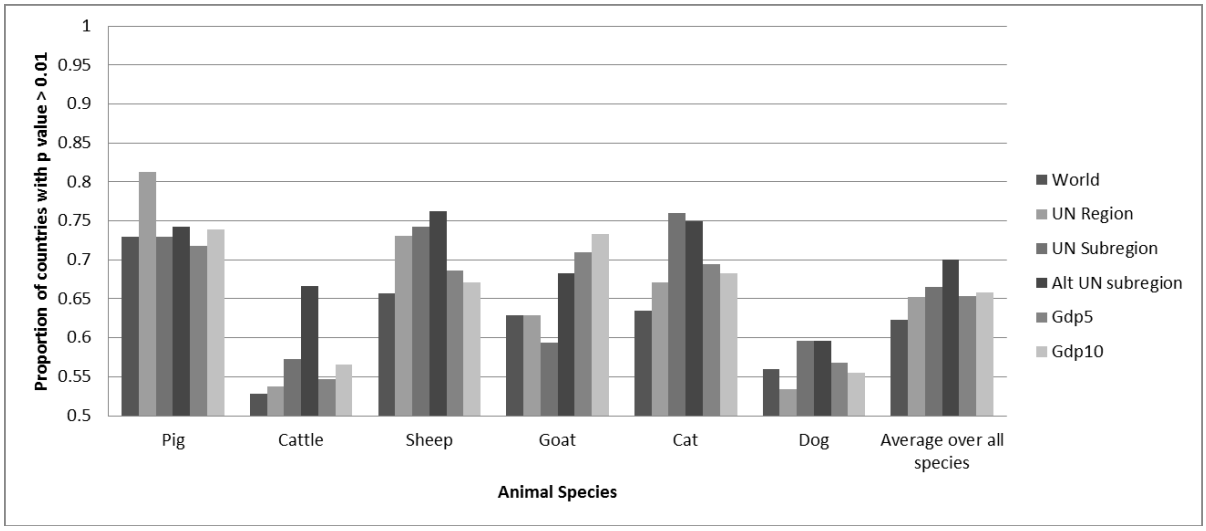

Fig. 1 Percentage of countries with a Kolmogorov-Smirnov *P*-value >0.01, by animal species and grouping method, for rabies. A higher proportion means that more countries have a distribution of historical outbreaks that could be considered to come from the same distribution as the one fitted to the grouped data

Figure 2 shows results for individual countries with the most observed data, using cattle as an example. It can be seen that for individual countries, the median of the observed data often falls between the 25th and 75th percentiles of the fitted distribution, which suggests both methods provide a reasonable fit.

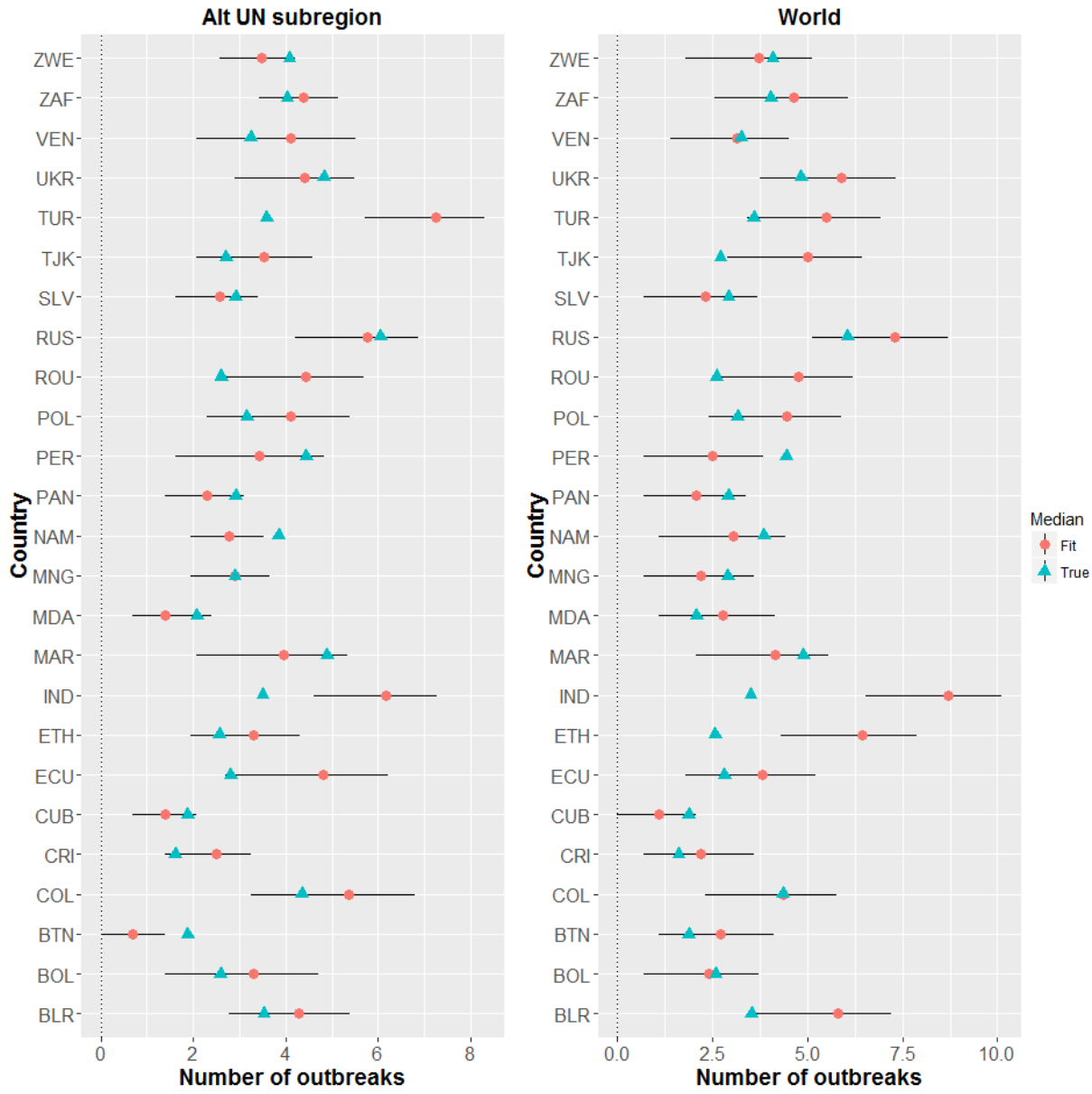

Fig. 2 Comparison of observed and predicted number of rabies outbreaks in cattle for *AltUNsubregion* (predicted values estimated by fitting different distributions to groups of countries based on geographical region and specific epidemiological considerations (Adkin et al., 2004)) and *World* (predicted values estimated using the same distribution for every country), where there were at least 15 observed historical outbreaks. Triangle= median of country-specific historical outbreaks, circle= median of fitted distribution, Line= 25^{th} – 75^{th} percentiles of country-specific historical outbreaks. Country names are the official UN IS03 codes (UN, 2016)

Further analysis on all the countries showed that the difference between the predicted and observed medians was smaller for the *AltUNsubregion* (compared to the whole world) for 58 countries, and larger for only 29 countries. In addition, the squared sum of the differences between the medians was larger for the whole world than for *AltUNsubregion*. A similar pattern was observed for the other species, suggesting that the *AltUNSubregion* grouping provided the better fit (Table 2).

Table 2. Comparisons between two grouping methods, *AltUNSubRegion (ALT)* and *World* (*Wrld*). The number of countries where the squared difference between observed and predicted median number of rabies outbreaks by species, *s,* and country, *k,* was smaller for *ALT* than for *Wrld, is depicted by* $N_{diff}(s,ALT)$ and the opposite is depicted by $N_{diff}(s,Wrld)$. The total sum of the squared difference over all countries for *ALT* is depicted by $S_{diffTot}(s,ALT)$ and for *Wrld is* depicted by $S_{diffTot}(s,Wrld)$

	Pig	**Cattle**	**Sheep**	**Goat**	**Cat**	**Dog**
$N_{diff}(s,ALT)$	23	58	30	25	47	67
$N_{diff}(s,Wrld)$	8	29	13	14	18	34
$S_{diffTot}(s,ALT)$	41	168	70	80	116	287
$S_{diffTot}(s,Wrld)$	148	319	137	164	244	420

DISCUSSION

Risk assessments for animal and public health require good quality data in terms of trade movements, animal health status and production systems (Rodgers et al. 2011). An essential prerequisite for estimating animal health status or disease burden is the availability of comprehensive national-level data on the prevalence of the disease of interest (McDonald et al. 2015). This is not always possible, however, and the treatment of missing values may lead to biased results. An alternative method, as presented here, is to estimate pathogen prevalence for countries with missing national-level data (McDonald et al. 2015). As risk assessments are data driven, with the value of the results being dictated by the quality of the data inputs, filling data gaps will reduce the occurrence of biased results and allow for more usable outputs giving more power to the risk assessment conclusions. Intrinsically, it is essential that the results of these methods provide transparent and robust estimates for the missing data.

To assess the risk of exotic disease incursion and spread through Europe, the OIE database of global animal disease prevalence was used and different imputation methods, based on country groupings, were compared to investigate whether model output accuracy could be improved. In the case study example of rabies, the results presented suggest that the methodology employed provided reasonable estimates for most countries in the world, but was not always accurate at country level. Results suggested that grouping countries before fitting a distribution was more accurate than just using a distribution fit to the whole world, but no grouping method was accurate for every country. As such, it is believed that the methodology is appropriate when estimating missing data, but it would be inadvisable to use the method in order to replicate an observed dataset.

An interesting result of these analyses is that they have the additional benefit of providing a statistic by which to determine the grouping method and/or proxy variables that are best to consider for a given situation; the results suggest that this varies between both animal host species and pathogens of interest. For the case study of rabies, a comparison of the average score across species suggested that the *AltUNsubregion* would be the best grouping measure to use. However, it is acknowledged that there are limitations to the analyses presented here. The metrics of comparison used here do not comprehensively describe everything about the goodness of fit; other metrics such as the average KS score may give different results. There are also other factors such as the proportion of MCs that fall into groupings where there are no observed data, so that no estimate can be determined: it should be considered whether a grouping that gives a higher KS score is still the best option if an alternative grouping with a

slightly lower KS score can provide estimates for more individual countries. This methodology could be further expanded to consider these issues, as well as test for other proxy variables or combinations of variables to evaluate whether they would provide a better fit. Another area where this methodology could be expanded would be to evaluate the fits for distributions other than the gamma (e.g. lognormal or Weibull).

The analysis presented here is designed to fill specific data gaps in global datasets, but is still reliant on good quality input data. While the OIE database is one of the most comprehensive in the world, there are still data gaps that lead to uncertainty about the model results, particularly with regard to the number of animal establishments. The assumption to treat cats and dogs as single entities could underestimate the risk, as it does not account for households that have multiple pets or institutions such as dog homes.

In conclusion, while there is a growing number of available global datasets providing information that could be used for risk assessments, there are currently assumptions that must be made regarding the data gaps before datasets can be used. Using global datasets as they stand, with missing data treated as a zero, may inadvertently penalise those countries that report disease outbreaks as opposed to those affected by a pathogen but that do not report the outbreak data. The methodology for estimating animal disease prevalence presented here allows for an objective, transparent approach to fill the data gaps and provide a more comprehensive base for data input to risk assessments.

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INFECTION DYNAMICS OF SCHMALLENBERG VIRUS IN CATTLE IN THE NETHERLANDS: ARE WE FACING AN ENDEMIC STEADY-STATE?

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SUMMARY

Since the outbreak of Schmallenberg virus (SBV) in 2011, several serological surveys have been carried out in the Netherlands that provided insight into the spread of SBV throughout the cattle population and the infection dynamics thereafter. The first serosurvey, conducted on archived serum samples from 2011-2012, demonstrated that SBV had rapidly led to herd prevalences >95%. Hence, new cases following overwintering of SBV were deemed unlikely. However, when herd immunity declines over time, re-emergence of SBV might result in a new epizootic. Therefore, the presence of SBV-antibodies in naïve youngstock was investigated in two surveys in 2013-2014 and 2015-2016, which revealed an increase in seroprevalence over time, indicating circulation of SBV. In most of the herds, however, the complete sample was seronegative. More importantly, if SBV has been circulating at a low level since 2012, the current level of herd immunity is likely to be sufficient to prevent large outbreaks.

INTRODUCTION

In the late summer of 2011, a new virus emerged in cattle, with fever, drop in milk production and diarrhoea as presenting clinical signs. A sudden rise in the incidence of these non-specific clinical signs was observed in dairy cows in the eastern region of the Netherlands (Muskens et al., 2012) and in northwestern Germany (Hoffmann et al., 2012). In the Netherlands, several affected farms were visited, and faecal and serum samples from diseased animals and bulk milk samples were investigated for numerous endemic and exotic pathogens. No causal agent was found until 18th November 2011, when the Friedrich Loeffler Institut (FLI, Germany) reported the detection of a novel orthobunyavirus in German cattle. The virus was identified by metagenomic analyses in samples from acutely diseased cows on a farm near the German city of Schmallenberg, and was thereafter named Schmallenberg virus (SBV). SBV belongs to the Simbu serogroup of the genus Orthobunyavirus of the family Bunyaviridae (Hoffmann et al., 2012). Viruses of the Orthobunyavirus genus are mainly transmitted by mosquitoes and *Culicoides* biting midges. After the identification of SBV in northwestern Europe, concerns were raised regarding the congenital defects SBV might induce in newborn ruminants, as several viruses of the Simbu serogroup are known to be teratogenic. These concerns were warranted, as from early December 2011 onward, congenital malformations, designated as arthrogryposis-hydranencephaly syndrome, were observed in SBV-infected newborn lambs and calves in the Netherlands (ProMED-mail,

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2011). Since its first detection in northwestern Europe, SBV spread rapidly throughout the rest of the continent in 2011-2013 (Afonso et al., 2014).

After the primary phase of an emerging disease outbreak, the objective of surveillance alters from early-detection to monitoring changes in prevalence of infection, monitoring the impact of control measures (such as vaccination or movement restrictions) and eventually demonstrating freedom from infection. The latter is of particular importance if trade restrictions are involved as long as disease freedom is not substantiated. To do so, cross-sectional surveys are often carried out. In the Netherlands, several serological surveys have been carried out that demonstrate the rapid spread of SBV throughout the ruminant population in 2011 and the infection dynamics thereafter. In the current study, the level of virus circulation inferred from these surveys is described with the aim to better understand the infection dynamics of SBV in a cattle population with a high replacement rate (about 25-30% in dairy cattle herds).

MATERIALS AND METHODS

Study population

The cattle population in the Netherlands comprises approximately 18,000 dairy herds and 18,000 non-dairy herds. Most dairy herds can be found in the northern and eastern region of the Netherlands. Most non-dairy enterprises (i.e. veal herds, beef suckler herds and cattle traders) are located in the eastern and southern part of the Netherlands.

Study design

Between early 2012 and late 2015, three serological surveys were carried out to gain insight into the prevalence of SBV in the cattle population. The primary objective of the first survey ('survey 2011') was to determine the true rate of infection after cessation of virus circulation in 2011, and to establish the proportion of naïve animals and therefore the risk of infection after potential overwintering of the virus. Over the following years, the presence of SBV-specific antibodies in naïve cattle was investigated in two subsequent surveys in autumn/winter 2013-2014 ('survey 2013') and in autumn/winter 2015-2016 ('survey 2015'), aiming to determine whether and to what extent SBV was still circulating/circulating again. Details regarding the design of the three surveys are described below. In all surveys, a sample size of five animals was deemed sufficient to detect SBV circulation in a herd at an expected animal prevalence of 50%, with 95% confidence. Where other assumptions were used, the sample size is further specified per survey.

Survey 2011: Archived serum samples from dairy heifers and adult non-dairy cattle submitted to GD Animal Health (GD) for monitoring purposes between November 2011 and March 2012 were selected. It was assumed that SBV virus circulation was very limited or had ceased in this period as a result of reduced activity of the vector. Therefore, a reliable estimate of the seroprevalence at the end of the 2011 epidemic could be made. SBV seroprevalence estimation for non-dairy adult cattle was performed based on serum samples that were originally collected to estimate Infectious Bovine Rhinotracheitis (IBR) seroprevalence in non-dairy herds in the Netherlands in autumn/winter 2011-2012. Based on the assumption that most of the SBV transmission had taken place before 1st November 2011, only serum samples collected after 31st October 2011 were included. In order to estimate regional differences, a maximum of 150 sera were randomly selected per province. This was

the sample size deemed sufficient to estimate animal prevalences of SBV in heifers with 95% confidence and a maximum accepted error of 8%, assuming an expected prevalence of 50%. In total, 1,373 samples from 276 herds were selected (Table 1). Non-dairy herd prevalences were estimated based on a mean number of five serum samples per herd (median 3). For dairy herds, sera from cattle that were initially sampled for national bluetongue monitoring in autumn/winter 2011-2012 were selected. Samples were originally selected at geographical compartment level according to EU regulation 1108/2008/EC. The selected animals had to be at least 8 months old at the time of sampling to exclude maternally derived antibodies against BTV. Only serum samples collected after 31st October 2011 were selected for SBV seroprevalence estimation, resulting in a total of 3,066 samples from 247 herds (Table 1). Dairy herd prevalence was estimated based on a mean number of 12.3 serum samples per herd (median 12).

Survey 2013: There was reason to believe that the level of SBV circulation was low in autumn 2013, as passive surveillance systems did not pick up signals of clinical disease in susceptible cattle during the most recent vector-active season in 2013. Therefore, the aim of the 2013 survey was to determine whether and to what extent SBV had circulated amongst dairy herds in the Netherlands in 2013. For this purpose, the presence of SBV-specific antibodies in naïve cattle (youngstock) was investigated. The study was conducted in two stages. In the first stage, a total of 394 randomly selected dairy farms were sampled between 1st October and 31st December 2013 (Table 1). This sample size was sufficient for the demonstration of freedom from SBV circulation using a design herd prevalence of 1%, with 95% confidence. In addition, if SBV did circulate in 2013, the sample size enabled an estimation of the herd prevalence with an approximate error of at most 5% (i.e. in the worst-case scenario with an expected prevalence of 50%; WinEpiscope 2.0 – De Blas et al., 2000). Participating farmers were requested to have their private practitioner collect blood samples from five randomly selected youngstock born between 30th October 2012 and 30th April 2013, being 8-12 months of age at sampling. It was assumed that animals with maternal antibodies or immunity following direct infection in 2011 or 2012 were excluded by these sampling restrictions. As the results of the first stage of the study were inconclusive in demonstrating freedom from SBV circulation, a more in-depth investigation was initiated to provide more insight. Therefore, in the second stage of the study, an additional sample of 20 youngstock within the same age category (including the five initially sampled animals) was collected from herds that tested positive in the first stage, in order to rule out potential false-positive test results. A sample size of 20 animals per herd was chosen to detect circulation at herd level with 95% confidence when the within-herd prevalence was at least 12.5% (WinEpiscope 2.0 – De Blas et al., 2000).

Survey 2015: In the autumn of 2014, SBV was detected in the Netherlands in a number of heifers following pre-export testing (ProMED-mail, 2014), indicating circulation of the virus. Therefore, in 2015, a survey was initiated to determine SBV seroprevalence amongst dairy and beef suckler herds in the Netherlands. For this purpose, the presence of SBV-specific antibodies in naïve youngstock was investigated in 193 randomly selected dairy herds and 149 randomly selected beef suckler herds (Table 1). These sample sizes enabled an estimation of an expected herd prevalence of 0.5% with an approximate error of at most 2%, with 95% confidence (WinEpiscope 2.0 – De Blas et al., 2000). Participating farmers were requested to have their private practitioner collect blood samples from five randomly selected youngstock born between 30th October 2014 and 30th April 2015, being 8-12 months of age at sampling. Blood samples were collected between 1st October 2015 and 31st December 2015.

Table 1. Overview of the study design of three serological surveys to estimate SBV prevalence amongst cattle herds in the Netherlands between 2011 and 2015

Year	Type of herd	Sample size	Time period	Age category
2011	Dairy	3,066 (247 herds)	1/Nov/2011 - 12/Dec/2011	8-24 months
	Non-dairy	1,373 (276 herds)	1/Nov/2011 - 31/Dec/2011	24+ months
2013	Dairy[a]	1,923 (394 herds)[a]	1/Oct/2013 - 31/Dec/2013[a]	8-12 months
2015	Dairy	951 (193 herds)	1/Oct/2015 - 31/Dec/2015	8-12 months
	Beef suckler	666 (149 herds)		

[a] In stage 1; in stage 2, another 316 samples from 17 herds were collected 2nd-23rd July 2014

Diagnostic methods

In each survey, samples were investigated for the presence of antibodies against SBV by means of an in-house indirect whole-virus ELISA (Van der Heijden et al., 2013) with a sensitivity of 98.8% (95% confidence interval (CI): 93.3-99.8) and a specificity of 98.8% (95% CI: 97.5-99.6). Test outcomes were expressed as a sample to positive percentage (S/P%) by comparing the net optical density (OD) of each sample with the average net OD of positive controls. Test outcomes were considered to be non-specific when the serum sample reacted with the control antigen (without viral antigen), resulting in an OD greater than 1.0, irrespective of the sample's gross optical density. Samples with an S/P% higher than 15% were considered positive. This cut-off was determined using a SBV virus neutralisation test (VNT; Loeffen et al., 2012) as gold standard. In the 2013 survey, seropositive samples from the first stage of the study were confirmed by VNT, as described by Loeffen et al. (2012).

Data analysis

Seroprevalences at animal level were estimated using multivariable logistic regression with a random herd effect in STATA 13.0 with the xtgee family (binomial) procedure. With this method, clustering of animals (or test results) within herds was taken into account. In the 2011 survey, herd prevalences were estimated based on a cut-off of one seropositive animal per herd as the whole population was assumed to be naïve. In the 2013 and 2015 surveys, herd prevalence was estimated based on a cut-off of two seropositive animals per herd to account for misinterpretation of results as a consequence of (long-lasting) maternal immunity. Results from dairy herds and non-dairy herds are presented separately due to differences in sampling design (in 2011) and the level of exposure to SBV (i.e. the amount of grazing).

RESULTS

Survey 2011

Results of the first survey indicated that SBV was rapidly transmitted throughout the ruminant population in 2011. An overall animal prevalence in cattle was estimated at 63.4% (95% CI: 57.9-68.5) in dairy heifers and 98.5% (95% CI: 97.2-99.3) in adult non-dairy cows. In 95.5% (95% CI: 92.3-97.7) of the dairy herds and 99.3% (95% CI: 97.4-99.9) of the non-dairy herds, at least one animal was seropositive (Table 2). No gradient spatial pattern in final

seroprevalence could be seen in the data and therefore no inferences about the site of introduction and spread of SBV in the Netherlands could be made.

Table 2. Overall estimated seroprevalences for SBV in cattle in the Netherlands between 2011 and 2015

Year	Type of herd	Seroprevalence (95% confidence interval)	
		Herd level	Animal level
2011	Dairy	95.5% (92.3–97.7)	63.4% (57.9-68.5)
	Non-dairy	99.3% (97.4-99.9)	98.5% (97.2-99.3)
2013	Dairy[a]	0.0% (0.0-0.9)[a,b]	1.1% (0.7-1.7)[a]
2015	Dairy	6.2% (3.3-10.6)	6.5% (5.0-8.3)
	Beef suckler	12.1% (7.3-18.4)	10.9% (8.7-13.6)

[a]In stage 1
[b]Zero herds were positive as the cut-off to classify a herd as positive was set at two seropositives

Survey 2013

First stage: ELISA testing indicated the presence of antibodies in 21 samples from 21 herds, resulting in an apparent animal level prevalence of 1.1% (95% CI: 0.7-1.7) and a herd prevalence of 0.0% (95% CI: 0.0-0.9; Table 2). Virus neutralisation testing confirmed the presence of antibodies in 13 out of the 21 ELISA-positive samples, with VNT titres ranging from 12 to ≥48 (median: 24). All 21 seropositive calves were single reactors within the sample obtained from each farm. They were born between 26th July 2012 and 16th March 2013, and the age at sampling ranged from 8.4 to 15 months (median: 12.2).

Second stage: To rule out false positive results, a more in-depth investigation was initiated in a second stage of the study. Seventeen out of the 21 farmers agreed to participate in the second stage of the study and serum samples were collected from an additional sample of youngstock (including the initially sampled youngstock). From those farms, a total of 316 animals were sampled between 2nd and 23rd July 2014. Antibodies were detected in 9 out of 316 samples, from nine herds. This suggested that these seroconversions were unlikely to be the result of natural infection in 2013, as SBV circulation is known to lead to high within-herd seroprevalences. An overview of the combined test results of the 17 herds is provided in Table 3.

Eleven out of 12 animals that were confirmed positive in the initial investigation were included in the second stage of the study. In six of them (herds 4-9) aged 9-15 months at first sampling, no SBV-specific antibodies were detected in the second investigation. The remaining five animals (herds 12-15 and 17) repeatedly tested positive in both investigations. These five animals were born between the end of August 2012 and mid-December 2012, and were at least 1 year old in both investigations. The five animals that were initially confirmed negative (herds 1-3, 10-11) also tested negative in the second stage, yet in two of these herds (10 and 11), another animal tested positive in the second stage. In one herd (no. 16), an animal that tested negative in the first stage tested positive in the second stage.

Table 3. SBV-specific ELISA and VNT test results in youngstock in 17 dairy herds, with sample size per herd, confirmation virus neutralisation test (VNT) result and titre of seropositive animal and date of birth of animals with a positive test result

	Stage 1				Stage 2		
Herd no.	Sample size[a]	VNT result[b]	VNT titre	Date of birth[c]	Sample size	ELISA-positives (n)	Date of birth[c]
1	5	N	<8	24-Nov-12	20[d]	0	-
2	5	N	<8	24-Nov-12	20	0	-
3	5	N	<8	25-Oct-12	16	0	-
4	5	P	12	03-Oct-12	20	0	-
5	5	P	24	12-Jan-13	20	0	-
6	5	P	12	11-Nov-12	21	0	-
7	5	P	≥48	01-Oct-12	21	0	-
8	5	P	≥48	18-Nov-12	20	0	-
9	5	P	16	11-Oct-12	20	0	-
10	3	N	<8	26-Jul-12	23	1	25-Apr-12
11	5	N	<8	28-Jan-13	20	1	21-Jun-12
12	5	P	12	14-Dec-12	14	1	14-Dec-12
13	5	P	≥48	20-Aug-12	9	1	20-Aug-12
14	5	P	12	11-Dec-12	20	1	11-Dec-12
15	5	P	≥48	30-Nov-12	23	1	30-Nov-12
16	5	P	24	05-Nov-12	9[d]	1	03-Oct-12
17	5	P	12	08-Nov-12	20	2	08-Nov-12[e]
17							29-Aug-12[e]

[a]Each sample yielded one ELISA-seropositive animal in stage 1
[b]VNT result of the ELISA-seropositive animal
[c]Date of birth of the ELISA-seropositive animal
[d]In this sample, not all animals from stage 1 could be included
[e]Both belonged to herd 17

Survey 2015

An overall true animal-level seroprevalence of 6.5% (95% CI: 5.0-8.3) and 10.9% (95% CI: 8.7-13.6) was found in dairy herds and beef suckler herds, respectively (Table 2). In dairy herds, the age of the seropositive calves was no different from the age of the seronegative calves (*P*-value: 0.47). In beef suckler herds, the age of the seronegative calves was slightly lower than the age of the seropositive calves (*P*-value: 0.04). It was hypothesised that calves born from dams that were exposed during the 2011-2012 epidemic were more likely to benefit from maternally derived immunity than calves born from mothers born after the 2011-2012 epidemic. If so, seronegative calves were born from older dams than seropositive calves. In dairy herds, the age of the dams of seropositive calves (median 53 months) was no

different from the age of dams of seronegative calves (median 56 months; *P*-value: 0.49). In beef suckler herds, the age of dams of seropositive calves (median 56 months) was no different from the age of dams of seronegative calves (median 57 months: *P*-value: 0.74).

At herd level, 12 out of 193 dairy herds were classified as positive (6.2%, Table 2). In two of these herds, all five samples were seropositive. In 42 dairy herds, one sample tested positive (single reactor; Fig. 1). In beef suckler herds, 18 out of 149 herds were classified positive (12.1%, Table 2). In one of them, all five samples were seropositive. Thirty-eight beef suckler herds had single reactors (Fig. 1).

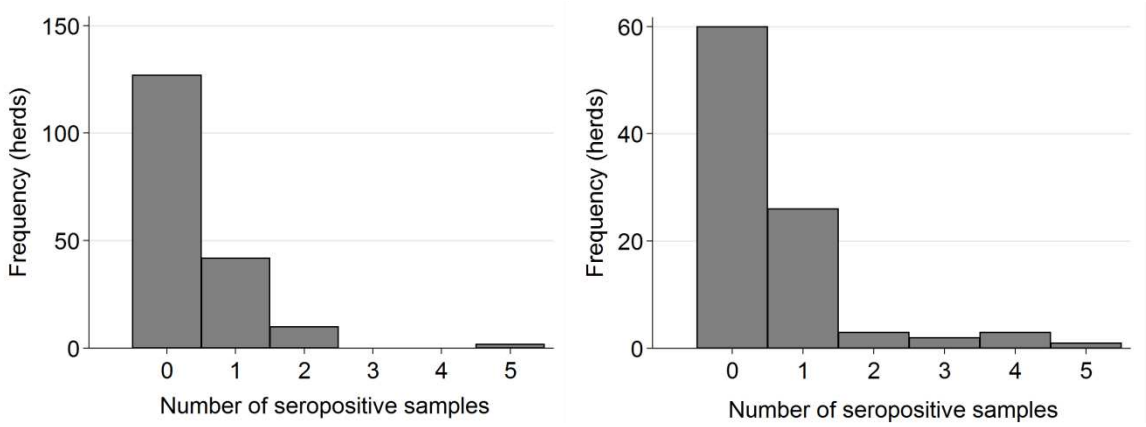

Fig. 1 Frequency distribution of number of youngstock with SBV-specific antibodies from a sample of five serum samples per herd in 193 dairy herds (left) and 149 suckler herds (right). Serum samples were obtained from calves with an age of 8-12 months and were collected between 1st October and 31st December 2015

DISCUSSION

When SBV was identified in 2011, very little was known about this emerging virus. The large-scale serosurvey performed in 2012 in the Netherlands provided evidence that the introduction of SBV had rapidly led to high seroprevalences in the cattle population. This finding had two important implications. Firstly, due to the small proportion of (remaining) naïve animals after one vector-active season, it was likely that the incidence of new cases following potential overwintering of SBV was going to be low. A lower seroprevalence might have been a justification for a vaccination campaign or other protective measures. Secondly, this novel virus was apparently transmitted throughout the ruminant population very efficiently, affecting >95% of the cattle herds.

As a consequence of the extensive spread in 2011, the herd immunity after one vector-active season was high. In the subsequent vector active season (2012), SBV was still circulating in the Netherlands and in other primary affected regions (Conraths et al., 2013; Elbers et al., 2015; Méroc et al., 2013), but at a low level. In time, when herd immunity declines in Europe, reintroduction or re-emergence of SBV in Europe (or parts thereof) might result in a new epizootic. The question was when herd immunity would drop to a critical level where the risk of a new epidemic was substantial. Therefore, the presence of SBV-specific antibodies in naïve cattle was investigated in the 2013 survey. The combined results of this survey revealed a low level of SBV seroconversions in the sampled youngstock,

although the results were somewhat surprising as all but one of the seropositive calves were single-reactors in the 394 study herds. This suggested that these positive test results were unlikely to be the result of natural infection in 2013, as SBV circulation in a herd is known to result in high within-herd seroprevalences. Therefore, assuming the single-reactors to be false-positives, this survey showed that in 2013, SBV circulated in less than 1% of the dairy cattle herds in the Netherlands. This result was in agreement with the fact that the passive surveillance component in place ('GD Veekijker') had not picked up any signal of clinical disease suspected to be caused by SBV during the vector-active season in 2013. Single reactors have been found in other European surveys as well (Stokes et al., 2016, Collins et al., 2016). One effective (but costly) alternative to explain the true nature of seroconversions is to set up a longitudinal study to follow-up immune responses of (sentinel) animals over time during months of vector activity.

In the 2015 survey, a low level of circulation of SBV was found, based on SBV-specific antibodies in youngstock born in 2015 and at least 8 months of age at the time of sampling, in dairy and suckler herds. The results of the survey confirmed that the virus was again circulating in the Netherlands, but still at a low level; in 72% of the dairy herds and 64% of the beef suckler herds all of the tested youngstock were seronegative (results not shown). In Belgium and Luxemburg, SBV was confirmed as the causative agent in two malformed bovine foetuses in February and April 2016, indicating SBV circulation in 2015 (DGZ, 2016a). Whether SBV circulated in 2015 in other European countries that were part of the primary outbreak area is unknown. In 2015 and early 2016, no calves with the arthrogryposis-hydranencephaly syndrome or other malformations typical of SBV infection were submitted to GD for post-mortem examination. In one malformed lamb, submitted to GD for post-mortem examination in February 2015, SBV was detected in brain tissue and SBV-specific antibodies were found in the serum (GD Animal Health, 2015). However, the number of phone calls to GD Veekijker regarding SBV was no different in 2015 than 2014 or 2013 (2% per year; unpublished data). This may be because farmers knew or suspected that clinical signs were caused by SBV and therefore did not call, but it might also suggest that SBV did not cause significant health problems in cattle in 2015.

The low-level transmission as inferred from the 2015 observations was rather surprising, as the average yearly replacement rate of the cattle population and the absence of preventive measures such as vaccination, is likely to have resulted in a ruminant population with the majority susceptible to SBV infection. Assuming a basic reproduction number of 6.2 in a fully susceptible cattle-only population, as estimated by Gubbins et al. (2014), the effective reproduction ratio would be above 1 in 2015, allowing for extensive transmission of SBV. However, it is important to note that the effective reproduction number of vector-borne pathogens is strongly influenced by climatological and ecological conditions that determine vector abundance and competence, leading to seasonal fluctuations in transmission rates. One can only speculate about other reasons for the lower than expected transmission, such as the virus might have mutated to a strain causing less rapid spread throughout the ruminant population, or the possibility that the infection in the population is currently settling towards an endemic equilibrium, with fluctuating numbers of susceptible, infectious and immune animals until the equilibrium is stable.

Since the summer of 2016, GD Veekijker has received a number of phone calls about fever and diarrhoea in cows. In several of these herds, SBV or SBV-specific antibodies have been demonstrated in collected blood samples (ProMED-mail, 2016). At the same time, in Belgium, several malformed aborted calves with typical signs of SBV infection were

submitted for autopsy, yet the presence of the virus could not be demonstrated (DGZ, 2016b). These signals indicate that SBV is indeed circulating in some of the countries in the primary SBV outbreak area. However, if SBV has circulated at a low level since 2012, the current level of herd immunity is likely to be sufficient to prevent large outbreaks.

The three successive surveys resulted in complementary information about SBV circulation in the Netherlands. However, the surveys were not sufficient to provide definite answers about the infection dynamics of SBV. Other study designs, such as longitudinal studies in sentinel herds would be able to add information. In addition, experimental infections may provide insight into the fluctuations in transmission rates that seem to occur in the field. Nevertheless, the surveys provided adequate information to assess the expected impact of SBV circulation for the cattle industry.

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SURVEILLANCE AND IMPORT RISK

SYNDROMIC SURVEILLANCE OF PHONE CALLS CONCERNING DAIRY CATTLE HEALTH PROBLEMS FOR EARLY DETECTION OF DISEASE OUTBREAKS IN THE NETHERLANDS

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SUMMARY

The aim of this study was to develop a real-time syndromic surveillance system based on calls to helpdesk 'Veekijker' concerning non-specific health problems in dairy cattle for early detection of emerging infectious diseases. The 2011 Schmallenbergvirus (SBV) outbreak in the Netherlands was used as case study, and a time series and spatiotemporal cluster analysis were run. At least four consecutive weeks with one or more alerts of increased phone calls were considered to require follow-up. The results showed that syndromic surveillance of phone calls would have resulted in follow-up actions in mid-July, whereas in reality with no syndromic surveillance, actions to verify whether a disease had emerged started on 25th August 2011. This study showed that real-time syndromic surveillance of phone calls about cattle health problems provides quantitative information to 'Veekijker' veterinarians, and may increase the sense of urgency in the initial phase of an emerging disease outbreak.

INTRODUCTION

Since 2002, a national surveillance system has been operational to monitor trends and developments in animal health, and for the early detection of (re)emerging diseases in livestock in the Netherlands. Concerning the latter, a telephone helpdesk 'Veekijker' is operational, staffed with veterinary experts. This helpdesk receives approximately 4,000 calls about cattle each year. The aim of this helpdesk is to provide free veterinary advice and in return, the helpdesk gains information on animal health issues that may be related to (re)emerging diseases, which is valuable for early detection of animal health disorders and diseases (Van Wuijckhuise et al., 2011; Santman-Berends et al., 2016). All telephone calls are registered in a central database. Most phone calls concern non-specific symptoms, such as fever, diarrhoea, drop in milk production and fertility disorders in dairy cattle. However, many (re)emerging diseases show non-specific symptoms at the onset of the disease that may be misinterpreted as endemic diseases. Misinterpretation of non-specific symptoms extends the high-risk period of (re)emerging diseases, giving the infection more time to spread to other farms and increasing the costs needed to control the outbreak. Therefore, a tool was needed to quantify and combine phone calls about non-specific symptoms among dairy cattle. The sense of urgency would increase with a signal from the syndromic surveillance system.

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This signal would be triggered by deviations on phone call data, possibly associated to initial symptoms of the disease. The aim of this study was to develop a real-time surveillance system based on phone calls about non-specific symptoms in dairy cattle providing quantitative information to veterinary experts concerning cattle health problems. The Schmallenbergvirus (SBV) that emerged in Dutch ruminants in 2011 causing a drop in milk production, fever and diarrhoea at the early onset of the epidemic was used as case study.

MATERIALS AND METHODS

Phone calls

Phone call data about symptoms including a drop in milk production, fever, diarrhoea and fertility disorders were available from 1st January 2009 to 31st December 2011. These data included unique farm identification or unique veterinary practice numbers, the date of the phone call, cattle category (e.g. dairy cows, youngstock, bulls, veal calves, suckler cows), and symptoms (drop in milk production, fever, diarrhoea or fertility disorders, including abortions). Geographic location (x, y coordinates) of Dutch dairy herds and veterinary practices were provided by GD Animal Health.

Statistical analysis

Statistical analyses were performed using STATA/SE version 14 software (StataCorp. 2015). Firstly, for each of the reported symptoms, the number and percentage of phone calls per year were determined for 2009, 2010 and 2011. Statistically significant differences between years were determined using two-sample test of proportions (prtesti) with *P*-value ≤ 0.05.

Secondly, for each of the reported symptoms, a time series analysis was run for which the data were aggregated at week level. For each week and each reported symptom, a two-weekly moving sum of phone calls was determined to have sufficient numbers of phone calls per week. In addition, a time series of the two-weekly moving sum of phone calls per week was constructed using a linear regression model (regress; Eq. (1)). Seasonality was taken into account by including sine/cosine harmonics as predictors. The number of harmonics was chosen according to the AIC criterion to best fit the observed number of phone calls.

$$y_i = \beta_0 + \sum_{n=1}^{7} \left(\alpha_n \cos \frac{2\pi * t * n}{52} + \beta_n \sin \frac{2\pi * t * n}{52} \right) + \varepsilon_i \quad (1)$$

where:

y_i	= the expected two-weekly moving sum of phone calls in week *i*
β_0	= intercept (constant)
α_n/β_n	= phase and amplitude parameters
n	= harmonic number
t	= the number of weeks since the first week in the dataset
ε_i	= random error in week *i*

For each reported symptom, the analysis was repeated for each week in 2011 with an updated moving baseline period of the previous 104 weeks (i.e. the use of a weekly time series analysis was simulated, as if applied in real-time). The difference between the observed

and predicted number of phone calls (the residual) was determined for weeks 1 to 52 in 2011. Weeks in which the residual exceeded the threshold value (95th percentile of the distribution of residuals from the baseline period) were considered alert weeks.

Thirdly, for each of the reported symptoms, a spatiotemporal cluster analysis was run to identify space-time clusters of increased number of phone calls about drop in milk production, fever, diarrhoea and fertility disorders. Phone calls on each of the reported symptoms were aggregated on postal district-week level and uploaded in SaTScan (Kulldorf, 2009). Unique farm identification or unique veterinary practice numbers were missing for 9.0% of the phone calls, and the location of these phone calls was therefore unknown and they were excluded from the spatiotemporal cluster analysis.

For each of the reported symptoms, a prospective analysis was carried out using a space-time permutation model (Kulldorf, 2005). A circular window shape was chosen. 'High rate' clusters (i.e. increased number of phone calls on the reported symptom than would have been expected) were scanned. For each window, a likelihood-ratio test statistic was calculated and the window with the maximum value was considered as the cluster least likely to have occurred by chance. The analysis was repeated for each week in 2011 with an updated moving baseline period of the previous 104 weeks. The maximum spatial cluster size was set at 10% of the population at risk. A *P*-value was assigned to each cluster using Monte Carlo hypothesis testing (999 simulations). Clusters of increased numbers of phone calls were defined as windows with a *P*-value $\leq$0.05 and considered alerts.

For both analyses, the threshold to trigger an alarm was set at a minimum of four consecutive weeks with one or more alerts of increased numbers of phone calls on drop in milk production, fever, diarrhoea and/or fertility disorders. These alarms were considered as signals requiring follow-up actions. It was thought that the SBV epidemic started between mid-July and mid-August and the first signal obtained by 'Veekijker' was on 25th August 2011 (Veldhuis et al., 2013). However, because of the delay between infection and appearance/absence of clinical signs and limited awareness of SBV in the initial stage of the epidemic, it was assumed that reporting of clinical suspicions was probably delayed. Therefore, alarms were considered true up to 4 weeks prior to suspicion (Veldhuis et al., 2016). Alarms that were found from mid-July 2011 onwards were considered alarms that could have been associated with the SBV epidemic.

RESULTS

Descriptive analysis

The percentage of phone calls concerning drop in milk production, fever, diarrhoea and fertility disorders were significantly higher in 2011 compared to 2009 and 2010 (Table 1, two-sample test of proportions, *P*-values $\leq$0.01). No significant differences in the percentage of phone calls on each of the reported symptoms were found between 2009 and 2010 (Table 1, two-sample test of proportions, *P*-values >0.05).

Table 1. The number and percentage of phone calls concerning a drop in milk production, fever, diarrhoea or fertility disorders in dairy cattle and the total number of phone calls concerning cattle health problems in dairy cattle to the telephone helpdesk 'Veekijker' in 2009, 2010 and 2011

Phone calls	2009		2010		2011	
	Number	%	Number	%	Number	%
Drop in milk production	172	4.8	137	4.4	280	7.9
Fever	70	2.0	77	2.5	198	5.6
Diarrhoea	80	2.3	71	2.3	132	3.7
Fertility disorders	229	6.4	167	5.4	293	8.3
Total number of phone calls	3,554	100.0	3,089	100.0	3,542	100.0

Time series analysis

The results from the time series analysis showed four signals with an increased number of phone calls about a minimum of one of the four symptoms in 2011, namely: i) 6th August (alerts in weeks from 9th July until 5th August) and ii) 10th September, 17th September and 24th September (alerts in weeks from 13th August until 23rd September; Fig. 1). The first signal could have been associated with the start of the SBV epidemic. When this signal was discussed with veterinary experts at that time, it could have resulted in follow-up actions that were 19 days earlier than when the veterinary experts of 'Veekijker' signalled the unusual events in the absence of the real-time syndromic surveillance. The signals found in September were in the period during which SBV quickly spread among Dutch dairy cattle.

Spatiotemporal cluster analysis

The spatiotemporal cluster analysis showed ten signals. Follow-up actions would have been taken on: i) 9th April and 16th April (alerts in weeks from 12th March until 15th April) and ii) 16th July until 3rd September (alerts in weeks from 8th June until 2nd September; Fig. 2). The signals in April were found in a period in which it was unlikely that SBV had emerged. The signals from 16th July until mid-August could have been associated with the start of the SBV epidemic. The signal found on 16th July would have been discussed with veterinary experts from the 'Veekijker' when the real-time syndromic surveillance was run during the SBV epidemic. This could have resulted in follow-up actions that were 40 days earlier than the first signal from the 'Veekijker' experts without a real-time syndromic surveillance. The consecutive signals found from mid-August until the beginning of September were in the period that SBV was quickly spreading among Dutch dairy cattle.

Clusters of increased phone calls about a drop in milk production and fever were found during the same time period in the same area, the eastern part of the Netherlands. In addition, overlapping clusters of increased phone calls about a drop in milk production and fertility disorders were found in the northern part of the Netherlands. Clusters of increased telephone calls about a drop in milk production and diarrhoea in the southern part of the Netherlands and clusters of fertility disorders in the middle and western part of the Netherlands increased over time (i.e. the radius of the clusters increased). These increasing space clusters over time could have been associated with the spread of the SBV virus to farms in the vicinity.

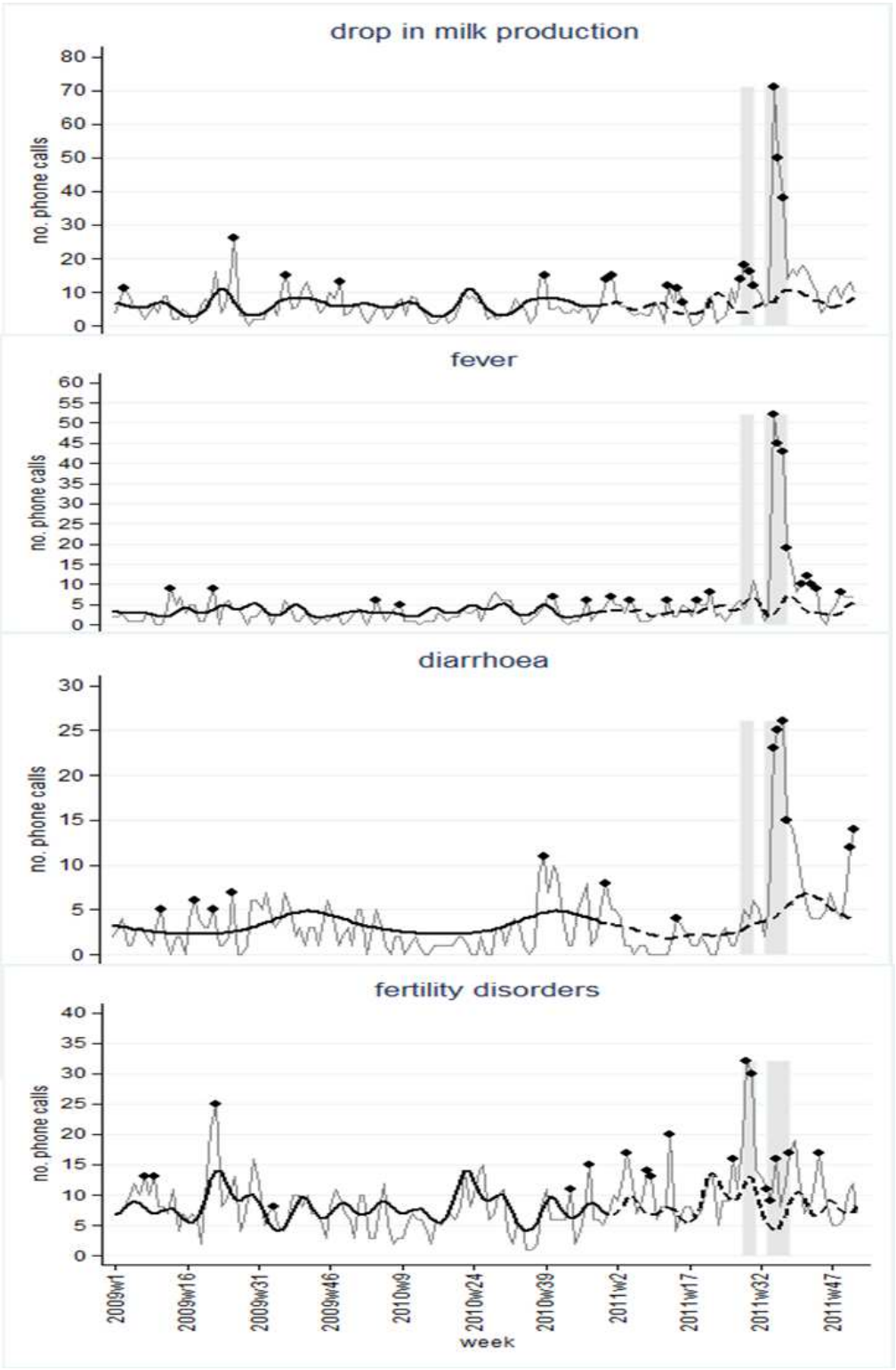

Fig. 1 Observed (solid grey), fitted (solid black) and predicted (dashed black) 2-weekly moving sum of phone calls concerning drop in milk production, fever, diarrhoea and fertility disorders in dairy cattle for the period 1st January 2009 until 31st December 2011. The black squares represent alert weeks, i.e. weeks in which the residual exceeded the threshold value (95th percentile of the distribution of residuals from the baseline period). The grey bars represent the weeks in which the time series analysis would have given a signal requiring follow-up actions

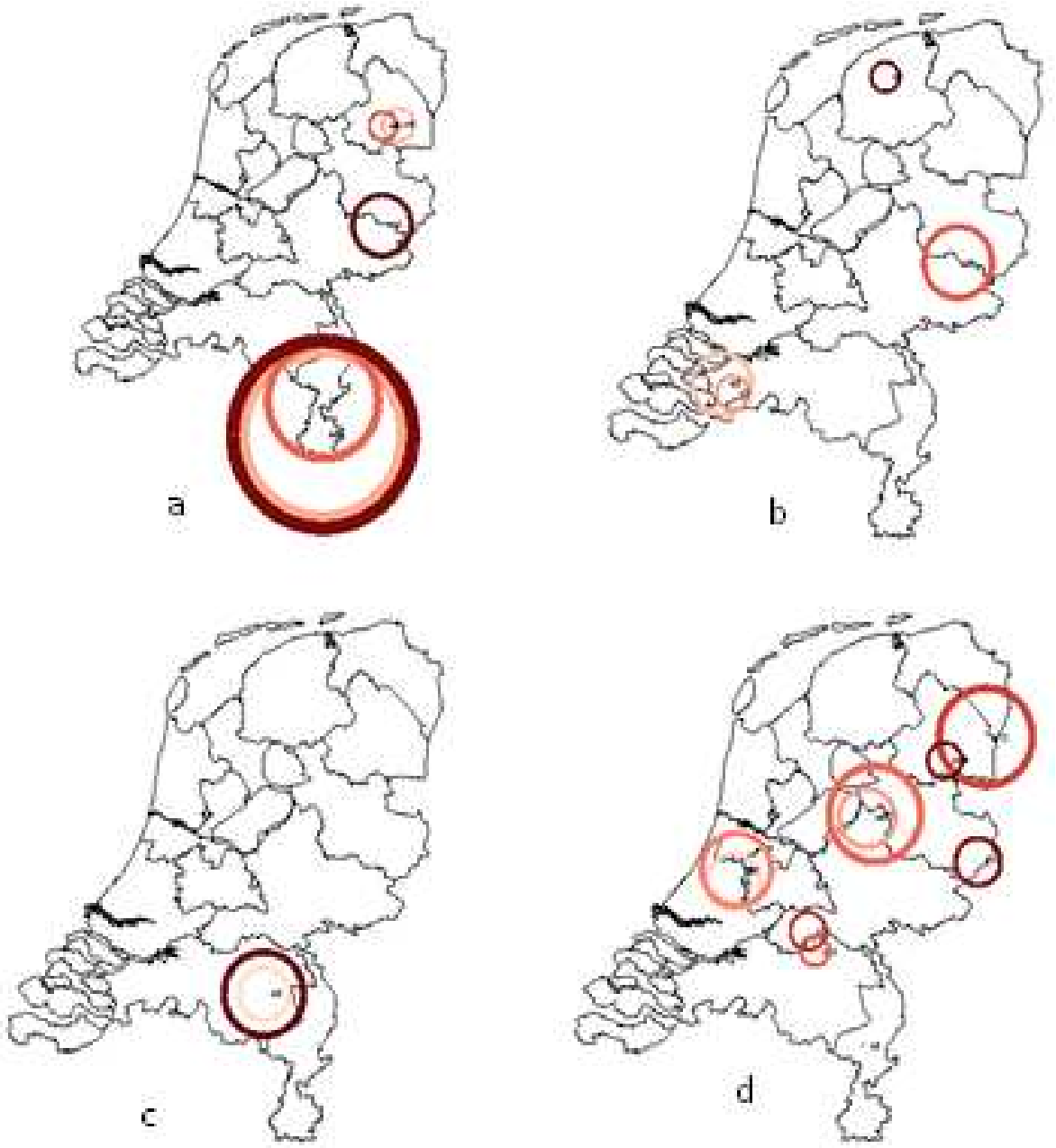

Fig. 2 Space-time clusters of increased phone calls concerning drop in milk production (a), fever (b), diarrhoea (c) and fertility disorders (d) in dairy cattle in the Netherlands in the period between 8th June and 31st December 2011 in agreement with the SBV epidemic. The darker the circle, the more recent the cluster

DISCUSSION

As many emerging diseases show non-specific symptoms at the start of the epidemic, these signs may be interpreted as endemic diseases, and the start of an epidemic may go unnoticed. This study showed that combining the signals from a real-time syndromic surveillance of phone calls concerning four non-specific symptoms provided quantitative information to veterinary experts and, combined with their expertise, might increase the sense of urgency in the initial phase of an emerging disease outbreak. If the syndromic surveillance

of phone calls ran in real-time in 2011, it could have resulted in follow-up actions in mid-July, whereas without syndromic surveillance, the follow-up actions to verify whether a disease had emerged actually started at the end of August 2011.

Data on phone calls to 'Veekijker' from practitioners and farmers concerning cattle health problems were used. These data are uniformly collected and registered in a central database. However, registration of unique farm identification or veterinary practice number is voluntarily and in 9.0% of the registered phone calls, this number was missing. The origin of these phone calls is unknown and therefore these calls were excluded from the space-time cluster analysis, which could have resulted in decreased sensitivity and timeliness of the analysis. Complete registration of unique farm identification or unique veterinary practice number is recommended to increase the performance of the syndromic surveillance of phone calls.

In this study, data on phone calls concerning four non-specific symptoms were combined into a syndromic surveillance tool and implemented at GD Animal Health in March 2016. Recently, the syndromic surveillance tool for phone calls has been extended, with phone calls concerning respiratory problems, sudden death and increased mortality among dairy cattle. In addition, phone calls concerning non-specific health problems among youngstock have been included. On the other hand, routinely available data on bulk milk and fertility have been shown to be suitable for syndromic surveillance to increase the sense of urgency in the initial phase of an emerging disease outbreak (Veldhuis et al., 2016). If routine census data, such as bulk milk collection recordings and fertility records, are available, these data can also be included to increase the sensitivity of the syndromic surveillance for early detection of disease outbreaks.

An appropriate threshold to trigger an alarm was set when at least four consecutive weeks with one or more alerts of increased number of phone calls about a drop in milk production, fever, diarrhoea and/or fertility disorders were found. The choice of an optimal threshold to trigger an alarm is an important issue when setting up a syndromic surveillance, and might differ between countries. For example, lowering the threshold to trigger an alarm to two consecutive weeks would increase the sensitivity and timeliness of the surveillance system but decrease the specificity due to more false alarms. The costs of follow-up actions have to be taken into account, and to keep them at an acceptable level, only combined alerts could be investigated. However, the choice of the temporal and geographical unit might differ per health problem and between countries, depending on the availability and frequency of data collection and the occurrence of health problems after the onset of a disease outbreak.

The results from the space-time cluster analysis showed that clusters with increased phone calls concerning more than one symptom from the same area and the same time period were detected. Some space clusters of non-specific health problems increased in time, which could have been associated with the spread of the SBV virus to multiple farms in the vicinity. The detection of multiple clusters of different symptoms from the same area and the increase of space clusters in time are important signals. These should therefore be taken into account when setting an optimal threshold to trigger an alarm.

ACKNOWLEDGEMENTS

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QUANTIFICATION OF THE IMPORT RISK OF BVD AND IBR: SIMILARITIES AND DIFFERENCES

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SUMMARY

The eradication of bovine viral diarrhoea (BVD) and infectious bovine rhinotracheitis (IBR) has been considered in the Netherlands. Therefore, insight into the risk of introducing these viruses through cattle imports is important. This study presents a quantification of import risks using stochastic simulation models. Intervention scenarios involving testing, import restrictions and vaccination were also evaluated. Both BVD and IBR are imported on a regular basis, especially by veal herds. For BVD, the import risk in non-veal herds is limited, while the import risk for IBR is substantial. For BVD, virus testing combined with antibody testing in pregnant cattle was the most effective preventive measure. For IBR, the most effective intervention involved vaccination of calves combined with testing of older cattle. Similarities between the models were the input parameters on import numbers and transport, and risk factors such as maternal protection. Nevertheless, the models also differed due to disease-specific characteristics.

INTRODUCTION

Quantitative risk assessments are frequently used to evaluate the risk of importing infectious diseases (De Vos et al., 2015). The feasibility of national control programmes for bovine viral diarrhoea (BVD) and infectious bovine rhinotracheitis (IBR) are currently being discussed, with the goal to eradicate both cattle diseases from the Netherlands. BVD virus can be transmitted both horizontally leading to transiently infected cattle (TI) and vertically. Vertical transmission results in Trojan (TR) cows that carry a persistently infected calf (PI) (Van Oirschot, 1983; Houe, 1995). PI cattle are the most important source of virus spread as they continuously shed large amounts of virus (as reviewed by Lindberg & Houe, 2005). IBR is caused by Bovine Herpes virus type 1 (BoHV1). After infection with BoHV1, the animal will shed the virus during a short period, after which it becomes seropositive (Bosch et al., 1996). Seropositive cattle remain latently infected for the rest of their lives and stress can induce reactivation and intermittent excretion of the virus into the environment (Kaashoek et al., 1996a; Muylkens et al., 2007).

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Import of cattle is considered the largest threat for introduction of BVD and IBR into the Netherlands. The exact risk of import is unknown and more information is required to evaluate whether risk-mitigating actions will be effective. For the risk analysis of both infections, the same approach could be applied. Nevertheless, the transmission and impact of BVD and IBR are not similar and therefore the structure of the respective models differed.

In this study, a stochastic simulation approach was used to quantify import risks for BVD and IBR. For both infections, a number of intervention scenarios were evaluated. Similarities and differences between the simulation models assessing the import risk of BVD and IBR in Dutch cattle herds are discussed.

MATERIALS AND METHODS

A risk-release pathway described the risks of cattle imports for both BVD and IBR in which the initial risk for both viruses was the same (Fig. 1). In this model, the risk of cattle imports was quantified and the information of imports at animal level were derived from Identification and Registration data (I&R; RVO, Assen the Netherlands). In this database, all imports are registered at animal level with the country of origin, the birth date of the imported animal, the date of import and the unique herd number of the receiving herd.

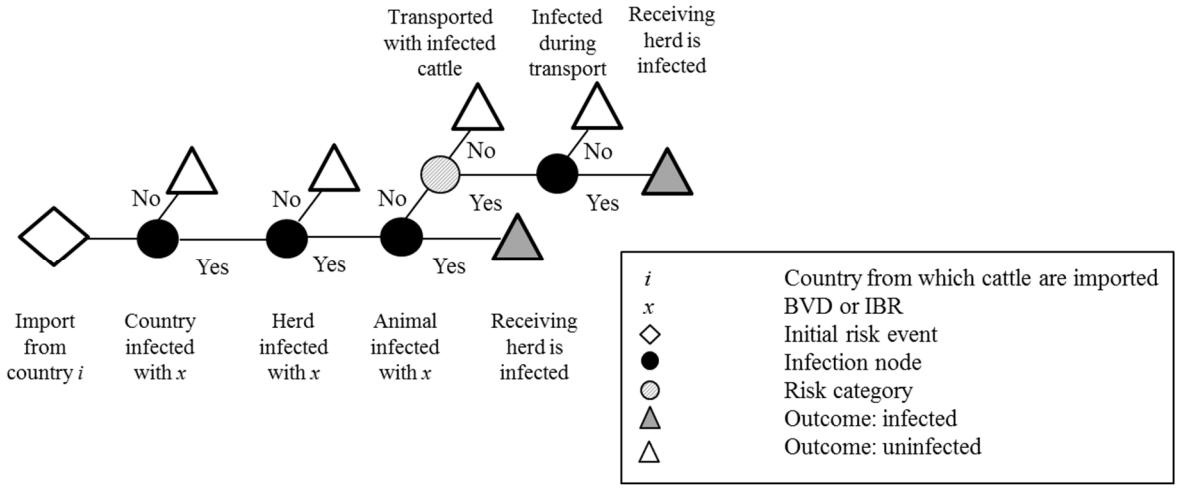

Fig. 1 Risk-release pathway for cattle imports

Imports

Annually, about 900,000 cattle originating from 21 different European member states (EU MS) are imported into the Netherlands (I&R data from 2011 until 2015). Most imported cattle are calves <4 months (mo) of age and originate from Germany (55%), Poland (9%) and Belgium (7%; Table 1). The probability of importing BVD or IBR could be estimated based on, among other things, information on the number of imports and the disease status of the country of origin (free versus endemic), combined with demographic information of the country of origin (i.e. number of cattle herds and total number of cattle).

Table 1. Imported cattle (total number (N) and number of cattle <4 months of age) in the Netherlands, by country of origin

Country	Total imported cattle (N)	N <4 months of age	Country (continued)	Total imported cattle (N) (continued)	N <4 months of age
Austria	448	282	Latvia	33,787	33,575
Belgium	63,951	42,242	Lithuania	48,226	48,008
Bulgaria	15	15	Luxembourg	10,809	10,362
Czech Republic	39,405	37,094	Poland	76,267	75,632
Denmark	37,449	35,730	Portugal	8	3
Estonia	23,464	22,980	Romania	1,608	1,540
France	6,074	497	Slovakia	10,851	10,736
Germany	495,328	487,071	Spain	72	22
Hungary	157	83	Sweden	18	0
Ireland	45,409	45,218	United Kingdom	38	1
Italy	8,690	7,873			
			Total	902,074	858,964

Disease-specific information

For BVD, an important input parameter was the percentage of herds with an indication of virus circulation and thus presence of a PI animal in the country of origin (Santman-Berends et al., 2017). For IBR, all herds with seropositive cattle were assumed to be a risk and thus the seroprevalence at herd level for each of the source countries was obtained. Initially, information on the BVD and IBR herd prevalence was evaluated based on the literature. In addition, more recent information was gathered through personal contacts (either in person or by phone) with BVD and IBR experts from the source countries.

Within an infected herd, cattle could have several disease statuses, which differed between BVD and IBR. For BVD, the import risk depended on the animal status, and six statuses were distinguished: maternally protected calves (M), susceptible cattle (S), recovered cattle (R), PI, TR or TI cattle. For IBR, cattle from infectious herds could have one of four risk statuses: M, S, acutely infected (AI) or latently infected (LI).

For BVD, a PI or TR is known to pose a higher risk for infection compared to a TI. For IBR, an acutely infected cow is known to pose a higher risk for infection when imported compared to a latently infected animal. In addition, the probability of occurrence of each of these infection statuses depends on the age of the animal, the presence of maternal antibodies and the pregnancy status of the animal. Because the animal infection status within infected herds differs greatly between BVD and IBR, the risk pathway described in Fig. 1 had to be expanded. This resulted in two separate risk pathways to describe the import risk for both diseases (Fig. 2a and 2b).

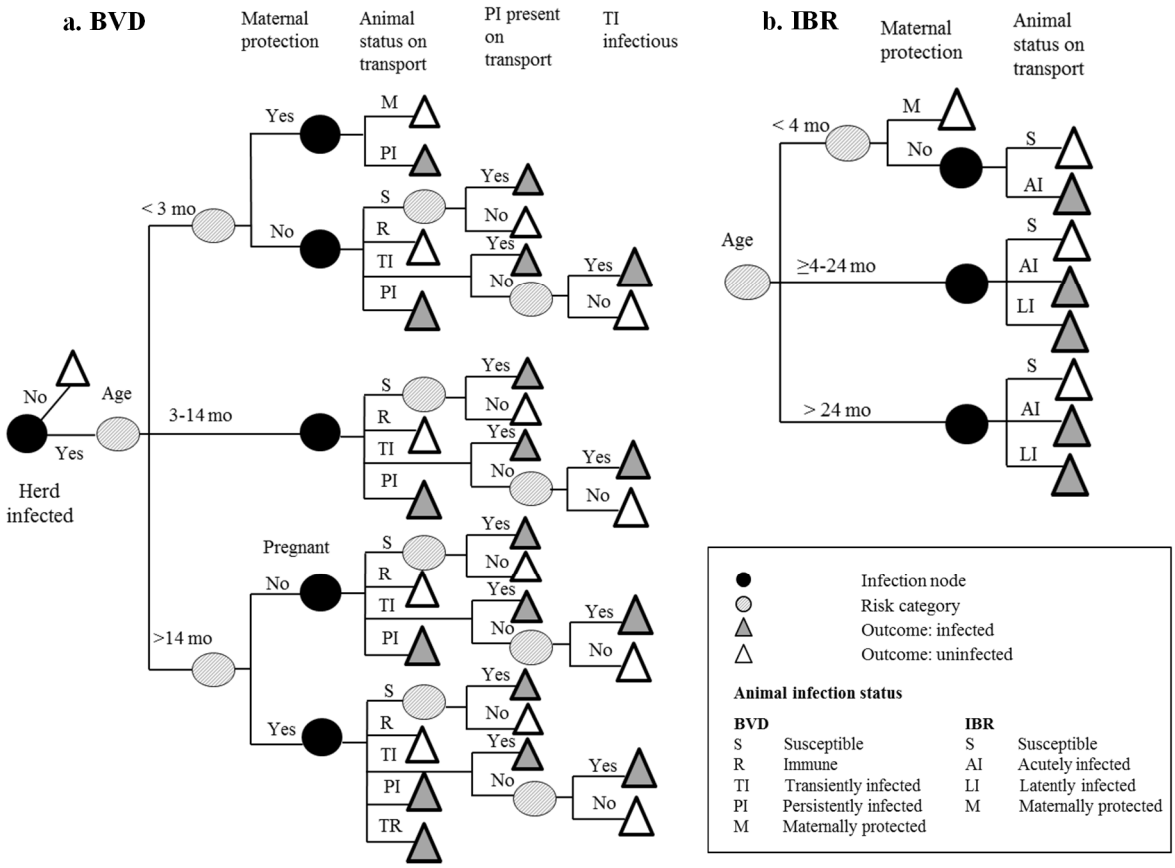

Fig. 2 Risk pathway for cattle imported from a herd infected with BVD (a) or IBR (b)

The risk pathway, risk factors and assumptions for BVD are described in detail by Santman-Berends et al. (2017). Therefore, the detailed pathway, risk categories and assumptions will be described for IBR in this paper.

The percentage of cattle infected with IBR (either acute or latent) in infected herds differs per age category, and was estimated as 55% in adult cows (Mars et al., 2001; Lassen et al., 2012), 18% (uniform distribution between 15% and 20%) in youngstock between 4 and 24 months of age (Mars et al., 2001; Sayers et al., 2015) and 1.5% in calves <4 months of age without maternal antibodies (data GD Animal Health, Deventer, the Netherlands). Given that 67%-98% (expert opinion) of newborn calves receive sufficient amounts of colostrum, the probability that import calves originating from IBR-positive herds are infected with IBR was assumed to be 0.8%. Assumptions were also included for the ratio acute versus latent infected cattle and the infectious period (Table 2).

Risk associated with transport

To evaluate the number of times IBR was introduced due to cattle imports, the number of imported cattle were aggregated to the number of transports with imported cattle per year, as described by Santman-Berends et al. (2017). In short, a transport with imported cattle was imported on a specific date to one specific herd. Based on I&R data in accordance with EU and national regulations with regard to capacities of trucks (EC, 2005; IKB, 2008), the number of imported cattle per transport was estimated. According to the same regulations, it was assumed that a transport unit (such as a truck or trailer) was allowed to transport cattle to one, or at most two locations.

Table 2. Input parameters for the cattle import risk analysis for IBR in the Netherlands

Parameter	Most likely estimate	Source
Ratio acute versus latent IBR infections (calves <4 months of age)	100% acute 0% latent	Expert opinion
Ratio acute versus latent IBR infections (youngstock 4 to 24 months of age)	1% acute 99% latent	Expert opinion
Ratio acute versus latent IBR infections (cows>24 months of age)	1% acute 99% latent	Expert opinion
Average infectious period of acute infected cattle (tI)	10 days[a]	Bosch et al., 1996; Kaashoek et al., 1996a,b,c; Kaashoek et al., 1998

[a]Included as discrete distribution

The stochastic simulation model

A stochastic simulation model was built to evaluate the risk of cattle imports for introduction of BVD and IBR using MS Excel (Microsoft Corp., 2013) and @Risk 6.2.0 (Pallisade, 2013).

For BVD, the model distinguished the risk of importing PI, TR or TI cows (Santman-Berends et al., 2017). For IBR, the risk of cattle imports depended on the number of transports with imported cattle that were AI or LI with IBR.

Imported cattle with an acute IBR infection at arrival in the receiving herd were defined as cattle that were infected within 10 days prior to the moment of import and were infectious during transport. For each of the EU MS (i) from which cattle were imported, the probability that a random animal was either acutely or latently infected with IBR ($pINF_c$) was determined from the between- ($Hprev$) and within-herd IBR prevalence ($INFprev_c$) combined with the probability that an IBR-positive animal was in the acute or latent phase of the infection at the moment of import ($pINF_c$). The subscript letter c represents the infection status which can be either acute (a) or latent (l). The within-herd prevalence differed between each of the age categories (age) in the model and thus $pINF$ was calculated per specific age class, Eq. (1):

$$pINF_{c,age,i} = Hprev_i * INFprev_{c,age} * pINF_{c,age} \quad (1)$$

Latently infected imported cattle were defined as cattle that were infected more than 10 days prior to the moment of import and were antibody positive. The probability that a random imported animal was either acutely or latently infected with IBR was multiplied by the total number of imported ($nIMP$) cattle for each of the stratified age categories (age) and EU MS (i). This was summed and subsequently resulted in the number of acutely and latently infected imported cattle ($nimp_{c)}$ per age category, Eq. (2).

$$nimp_{c,i} = \sum_{n=1}^{age} (pINF_{c,age,i} * nIMP_{age,i}) \quad (2)$$

To translate the number of imported cattle that were infected with IBR into the number of infected transports, the probability that a transport was free from IBR-infected animals ($pTrans\_free_i$) was calculated as the probability that all cattle in the transport unit ($nTrans$) were not infected with IBR (1-pinf) for each type (c) of infection i.e. acute or latent, Eq. (3).

$$pTrans\_free_{c,age,i} = (1 - p\inf_{c,age,i})^{nTrans} \quad (3)$$

Based on $pTrans\_free_i$, the probability that a transport was infected with at least one IBR-infected animal was estimated. It was assumed that each transport unit would be most likely to infect one, and at most two, importing herds. Cattle that became infected with IBR during transport did not generally lead to any additional infected herds because the receiving herd would have already become infected due to the initial infected animal present in the transport. In the model, the Netherlands was assumed to be free from BVD and IBR. Thus every import of infectious cattle would lead to newly infected herds. In the Netherlands, the cattle industry consists of seven different cattle herd types: dairy, suckler, veal, beef, trade, youngstock and small-scale herds. Because both the risk of import and the risk of subsequent spread to other herds differed per cattle herd type, the import risk was stratified to each of the seven cattle herd types.

The stability of the model outputs was evaluated by comparing the outputs of different numbers of iterations and was determined to be stable after 5,000 iterations when the mean and variation of the output were stable.

Intervention scenarios that reduce the risk of import

For both BVD and IBR, a number of intervention scenarios were evaluated that could reduce the risk of import. For both infections, test scenarios were included. For BVD, imported cattle were either tested for the presence of virus (sc BVD1) or were tested for virus and, if pregnant, also for antibodies (sc BVD2; Table 3). For IBR, imported cattle were tested with a gE ELISA test for IBR wild-type antibodies (sc IBR1) prior to import.

Table 3. Description of the intervention scenarios included in the stochastic simulation model of BVD or IBR

	Scenario	
BVD	1	Testing of all cattle for presence of virus (99% sensitive, Mars & Van Maanen, 2005)
	2	Sc 1, combined with testing all pregnant cattle for antibodies (98% sensitive, Mars & Van Maanen, 2005)
	3	Imports from countries with a BVD herd prevalence >15% are prohibited
IBR	1	Testing imported cattle (≥4 months of age) with a gE test prior to import (87% sensitive, Wellenberg, 1998)
	2	Imports are only allowed from countries with an article 9 or 10 status, and cattle (≥4 months of age) from countries with an article 9 status are tested with a gE test prior to import.
	3	Imported calves (<4 months of age) are vaccinated with live vaccine, reducing the risk of infection by 50%, imported cattle (≥4 months of age) are tested with a gB test prior to import (98% sensitive, Wellenberg, 1998)

In these scenarios, test sensitivities were included to correct for variability in the test results (Wellenberg et al., 1998; Mars and Van Maanen, 2005) and it was assumed that cattle with a positive test result would not be imported. In addition, for both infections, a scenario was included in which import from high-risk countries would be prohibited (sc BVD3 and sc IBR2). For BVDV, no official national free status exists, for IBR two national statuses can be obtained: article 9 (control programme in place) or article 10 free status. In this scenario for BVD, only imports from countries with a BVD herd prevalence at or below 15% were allowed, and for IBR, only imports from countries with an EU article 9 or 10 status were allowed. Cattle originating from countries with an article 9 status were additionally screened with a gE test. Finally, for IBR, a third scenario was included in which calves (<4 months of age) would be vaccinated, and older cattle (≥4 months of age) would be tested with a gB ELISA test prior to import (Table 3). A scenario involving vaccination was not included for BVD because this was assumed not feasible.

RESULTS

Both BVD and IBR-infected animals are regularly imported in cattle herds in the Netherlands. Annually, 334 Dutch cattle herds (5th and 95th percentile: 65-902) are infected with BVD due to import (Fig. 3). Of these, 75% are associated with import of PI cattle, 8% with import of TR cows and 17% with import of TI cattle. The vast majority of BVD-infected cattle are imported by veal calf herds (n=290). Only 44 herds of other types are infected each year through cattle imports (Fig. 3).

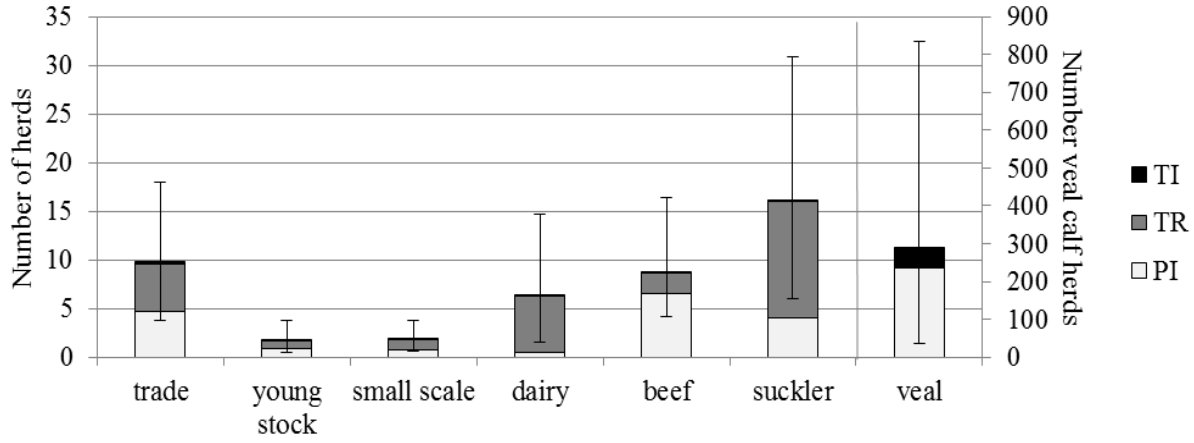

Fig. 3 The average number of cattle herds newly infected with BVD per year (5th and 95th percentile presented with the error bars) in a situation in which the Dutch cattle sector is free from BVD

Annually, 571 Dutch cattle herds (5th and 95th percentile: 431-781) import IBR-positive cattle. Of these, 437 (77%) herds import latently infected cattle and 134 (23%) import IBR acutely infected cattle (Fig. 4). Veal herds import the majority of IBR-infected cattle. In contrast to BVD, a substantial number of non-veal herds also import IBR-infected cattle each year (n=418 herds; Fig. 4).

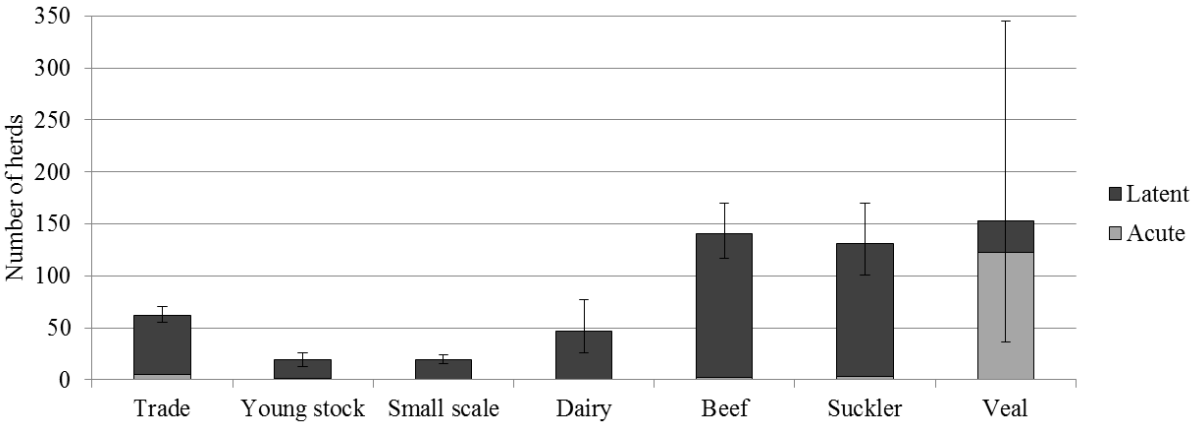

Fig. 4 The average number of cattle herds newly infected with IBR per year (5th and 95th percentile presented with the error bars) in a situation in which the Dutch cattle sector is free from IBR

When the intervention scenarios were applied in the model, the number of cattle herds that were infected through import of BVD-infected cattle decreased from 334 to 81, 58 and 88 in scenario 1, 2 and 3, respectively (Table 4). Scenario 2 was most effective because it reduced both the number of imported PI and TR cattle, while scenario 1 mainly reduced the risk of imported PI cattle (Table 4). In the second scenario, the import risk for non-veal herds reduced to only one infected cattle herd per year. In scenario 1 and 2, import of TI cattle remained the most important risk. The risk of these cattle was assumed not to be influenced by virus testing prior to import (worst case) because the testing was assumed to be conducted before the moment that they were infected, which was only a few days before import.

Table 4. The average, 5th and 95th percentile of the total number of BVD- and IBR-infected cattle herds associated with cattle imports per year in the default model and with the intervention scenarios in the Netherlands

Infection	Scenario	Type of infection			Total (5th and 95th percentile)
		PI[a]	TR[a]	TI[c]	
BVD	Default	252	27	56	334 (65-903)
	1. Virus testing	3	22	56	81 (6-476)
	2. Virus and antibody testing	3	0	55	58 (1-432)
	3. Import from high-risk countries is prohibited	57	14	16	88 (12-309)
		Acute	Latent		
IBR	Default	134	437		571 (431-781)
	1. Testing imported cattle (gE)	134	60		196 (74-402)
	2. Import from article 9 or 10 countries only combined with testing	34	36		70 (41-119)
	3. Vaccination combined with testing	73	9		82 (21-184)

[a]BVD persistently infected
[b]BVD trojan cow
[c]BVD transiently infected

The number of cattle herds infected with IBR through import of cattle would decrease from 571 to 196, 70 or 82 with scenario 1, 2 and 3, respectively. Although scenario 2 was most effective, scenario 3 seemed more feasible to implement and was also effective in reducing the number of infected herds associated with cattle imports. Scenario 1 exclusively reduced the import risk of non-veal herds because in this scenario, only imported cattle (>4 mo) were tested and no risk-mitigating scenarios in veal calves were applied. Scenarios 2 and 3 reduced the import risk in both veal and non-veal herds. In the IBR scenarios, the risk in non-veal herds remained substantial.

DISCUSSION

A stochastic simulation model that was initially built based on a generalised risk pathway, could be used to assess the import risk of two different viral infections: BVD and IBR. Part of the input parameters was similar for both diseases and would also be the same for other infectious diseases. Using this approach, synergy was obtained for both risk assessments because the number of imported cattle, the demographic information from the source countries and the herd prevalence estimations were obtained only once. The definitions of an infected herd were different for the two diseases and thus in the models. For BVD, PIs were the main risk for transmission, with only the presence of a PI constituting a risk. This was different for IBR as all cattle with antibodies could reactivate and spread BoHV1. Therefore, the models were designed differently from the moment the risk of each individual animal had to be determined. The risks involved with certain types of infections differed greatly between BVD and IBR. For BVD, PI animals were the main and most important source of transmission. TR cows can only occur when cows are infected during their foetal stage in pregnancy (i.e. between 30 and 120 days, McClurkin et al., 1984), and the exact risk of TI cattle in transmission of BVD remains unclear. The probability that one TI animal would cause a BVD infection in the receiving herd was estimated at an average of 1.1% based on the study of Sarrazin et al. (2014), who found an R0 value for TI of 0.24. Because the large confidence interval and the uncertainty of the role of TI in transmission of BVDV, this parameter was varied in a sensitivity analysis. The model output changed considerably by changing the R0 value to 1.95 (the upper limit of the 95% CI estimated by Sarrazin et al., 2013). However, given that these increased infection rates are not observed in reality and because the results of the default model agreed with the expectations of the BVD experts, it is expected that the true risk of TI would not be higher than assumed in the default model.

For IBR, the impact of importing acute or latent infected cattle was not differentiated. However, there might be a major difference in impact between the two types of imported IBR-positive animals. Importing an animal with an acute IBR infection that is still infectious at arrival is likely to result in a major IBR outbreak in a susceptible receiving herd. An imported latently infected animal would first need reactivation before spreading the virus. Nevertheless, because seropositive cattle can reactivate throughout their lives, and reactivation is induced by stress factors such as transportation, they pose a risk of introduction of IBR.

The percentage of calves without maternal antibodies that were housed in IBR-infected herds and assumed to be infected with IBR within 10 days prior to import was estimated at 1.5%. The value of this parameter was varied in a sensitivity analysis between 1% and 5.2% based on expert opinion and literature (Sayers et al., 2015; Pers. Comm. Belgium). Changing the probability of an acute infection at the moment of import in calves originating from

infected herds and without maternal antibodies to 1% resulted in a decrease in the number of infected herds through imports (decrease of 39 herds). Increasing this probability to the most likely value of 5.2% resulted in an additional 248 IBR-infected importing herds (results not shown). According to expert opinion, it was expected that the probability that calves without maternal antibodies in IBR-infected herds were infected with IBR during the first 20 days of their lives would be lower than the 1.5% default value. It was therefore expected that the presented results slightly overestimated the import risk of IBR in veal herds.

The risk of infections with BVD or IBR through other routes (such as import of semen, embryos or contaminated trucks) were not included in this risk assessment. They were hypothesised to play a negligible role compared to the large number of imported cattle in the Netherlands. In earlier risk assessments, semen, embryos or contaminated trucks were also found to be associated with a negligible risk for import of BVD in Denmark (Foddai et al., 2014).

The model did not consider the additional risk of collection prior to transport. This may have resulted in a slight underestimation of the total import risk for both infections, although this may have a larger effect on IBR than on BVD, for which no additional PIs could occur through horizontal transmission. Nevertheless, given the R0 value of 2.1 for IBR (Mars et al., 2001) and the average infectious period of 10 days (Kaashoek et al., 1996a) combined with the short stay at a collection centre, it was assumed that underestimation due to the exclusion of this factor would be very low.

This import risk assessment only considered the risk of introduction of BVD and IBR into the Netherlands. The impact of an introduction, which may be large because of clinical signs (BVD) or trade restrictions (IBR), was not assessed. The quantitative risk analysis showed that the import risk differed greatly between the two viruses, but also between herd types. Veal calf herds had the largest probability of importing both BVD and IBR. For the other herd types, infections due to import of BVD-infected cattle occurred only 44 times per year, while IBR was imported more regularly in these herds (418 times per year). When either of these infections is introduced through import, the risk of spread to other cattle herds will be higher in herds with calves (Gates et al., 2014), herds that graze their cattle and herds that trade (Van Schaik et al., 2002). The risk of transmission of the virus from veal herds to other cattle herds is considered to be limited, as veal herds do not have any of these risk factors and are slaughtered at an age of 6 mo. In order to minimise the probability that these viruses will spread from veal herds to other herds, additional management measures such as enhanced biosecurity measures could be implemented.

Although this study was conducted for the Netherlands, which imports high numbers of cattle, the applied methodology can also be used for quantitative risk assessments of other diseases in other countries. Synergies between the models for both diseases were the selection of the risk factors (i.e. age, maternal protection), quantification of import movements and the stochastic framework. Differences were related to disease-specific characteristics that influenced the probability of transmission and possible risk-mitigating measures that could be applied. It was therefore concluded that although many similarities between the stochastic models existed, it is also necessary to take the disease-specific characteristics into account.

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ASSESSING FUTURE CONTROL OPTIONS FOR THE BVD ERADICATION PROGRAMME IN IRELAND

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SUMMARY

Bovine Viral Diarrhoea (BVD) is an important infectious disease of cattle. Infection is primarily transmitted by direct animal contact. In most cases, infection is transient, leading to lifelong immunity. However, during a defined window of pregnancy, *in utero* infection leads to the creation of persistently infected (PI) calves. These animals represent both the major risk for BVD spread and an efficient target for effective infection control efforts. Several broad approaches to BVD control are being applied in different countries, and there is ongoing debate about appropriate strategies during the latter stages of eradication. An explicit model of 6 million Irish bovine animals including dynamic within-farm management and individual-oriented BVD epidemiology is used. The model outcome shows the importance of timely removal of PI calves. The two main options for BVD control – tissue testing and serology – were found to be equally efficient as strategies during a programme through to final eradication.

INTRODUCTION

Bovine Viral Diarrhoea virus (BVDV) causes high production losses due to reproductive, enteric and respiratory disease in many cattle sectors of the world (Moennig et al., 2005). The infection is predominantly transmitted by animal contact. In most animals, exposure leads to transient infection, followed by immunological protection. However, during a defined window of gestation, *in utero* infection results in the production of persistently infected (PI) calves (Coria & McClurkin, 1978, McClurkin et al., 1984, Moennig & Liess, 1995). PI animals shed large amounts of BVDV throughout their lives (Coria & McClurkin, 1978, Houe, 1995), and are considered much more infectious than animals with transient (non-persistent) infection (Cherry et al., 1998, Viet et al., 2004).

The early detection and elimination of PI animals is the key to effective control, leading to eradication of BVD. A number of national BVD control or eradication programmes have been initiated, leading to reduced PI animal occurrence and a substantial decrease in herd-level incidence (Lindberg & Houe, 2005, Presi et al., 2011, Norström et al., 2014). The two primary diagnostic methods applied in BVD control programmes were reviewed previously by Graham et al. (Graham et al., 2014). The principle difference is whether the presence of PI animals is detected directly or indirectly. Direct strategies address the individual animal status by detecting viral antigens ("Swiss model"; Presi & Heim, 2010). The approach is

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commonly referred to as tissue tagging or tag testing. Testing is conducted on all newborn calves in order to directly identify PI animals. Indirect strategies use preliminary serological screening tests to determine herd BVD status ("Scandinavian model"; Lindberg & Alenius, 1999). This approach, commonly called serological surveillance, relies on serum testing of animals of a certain age, or milk samples.

In a number of countries, mandatory control programmes have proved effective in rapidly reducing the number of newborn PI animals (Ståhl & Alenius, 2012). Consequently, BVDV has been eliminated from the majority of cattle herds within a few years after programme initiation. The present study addresses the well-recognised problem of how a national BVD control programme can achieve eradication once initial measures have brought the burden of infection to very low levels. In a programme reliant on direct detection of PI animals, it should be possible in this latter phase either to change to surveillance using indirect approaches (antibody detection) or to continue tag testing to the point of eradication. A final decision concerning which of these two options should be implemented will be influenced by expectations, previous experiences and the interpretation of the risk profile with regards to costs, benefits and practical constraints. Assessments in support of such decisions in advance are only possible by projecting recent efforts and successes into plausible future outcomes for the alternative strategies. It has been shown that epidemiological expert system modelling is a useful methodology in such circumstances. Nevertheless, although numerous BVD models exist (Cherry et al., 1998, Tinsley et al., 2012, Damman et al., 2015, Santman-Berends et al., 2015), there are few reported applications in the literature addressing the predicted future of real world BVDV control programmes. This is understandable, given the difficulties in working at a national level whilst modelling the particularities of cattle management, BVD transmission and the wider impact of control measures. Here, an expert system model (FarmNet-BVD) is proposed, in support of the Irish national BVD eradication programme to study alternative control methods.

The Irish eradication programme was broadly based on the "Swiss model" using direct tests to identify BVDV-positive animals. After a voluntary phase in 2012 (Graham et al., 2014, Graham et al., 2015b), the programme has been compulsory since the start of 2013. By the end of 2015, an increasing number of farms achieved negative herd status (NHS) based on a demonstrated absence of PI animals in the herd for the preceding 12 months and a known direct or indirect negative status for all animals in the herd. Concern had been raised by some stakeholders about the efforts spent in continued tag testing and programme-related on-farm costs, particularly in light of the steadily increasing cohort of NHS farms along with the ongoing compulsory controls. In this context, it has been suggested that serosurveillance, applying indirect testing of a subsample of animals in each herd, could be an equally effective but more efficient way to drive BVDV to eradication. Consistent with approaches taken in other BVD control programmes, serosurveillance was assumed to be conducted once-a-year on each epidemiologically distinct group of calves aged 6-9 months providing 10 individual serological samples.

Problems of PI retention have been identified previously in the Irish programme (Graham et al., 2015a, Graham et al., 2015b, Clegg et al., 2016). The problem was first recognised during the voluntary phase of the programme (Graham et al., 2014). A PI animal is considered to be retained if it is present more than 7 weeks after the date of the initial positive test, with this period being considered sufficient to perform a confirmatory test and to allow subsequent removal. Research has highlighted the adverse influence of PI retention on both the index farm (Graham et al., 2015a) and on neighbouring farms (Graham et al., 2016).

The objective of the present study was to compare the relative impact on 'time to eradication' of retention behaviour and alternative BVD eradication methods after 3 years of compulsory tag testing. Hence the scenarios were either continued tissue tagging of all newborn calves or an agreed national shift to serosurveillance in all herds having no BVD detection over the last year (NHS).

MATERIALS AND METHODS

Data

The expert system model is built on input data and knowledge from the scientific literature. The data input is general and applicable to other national industries if the following information can be derived: (i) Production type and seasonal calving pattern of each herd, including the number of animals of breeding age and the dominant purpose (dairy, beef or mixed farming). Additionally, records of seasonal calving date distributions determine calving regimes of model herds (Irish data accessed from the Irish Cattle Breeding Federation (ICBF) database); (ii) Spatial location and contiguity structure of each herd, which determines the landscape geography of herds in the model and possible contiguity structures different from distance-based approaches (Irish data accessed from the national Land Parcel Information System (LPIS) database); (iii) Movement records by source, destination and age cohort per animal movement, including passages through cattle markets or other dealers (Irish data accessed from the national Animal Identification and Movement (AIM) database).

Model credibility

The development of the expert system model is consistent both with the work of Kopéc et al. (2010), who outlined three main criteria for evidencing credibility of models for decisions (model development, performance and usability), and available published research on epidemiological modelling of BVD in cattle (scientific literature and expert knowledge).

Viet et al. (2007) have presented the conceptual framework of modelling BVD spread and control at the population level. The respective conceptual model formulated by Damman (2015) was used to represent the necessary complexity of within-herd BVD transmission (Viet et al., 2007). This framework was then extended to represent the individual animal-level aspects of *in-utero* PI movement and related mitigation measures, as well as the details of a transportation network as proposed by Tinsley et al. (2012). In relation to the critical issue of farm-to-farm (over-the-fence) transmission (Graham et al., 2016), our model was coupled with the spatial structure of Irish farms to accurately reflect the heterogeneous contact landscapes for local transmission between neighbouring herds. Model performance tests (Kopec et al., 2010) were conducted to critically evaluate independent observed patterns derived from the Irish BVD programme against model output, including the observed early PI decrease, resulting spatial distribution of infection, and duration of individual PI survival, as well as taking into account within-farm BVD dynamics across cohorts and control methods. Evidence of usability was demonstrated (Kopéc et al., 2010) by the influence of the model-based insights on the Irish BVD programme implementation into the fourth year.

Model description

The model is documented and appended as online supplementary material (www.ecoepi.eu/FarmNet-BVD) following the ODD-protocol (Objective, Design, Details;

Grimm et al., 2006, Grimm et al., 2010) prescribing documentation standards for system models.

The model is a hybridisation of a spatial-explicit network model, a discrete-time cohort-based SIR and event-based modelling of PI animals. Network nodes are individual herds. Model herds comprise multiple cohorts representing management stage: Cows (CO), Calves (CA), Grazing animals for slaughter (GR), Heifer first year (H1), Heifer second year (H2), Heifer breed (HB) and Cattle for fattening prior to culling (FF). Every management cohort is sub-grouped according to the infection status: Susceptible, Immune, Transient, PI and (for calves) the status maternal antibodies positive (MAB+). If all combinations are in use, model herds will have animals in 29 sub-cohorts i.e. 7 management x 4 infection + 1 MAB+. The individual animal perspective was taken for gestation schedules, movements of animals pregnant with a PI, and in order to manipulate the fate of the PI.

Cattle management is represented as an annual timeline of events, as proposed by Damman et al. (2015), with amendments as necessary to cover data-driven differences in the calving season and rearing principles in different herd types (Fig. 1).

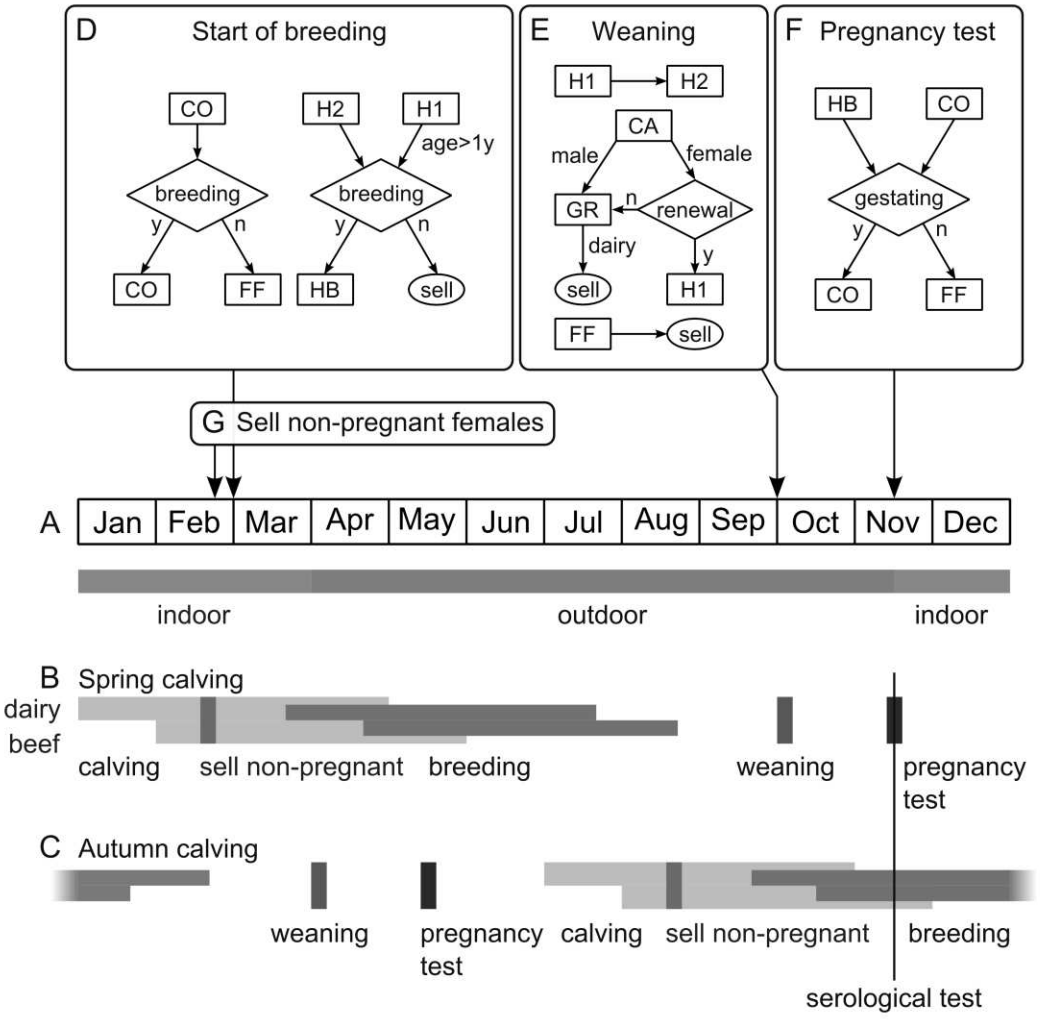

Fig. 1 Graphical representation of event and process scheduling in the farm sub-model. For a detailed explanation of the annual timeline, see the complete model documentation appended as online supplement (www.ecoepi.eu/FarmNet-BVD)

The model was geared to allow two alternative approaches with respect to PI retention, occurring after PI detection in individual herds. One approach was to describe the percentage of herds in each production type that did not remove identified PI animals, assigning to these the survival probability associated with PI status (Damman et al., 2015). The retention characteristic was then assigned as a fixed attribute to respective farms during simulation. Alternatively, retention was implemented as event driven, following given survival curves across all detected PI animals i.e. including early and late removals as reported from data (Clegg et al., 2016).

The direct control option was implemented according to the given protocol for tissue tag testing applied to the Irish national programme i.e. following birth, each calf must be tested within 3 weeks (20 days) and removed at latest following a positive confirmatory test 3 weeks later. In the model, compliance with the prompt test and removal requirement was represented by a testing rate parameter applied as an event probability to untested calves in a herd. Indirect testing follows a defined protocol in all herds free of BVD from the previous year (i.e. NHS, no BVD+ animals present in preceding 12 months and known negative for all animals in the herd). Independent of their epidemiological status, a fixed number of young animals (older than calves but having not yet bred) was sampled from each management group in the herd and tested using serology. The model test would be positive for any sample taken from the immune cohort. After detection of seropositive animals, the complete age-cohort was tested using direct tissue testing. If further infected animals were detected, the remaining animals in the herd were subjected to direct tissue testing. As an additional scenario option and in advance of the regular indirect test season, the indirect strategy can be accompanied by additional tissue testing of untested calves prior to being sold from their herd. For this study, test performance was assumed to be perfect.

Simulation experiments were conducted comparing the development of the PI cohort on the global scale for each of the two contrasting control strategies combined, both with and without retention. For each run, a 30 year burn-in period was simulated, ending in an endemic situation with sector-wise herd-level prevalence similar to that before the start of the control programme in Ireland, as well as the resulting share of herds with immune animals only. The direct control strategy was subsequently applied, mimicking the first 3 years of the Irish national programme. During the following 7 years of simulations, either the direct control strategy was continued for all herds or the indirect testing strategy was applied using serology on all BVD-free farms and tissue testing on herds with BVD detections for the year of detection plus the following year. Outputs included time-series of the number of PI animals, as well as the number of tissue and serology tests applied.

In this analysis, the costs reflect those of the Irish BVD eradication programme, and are used for illustration purposes. The cost of veterinary herd visits (serology) and postage (tissue tag) is not included. A cost for tissue testing of €2.50 was used, which reflects the minimum cost per test unit in BVD-free herds. Serological tests cost €7.66 per sample including the cost of sampling by a veterinary surgeon and the laboratory test cost being the minimum cost available on the market, but excluding logistics. In other national programmes, the costs will vary, including the cost difference between individual tissue and serological testing.

RESULTS

The results of the model highlight the dramatic effect of both eradication strategies on the number of PI herds when compared to the simulation without controls (Fig. 2). The future of

the simulated control programme from year 4 onwards did not differ between different strategies. Without altering the assumed retention behaviour (i.e. it was modelled as a permanent attribute assigned to selected herds), the eradication programme followed a power law decline in the number of PI herds (or animals; not shown). This outcome is in agreement with a constant herd-level reproductive ratio of less than one, as deemed necessary for the successful control of infectious diseases.

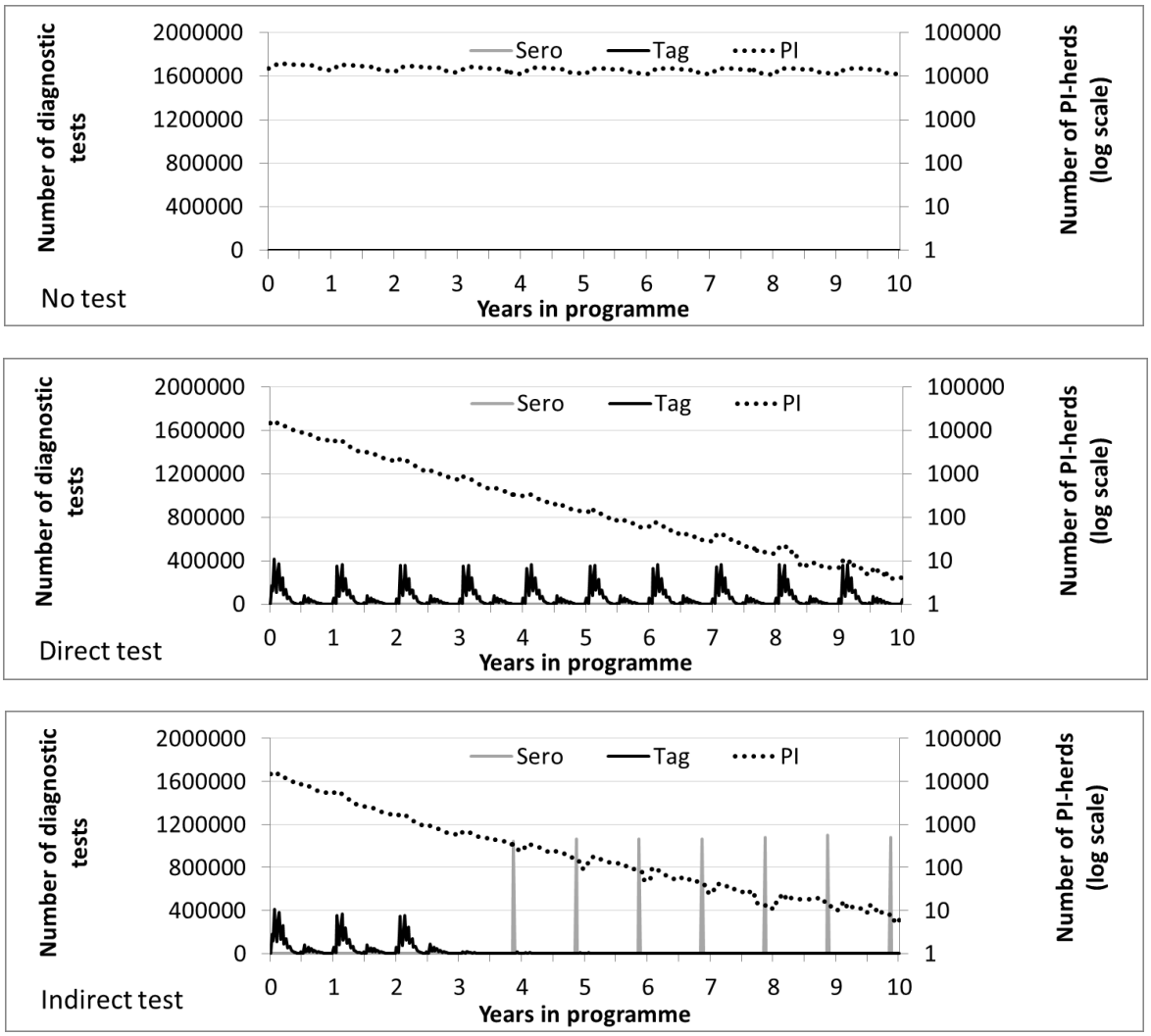

Fig. 2 Comparative individual simulation runs of the PI situation in the Irish cattle population, using Irish cattle herd and movement data and assuming previously recorded retention levels (fraction of herds possibly demonstrating retention behaviour). The different panels reveal the difference between endemic infection and alternative control approaches, including differences in eradication performance. Dotted line – right axis: PI-herd numbers log scaled; black line – left axis: weekly number of tissue tag tests applied; grey line – left axis: weekly number of serological tests applied. Top panel: without control; middle panel: 10 years of direct test strategy; bottom panel: 3 years of direct test strategy followed by switch to indirect testing strategy (NB: in the bottom panel there are tissue tag tests applied from year 4 onwards due to further detections, however, the numbers are too small to be observed given the resolution used on the y-axis)

The steepness of the decrease in the PI-cohort at both herd and animal level (the latter is not shown) was sensitive to the assumption on retention levels with steeper decrease associated with less retention. Under the extreme assumption of no retention (Fig. 3), the programme will end substantially sooner because the herd-level reproductive ratio will reach its theoretical minimum. Nevertheless, both control strategies performed equally well with, as before, slightly greater noise in the indirect test strategy, which reflects the stochastic nature of finding particular herds by subset sampling compared to algorithmic identification via the direct test of all newborns.

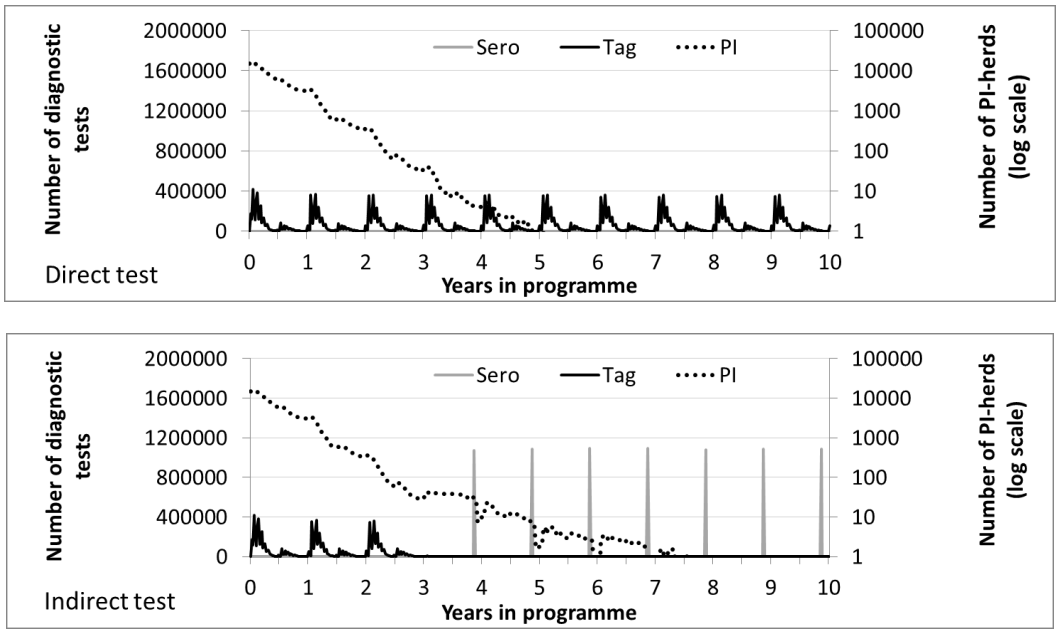

Fig. 3 Average of five runs of simulated PI situation in the Irish cattle population using Irish cattle herd and movement data. In contrast to Fig. 2, retention levels were set to zero (i.e. all identified PI animals were removed subsequent to positive confirmatory testing). The two panels reveal the differences between the alternative control approaches, including differences in eradication performance. Dotted line – right axis: PI-herd numbers log scaled; black line – left axis: weekly number of tissue tag tests applied; grey line – left axis: weekly number of serological tests applied. Top panel: 10 years of direct test strategy; bottom panel: 3 years of direct test strategy followed by switch to indirect testing strategy. (NB: in the lower panel there are tissue tag tests applied in years 4 and 5 due to further detections, however, the numbers are too small to be observed given the resolution used on the y-axis)

Figure 4 shows the cumulative numbers of tests that were applied in the model during the simulation of 10 years of control, including the application of alternative testing strategies after the third year of the programme. The left panel in Fig. 4 is the null model, which refers to ongoing application of the direct test strategy. Approximately 30 million tissue tag tests would have been applied over the 10-year period, corresponding to ten times approximately 2.3 million calves plus occasional testing in older age groups. If the strategy is changed after 3 years of direct testing (middle panel) and continued by the indirect test approach, the number of tissue tests applied will, logically, be reduced to very low levels (i.e. for years after diagnostic clearance, tissue tagging will only be triggered in herds with positive serological

BVD results). Indirect test numbers did increase, logically, but with a lower gradient than tissue tag test numbers in the direct scenario. Of various possible management group size scenarios, the one assuming a maximum of 50 calves per group shown here would be the one entailing the highest number of serological tests (i.e. about 8 million tests in 7 years). The right panel in Fig. 4 illustrates further action to close the safety gap of the indirect strategy, whereby all calves sold each year in advance of serological screening of the herd are required to be tissue tag tested. The additional option will increase necessary testing costs and hence lead to a larger difference between the total efforts needed compared to the direct test strategy.

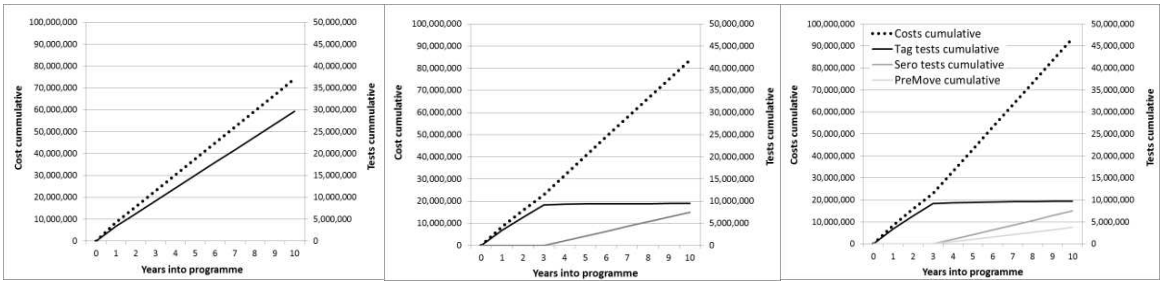

Fig. 4 Cumulative count of tests applied in model simulations (right axis) in accordance with alternative control strategies. Left panel: direct strategy; middle panel: 3 years of direct testing followed by 7 years of indirect testing; right panel: as middle but with additional testing of any untested calf leaving the herd. Black line: tissue tests; grey line: serological tests; light grey line: tissue tests on outgoing animals. Left axis shows the cumulative cost of the test (dotted line) by multiplying tissue tests by €2.50 and serology tests by €7.66 (see methods)

DISCUSSION

Compulsory or high-coverage BVD control programmes are effective in reducing the continued presence of the pathogen in the cattle population. The present model outcome is in agreement with observations from real-world national programmes. The model reveals the comparable performance of the direct and the indirect test strategies independent of the level of retention (Fig. 2 and Fig. 3). The insight is validated by comparing successful national control programmes based on different strategies, e.g. Switzerland (Zimmerli et al., 2009, Presi & Heim, 2010, Presi et al., 2011) and Sweden (Lindberg & Alenius, 1999, Alenius et al., 2005, Houe et al., 2006). In the present study, the two strategies were predicted equally useful in the context of finalising the Irish eradication programme. Interestingly, the historic choice of the Swiss programme being tagging-based had been reasoned by high levels of cattle movement and contacts; both of which also apply in Ireland.

On an initial review of the two testing strategies, the model outcomes do not appear to be comparable with other programmes' observations regarding the related financial burden of implementing the foreseen diagnostic testing protocols. Using Irish data, it is clear that there would be a substantial reduction in the absolute number of tests applied per annum when moving between the two programme alternatives. As reflected in Fig. 4, over the latter 7 years of the simulated programme, about 8 million indirect tests would be used compared to about 20 million direct tests (i.e. an effort ratio of 1:2.5). Therefore, one would anticipate a

substantially reduced testing budget if the programme was continued using the indirect testing strategy. However, the programme costs attributable to the price of testing are at odds with what would intuitively be expected. This is explained, in the Irish setting, by the estimated cost of the two tests, which differ by 3:1 for serological vs. tissue tag tests. Furthermore, one might argue that the comparable model output shown in Fig. 4 is misleading because of the technical assumption of a maximum management group of 50 calves. This leads to an inflation in the number of indirect tests required because larger farms would need to test multiple management groups to satisfy the required diagnostic herd sensitivity of the programme. The alternative of a single sample-set, independent of herd size, was simulated in order to test the plausibility of the model outcome. The results are not shown, but can be predicted from what is already presented. In the middle panel of Fig. 4, approximately 8 million indirect tests were conducted per 7 years after the scheduled change in strategy assuming a management group of no greater than 50 calves per year. Calculating the testing numbers purely on the basis of ten calves per herd and not taking account of herd size, about 850,000 samples would be collected each year from 85,000 herds (ignoring very small herds with fewer than 10 calves per year); cumulated over 7 years of simulation, this would give about 6 million indirect tests instead of the previous 8 million. Hence, the 7-year cumulative costs of indirect tests would differ by about €15 million (2 million * €7.66). These savings would bring down the total testing costs in the middle panel of Fig. 4 (i.e. from €85 million using 50 head management groups to about €70 million if a single sample-set were collected independent of farm size). Interestingly, even with the cheaper but simplistic version of the indirect strategy, the expected overall test costs remain comparable for both strategies. Assuming a limited management group size, in agreement with expert knowledge, the testing costs of the changed strategy exceeded those of continued direct testing.

Therefore, decisions in favour of implementing the indirect strategy to finalise BVD eradication would need to be supported by arguments other than cost savings or time to eradication, which are essentially equivalent for both testing strategies.

The next lesson learnt from the application of the model was the size of the threat to national eradication efforts of PI retention, even by a minority of stakeholders. Although well understood conceptually, the impact of retention on time to eradication was not previously quantified. Programme non-compliance, presenting as PI retention, is present throughout Ireland (Fig. 5), but more frequently in beef herds, in smaller herds, and among farmers with no registered mobile phone (Clegg et al., 2016). This is despite a clear understanding by stakeholders of its adverse effects on the whole programme (Clegg et al., 2016). Future efforts at reducing retention should therefore take these characteristics into account.

In Ireland, PI animals have been retained in herds for more than 100 weeks following identification. Model sensitivity towards details of retention in the cattle population highlights again the acknowledged importance of addressing the issue of retention during a well-controlled eradication programme. PI identification and removal is a well-recognised tool for successful eradication of BVD and the year-on-year improvements are worth noting (Clegg et al., 2016).

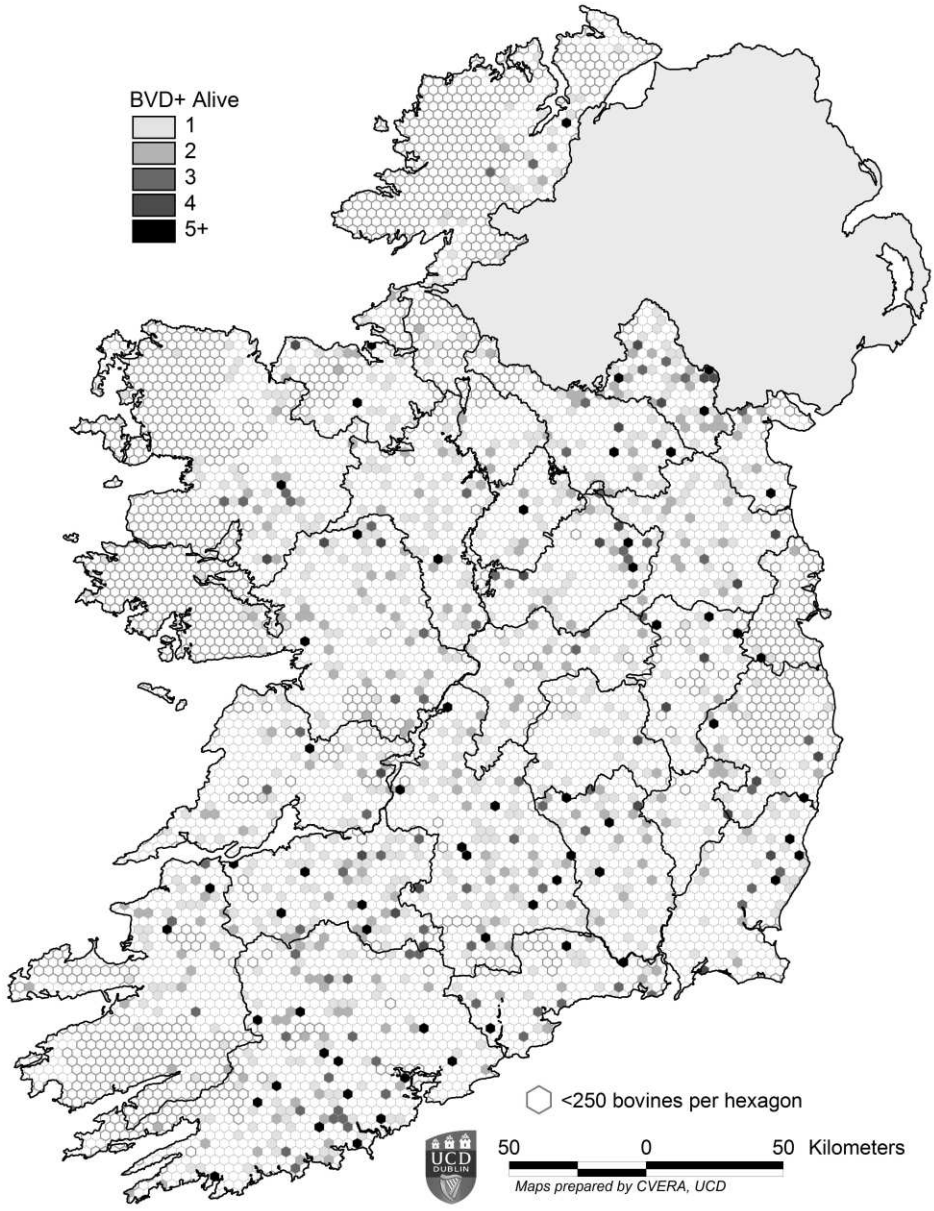

Fig. 5 Spatial distribution of PI animals as of 1st July 2015, in the middle of the third year of the compulsory Irish BVD eradication programme, when a decision on further strategy was addressed. Herd data were aggregated to a summary spatial scale and cannot be applied to individual point locations. PI presence is shown by the total number per hexagonal spatial unit (source UCD Centre for Veterinary Epidemiology and Risk Analysis using ICBF data)

Although compliance with this strategy is high, it is not yet universal. Stakeholder compliance in disease control programmes is a critical issue, and may require interventions on a societal level, or at best internally by the beneficiaries of a control programme (Thulke et al., 2011), Del Rio Vilas et al., 2017). The BVD Implementation Group, which oversees

the Irish eradication programme, has introduced a number of measures for calves born in 2017 to address the problem of retention (see http://animalhealthireland.ie/?page_id=227).

The model outcomes suggest equivalence with respect to eradication performance for both of the proposed testing strategies towards the end of a BVD programme. These outcomes confirm observations from different national programmes that either testing approach can be used to control the PI population. Hence, following Kopéc et al. (2010), this conclusion further contributes to the credibility of the BVD model using evidence based on model performance (i.e. plausibility and comparison with external data, in this case). This point is important given the central role of this model in national decision-making.

In conclusion, the findings of this study do not distinguish between the alternative testing strategies in terms of their effectiveness, at least in an Irish context. Nevertheless, stakeholders argue that it would be beneficial to the overall programme if there were a change at some time from the direct to the indirect testing strategy. Without logical and quantitatively convincing reasons, the principle of not changing a working system may be seen as pivotal in questioning any efforts to bring into effect a change in strategy. Some additional issues to be considered, with respect to a comparison of the alternative testing strategies, include the logistics of simultaneously managing two testing strategies (3 million tissue tag test plus approximately 1 million serological tests), and of managing a programme where herd owners may be subjected to a change in test protocol with any new detection. These changes would occur without any evidence that eradication would be achieved more quickly. That is, concerns about fatigue after programme prolongation are similar, regardless of the used testing strategy. So on what criteria should decisions be based? It might be appropriate to consider the question where the benefit from the strategy change could actually come from, and to quantify this. In order to provide relevant decision support, the credibility of the Irish BVD model has been demonstrated based on the principles outlined by Kopéc (2010).

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POLICY AND DECISION SUPPORT

THE FEEDBACK LOOP BETWEEN ENDEMIC DISEASE AND PIG FARMER DECISION MAKING: AN AGENT-BASED SIMULATION STUDY

E.A.J. FISCHER*, T. VERWAART, N.I. VALEEVA AND T.J. HAGENAARS

SUMMARY

The use of antimicrobials is complex and involves farmers making decisions based on economic, social and personal motivators. An agent-based model was developed, capturing the dynamics of endemic animal diseases and decision making of fattening-pig farmers. Farmers could take three measures to reduce antimicrobial usage and endemic disease burden on their farm: long-lasting investments in housing, reversible changes in management practice and reversible changes in antimicrobial policy.

When farmers could choose to adopt any of the measures, the variation between model runs in the proportion of farmers was larger compared to a situation where only one measure was available. Antimicrobial reduction policies may produce the unwanted effect of increased group treatments. Investment in better housing is a measure only adopted when the expected endemic disease reduction exceeds a threshold value. These model outcomes emerge from the individual farms' characteristics showing the strength of agent-based models.

INTRODUCTION

The prevalence of antimicrobial resistance (AMR) in livestock in the Netherlands increased dramatically in the decades up to 2009 (Mevius et al., 2015). Reduced antimicrobial usage is seen as the main approach to decrease the prevalence of AMR.

Measures against antimicrobial usage in livestock in the Netherlands, including legislation to reduce the prescriptions by veterinarians and the demand for usage by farmers, were taken with high priority in 2009 (Speksnijder et al., 2015b). Legislation includes a farm health plan (FHP) and farm treatment plan (FTP) dedicated to a specific farm so that it can change the management practices in order to improve animal health (Speksnijder et al., 2015b). This has led to a substantial decline in the use of antimicrobials ($DDDA_{nat}$) in the Netherlands (Mevius et al., 2015). The reduction does slow down or even stale in fattening-pig production.

The use of antimicrobials in livestock production is a complex issue with many natural feedback loops between biological processes in farm animals, bacteria and farm management. Additionally, farmers adapt their decisions on antimicrobial use and farm management

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practices in response to governmental and supply-chain policy interventions, such as a quota system for antimicrobials, price increase for antimicrobials and price differentiation for antimicrobial-free products. Farmers' decisions are not only driven by economic considerations, but also other motivations, perceptions and professional and personal networks (Valeeva & Verwaart, 2011). The animal production systems facing the problem of reducing antimicrobial usage are thus characterised by both complexity and adaptation.

Although legislation is an important driver for reduction of antimicrobial usage, in essence the decisions of farmers determine the usage of antimicrobials at their farms, and thus the overall antimicrobial usage at population level. This study describes an agent-based model developed to investigate the effects of individual farmer decision-making on the overall antimicrobial usage. Three measures adopted by farmers were evaluated. These measures were: change of management practice, change in antimicrobial usage policy and investment in "healthier" housing. The agent-based model was used to investigate the relationship between the expected effect of a measure and the adoption of the measure by farmers. Furthermore, the agent-based model was used to study the relationship between the effect of adopted measures on the endemic diseases and antimicrobial usage.

MATERIALS AND METHODS

The agent-based stochastic model is described according to the ODD-protocol (Grimm et al., 2006, 2010).

Purpose of the model

The purpose of the model is to understand how the adoption level of measures to reduce antimicrobial usage by pig farmers depends on endemic disease epidemiology and socio-economic decision making. The farmer can invest in better stables or equipment to have a lasting mitigating effect on transmission of endemic disease. This measure will be referred to as *investments*. The farmer can adopt a change in management practice, which can be changed each round. This measure will be referred to as *practice*. The last option is to reduce the policy in the application of antimicrobials, in order to reduce their use. This measure will be referred to as *policy*.

Entities, state variables and scales

The model consists of two entities: farmers and pens. Farmers decide on antimicrobial usage within the pens on their farm. Pens are inhabited by pigs grouped into departments and departments are grouped into farms.

Farmers were determined by state variables for: farm characteristics, antimicrobial usage, decision making and social network with other farmers. Farm-characteristic variables include the number of pens in the farm, the annual cost of disease and measures taken, the probability of preventive antimicrobial use and the probability of treating an infected pig. The contact network of a farmer of other farmers and the degree of this network were farmer state variables. Decision-making variables included current utility and satisfaction, as well as the perceived norm with respect the adoption of a specific measure. The state variable for intention to adopt a measure was determined by the utility, satisfaction and perceived norm weighted by the relative importance given to these components by the farmer. The precise definition of utility, satisfaction and norm are described in the sub-model section. These

components were calculated using state variables for the degree of belief in the potential effectiveness of applying new measures, openness to communication and risk aversion.

The unchangeable state variables of a pen were the farmer and department to which the pen belonged. Changeable state variables were the age, weight and number of pigs, the number of pigs in each of the disease states and the antimicrobial usage in the pen.

Process overview and scheduling

Every time step process is scheduled in the following order:

1. Calculate the force-of-infection in each pen
2. Determine the number of new infections and recoveries per pen
3. Ageing and growth of the pigs, and slaughter (if the slaughter weight has been reached)
4. Determine the start and end of antimicrobial treatment for individual animals and for group treatment of pens
5. Process farm, network and global information
6. Calculate utility and satisfaction
7. Update the intention and schedule actions taken in the next step
8. Repopulation of empty pens

Design concepts

Basic principles: The model used basic modelling principles in infectious disease epidemiology (Diekmann et al., 2012) and decision-making theory (Verwaart & Valeeva, 2011), but introduces novel complexities by making these interact at farm level.

Emergence, adaptation and farmer objectives: The farmers explicitly improved their utility and satisfaction by taking or stopping specific measures. They adapted to the previous round of production and optimised their farm by taking certain measures. The proportion of other farmers in the network applying a measure (i.e. the perceived norm) partly determined the intention to adopt a measure. The farmers did not, however, attempt to have a higher yield than other farmers, and did not adapt to outcompete them.

Prediction and sensing: Farmers predicted their future satisfaction and utility based on their beliefs and the current situation of the farm. Farmers obtained information on the global parameters determining the effect of measures on antimicrobial usage and on endemic diseases. The information was received at random events determined by the openness to communication and the frequency of broadcasts. The information decreased if the farmer did not receive information.

Interaction and collectives: Each farmer had a group of contacts (the network) from which they determined the norm of using a measure. The network was a regular network in which each farmer was placed in a regular grid and linked to the farmers directly adjacent. In order to reduce finite-size effects, periodic boundary conditions were used.

Stochasticity: Some state variables of the farmers were drawn from a uniform distribution: the weights, probability to treat, risk aversion, openness to communicate and intention persistence. Stochasticity was introduced here to model the variation between farmers.

The infection and recovery process was a stochastic SIS model to enable variation in disease load between farms and pens. The decision to treat (both individually and by group

treatments) was modelled stochastically to account for variation in observations on pigs. Individual treatment ended at a randomly chosen time to allow for difference in treatment time due to difference in clinical disease.

Observation: The overall antimicrobial usage by individual treatments and group treatments, aggregated over all farmers, was determined by both the prevalence of endemic disease on each of the farms, as well as by the decisions made by individual farmers. The individual farmer decisions on adoption of measures resulted in the level of measure implementation in the whole farmer population.

Initialisation

A simulation started in the deterministic equilibrium of the demographic and the epidemiologic sub-model and ran for a predefined burn-in period to allow the model to settle to a quasi-steady state. During the burn-in period, no measures were implemented. At the end of the burn-in period, the farmers began updating their intention to act as shown by the decision-making sub-model.

Input

Te model was calibrated to the situation of 2008 in the Netherlands, prior to the major decrease in antimicrobial usage, because this is a relevant situation to start studying the adoption of measures.

The demographic sub-model was calibrated to average pig production data (Vermeij, 2014) in order to resemble the mean weight of pigs at slaughter as well as their age. A parameter for the maximum weight was introduced to avoid unrealistically heavy animals in the simulation.

The epidemiological sub-model was calibrated on a prevalence of 0.22 for the most important endemic infections for fattening pigs (Bergevoet et al., 2012) in the Netherlands: *Lawsonia* infections, *Streptococcus* infections, Enzootic pneumonia, Influenza infections, and Circo infections. (Bergevoet et al., 2012). Additional experimental data (Andraud et al., 2008; Dekker et al., 2013) yielded within-pen transmission rate $\beta_{withinpen}$and recovery rate λ. The number of infected piglets at weaning was 1 out of 50 in an infected batch given a probability of 0.3 that a specific batch was infected (Stege et al., 2001; van der Heijden et al., 2004; Wellenberg et al., 2004; Engel et al., 2006; Van Reeth et al., 2008; Loeffen et al., 2009).

The exact reduction in transmission of the proposed measure was not known and because this was the object of interest in this study the values were evaluated between 0% (no effect) and 100% (complete prevention of transmission).

The antimicrobial usage parameters were calibrated to the antimicrobial usage in fattening pigs in 2008 by calibrating the probability of preventive usage and parameters for individual treatment, and the threshold for group treatments (Mevius et al., 2009; Broens et al., 2012).

The decision-making sub-model with the economic evaluation by the farmer was based on the annual costs for disease, calculated by the loss of yield from disease and the costs of antimicrobials. The costs of measures were calculated based on expert consultation at Wageningen Economic Research. Information to parameterise other processes in the

decision-making sub-model was scarce and based on previous studies in dairy cattle farmers (Valeeva & Verwaart, 2011; Verwaart & Valeeva, 2011).

Sub-models

Pig demographics: New piglets entered an empty pen. These pigs grew with a net growth rate r. The net growth rate r is a function of prevalence of disease and the use of antimicrobials, because antimicrobials act as growth promotors (Eq. (1)):

$$r = r_0\left(\frac{xc}{n_{pen}} + growthreduction\frac{yc}{n}\right) + r_{ab}(age)\left(\frac{xa}{n_{pen}} + growthreduction\frac{ya}{n_{pen}}\right) \quad (1)$$

where r_0 is the default growth rate, $r_{ab}(age)$ is the growth rate when given a growth promotor, and $growthreduction$ is the reduction in growth due to disease. The pen-state variables for the number of pigs depict the number of susceptible (*xc* and *xa*), infectious (*yc* and *ya*) and total (n_{pen}) pigs in a pen, where *c* describes pigs that were not on antimicrobial treatment and *a* describes those that were on treatment. The growth-promotion effect of antimicrobials increased linearly with the age of the pigs.

When the average weight of all pigs in a pen reached the minimum slaughter weight, a fraction was removed. The remainder of pigs were removed after a short fixed period, or when the maximum slaughter weight was reached. An empty pen was repopulated with new pigs after a fixed period of being empty.

Epidemiologic model: The endemic diseases in a pen followed a SIS model (Diekmann et al., 2012) in which pigs moved from susceptible to infectious (and diseased) to susceptible. At repopulation, each batch of pigs had a probability of being infected and each pig in an infected batch had an individual probability of being infected. These pigs seed the disease after repopulation. Subsequently, the probability of becoming infected during a time step was based on the force of infection, $Pr(S \rightarrow I) = 1 - e^{-foi}$. The force of infection in a pen was determined by the number of infections in the pen and the transmission rate. During a time step, pigs recovered with cure rate, λ, after which they became susceptible again.

The influence of antimicrobials on the transmission of endemic diseases was modelled by lower transmission rate for the fraction of pigs receiving antimicrobial treatment. Another specific effect of antimicrobials was an increased recovery rate for treated animals.

Application of antimicrobials for individual pigs depended on the probability that a farmer would treat a diseased pig and the inclination to use preventive treatment. The length of an individual treatment depended on the progression of a pig from diseased to non-diseased ($I \rightarrow S$). Treatment ended with specific daily probabilities for susceptible pigs and for diseased pigs. Stopping treatment in diseased pigs took into account that even for infected pigs, a treatment would be stopped if no improvement was seen.

A farmer will apply group treatment to all pigs in a pen when the within-pen prevalence of infection exceeds a random threshold value drawn from a uniform distribution between GT-threshold-minimum and GT-threshold-maximum. Group treatment was maintained for a fixed period.

If a farmer implemented the measure *practice* or *investments*, the transmission of endemic diseases decreased, accordingly to the magnitude of the effect of the measure. Adoption of the antimicrobial reduction *policy* was modelled by a decrease in the daily-probability of treating individual diseased pigs and the farmer no longer providing preventive treatment to his pigs.

Decision making: The decision of the farmer with respect to adopting measures was based on the theory of planned behaviour (Ajzen, 1991). The intention of a farmer $I(t)$ to change (Eq. (2)) is determined by the current situation and the expected situation given the adoption of the measure in the next round *t*:

$$I(t+1) = \begin{cases} -1 & t = 0 \\ p\,I(t) + (1-p)(w_1\Delta U + w_2\Delta S + w_3 N) & t > 0 \end{cases} \quad (2)$$

where p is the intention persistence to change given the observed and expected situation. The change in utility (ΔU), the change in satisfaction (ΔS) and norms (N) are weighted using farmer-specific weightings.

When the intention exceeded 0, the farmer applied the measure in the next round. Changes in management practice and in applying antimicrobial usage policy were reversible. The farmer implemented the measure when in a future round the intention dropped below 0. In this situation, not applying the measure would have a better expected outcome. Application of investments was irreversible and the corresponding benefits lasted until the very end of the simulation. The change in utility (Eq. (3)) was the expected economic gain of implementing a measure given the farmer's risk aversion (Valeeva & Verwaart, 2011):

$$\Delta U = \begin{cases} e^{-\phi\ (BD\cdot K - C)} - 1 & \text{if measure is currently not implemented} \\ 1 - e^{-\phi\ \left(\frac{BD\cdot K}{(1-BD)} - C\right)} & \text{if measure is currently implemented} \end{cases} \quad (3)$$

The measure was expected to reduce the costs K of disease with a fraction BD, and implementing the measure implied a fixed cost C.The costs of disease K were calculated by the farmer based on the number of diseased pigs and antimicrobial usage in the pre-set observation period. The parameter ϕ was an individual state variable of farmers and determined their risk aversion of farmers. A higher value of ϕ translated into a larger change in utility with smaller changes in the difference between disease costs and measure-implementation costs.
Change in satisfaction (Eq. (4)) was determined by the antimicrobial usage in the last round, and the minimum and mean antimicrobial usage, $\min(ABuse)$, recalled by the farmer, and the expected change in antimicrobial usage in the next round ($ABuse(t+1)$):

$$\Delta S = \frac{ABuse(t) - mean(ABuse)}{\min(ABuse) - mean(ABuse)} - \frac{ABuse(t) - ABuse(t+1)}{ABuse(t)} \quad (4)$$

In Eq. (5), BA is the farmer's belief (or expectation) in the ability to reduce antimicrobial usage by implementing the measure.

$$ABuse(t+1) = \begin{cases} ABuse(t) \times (1 - BA) & \text{if measure is currently not implemented} \\ \frac{ABuse(t)}{(1 - BA)} & \text{if measure is currently implemented} \end{cases} \quad (5)$$

Both satisfaction and utility were based on the beliefs of farmer (BA and BD) regarding the effects of the measures. Farmers need information to assess potential benefits of measures to reduce antimicrobial usage and endemic disease costs. The information about the cost of measures was fully known to farmers in the model. The information about effects was modelled as being distributed by an information service communicating information about expected effects $E_{Ti}, T \in \{D, A\}$ of measure i on disease (E_D) and antimicrobial usage (E_A). The communication intensity resulted in a probability q that a farmer received the information. For each measure i, each farmer was modelled to maintain a temporal belief $B_{Ti}(t)$ about each effect E_{Ti}, which was updated in each time step (Eq. (6)):

$$B_{Ti}(t) = \begin{cases} \gamma E_{Ti} + (1 - \gamma) B_i(t-1) & \text{if } X(t) < q \\ (1 - \delta) B_i(t-1) & \text{if } X(t) \geq q \end{cases} \quad (6)$$

where $B_{Ti}(0) = 0$; $X(t)$ is a random variable drawn from the uniform distribution between 0 and 1 for each farmer and in each time step; δ is a globally defined belief decay factor, typically set; γ represents a farmer's responsiveness to new information. Each farmer has a specific value of γ drawn from unif(a_γ, b_γ) during each simulation setup.

The last factor determining the intention of a farmer to adopt a measure was the norm, which was the fraction of his contacts that adopted the measure in the current round.

RESULTS

Measures

The uptake of measures was simulated to allow farmers to implement each of the three measures alone or simultaneously, and simulations were done in which only one of the measure could be chosen (Fig. 4). The measure *investments* was shown to have a threshold, below which this measure would not be implemented by the farmers. This threshold was not influenced by the possibility to apply the measures *practice* or *policy*. The variation in and average of the number of farmers adopting measures *practice* and *policy* (compare top and bottom panel of Fig. 4) was reduced by the adoption of measure *investments*.

To analyse the effects of the three components Utility, Satisfaction and perceived Norm in the decision-making process, the model was run with only one component at a time influencing the intention (data not shown). Interestingly, the height of the threshold for adopting the investments was reduced when the farmer also took into account his satisfaction and the perceived norm next to utility. The perceived norm as a single component did not produce any change, because none of the farmers started implementing a measure. On the other hand, the satisfaction of farmers as a single component led to a 30% – 35% adoption of each of the measures (data not shown). This is an emergent balance between the expected benefits of a higher satisfaction and the epidemiological dynamics. The disease occurred in 30% of the pens, which were the only pens in which antimicrobials would be used. A farmer

could not increase satisfaction by implementing a measure when no disease and no antimicrobial usage were present. This reduced the intention to apply this measure in the coming round.

After analysing the dynamics of the uptake of the measures, it was possible to show the effect of the implementation of measures on antimicrobial usage, which was the most important outcome of the model (Fig. 5). Neither *investments* nor *practice* decreased the overall antimicrobial usage, but resulted in a decrease of group treatments. There was, however, a steady increase in group treatments with implementation of the reduction policy. The reduction of antimicrobial usage by *policy* led to an increase in the force of infection (data not shown), such that more pens crossed the threshold for group treatment. Adoption of changes in management practices slowed the spread of the disease. This decreased the number of group treatments (Fig. 5).

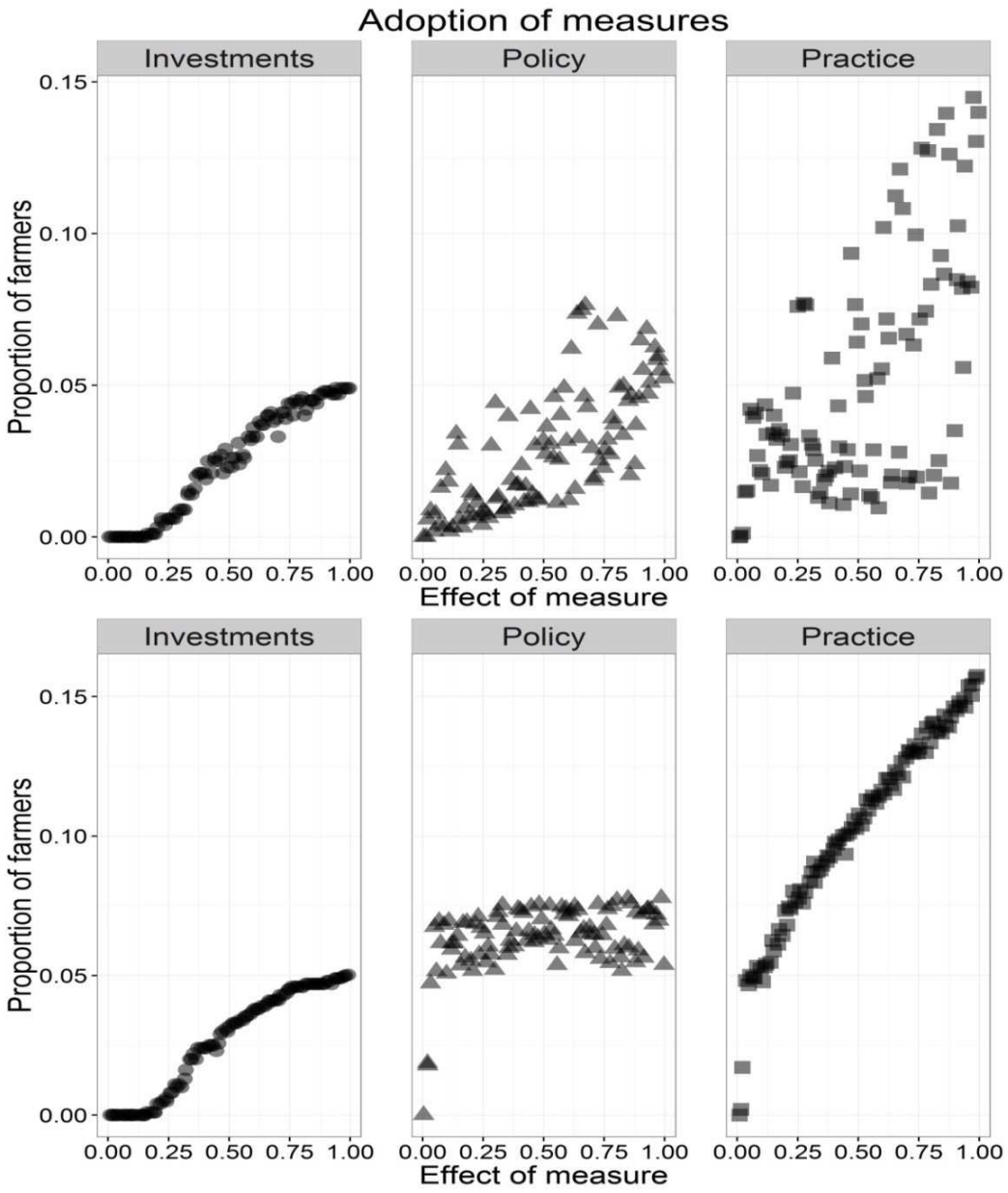

Fig. 4 Adoption of measures by farmers by the (expected) effect of the measure when able to choose other measures (top) or not able to choose other measures (bottom)

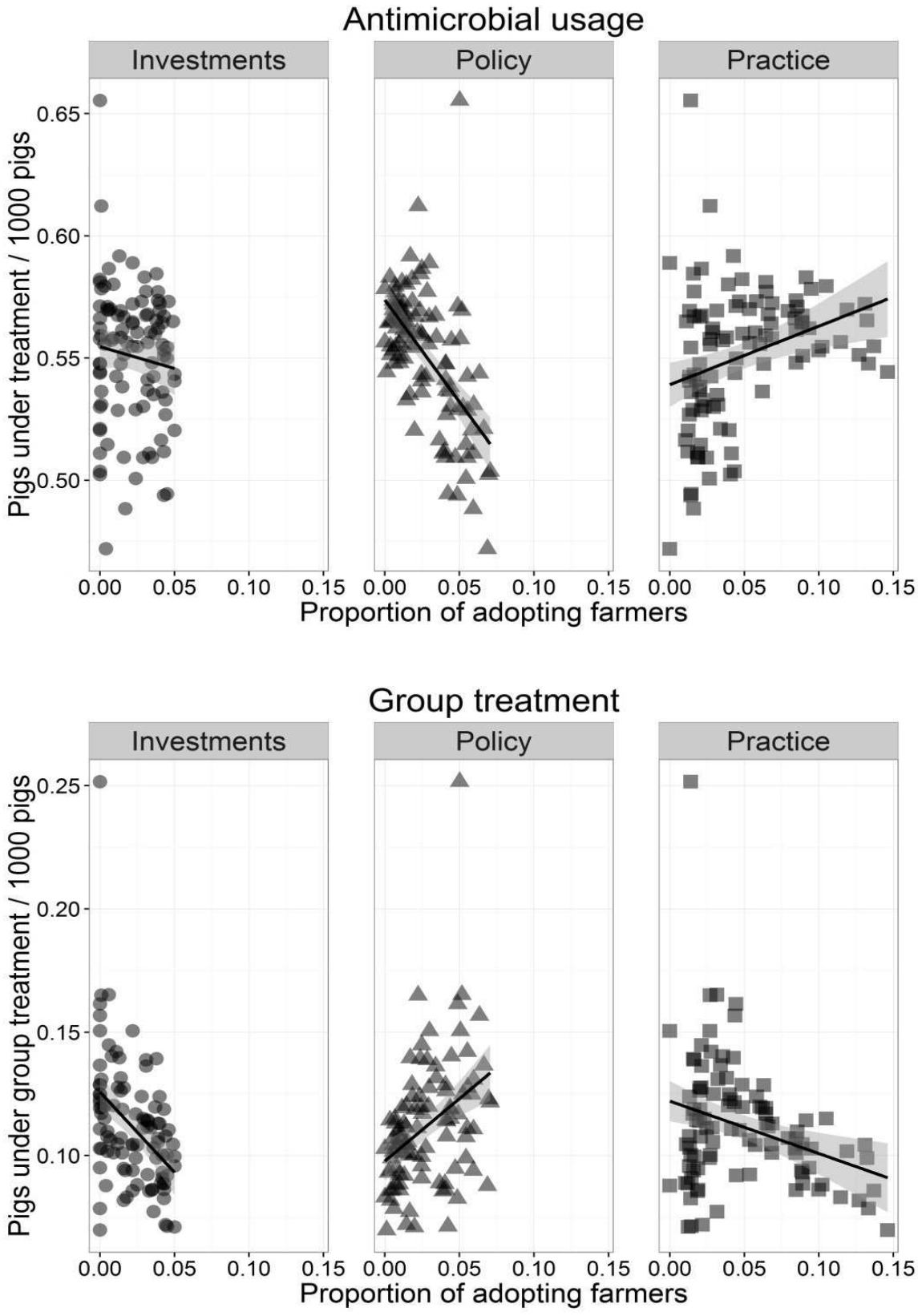

Fig. 5 Effect of proportion of farmers adopting measures on the total antimicrobial usage (top) and number of group treatments (bottom)

DISCUSSION

A threshold property for adopting investments to reduce endemic disease transmission was found using an agent-based model. This threshold was expected given the high costs of such investments. Interestingly, although intuitively logical, the threshold for investments decreased when satisfaction and perceived norm were taken into account. In addition, the model results gave an insight into the interaction between the adoption of different measures. The adoption of changes in management practice and the adoption of reduced antimicrobial policy decreased and were more variable when a farmer was also able to control endemic diseases by investments. These effects might direct field studies to identify reasons for applying certain measures.

This study focused on the situation in 2008 and simulated from that point onwards. The year 2008 may be seen as a turning-point in the Netherlands for the use of antimicrobials in livestock (Speksnijder et al., 2015b). From 2009 onwards, farmers were not allowed to consult more than one veterinary practice, and needed to implement farm-health and farm-treatment plans (FHP and FTP: Speksnijder et al., 2015b). A 56% decline in antimicrobial usage was observed in the Netherlands, which is partially due to a ban on the preventive use of antimicrobials and the implementation of FHP and FTP (Speksnijder et al., 2015a). This model can help to distinguish how these changes at individual level can be translated to the population level.

At population level, the model predicts an increase in group treatments without mitigation of endemic diseases if antimicrobials are used more restrictively. There is no evidence that this actually happened in the Netherlands, but this effect could be of concern to farmers, veterinarians and policy makers. This effect might not have occurred due to the enforcement of FHP and FTP, such that antimicrobial reduction policies were implemented alongside other measures (Speksnijder et al., 2015a).

Infection equals disease in the current model. However, this only applies to very few diseases. The endemic diseases on which the parameters are based include diseases that are present asymptomatically (Stege et al., 2004; Loeffen et al., 2009; Dekker et al., 2013). The effectiveness in reducing transmission might therefore be exaggerated in the epidemiological sub-model. The effect is hard to predict, because a weaker association between infection and antimicrobial usage would affect the transmission dynamics, the growth promotion effects and the expected gain by implementing other measures.

The social network is currently completely regular. Each farmer has the same number of contacts and no clustering of contacts occurs. Social networks are irregular, clustered and contain a number of influential members. This structure is important for the adoption of innovations (Delre et al., 2010; Eck et al., 2011).

The decision-making sub-model in this study was based on the theory of planned behaviour (Ajzen, 1991). This theory states that three cognitive components (satisfaction, perceived norm and utility) will shape the intention of a person. The intention is assumed to map directly to the actions or behaviour of an individual. This theory has been widely criticised, because it seems hard to predict the effect of interventions on the separate components, the intention and on behavioural change (Sniehotta et al., 2014). These criticisms are mainly directed at decision-making models for health interventions. The theory does explain the actions of people in an economics-related decision-making context, i.e. starting one's own business (Kautonen et al., 2013, 2015). Nevertheless, future exploration of

other decision-making models would enhance the robustness of the model to predict the qualitative effects of the adoption of measures.

Important future directions include modelling a more detailed disease sub-model, a better-defined social network, and implementating other decision-making models; each of which is currently being investigated. The complex-adaptive systems approach shows that using individual-based description of the farmer's behaviour can derive emergent properties at the population level. This comes with the cost of more complicated models (Grimm et al., 2006). The strength of agent-based modelling is the ability to specify actions at the individual level and study their aggregated effect in terms of population-level dynamics, This assists the understanding of systems as well as to evaluate different intervention strategies (Fischer et al., 2010, 2011), including the use of antimicrobials in livestock.

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GOVERNMENT PREPAREDNESS FOR EXOTIC INCURSIONS OF SWINE FEVERS IN SCOTLAND: HOW CAN RESEARCH HELP?

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SUMMARY

The results of an ongoing policy-linked research programme aiming to improve evidence used for prevention and control decisions against swine fever outbreaks in GB are presented. This programme has generated invaluable knowledge for developing more robust biosecurity and surveillance plans for emerging swine diseases. The predominant benefit, however, has been to facilitate collaborations and discussion between researchers and various actors, for better uptake of coherent industry-level emerging swine disease control.

INTRODUCTION

The pig industry is one of the major agricultural livestock sectors for the United Kingdom (UK), as well as one of the largest in the European Union (EU), occupying the 10th position in terms of meat production (Eurostat, 2015). Overall, there are 4.7 million pigs on 11,500 currently listed pig farms in UK (UK Agricultural Census 2015). The pig industry sector is mostly concentrated in England, with 82% of the UK pig herd, whereas the Scottish pig sector accounts for nearly 7% of the UK pig herd (PHWC, 2015). Although the pig industry is associated with exports of pigs and pork products worth £300 million (GTIS, HM Revenue and Customs, 2014), the profitability of pig farming in the UK has declined, leading to a progressive reduction in the number of pigs. As such, the industry is vulnerable to threats such as exotic or emerging diseases that, if introduced into the pig population, would undoubtedly have major economic consequences.

The recent epidemic of African swine fever in the eastern European region has highlighted the threat posed by incursions of swine fevers (SF) to the UK swine industry, despite regulations in place to reduce the risk of SF spread within Europe (e.g. regarding the import of live animals and the feeding of food waste). In response to these concerns, a national response planning exercise, "Exercise Walnut", was carried out in 2013 to test the preparedness of the relevant government bodies to incursion of SF in the UK (AHVLA, 2014). From the outcomes of this exercise, it was apparent that important knowledge gaps existed in the UK, particularly around the level of interaction between the different actors in the pig industry (i.e. commercial and non-commercial) and the potential role of small-scale pig producers in the risk of SF spread. Such knowledge is critical for identifying likely routes of disease incursion and transmission prior to disease detection, and identifying weaknesses

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in biosecurity, surveillance and contingency planning. These are key components in the development of a representative, and therefore more robust, biosecurity and surveillance plan.

To improve the evidence base for rapid disease control decisions in the face of SF outbreaks, an extensive programme of research was initiated by Scottish Government, through its Centre of Expertise on Animal Disease Outbreak (EPIC) and its Strategic Research Programme (SRP). The programme aims to (1) improve knowledge on the structure of the pig industry in Scotland and for the whole of Great Britain (GB); (2) clarify the role of small producers in the spread of SF, whether caused by classical swine fever (CSF) or African swine fever (ASF); (3) develop more robust surveillance and control plans against SF.

The objective of this paper is to review the activities of this programme since 2013.

THE ROLE OF EPIC AND THE SRP

EPIC and the SRP are both multidisciplinary Research Programmes funded since 2011 by the Scottish Government to provide research-based evidence on relevant issues to Scotland's communities, its people and the rural economy. The primary objective of the SRP is to support research that is relevant to climate change, land use and food security. As such, the SRP has a strong commitment to improving animal health and welfare as well as the management of livestock production in Scotland. In contrast, EPIC focuses on best preparing Scotland's livestock industry and stakeholders for disease outbreaks. Both Research Programmes have strong commitments to providing applied, interdisciplinary, policy-responsive science whilst responding to the need for cross-cutting evidence to inform policy.

Together, they are resources unique to Scotland, and involve multiple Scottish animal health research institutions in the form of the University of Edinburgh/Roslin Institute, the University of Glasgow and the Main Research Providers (MRPs), particularly Biomathematics and Statistics Scotland (BioSS), the James Hutton Institute, Moredun Research Institute (MRI), and Scotland's Rural College (SRUC).

Commitment of the industry to research-based policy is critical to enable research and to evaluate the success of a programme. Both EPIC and SRP have therefore built bridges in common to coordinate and communicate research outputs with the industry. Both regular interface meetings and ad hoc policy-specific meetings have been carried out throughout the programme between researchers and stakeholders, presenting assumptions and discussing inferences from research outputs. These meetings enable researchers to gather relevant contact with stakeholders and allow them to better understand research objectives and motivations, facilitating the transfer of important industry-owned data.

RESEARCH ACTIVITIES

Preliminary activities

A gap analysis was carried out to review significant knowledge gaps regarding the role and importance of small-scale pig producers in Scotland. This gap analysis reinforced the perception that the lack of knowledge on small-scale pig producers was a critical barrier to the accurate assessment and estimation of risk of disease entry and spread, and would affect

policy-makers' ability to prepare for future SF outbreaks. Gaps were mainly in the size and distribution of the non-commercial sector of the industry, the trading links between commercial and non-commercial sectors of the industry and the level of biosecurity carried out in both sectors of the industry.

Over the course of the programme, studies integrating pig movement databases with industry-relevant information were carried out to describe the structure of the British swine industry and unravel trading behaviours between different sectors of the industry. These descriptive studies were further completed with surveys informing on the current implementation of biosecurity practices in key sectors of the industry.

The structure of the industry: As there are no unique centralised databases recording details of all pig-holding farms and their movements within the UK, an important activity of the programme was to gather various databases held by governments and the industry to inform on the structure of the pig industry in Scotland and GB. In particular, efforts were made to collate all data relevant to the movements of pigs, the size and location of pig farms, the type of production of these farms, their membership in various government- and industry-led schemes, as well as the location and type of all gathering places (i.e., markets, show grounds, ferry collection centres and slaughterhouses) involved in the trade of pigs. Using these datasets, farms were categorised into seven *production types*: boar stud (BS), breeders (B), weaner (W), finisher (F), breeder-to-weaner (B2W), breeder-to-finisher (B2F) and weaner-finisher (W2F). Further discussions with the pig industry also enabled us to define topological groups of farms relevant to disease spread and control according to their pig population size, movement activity and quality assurance scheme membership. Throughout the whole programme, farms were classified into three *producer types*: small non-commercial producers, non-assured commercial producers and assured commercial producers.

Trading behaviours: The electronic pig movement databases of Scotland (i.e. ScotEID, www.scoteid.com) and England/Wales (i.e. eAML2, www.eaml2.org.uk) were independently analysed prior to being merged into a single, integrated and comprehensive movement dataset. The analysis of ScotEID was focused on characterising all movements between pig premises in Scotland (specifically, quantifying the amount, frequency and distance of movements between farms of different producer types) and in quantifying the frequency of usage of livestock hauliers within the Scottish pig industry. In contrast, the analysis of eAML2 focused on describing the pig trade network in England/Wales using social network analysis methods, characterising key features that may guide surveillance activities.

Biosecurity behaviours: Two surveys were carried out to gather information on farm practices in both small and commercial pig producers in GB. The first questionnaire was designed to capture information from small pig holders in Scotland, including questions on transport, husbandry, pig health management and biosecurity. This questionnaire was available online and also posted to 610 randomly selected non-commercial pig producers. In total, 135 completed questionnaires were gathered from small-scale pig producers. The second questionnaire consisted of a biosecurity audit of 554 English commercial pig holdings belonging to the Red Tractor quality assurance scheme that are part of the Pig Health Improvement Project. This questionnaire included questions related to biosecurity risks directly related to pigs, farm personnel and visitors, and associated with fomites. Both surveys showed a low non-response rate (<12%) for most of the variables and were deemed to be a good representation of practices in these two different sectors of the pig industry in GB.

Modelling capacity building

Mathematical models that simulate the course of epidemics are regularly used to provide guidance for decision-making to control outbreaks of notifiable diseases, and therefore represent a key asset to develop robust surveillance and control plans against SF. Over the course of the programme, a spatially explicit, premises-based simulation model was developed to model the spread of SF over all movements of pigs recorded in GB between January 2012 and December 2013.

Development of GB wide simulation model: The premises-based simulation model developed in the programme comprises modules accounting for (1) the transmission of the disease between premises, (2) the influence of the within-farm prevalence on disease transmission between premises, and (3) mitigation and surveillance activities carried out to control epidemics. As a first step, it was considered that between-farm transmission processes were driven by pig movements alone. In this situation, we evaluated the spread of ASF within GB from incursions in two major pig-rearing areas in GB (i.e. East Anglia and Aberdeenshire/Moray). In this study, ASF spread within British farms was assumed to be similar to the spread observed in nine commercial farms from the Russian Federation (Guinat et al., 2016). Because the spread through pig movements is not the only route of SF spread between premises, efforts were then made to account for the local between-farm spread of SF in the simulation model by incorporating a distance-dependent transmission kernel. Parameterisation of the shape of transmission kernels can be challenging, and informing policy based on unrepresentative parameters could have negative consequences for disease management. In particular, important determinants in between-farm spread of disease (Mintiens et al., 2003; Boender et al., 2014), such as pig density in the high production regions, national average pig farm density and farming structure vary between countries. Available historical SF outbreak data from GB were therefore used to develop country-specific data-derived parameters, thereby allowing the robust evaluation of how SF may spread from single incursion events in the British pig industry.

Within-herd spread of ASF: In collaboration with the Pirbright institute and the Royal Veterinary College, a stochastic compartmental epidemiological model was fitted to mortality records of nine infected commercial herds to quantify the transmission dynamics of ASF within pig farms (Guinat et al., 2016). Epidemiological parameters were estimated using the Approximate Bayesian Computation (ABC) method based on sequential Monte Carlo (SMC) (Toni et al., 2009).

Analysing the 2000 CSF outbreak in the UK: In GB, the last epidemic of SF occurred in 2000, during which 16 farms were detected as being infected with CSF in East Anglia (Paton, 2002), a major pig-rearing area in GB. Based on these data, models were applied based on continuous time semi-Markov processes, using data augmentation Markov chain Monte Carlo techniques within a Bayesian framework (Jewell et al., 2009) to infer the spatial transmission kernel (describing pathogen spread between farms) and the distribution of times between the (unobserved) introduction and detection of SF. In particular, attention was paid to model evaluation and selection to ensure the inferred parameter estimates were reliable and representative of the underlying system even when using data from relatively small outbreaks.

RESEARCH OUTCOMES

The structure of the British pig industry

The size of the industry was found to be clearly underestimated by the 2015 UK Agricultural Census, and captures only a third of the number of pig farms. Combining all sources of available information showed that 34,294 pig holders are present in GB alone (of which 81% are small-scale producers), gathering more than 5.4 million pigs mainly distributed around three production hotspots (East Anglia, North-East Scotland, and Yorkshire and the Humber, Fig. 1A). Although the spatial distribution of the national herd is consistent with prior knowledge, areas of high farm density outside these production hotspots were also found, particularly in the South of England (Fig. 1B). These areas of low density of pigs but high density of pig farms participate actively in the trade of pigs in GB (Figs. 1C and 1D), and confirm the results of the biosecurity survey on Scottish small-scale producers (this survey showed that a substantial proportion of respondents move pigs for trade). Small-scale pig holders also keep other species on their properties (Fig. 2), potentially increasing the number of vehicles moving on and off farms. Together with their relatively low uptake of biosecurity activities (such as cleaning and disinfection of vehicles before or after movements or boot dipping), these findings highlight the potential of small producers to generate widespread SF epidemics.

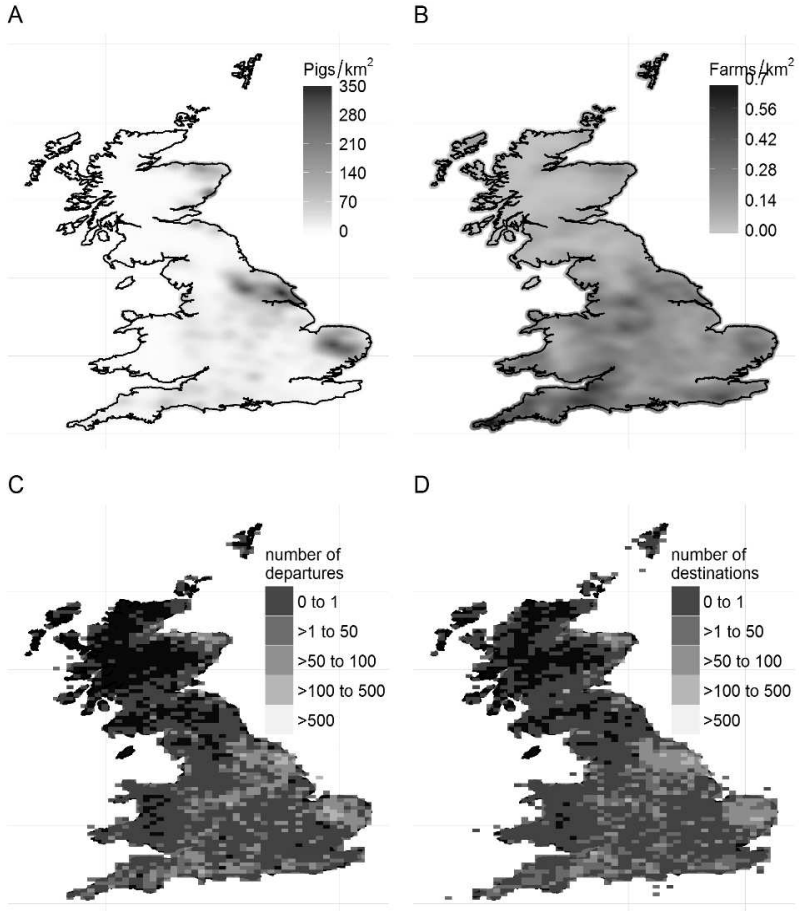

Fig. 1 The spatial layout of the pig industry. Maps showing the smoothed number of (A) pigs and (B) pig farms per squared kilometre. Maps showing the number of (C) departures and (D) destinations for all movements of pigs between farms as recorded between January 2012 and December 2013 in Great Britain

In England/Wales, most movements are typical of a vertical industry, progressively going from breeding to finishing units. However, the importance of breeding pyramids is limited in GB. Instead, movements of pigs are organised into communities of farms. In total, there are 44 communities of more than 50 farms in England/Wales. These 44 large communities (LC) cover surface areas ranging from 0.5% to 60.2% of GB surface area, with the largest encompassing an area from South-West England to Scotland. There is an association between the spatial area of LC and the proportion of the commercial farms within LC (R2=0.20, LRT P-value = 0.002), with the surface increasing with an increasing proportion of commercial farms. However, LC cover overlapping areas, regardless of whether they show a high proportion of commercial farms.

Although the trade between these LC is limited, they are not isolated and movements occur between farms of different communities. Among all farms that trade pigs, 1,000 farms can be targeted to affect the cohesion of the pig trade network efficiently and improve the cost-efficiency of prevention and mitigation measures against SF. Interestingly, only 35% of these farms are commercial producers, highlighting that the commercial and non-commercial sectors of the industry are not isolated from each other. Although they are assumed more bio-secure than the non-commercial producers, assured producers do trade (albeit infrequently) with small-scale producers. However, isolation of new stock, implementation of "All-In–All-Out" production systems and restriction of entry for vehicles coming from other pig units were not widely implemented practices according to the English assured producers' biosecurity audit.

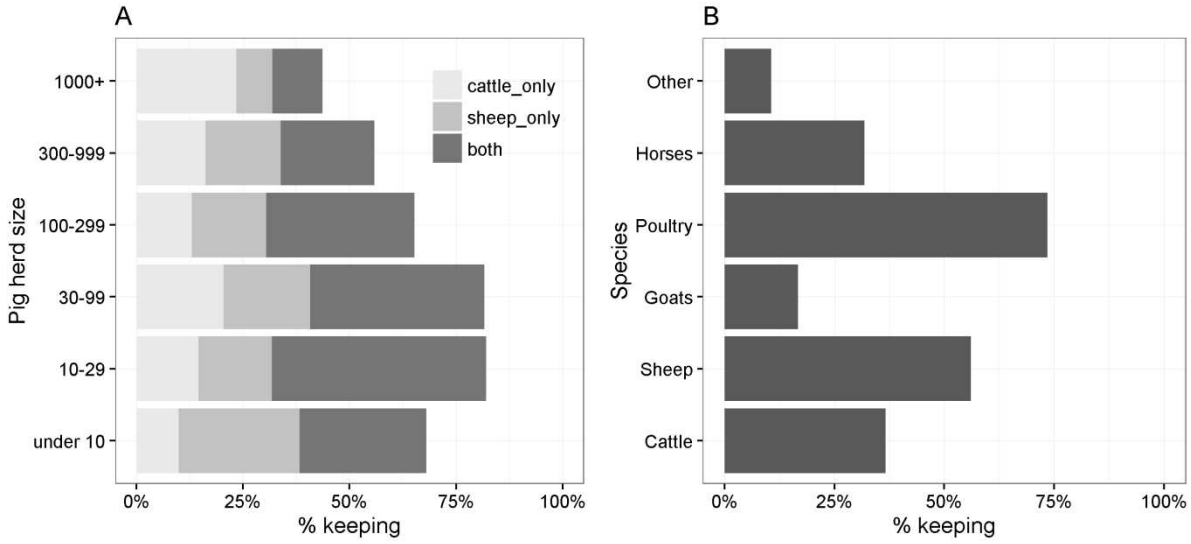

Fig. 2 Proportion of farms with pigs that also kept other livestock species. (A) Proportion of farms with pigs, stratified by herd size, that also kept cattle and sheep, as reported by the Scottish Agricultural Census June 2011. (B) Proportion of respondents with other livestock species, as reported in the survey on small-scale pig holders in Scotland. While the Scottish Agricultural Census June 2011 reveals that up to 80% of pig herds also had cattle or sheep on the same property, 91% respondents of the biosecurity survey on small-scale Scottish pig holders indicated keeping at least one other species

Disease spread and risk areas

The results of our modelling exercises showed that incursions of ASF in the industry might spread widely via movements of pigs if left undetected for 8 weeks (Fig. 3A). The

spatial extent of this spread varies as a function of the location of the incursions and involves numerous LC. However, the chance for large widespread epidemics is low: the median probability that a primary case generates epidemics involving at least two other farms varies from 0.04 (95% CI: 0.03-0.05) to 0.13 (95% CI: 0.09-0.14) from primary incursions located in East Anglia and Moray/Aberdeenshire, respectively.

The role of local spread was found to be critical in the spread of CSF, particularly in England/Wales. However, it remained constrained to areas within 5km of infected farms in GB, with a rate of infection at 1km and 5km of 0.073 (95% Cr. I.= 0.014 – 0.229) and 0.005 (95% Cr. I.= 0.0004 – 0.028) per 1,000 infectious premises per day, respectively. Together with transmission via movements and control activities, outbreaks would be restricted to the primary case in 81.3% of the incursion events and 8.0% would last long enough to be detected if SF were left undetected for 8 weeks. In this situation, detected outbreaks would involve a median of three farms (95% range 1 – 30 farms) and last 21 days (95% range 1 - 109 days). Substantial disease spread may still occur and could involve as many as 107 farms. Although outbreaks may be generated from anywhere in GB, the risk of generating widespread CSF epidemics is higher within a limited number of areas in GB (Fig. 3B). These areas are characterised by their greater density of pig producers, whether commercial or non-commercial.

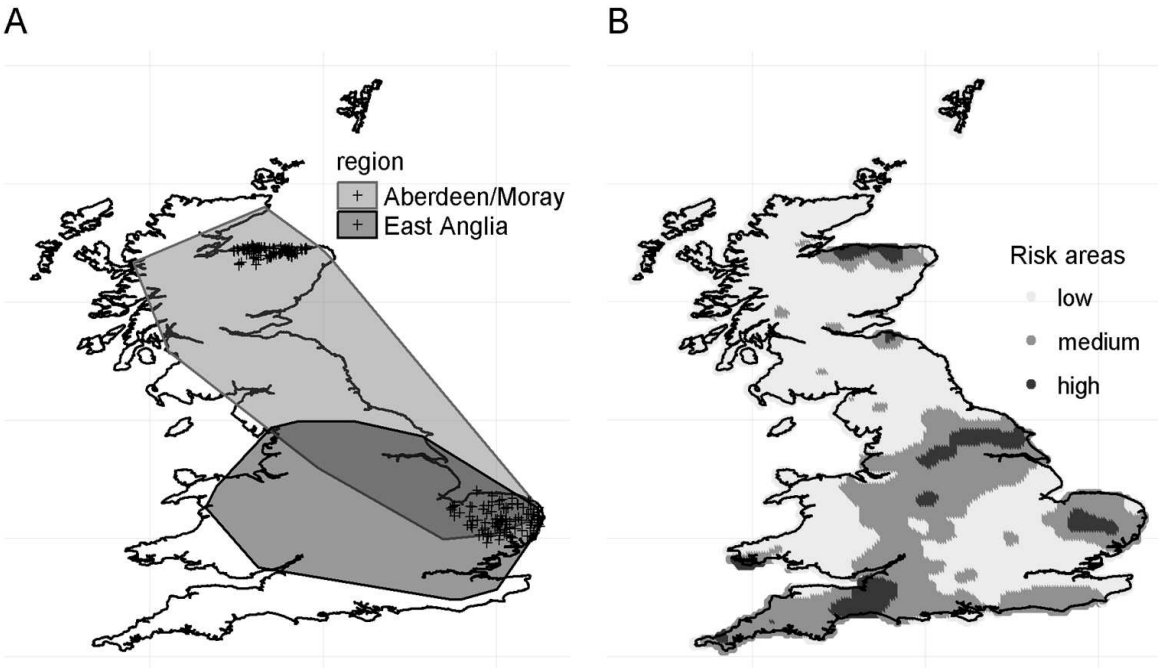

Fig. 3 Spatial risk of SF spread in Great Britain. (A) Extent of the areas including 95% of the secondary infected pig premises (i.e. farms and gathering places) that were susceptible to infection by ASF epidemics in GB subsequent to primary incursions (black crosses) into Moray/Aberdeenshire or East Anglia. (B) Smoothed spatial distribution of the low-, medium- and high-risk areas for generating epidemics of CSF. Risk areas in B are defined based on the local probability of epidemic take-off generated from a single incursion in which the probability of epidemic take-off is defined as the probability that a primary case generates epidemics involving at least two other farms

It is clear that the role of commercial producers is of utmost importance and that good biosecurity measures and compliance to mitigation procedures of this sector of the industry are necessary (and need to be improved) to limit the spread of SF. However, the risk of SF spread in GB when incursion occurs in small-scale producers is not negligible, with 1% and 0.02% of the outbreaks from high- and low-risk areas, respectively, involving at least 50 infected farms.

DISCUSSION

In order to improve preparedness for disease incursion and assure sustainability of the British industry, it is critical to have some understanding of its structure and its resilience to incursions of exotic notifiable diseases. In this collaborative programme involving EPIC and the SRP, a whole collection of studies have been carried out to increase insights into the pig industry in GB and increase Scotland's capacity to reactively support decisions in the face of an outbreak of an infectious disease in pigs. Swine fever was chosen as an exemplar, not only because the spread of ASF in Eastern Europe poses an imminent threat to the industry, but also because the availability of historical information enabled models to be parameterised with country-specific information. However, the scope of this programme goes beyond SF and aims to pro-actively respond to other threats (present and future) to the industry, such as porcine epidemic diarrhoea and foot-and-mouth disease.

Overall, the low vulnerability of the British pig industry to large SF outbreaks was highlighted, but concerns were identified with respect to the role played by the non-commercial sector of the industry. In particular, it was shown that, in contrast to what is commonly thought, this sector is a large and active trading population and is not isolated from the commercial sector. Furthermore, the general low standards of biosecurity practices observed in non-commercial pig holdings, together with the insufficiencies of preventive measures against fomite-mediated disease introduction and spread in assured producers, further highlights the potential of the non-commercial sector to the widespread dissemination of directly transmitted viral diseases.

It is important to note that researchers from EPIC and SRP worked closely together to provide a comprehensive collection of research outcomes, avoid duplication and increase synergies between activities carried out in these two research programmes. Together, they also acted as knowledge brokers between industry stakeholders and policy-makers, allowing critical issues and limitations to be identified and ground-truthing assumptions, as well as research results. These coordinated and continual interactions built trust between all partners, not only easing the flow of critical data but also ensuring the uptake of evidence-based policy measures by the key stakeholders.

Notably, livestock information and data in GB are fragmented across all actors of the sector and between administrations. Although efforts were taken to automatically collate all available data into a single, centralised and clean database, errors may still remain to limit our ability to provide evidence robust enough to usefully inform policies. However, the emphasis of researchers to integrate both policy-makers and industry actors into the data management processes facilitated the identification of errors in datasets, clarified observed dangerous behaviours, and drove improvements in data-recording systems. Therefore, this was perceived as key in the success of the programme.

CONCLUSION

In conclusion, this paper reviews the results and challenges of an ongoing policy-linked research programme aiming to help enhance veterinary surveillance and concurrently provide reactive decision support in the face of an outbreak of swine fever in GB. Beyond its academic and policy benefits, this programme has facilitated collaborations and discussion between researchers and various stakeholders, which improved the uptake of coherent industry-level emerging pig disease control. Developing trust between researchers, policy-makers and stakeholders has been critical for the current success of this programme and constitutes a valuable stepping stone for its future activities and in guiding industry-led policy on SF surveillance and control in GB.

ACKNOWLEDGEMENTS

This work was commissioned and financed by the Scottish Government through its Programme of Research 2011-2016 (no. UEH/851/11) and its Strategic Research Programme 2011-2016 (Programme 2 - "Food, Land and People", Theme 6 - "Health and Welfare"). We would like to thank Dr. Claire Guinat (RVC/Pirbright), Dr. Simon Gubbins (Pirbright), Dr. Harriet Auty (SRUC) and Dr. Lisa Boden (University of Glasgow), Dr. Kokouvi Gamado and Dr. Glenn Marion (BioSS) for their input into the research carried out in the programme. The authors also thank all the different industry and government partners in both Scotland and England/Wales for providing intellectual input in the conduct of the research project and for providing commercial data: Scottish Government, Quality Meat Scotland (QMS), Scottish Pig Producers, Scottish EID Livestock Traceability Research (ScotEID), Red Tractor, the British Pig Association (BPA), the Agriculture and Horticulture Development Board for pigs (AHDB-pork), the Animal and Plant Health Agency (APHA).

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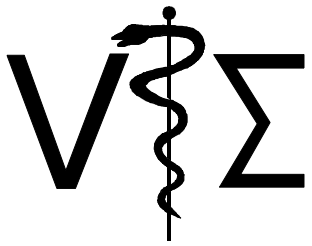

SOCIETY FOR VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

PAST VENUES AND ORGANISERS OF ANNUAL MEETINGS

Year	Venue	Organiser(s)
1983	Southampton	Davies & Thrusfield
1984	Edinburgh	Thrusfield
1985	Reading	Thrusfield
1986	Edinburgh	Thrusfield
1987	Solihull	Thrusfield
1988	Edinburgh	Thrusfield
1989	Exeter	Howe
1990	Belfast	McIlroy
1991	London	Jones
1992	Edinburgh	Thrusfield
1993	Exeter	Howe
1994	Belfast	Menzies
1995	Reading	Paterson
1996	Glasgow	Reid
1997	Chester	Clarkson
1998	Ennis, Ireland	Collins
1999	Bristol	Green
2000	Edinburgh	Thrusfield & Mellor
2001	Noordwijkerhout	van Klink
2002	Cambridge	Wood & Newton
2003	Warwick	Green
2004	Martigny	Stärk
2005	Nairn	Gunn
2006	Exeter	Peeler
2007	Dipoli	Virtala & Alban
2008	Liverpool	Pinchbeck & Robinson
2009	London	Verheyen & Pfeiffer
2010	Nantes	Fourichon & Hoch
2011	Leipzig	Thulke & Lange
2012	Glasgow	Parkin & Others
2013	Madrid	Martínez-López & Vargas Alvarez
2014	Dublin	More & Collins
2015	Ghent	Mintiens & Dewulf
2016	Elsinore	Nielsen & Halasa

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
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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
2010-'11	L.A. Kelly
2011-'12	C. Fourichon
2012-'13	T.D.H. Parkin
2013-'14	K.L.P. Verheyen
2014-'15	K. Mintiens
2015-'16	H.H. Thulke
2016-'17	A. Lindberg

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Honorary Auditors: Fraser Menzies & Keith Howe

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PLENARY TALKS

Year	Gareth Davies Lecture	Conference Opening Plenary
2017	Theresa Bernado TRENDS: Technology, Research, Epidemiology, Networks, Data & Surveillance	Tine Hald Source attribution: Translating science into public health action
2016	Bernhard Url The foundation of science-based risk assessment for decision support on food safety and animal health in EU	Mirjam Nielen Evidence-based veterinary medicine needs clinical epidemiology
2015	Piet Vanthemsche Preventive Veterinary Medicine as an essential part of sustainable animal production	Crawford Revie Hype and Hysteria: Should veterinary epidemiologists really care about Big Data?
2014	Ian Gardner Bridging the gap in infectious disease epidemiology between aquatic and terrestrial food animals: challenges and future opportunities	Nils Toft Confessions of a wannabe Bayesian
2013	Andreas Hensel Dioxins, EHEC and strawberries: Risk assessment and risk communication in practice	José Manuel Sánchez-Vizcaíno The Spanish experience on the control and eradication of infectious diseases: from the old to the current system
2012	Stuart Reid Evidence-based prevention: well done or rare	Didier Boichard Genomic selection: an opportunity for improving health of farm animals
2011	Karin Schwabenbauer From science to policy - the case of classical swine fever (CSF) control	Dominic Mellor The trouble with epidemiology: the tyranny of numbers
2010	David Waltner-Toews Beyond one world, one health and ecohealth…what's out there?	James Wood From pathogen adaption to host ecology: epidemiological and experimental contributions to the understanding of emerging infectious diseases
2009	Jørgen Westergaard The interaction between veterinary science, legislation and management in animal disease control in the European Union	Katharina Stärk Food safety challenges in a global market – are we ready?

2008	Paul Fine Infectious disease eradication – meanings and implications	Kenton Morgan For the benefit of Mr Kite
2007	Yrjö Gröhn Food supply veterinary medicine: Modelling of production, health and food safety	Laura Green Improving Animal Health
2006	David Galligan From partial budgets to real options - concepts in animal health economics	Nigel French Understanding human exposure to zoonoses from food and the environment: The application of molecular tools and modeling
2005	Bill Reilly From TB to VTEC: The changing epidemiology of foodborne zoonoses	Simon More Towards eradication of bovine tuberculosis in Ireland: A critical review of progress
2004	Ulrich Kihm BSE and the stable to table concept	Gary Smith Spatial models of infectious disease in the USA: a crisis of conference and confidentiality
2003	Sir David Cox The current state of statistical science	Ynte Schukken Molecular and mathematical epidemiology of bovine mastitis
2002	George Gettinby Informatics and epidemiology – the first 400 years	Bryan Grenfell Deterministic and stochastic influences on the dynamics and control of infectious diseases
2001	Will Houston Science politics and animal health policy: epidemiology in action	Mart de Jong Design and analysis of transmission experiments
2000	Jim Scudamore Surveillance – past, present and future	Dirk Pfeiffer Spatial analysis – a new challenge for veterinary epidemiologists
1999	Aalt Dijkhuizen The 1997/98 outbreak of classical swine fever in the Netherlands: lessons learned from an economic perspective	Mark Woolhouse Understanding the epidemiology of scrapie
1998	Wayne Martin Art, science and mathematics revisited: the role of epidemiology in promoting animal health	

SOCIETY FOR VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

APPLICATION FOR MEMBERSHIP

Name .....

Address .....

.....

.....

.....

Telephone: .....

Fax: .....

E-mail: .....

Signed ..... Date .....

Please enclose the membership fee (£40 sterling to cover two years' membership) along with this application form. Overseas members without British bank accounts are requested to pay 2 - 3 years in advance. Cheques should be in £ sterling and drawn from a British bank. British members should pay future dues by standing order (forms are available from the Secretary or Treasurer). Payment can also be made by credit card; the appropriate form is available from the Society's website, http://www.svepm.org.uk/, or from the Secretary or Treasurer.

Please send this form to the Society's Treasurer:

Dr Marnie Brennan
School of Veterinary Medicine and Science
Sutton Bonington Campus
College Road
Sutton Bonington
Leicestershire LE12 5RD
UK

TEL +44 (0) 115 951 6577
FAX +44 (0) 115 951 6415
Email: marnie.brennan@nottingham.ac.uk

Please turn over

INTEREST GROUPS

Please tick appropriate boxes to indicate your interests:

- ☐ Analytical Epidemiology (Observational Studies)
- ☐ Quantitative Epidemiology & Statistical Techniques (Incl. Modelling)
- ☐ Herd/Flock Level Disease Control Strategies
- ☐ National/International Disease Control Policy
- ☐ Sero-Epidemiology
- ☐ Herd Health and Productivity Systems
- ☐ Disease Nomenclature and Epidemiological Terminology
- ☐ Economic Effects of Disease on Animal Production
- ☐ Veterinary Public Health and Food Hygiene
- ☐ Computing, including data logging
- ☐ Computer Programming *per se*
- ☐ Population and Animal Disease Databases
- ☐ Information System Design
- ☐ Geographical Information Systems (GIS)
- ☐ Risk Analysis

CONSTITUTION AND RULES

NAME

1. The society will be named the Society for Veterinary Epidemiology and Preventive Medicine.

OBJECTS

2. The objects of the Society will be to promote veterinary epidemiology and preventive medicine.

MEMBERSHIP

3. Membership will be open to persons either actively engaged or interested in veterinary epidemiology and preventive medicine.

4. Membership is conditional on the return to the Honorary Treasurer of a completed application form and subscription equivalent to the rate for two calendar years at first application or subsequent application following an elapsed subscription. Subsequent annual subscriptions fall due on the first day of May each year.

5. Non-payment of subscription for six months will be interpreted as resignation from the Society.

OFFICERS OF THE SOCIETY

6. The Officers of the Society will be President, Senior Vice-President, Junior Vice-President, Honorary Secretary and Honorary Treasurer. Officers will be elected annually at the Annual General Meeting, with the exception of the President and Senior Vice-President who will assume office. No officer can continue in the same office for longer than six years.

COMMITTEE

7. The Executive Committee of the Society normally will comprise the officers of the Society and not more than five ordinary elected members. However, the Committee will have powers of co-option. Elected officers and ordinary members of the committee have normal voting rights at committee meetings but co-opted and ex-officio members (e.g. the proceedings editors) do not

ELECTION

8. The election of office bearers and ordinary committee members will take place at the Annual General Meeting. Ordinary members of the Executive Committee will be elected for a period of three years. Retiring members of the Executive Committee will be eligible for re-election. Members will receive nomination forms with notification of the Annual General Meeting. Completed nomination forms, including the signatures of a proposer, seconder, and the nominee, will be returned to the Secretary at least 21 days before the date of the Annual General Meeting. Unless a nomination is unopposed, election will be by secret ballot at the Annual General Meeting. Only in the event of there being no nomination for any vacant post will the Chairman take nominations at the Annual General Meeting. Tellers will be appointed by unanimous agreement of the Annual General Meeting.

FINANCE

9. An annual subscription will be paid by each member in advance on the first day of May each year. The amount will be decided at the Annual General Meeting and will be decided by a simple majority vote of members present at the Annual General Meeting.

10. The Honorary Treasurer will receive, for the use of the Society, all monies payable to it and from such monies will pay all sums payable by the Society. The Treasurer will keep account of all such receipts and payments in a manner directed by the Executive Committee. All monies received by the Society will be paid into such a bank as may be decided by the Executive Committee of the Society and in the name of the Society. All cheques will be signed by either the Honorary Treasurer or an elected Committee member.

11. Two auditors will be appointed annually by members at the Annual General Meeting. The audited accounts and balance sheet will be circulated to members with the notice concerning the Annual General Meeting and will be presented to the meeting.

MEETINGS

12. Ordinary general meetings of the Society will be held at such a time as the Executive Committee may decide on the recommendations of members. The Annual General Meeting will be held in conjunction with an ordinary general meeting.

GUESTS

13. Members may invite non-members to ordinary general meetings.

PUBLICATION

14. The proceedings of the meetings of the Society will not be reported either in part or in whole without the written permission of the Executive Committee.

15. The Society may produce publications at the discretion of the Executive Committee.

GENERAL

16. All meetings will be convened by notice at least 21 days before the meeting.

17. The President will preside at all general and executive meetings or, in his absence, the Senior Vice-President or, in his absence, the Junior Vice-President or, in his absence, the Honorary Secretary or, in his absence, the Honorary Treasurer. Failing any of these, the members present will elect one of their number to preside as Chairman.

18. The conduct of all business transacted will be under the control of the Chairman, to whom all remarks must be addressed and whose ruling on a point of order, or on the admissibility of an explanation, will be final and will not be open to discussion at the meeting at which it is delivered. However, this rule will not preclude any member from raising any question upon the ruling of the chair by notice of motion.

19. In case of an equal division of votes, the Chairman of the meeting will have a second and casting vote.

20. All members on election will be supplied with a copy of this constitution.

21. No alteration will be made to these rules except by a two-thirds majority of those members voting at an annual general meeting of the Society, and then only if notice of

intention to alter the constitution concerned will have appeared in the notice convening the meeting. A quorum will constitute twenty per cent of members.

22. Any matter not provided for in this constitution will be dealt with at the discretion of the Executive Committee.

Laid down April, 1982
Revised March, 1985; April, 1988; November 1994, March 2014
Corrected January 1997; April 2002