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EPIDEMIOLOGICAL METHODS 1

BAYESIAN LOGISTIC REGRESSION MODEL OF RISK FACTORS FOR INTRAMAMMARY INFECTION IN DAIRY GOATS

G. KOOP*, N. TOFT, I. A. GARDNER, T. VAN WERVEN AND M. NIELEN

SUMMARY

Infection of the goat udder may be diagnosed by bacteriological culture or by somatic cell count (SCC) of a milk sample, but both tests are imperfect. The objective of this study was to evaluate risk factors for the true (latent) infection status, accounting for imperfect measurement of the outcome.

Milk samples were collected and udder halves were classified as positive or negative for both tests. In a Bayesian logistic regression model, the latent infection status was linked to the joint test results, as functions of test sensitivity and specificity. The latent infection status was the dependent variable in the logistic regression model with risk factors as independent variables and random herd and goat effects.

Higher parity and low milk yield were significantly related to higher odds of the latent IMI status. Significant controllable risk factors were an udder base below the hocks and abnormal teat shape.

INTRODUCTION

To improve udder health, it is essential to know which factors increase the risk of intramammary infection (IMI). In cows, several risk factors (RF) have been identified. Several authors have studied risk factors for IMI in goats, using either bacteriological culture (East et al., 1987; El Idrissi et al., 1994; Sánchez et al., 1999; Moroni et al., 2005), the California mastitis test (CMT) (Montaldo & Martínez-Lozano, 1993; 2008), or mastitis indicator paper (Megersa et al., 2010) to differentiate infected from non-infected udders. In these previous studies, no distinction was made between IMI caused by major pathogens and IMI caused by other pathogens. In goats, however, the majority of IMI is caused by minor pathogens, such as coagulase-negative staphylococci (CNS) (Bergonier et al., 2003; Contreras et al., 2003), which usually induce a small increase in somatic cell count (SCC), rarely are the cause of clinical mastitis, and have minimal effect on milk yield (Koop et al., 2010). Major pathogens, in goats, most importantly *Staphylococcus aureus* (Bergonier et al., 2003; Contreras et al., 2003), can cause clinical mastitis and in subclinical cases, reduced milk yield. Therefore, RF for IMI by major pathogens are more relevant than RF for IMI by minor pathogens.

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Bacteriological culture as well as CMT or SCC are imperfect tests for IMI (Erskine & Eberhart, 1988; McDougall et al., 2001; Sanford et al., 2006). For example, conventional bacteriological culture has almost perfect specificity (Sp) but low sensitivity (Se) for diagnosis of *S. aureus* infection. Somatic cell count on the other hand, is much more sensitive, but reportedly lacks specificity (Koop et al., 2011). Therefore, the effect of a RF will be underestimated in a study that uses bacteriological culture as a diagnostic test for *S. aureus* infection (Magder & Hughes, 1997). On the other hand, studies based on the number of somatic cells in the milk (CMT or SCC) may be biased in an unknown direction, because both Se and Sp are less than 1.

When Se and Sp of the diagnostic test are known, this knowledge can be incorporated in the logistic regression model (Magder & Hughes, 1997). Rather than treating Se and Sp as fixed values, the uncertainty in these estimates can be included in a Bayesian analysis in the form of prior distributions (McInturff et al., 2004). In a Bayesian environment, it is relatively easy to include results of more than one test that measures the outcome (dependent) variable, as was done in a latent class model described by Hui and Walter (1980). This would allow for posterior inference of the regression parameters and the Se and Sp of both tests. The model would thus estimate the effect of the covariates on the odds or probability of occurrence of the true (latent) condition as measured by both tests.

In this paper, we describe the incorporation of a latent IMI status, as defined jointly by BC and SCC results, into a logistic regression model, based on the latent IMI status defined, and we use this model to evaluate possible risk factors for the occurrence of IMI with major pathogens in dairy goats.

MATERIALS AND METHODS

Study design and data collection

A prospective longitudinal study of udder health in dairy goats was performed in The Netherlands in 2009 and controllable and uncontrollable possible risk factors were recorded.

Five commercial dairy goat farms were selected based on willingness of the farmer to participate, accuracy of animal identification and registration, and expected dates of kidding, as previously described (Koop et al., 2010) . On 4 farms, 100 does were selected and on one farm, 130 does were selected. At the latter farm, yearling does kidded later, so 100 goats of parity ≥2 were initially selected and 30 yearling does (23%) were added to the sample after kidding of this group. On all other farms, approximately 30% of the selected goats were yearling does, approximately 30% were about to start a second lactation and the rest were older animals. However, on one farm, no parity ≥3 animals were available, so on this farm, about 70% of the selected animals was of parity 2. All animals were of the Saanen breed or closely related to this breed. Udder half milk samples were collected aseptically on 3 occasions during lactation: the first was between 4 and 42 days in milk (DIM), the second was 6 weeks later (around peak lactation) and the third was scheduled within 2 months after introduction of the breeding bucks. This latter sampling time was not the same for all herds and varied between 24 and 36 weeks after the first sample. Milk samples were cooled with ice packs and immediately transported to the Milk Control Center Flanders Laboratory (Lier, Belgium) for bacteriological culture according to National Mastitis Council (NMC)

guidelines (Hogan et al., 1999) and for SCC measurement with a Fossomatic 5000 machine (Foss, Hillerød, Denmark), calibrated with cow milk.

Information on kidding dates and parity was retrieved from the farmers' management system. Milk yield was recorded through regular dairy herd improvement (DHI) sampling, which was done in the two days before sampling for bacteriological culture. Caprine arthritis-encephalitis virus (CAEV)-status was assumed to be negative for all herds. Four of the five herds were certified free from CAEV, whereas the fifth herd had no certificate, but clinical signs of CAEV had not been seen for years. Data on udder, teat and teat-end characteristics were recorded once when the animals were in mid to late lactation. These farm visits were done between two and 87 days before the third sampling. During these visits, teat ends of the enrolled goats were inspected visually during the morning milking, after removal of the milking cluster. We used the classification system that was developed by Neijenhuis et al. (2000) for cows. Teat end callosity thickness (TECT) was scored in five classes: none, slight, moderate, thick and extreme. Teat end callosity roughness (TECR) was scored as smooth or rough. Teat end shape (TES) was scored as round, flat, pointed or inverted. After scoring of the teat ends, a picture of the udder was taken. Based on these pictures, udders were scored on depth (udder base below the hocks or not), teat size (longer or shorter than a fists width), teat placement (vertical or outward) and teat shape (cylindrical, funnel, bottle or balloon shaped) according to De La Fuente et al. (1996) and Montaldo and Martínez-Lozano (1993).

The data were organized as two datasets: the first contained longitudinal data from 3 sampling occasions and included only lactation stage, parity, milk yield and pregnancy status as risk factors. The second dataset contained information from the third sampling occasion only, but included parity, milk yield, pregnancy status as well as teat, teat-end and udder conformation measurements as risk factors.

Interpretation of bacterial culture results

Positive culture was defined as growth of ≥1 colony of the same species. Growth of ≥ 3 types of bacteria in a sample was considered to be attributable to contamination and these results were excluded from analysis. Culture results were classified as a binary variable: culture of a major pathogen (*Staphylococcus aureus*, non-staphylococcus gram-positive cocci, gram negatives and yeasts) or no culture of a major pathogen (culture negative samples or samples infected with coagulase-negative staphylococci, *Corynebacterium bovis* and *Bacillus* species).

Statistical analysis

Risk factor model: The effect of the possible risk factors on the odds of IMI caused by a major pathogen was estimated with logistic regression models. Because bacteriological culture and SCC are both imperfect tests for diagnosing IMI with a major pathogen (Koop, 2011), a combination of culture and SCC was used as the dependent variable for the logistic regression model. This was done in a Bayesian setting using OpenBUGS, version 3.0.8 (Thomas et al., 2006).

The four possible outcomes of the joint test results of the ith udder half of the jth goat of the kth herd at the lth sampling (BC+SCC+, BC+SCC-, BC-SCC+, and BC-SCC-) were modelled as discrete distribution with four values and linked to the latent IMI status in terms of sensitivity and specificity:

$$joint_{ijkl} \sim Discrete(p.joint_{ijkl}), \quad (1)$$

$$p.joint_{ijkl}[BC+SCC+] = IMI_{ijkl} \times Se_{SCC} \times Se_{BC} + (1 - IMI_{ijkl}) \times (1 - Sp_{SCC}) \times (1 - Sp_{BC}) \quad (2)$$

$$p.joint_{ijkl}[BC+SCC-] = IMI_{ijkl} \times (1 - Se_{SCC}) \times Se_{BC} + (1 - IMI_{ijkl}) \times (Sp_{SCC}) \times (1 - Sp_{BC}) \quad (3)$$

etc.

$$logit(IMI_{ijkl}) = X_{ijkl} + \gamma_{jkl} + \delta_k, \quad (4)$$

where $joint_{ijkl}$ = joint test result (of BC and SCC) of the ith udder half of the jth goat of the kth herd at the lth sampling, SCC = somatic cell count as a binary variable with a cut-off of $2{,}000 \times 10^3$ cells/mL, BC = bacteriological culture as a binary variable (culture of a major pathogen or not), IMI_{ijkl} = true (but latent) infection status, Se = sensitivity, Sp = specificity, X_{ijkl} represents the effects of the independent variables, γ_{jkl} represents the random effect of the jth goat in the kth herd at the lth sampling and δ_k represents the random effect of the kth herd.

The estimated fixed effects (for example the regression parameter for parity 2) in the above model correspond to cluster specific estimates, which essentially model the effect of the RF within a goat within a herd, something which is meaningless for say, parity. Thus, more informative inference can be made from population average (PA) estimates, i.e. the effect of an RF between goats, but within the same herd. Population-averaged odds ratios of the fixed effects were calculated in OpenBUGS by first transforming the obtained regression coefficients (betas) to population-averaged parameters, using the formula from Dohoo et al. (2009):

$$\beta^{PA} = \beta^{CS} / (1 + 0.346\, \gamma^2)^{0.5}, \quad (5)$$

where PA = population average, CS = cluster specific, and γ^2 is the variance of the random goat effect, and then calculating the odds ratio:

$$OR = \exp(\beta^{PA}) \quad (6)$$

Model building process: Two models were built. For the first model, the longitudinal dataset was used, containing all records, but no data on teat, teat-end and udder conformation; for the second model, the second dataset was used, containing only the records from the third sampling, but including teat, teat-end and udder conformation data. The effect of sampling occasion (3 classes: first, second or third) was only evaluated in the first model. The effects of parity (3 classes: first, second, and third or greater), milk yield (3 classes: <2.5 kg/day, 2.5 to 4 kg/d or >4 kg/day) and pregnancy (2 classes: up to 1 month pregnant (including non-pregnant) and ≥2 months pregnant) were evaluated in both models. The other variables were only evaluated in the second model: udder depth (2 classes: udder base below hocks or not), teat place (2 classes: vertical or outward), teat size (2 classes: large or normal), teat shape (2

classes: cylindrical or other), TES (2 classes: round or other), TECT (2 classes: none or slight versus moderate, thick and extreme) and TECR (2 classes: rough or smooth).

For both models, the process was as follows: first, each RF was entered in the model separately, and significance of the variable was assessed based on the 95% posterior probability interval (PPI, the Bayesian analogue of a confidence interval). Forward selection was used to identify significant RF. Variables were added to the model one by one and kept in the model based on the PPI of the beta estimates. During the model building process, only records without missing values were used (2771 records for the first and 860 records second model). The final model for the first dataset without the teat, teat-end and udder conformation data was:

$$\text{logit}(IMI_{ijkl}) = PAR_{jk} + MY_{jkl} + \gamma_{jkl} + \delta_k, \quad (7)$$

where PAR represents parity and MY represents milk yield and the random effects as defined above. The final model for the second dataset, containing only records from the third sampling but including teat, teat-end and udder conformation data was:

$$\text{logit}(IMI_{ijkl}) = PAR_{jk} + MY_{jk} + UD_{jk} + TS_{ijk} + \gamma_{jk} + \delta_k, \quad (8)$$

where UD represents udder depth, TS represents teat shape and the other variables as defined above. The OpenBUGS code of the model corresponding to Eq. (8) is given in the Appendix.

We used non-informative prior distributions, i.e. Beta(1,1) for Se and Sp of SCC and BC, Uniform(-10,5) for the regression coefficients (betas), and Uniform(0,5) for the standard deviation of the random effects (sigma). The sensitivity of posterior estimates to the selected domain of the Uniform distributions was assessed by trial and error, while keeping the domains narrow enough to force convergence towards plausible posterior distributions.

The Markov chain Monte Carlo model was usually run for 20,000 iterations and the first 10,000 iterations were discarded as the burn-in phase. Longer runs were performed if convergence had not been reached. Three chains with different initial values were run simultaneously and convergence was assessed by visual inspection of the time-series plots of the parameters (Toft et al., 2007).

RESULTS

Descriptive statistics

Data from 35 animals was lost because the exact kidding date could not be retrieved or because animals were missed during sampling. The total number of animals in the datasets was therefore 495. The apparent udder half-level prevalence of IMI was 2.6% (73/2815) based on bacterial culture, and 12.8% (367/2875) based on SCC (Table 1). Descriptive statistics of the relationship between the risk factors and both the dependent variables, high SCC and infection with a major pathogen, are given in Table 2.

Table 1. Joint test results for culture of a major pathogen (BC) and for somatic cell count >2,000 × 10^3 cells/mL (SCC) per herd

HERD	BC+SCC+	BC+SCC-	BC-SCC+	BC-SCC-
1	19	3	84	440
2	1	2	17	421
3	12	3	126	551
4	10	9	32	514
5	0	0	55	495

Logistic regression models

In general, models converged quickly, but had high autocorrelation, which could be solved by thinning. The final models for both datasets were as given in Eq. (7) and Eq. (8). The estimated Se and Sp of both tests were approximately the same in the univariable models. The results of the models are shown in Table 3.

Table 3. Results from Bayesian logistic regression for infection with a major pathogen, estimating the effect (odds ratio) of several risk factors, and the sensitivity (Se) and specificity (Sp) of both bacteriological culture (BC) and somatic cell count (SCC)

VARIABLE		DATA SET			
		ONLY 3RD SAMPLING OCCASION		ALL 3 SAMPLING OCCASIONS	
		MEDIAN	95% PPI	MEDIAN	95% PPI
Parity	1	Reference category		Reference category	
	2	3.09	1.61 – 5.85	3.86	2.16 – 6.74
	≥3	5.08	2.70 – 9.29	5.87	3.08 – 10.51
Milk yield	<2.5 kg/d	Reference category		Reference category	
	2.5 – 4 kg/d	0.26	0.14 – 0.48	0.35	0.23 – 0.51
	>4 kg/d	0.24	0.10 – 0.53	0.28	0.17 – 0.46
Udder base	Above hocks	Reference category			
	Below hocks	3.14	1.41 – 6.10		
Teat shape	Cylindrical	Reference category			
	Other	2.23	1.04 – 4.75		
Se SCC		0.83	0.64 – 0.98	0.69	0.57 – 0.85
Se BC		0.12	0.07 – 0.19	0.14	0.10 – 0.18
Sp SCC		0.99	0.96 – 1.00	0.99	0.98 – 1.00
Sp BC		0.99	0.97 – 0.99	1.00	0.99 – 1.00

Table 2. Descriptive statistics of the relationship between risk factors and BC or SCC status

VARIABLE		BC POSITIVE FOR MAJOR PATHOGEN			SCC > 2,000 × 10^3		
		NO	YES	FRACTION	NO	YES	FRACTION
Sampling	1	943	20	0.02	854	123	0.13
	2	910	25	0.03	859	103	0.11
	3	889	28	0.03	795	141	0.15
Parity	1	856	16	0.02	859	37	0.04
	2	1,336	42	0.03	1,216	184	0.13
	≥3	550	15	0.03	433	146	0.25
Pregnancy	Up to 1 month	2,584	67	0.03	2,364	344	0.13
	≥ 2 months	158	6	0.04	144	23	0.14
Milk yield	<2.5 kg/d	580	22	0.04	472	140	0.23
	2.5 – 4 kg/d	1,323	33	0.02	1,255	137	0.10
	>4 kg/d	805	17	0.02	751	84	0.10
Udder base	Above hocks	764	23	0.03	697	109	0.14
	Below hocks	73	5	0.06	54	24	0.31
Teat place	Vertical	467	18	0.04	429	65	0.13
	Outward	370	10	0.03	322	68	0.17
Teat size	Normal	706	24	0.03	636	111	0.15
	Larger than fist	131	4	0.03	115	22	0.16
Teat shape	Cylindrical	761	23	0.03	689	113	0.14
	Other	76	5	0.06	62	20	0.24
Teat-end	Round	759	26	0.03	677	125	0.16
Shape	Other	108	2	0.02	100	12	0.11
TECT[1]	None or slight	775	27	0.03	693	126	0.15
	Moderate, thick or extreme	92	1	0.01	84	11	0.12
TECR[2]	Smooth	580	19	0.03	535	82	0.13
	Rough	287	9	0.03	242	55	0.19

[1]TECT = teat-end callosity thickness
[2]TECR = teat-end callosity roughness

DISCUSSION

In the present study, a Bayesian logistic regression model was used to evaluate possible risk factors for the true (latent) IMI status in dairy goats, as evaluated by 2 imperfect tests (bacteriological culture and SCC). Higher parity and lower milk yield were significantly associated with greater odds of infection. Also an udder base below the hocks and abnormal teat shape were significantly associated with greater odds of infection. The latter risk factors are of practical importance because they can, to some extent, be controlled by management.

Parity was strongly related to increased odds of IMI, which is consistent with previous research findings. Several authors reported higher prevalence for higher parity goats (Boscos

et al., 1996; Sánchez et al., 1999; Ndegwa et al., 2000; Moroni et al., 2005). Moroni et al. (2005) hypothesized that the greater prevalence in higher parity animals was partially caused by chronic infections from the previous lactation that were not eliminated in the dry period. This is consistent with findings of Leitner et al. (2007) who described a low cure rate during the dry period, and also new infections of previously uninfected udder halves during the dry period. The aforementioned studies evaluated IMI with any pathogen, which was in contrast to our study which focused specifically on major pathogens. Most IMI in goats are caused by CNS, whereas *S. aureus* is probably the most important major pathogen (Contreras et al., 2003). The correspondence between our and prior studies may be explained by the fact that the pathogens under study were mostly staphylococci in both cases, which are all capable of producing persistent infections (Koop et al., unpublished data). The number of culture-positive udder halves and SCC typically increase with parity (Rota et al., 1993; Luengo et al., 2004; Paape et al., 2007). This is partially attributable to the increasing prevalence, but increasing SCC has also been reported in culture-negative goats, although this increase was much weaker than the effect of infection with a major pathogen (Luengo et al., 2004). We speculate that part of the increase in SCC in uninfected goats is explained by the low Se of bacteriological culture and that therefore some infected goats had been misclassified as uninfected goats in prior studies. In our study, IMI status was measured with both SCC and bacteriological culture and thus corrected for the imperfect measurement of IMI with culture.

Higher odds of infection was found for the late lactation sampling, but the beta estimate was not statistically significantly different from zero. Days in milk has a strong effect on SCC in goats through the effect of milk yield (Koop et al., 2010). Higher milk yield corresponds to lower SCC and vice versa. Therefore, the effect of milk yield was also included in the model, but no clear confounding effect was evident. El Idrissi et al. (1994) reported a significant effect of month of lactation on odds of infection, but did not specify the direction of this effect. Moroni et al. (2005) and Ndegwa et al. (2000) reported higher prevalences in late-lactation goats, in accordance with our findings. A low self cure-rate combined with new infections during lactation may explain this finding. Leitner et al. (2007) reported no cure of *S. aureus*-infected udder halves during lactation, but also no new infections. However, the majority of the goats in that study were at least 100 days in milk when first sampled. East et al. (1987) found a higher probability of infection in the first and last third of a standard 305-day lactation in Californian dairy goats. The higher risk of infection in early-lactation was explained by the occurrence of new infections during the dry period, as was also shown by Leitner et al. (2007), and the higher risk in late-lactation was attributed to overmilking, because of decreased milk yields in late lactation. Overmilking has been associated with poorer udder health in cows (Tamburini et al., 2010).

Milk yield was included in the models as a possible confounder of the relationship between stage of lactation and SCC, because of a hypothesized dilution effect (Koop et al., 2010). The regression coefficient (beta) for higher milk yield was negative. To our knowledge, no previous reports of the relationship between milk yield and risk for IMI in goats have been published, but in cows, Gröhn et al. (1995) reported a positive association between previous milk yield and risk for mastitis. In our study, however, previous milk yield was not included in the models, but milk yield was recorded at the same time as infection status, and therefore no causal relationship should be inferred. Lower milk yield probably does not increase the chance of udder infection and perhaps it is more plausible to interpret the observed negative association as the joint contribution of the abovementioned dilution effect and a milk yield loss resulting from IMI. The difference in milk yield between bacteriological culture-positive and culture-negative udder halves was smaller than the

difference between udder halves with SCC > 2,000 × 10^3 cells/mL and lower SCC (data not shown), indicating that the effect of dilution may be more important than the effect of milk yield loss.

In the final model, an udder base below the hocks was a significant controllable risk factor for IMI. This is in accord with the findings of Ameh and Tari (2000) who showed that prevalence of mastitis in goats increased (not significant), with decreasing distance between teat end and floor. Bhutto et al. (2010) reported more frequent isolation of *Strep. agalactiae* and *Strep. dysgalactiae* in cows with large pendulous udders. In their review, Seykora and McDaniel (1985) wrote that most studies support the conclusion that cows with higher, non-pendulous udders are more resistant to mastitis. More pendulous udders likely are in closer contact with the floor and the bedding and may therefore be exposed to more pathogens. Also, pendulous udders may be more easily damaged and thus be more susceptible to infection. In ewes, deeper and more pendulous udders were more often seen in older animals (De La Fuente et al., 1996), as was the case in our study (data not shown). However, inclusion of parity in the model did not have a strong effect on the estimate for udder depth. Abnormal teat shape was also significantly related to IMI. This is in agreement with Montaldo and Martínez-Lozano (1993), who found lower California mastitis test values for goats with non-balloon-shaped teats than goats with balloon-shaped teats. Udder conformation is heritable in cows (Seykora & McDaniel, 1985). If this is also the case in goats, selection may be a useful tool to improve mastitis resistance.

The combination of a logistic regression model with a latent class model in a Bayesian context allowed us to deal with imperfect measurement of IMI, the dependent variable. The use of SCC as a diagnostic test for IMI with a major pathogen in a latent class model has some potential problems, as discussed previously (Koop et al., 2011). Somatic cell count measures the immune response to the infection and is an indirect estimator of the disease of interest. Furthermore, it can be affected by factors other than IMI, such as parity and stage of lactation. The effect of these factors is generally not as strong as the effect of infection with a major pathogen (Paape et al., 2007; Koop et al., 2010), but still may have contributed to the relationships that were found in this study. Therefore, part of the effect of the RF that was seen may have resulted from the effect of these RF on SCC and not on the odds of infection.

The Se and Sp of both tests used in this study were comparable to those reported previously (Koop et al., 2011). In earlier work, we used a composite SCC threshold of 1,500 × 10^3 cells/mL versus udder-half SCC at a cut-off value of 2,000 × 10^3 cells/mL in the present study. In the model building process, we observed that when only the random effects were included in the model, the estimated test characteristics were quite similar to what we found when the same data were used in an latent class model without a logistic regression equation. With the inclusion of more regression parameters, the estimates for sensitivity of both culture and SCC decreased somewhat. This indicates that the estimation of test characteristics is influenced by covariates in the regression model. A study with simulated datasets may provide an explanation for this apparent effect.

In the present study, an udder base below the hocks and abnormal teat shape, which are controllable risk factors, were associated with infection of the mammary gland with a major pathogen. Non-controllable risk factors were parity and milk yield.

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APPENDIX

OpenBUGS code of the Bayesian logistic regression model:

```
model{

for(i in 1:860){

joint[i] ~ dcat(p.joint[i,])

p.joint[i,1] <- se[1]*se[2]*p[i] + (1-sp[1])*(1-sp[2])*(1-p[i]);

p.joint[i,3] <- se[1]*(1-se[2])*p[i] + (1-sp[1])*(sp[2])*(1-p[i]);

p.joint[i,2] <- (1-se[1])*(se[2])*p[i] + (sp[1])*(1-sp[2])*(1-p[i]);

p.joint[i,4] <- (1-se[1])*(1-se[2])*p[i] + (sp[1])*(sp[2])*(1-p[i]);

logit(p[i]) <- delta[herd[i]] + gamma[goat[i]] + beta[1] + beta[2] * par2[i] + beta[3] * par3[i] + beta[4] * my2[i] + beta[5] * my3[i] + beta[6] * ud[i] + beta[7] * ts[i]

}

 for(i in ngoats){gamma[i] ~ dnorm(0,tau[1])}

for(i in 1:nherds){delta[i] ~dnorm(0,tau[2])}

# beta priors

for (i in 1:7){

beta[i]~ dunif(-10,5)

}

#odds ratios

or[1]<-exp(beta[2]/pow((1+0.346*pow(sigma[1],2)),0.5))

or[2]<-exp(beta[3]/pow((1+0.346*pow(sigma[1],2)),0.5))

or[3]<-exp(beta[4]/pow((1+0.346*pow(sigma[1],2)),0.5))

or[4]<-exp(beta[5]/pow((1+0.346*pow(sigma[1],2)),0.5))

or[5]<-exp(beta[6]/pow((1+0.346*pow(sigma[1],2)),0.5))

or[6]<-exp(beta[7]/pow((1+0.346*pow(sigma[1],2)),0.5))
```

```
# test priors and sigmas

for (i in 1:2){

se[i] ~dbeta(1,1)

sp[i] ~dbeta(1,1)

sigma[i] ~ dunif(0,5)

tau[i] <- 1/pow(sigma[i],2)

}

}
```

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A GENETIC AND EPIDEMIOLOGICAL MODEL OF FOOTROT IN A SHEEP FLOCK.

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SUMMARY

A stochastic, individual-based, simulation model of footrot in a flock of 200 ewes was developed that included flock demography, disease processes, host genetic variation for traits influencing infection and disease processes, and bacterial contamination of the environment. Sensitivity analyses were performed using ANOVA to examine the contribution of unknown parameters to outcome variation. The infection rate and bacterial death rate were the most significant factors determining the observed prevalence of footrot, as well as the heritability of resistance. The dominance of infection parameters in determining outcomes implies that observational data cannot be used to accurately estimate the strength of genetic control of underlying traits describing the infection process, i.e. resistance. Further work will allow us to address the potential for genetic selection to control ovine footrot.

INTRODUCTION

Footrot is an infectious bacterial disease of sheep in which infection is transmitted between animals via contaminated pasture (Beveridge, 1941). Clinical signs include lameness and foot lesions which start in the interdigital space and can progress to cause separation of the hoof horn from the sensitive dermis (Beveridge, 1941). The disease is common, with a prevalence of 8 – 10% in England (Kaler and Green, 2009), detrimental to production (Wassink *et al*, 2010) and reduces both animal health and welfare (Fitzpatrick *et al*, 2006). Footrot has been estimated to cost the GB sheep industry approximately £24.4 million per year (Nieuwhof & Bishop, 2005), and in one survey sheep farmers rated it as the second highest threat to animal health and welfare, after only sheep scab (Morgan-Davies *et al*, 2006).

Field and experimental data suggest that susceptibility to footrot is partly under genetic control. A number of studies have estimated heritability of footrot severity and associated lameness, but only on data sets with short time scales or with limited observations (e.g. Skerman *et al.*, 1988 (New Zealand); Raadsma *et al.,* 1994 (Australia); Nieuwhof *et al*, 2008 (UK)). In New Zealand, there has been some success with breeding footrot resistance into Broomfield Corriedale sheep; selection for footrot resistance for 15 years resulted in greater resistance to clinical footrot than other breeds when introduced to contaminated pasture in field trials (Skerman & Moorhouse, 1987). In Australia, selective breeding has also been successfully used to reduce the prevalence of footrot (Mitchell, 2001; Egerton *et al*., 2004).

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Hence it may be possible, in principle, to use breeding programmes to reduce disease prevalence / incidence in the UK (Conington *et al.,* 2008). However, the climatic differences between Australia and New Zealand and the UK, where long, hot summers free from transmission do not occur, could mean that breeding for resistance requires a different approach in the UK.

Heritability estimates can be made using pedigree and phenotype data, however true underlying heritabilities for traits describing resistance to infectious disease can be difficult to estimate from field data because the data are confounded by environmental factors, such as exposure rates, disease dynamics and diagnostic ability (Bishop & Woolliams, 2010). Heritability estimates made when disease prevalence is low often differ from estimates made when disease prevalence is high (Nieuwhof *et al*, 2008) because expression of genetic differences between hosts is exposure dependent. Further, when prevalence is very low there are often scale effects, i.e. very few observed infections, limiting the phenotypic variation seen between individuals and thus ability to identify genetic effects.

To fully understand endemic diseases such as footrot, and work towards long term solutions for control, genetics, epidemiology and their interaction must be considered in detail and simultaneously. Modelling has been used in a limited way to explore the potential for a reduction in footrot prevalence, particularly in the deterministic mode of footrot produced by Nieuwhof *et al.* (2009). However, the complex nature of the disease has not yet been fully addressed in a simulation model.

In this study, a stochastic, individual-based, genetic-epidemiological model of footrot was developed that included sheep demography, individual host genetic effects and full flock life cycles. In this paper the model structure, assumptions and processes are presented, along with the results of a sensitivity analysis exploring the significance of variation in parameters whose values are unknown. The outcome of interest was the variation in disease patterns, not the mean value. This is important because it is variation between sheep that is the material observation for determining if genetic influences are important, and for selecting sheep for resistance.

MATERIALS AND METHODS

Population and demography

The model assumes continuous time and the rate of events stochastically determines the timing of infection/disease events; demographic events (birth and culling) occur at set times.

Each sheep in the model can be uniquely identified. Data recorded for each sheep include genetic parameters which are set at birth dependent on parents' genotypes (Table 1), current status (e.g. disease state and age) and disease history. Animal phenotype and genotype definitions are given below.

Rams do not participate in any disease events and only identification numbers and genetic information used to calculate genetic values for their lambs are recorded.

Table 1. Sheep information set at birth

Field name	Description
IDNum	Unique individual ID number
YearOfBirth	Year in which sheep was born
Mother	Dam ID number
Sire	Sire ID number
Female	0/1
Susceptibility	Applied susceptibility phenotype (≥0)
TrueSus	True susceptibility phenotype (may be <0)
GTSus	Genetic term for susceptibility
Recoverability	Applied recoverability phenotype (≥0)
TrueRec	True recoverability phenotype (may be <0)
GTRec	Genetic term for recoverability
Revertability	Applied revertability phenotype (≥0)
TrueRev	True revertability phenotype (may be <0)
GTRev	Genetic term for revertability

A base population of 200 ewes is simulated, with female lambs kept each year as replacements. The number of lambs born to each ewe is sampled from a Poisson distribution with mean 1.5, with a maximum number of lambs set at three. All lambs are assumed to be born on 1st March and on this date the population also ages by one year. Five different rams are used for breeding each year and each lamb is randomly allocated one of these rams as its sire. Any female lambs not required to maintain the population, all male lambs, and ewes aged 5 are removed on 1st September.

Disease states and parameters controlling transitions between states

The footrot infection process is modelled as shown in Figure 1. Only sheep in the infected states, i.e. those with visible clinical signs, are infectious and contribute to bacterial load in the environment. Mild and severe infection states may be considered to represent interdigital dermatitis and footrot respectively and sheep in both states contribute equally to bacterial contamination of the environment. Latently infected sheep represent the time between infection and the appearance of clinical signs. Following periods completely free from disease in a flock, disease may still recur (Egerton *et al.,* 2002; Abbott & Egerton, 2008), suggesting a role for carrier sheep. Transitions between states are driven by the rates given in Table 2.

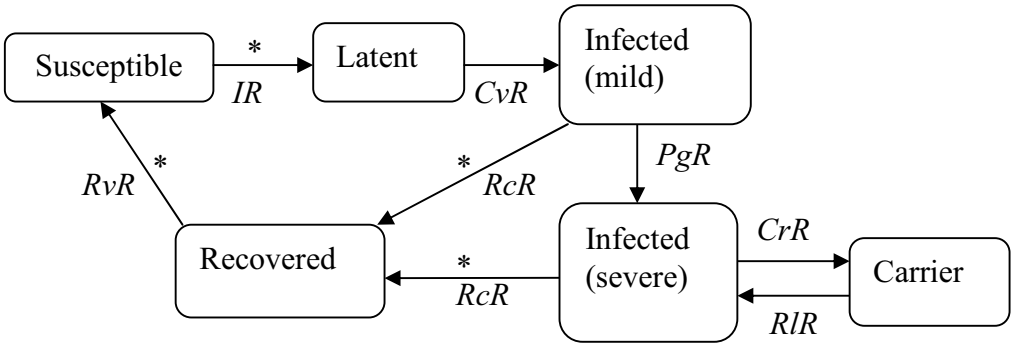

Figure 1. Footrot infection states. Transition rates are symbolised according to Table 2. Transitions influenced by host genotypes marked by *.

Table 2. Parameter values in the model. (FR = clinical infection)

Parameter	Definition	Source and notes	Base value	Variation in sensitivity analysis
IR	Infection rate	Unknown – tested on sample model runs to determine values for base and variations.	15×10^{-7}	5,7.5, 10, 15, 25 and 50 x 10^{-7}
CvR	Rate of conversion from latent to FR	Egerton, Roberts, Parsonson, 1969a, 1969b	0.14 (average duration 1 week)	constant
PgR	Rate of progression from mild to severe FR	Beveridge 1941 (inferred – sheep recover from mild infection after approx. 2 weeks so if not recovered by this point hypothesise that animals are likely to progress)	0.07 (average duration 2 weeks)	constant
RcR	Rate of conversion from FR to recovered	Beveridge 1941 Roberts, Egerton, Parsonson 1969a, 1969b Hawker, 2007	0.033 (average duration 4 weeks)	constant
RvR	Rate of reversion from recovered to susceptible	Egerton, Roberts 1971	0.029 (average duration 5 weeks)	constant
CrR	Rate of transition from FR to carrier	Treating this as the same as *RcR* – sheep hypothesised to recover as normal but harbour pockets of infection inside the hoof, becoming carriers instead of recovered sheep.	0.033 (average duration 4 weeks)	triangular distribution 0.01-0.07, peak at 0.03
RlR	Rate of conversion from carrier to FR	Base value set to be equal to *RvR*, however there is no explicit measurement of this parameter.	0.029 (average duration 5 weeks)	triangular distribution 0.01-0.07, peak at 0.03
ELR	Death rate of bacteria in environment	Beveridge 1941 There is evidence that this rate varies by environment.	0.14 (average duration 1 week)	triangular distribution 0.05 – 0.5, peak at 0.14
SR	Rate of shedding of bacteria from infected sheep	Unknown – using undefined units to include shedding processes but not representative of true bacterial load.	1	Constant
h^2	True heritability for genetically influenced traits	Unknown. The observed heritability for footrot occurrence is ~20%, suggesting that the true heritabilities of underlying traits are likely to be higher.	0.5	triangular distribution 0-1, peak at 0.5
σ^2	Variance of underlying genetic traits	Unknown.	0.1	uniform distribution 0.01-0.50

Host genetics

State transitions are also affected by individual host genotypes. Each sheep has three unique phenotypes, under partial genetic control, that affect its likelihood of becoming infected (susceptibility), recovering from infection (recoverability) and becoming susceptible again from an immune state (revertability). The transitions driven by these traits are marked in Figure 1.

All traits with a genetic component are assumed to be polygenic, i.e. affected by variants at many genes, and under partial genetic control. Under this situation, we may assume the central limit theorem, and sample animal genotypes from a normal distribution, the variance of which is a function of the trait variance and heritability.

For each trait the phenotype, *P,* for each sheep, *i*, may be defined as comprising the following components:

$$\boldsymbol{P_i = \mu + g_i + e_i} \quad (1)$$

where μ is the trait mean in an unselected population, g_i is the genetic component (expressed as a deviation from 0) and e_i is the residual component (expressed as a deviation from 0), which is also assumed to be normally distributed.

The variance of the P_i is the phenotypic variance, denoted by σ^2_P and the variance of the g_i is $\sigma^2_A=h^2\sigma^2_P$, where h^2 is the trait heritability. Assuming that g_i and e_i, are uncorrelated, then the variance of the residuals is $\sigma^2_e=(1-h^2)\sigma^2_P$.

The simulation procedure was as follows. The population comprises **founder** animals, i.e. those without recorded or known parents, and **progeny** whose parents are known. Each **founder** animal had a genotype, or breeding value, g_i, for each genetically controlled input trait randomly sampled from a normal distribution, $N(0, \sigma^2_A)$, where σ^2_A is estimated as defined above. The breeding values for each trait for each **progeny** were constructed as $(g_{sire}+g_{dam})/2$ plus a Mendelian sampling term. This term accounts for recombination events at meioses and it was randomly sampled from a $N(0, 0.5\sigma^2_A)$ distribution (Falconer and Mackay, 1996). The residual for each trait for each animal was sampled from $N(0, \sigma^2_e)$. The phenotype for each animal was then calculated from Eq. 1 being simply the sum of the trait mean, the breeding value and the residual term. All phenotypes for the traits considered should be positive values; on the few occasions when a negative value was obtained, it was set to zero.

Bacteria in the Environment

Bacteria are transmitted between sheep via contaminated pasture and the model includes two parameters to account for this. *ELR* determines the rate at which bacteria are lost from pasture as a result of bacterial death. *SR* determines the amount of contamination being added to pasture as a result of shedding from a single infected sheep. *SR* is multiplied by the number of currently infected sheep to determine the rate of contamination.

Sensitivity analysis

The model was run for 50 years with base parameter values (Table 2) to obtain a population at equilibrium and this population was used as the input for each run of the model in the sensitivity analysis. Sensitivity analysis was performed using ANOVA (Saltelli *et al.,* 2008) to examine the contribution to variance of outcomes for the non-constant parameters in Table 2.

Four areas (represented by 6 parameters) have been identified where little or no experimental data are available and these were examined in the sensitivity analysis. These four areas are:

1. Survival time of (viable) bacteria in the environment - *ELR*.
2. Carrier sheep properties - *CrR* and *RlR* determine the likelihood of sheep becoming carriers (no clinical signs and no bacterial shedding) and the rate at which they revert to an infectious state with clinical signs.
3. Host genetics - h^2 and σ^2 determine the proportion of phenotype determined by additive genetic effects and the variance of the trait of interest.
4. Infection rate - *IR* determines the probability of a susceptible sheep becoming infected.

Distributions of parameter values (Table 2) were divided into five sections of equal probability and the mid-point value of each section was calculated. Using Latin hypercube sampling these sections were sampled without replacement to give five combinations of five parameters (one lhs set) (Helton & Davis, 2003). This was repeated four more times to give five lhs sets – a total of 25 parameter combinations. Each of these 25 parameter combinations was run with *IR* values of 5, 7.5, 10, 15, 25 and 50 x 10^{-7}, a total of 150 simulations. Each sensitivity analysis model was run with a simulated real time of 20 years, with the first ten years' data discarded to allow the system time to approach equilibrium following the change in parameters from base values.

ANOVA was used to analyse the resulting output data of the model: Disease outcomes i.e. total number of new infections in year 20 (*numinf*), total number of lame days (mild or severe footrot) in year 20 (*tld*), and genetic outcomes i.e. heritability of number of episodes of lameness (*hepy*) in lambs (years 11-20) and heritability of number of lame days (*hldpy*) in lambs (years 11-20). For disease outcomes (*numinf* and *tld*) data from the final year (year 20) were used and for genetic outcomes (*hepy* and *hldpy*) data from the final ten years were evaluated. Sheep were defined as lambs for the first ten months of their life – until the end of the calendar year in which they were born.

ANOVA models were of the following form including factors related to persistence (*ELR*, *RlR*), host genetics (h^2, σ^2), and infection processes (*CrR*,*IR*):

$$Y = ELR + RlR + h^2 + \sigma^2 + CrR + IR + E \quad (2)$$

where *E* is the residual or error term and the other factors are input parameters as described in Table 2.

Observed heritability within each individual simulation was also calculated using results from an ANOVA model of the following form:

$$Y = sire + dam + E \quad (3)$$

where *sire* and *dam* are the two parents and *E* is the residual or error term.

Observed heritability was then calculated as:

$$Heritability = 2(Vsire + Vdam) / V_P \quad (4)$$

where *Vsire* and *Vdam* are the sire and dam variances from the ANOVA, and V_P is the total observed (phenotypic) variance, i.e. *Vsire + Vdam + residual variance*.

All model simulations were programmed in MatLab R2008b Student and ANOVA models were performed using constrained (Type III) sums of squares.

RESULTS

To illustrate the types of outputs obtained and the variability between simulations, results from five model runs with base parameters (see Table 2) are shown in Table 3.

In the sensitivity analysis, the number of new infections in year 20 with *IR* = 0.000005, ranged from 931 to 1340 (mean: 1145.80, or 3.14 new infections per day), and with *IR* = 0.0000025 from 739 to 1270 (mean: 1027.72, or 2.82 new infections per day). All other *IR* values resulted in some runs where no new infections occurred and no lame days were observed in year 20. This occurred in 23 out of 25 runs with *IR* = 0.0000005, 17/25 model runs with *IR* = 00000075, 11/25 with *IR = 0.000001* and 5/25 with *IR* = 0.0000015.

Table 3. Outcomes from five runs of the model with base parameters

Outcome	**Mean**	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**
Total lame days Yr20 (*tld*)	24109	24564	24372	22986	21989	26637
Mean lame days per infected sheep Yr20	74.3	78.0	75.2	74.4	68.2	75.5
Total new infections Yr20 (*numinf*)	1690	1635	1685	1739	1573	1820
Mean episodes per infected sheep Yr20	5.2	5.2	5.2	5.6	4.9	5.2
Heritability of episodes of lameness (*hepy*)	0.204	0.234	0.162	0.253	0.185	0.185
Heritability of number of lame days (*hldpy*)	0.246	0.269	0.195	0.296	0.199	0.270
Prevalence 1st Jan (%)	21.9	23.0	23.5	18.5	23.5	21.0
Prevalence 1st Apr (%)	36.9	43.0	41.1	34.4	26.1	39.8
Prevalence 1st Jul (%)	32.2	34.0	33.9	28.0	30.1	34.9
Prevalence 1st Oct (%)	24.1	22.5	24.0	25.0	20.0	29.0

Observed heritability for the number of lameness episodes per year ranged from 0.014 to 1 (mean 0.25 excluding runs where heritability was non-estimable), and for the number of days spent lame from 0.04 to 0.74 (mean 0.28 excluding runs where heritability was non-estimable).

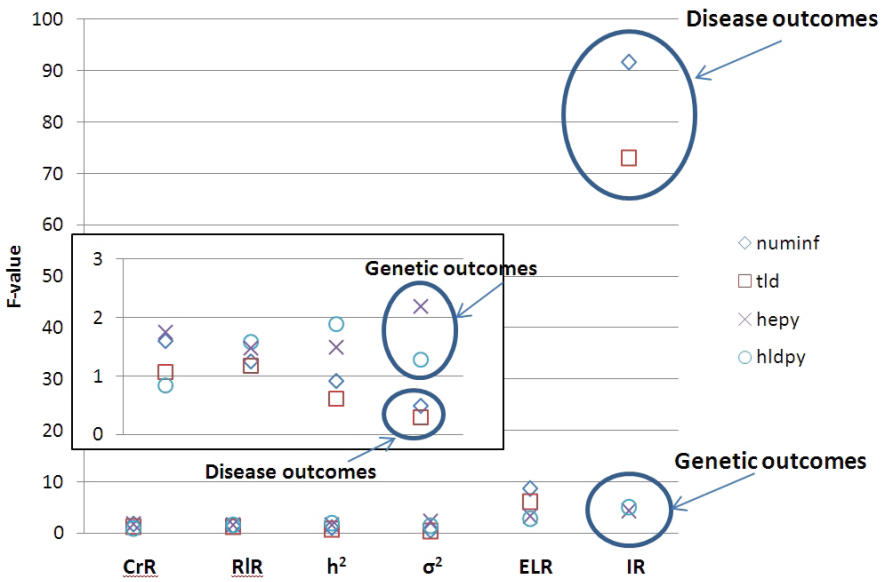

Fig. 2. Influence of variation in input parameters on disease (*numinf* & *tld*) and genetic (*hepy* & *hldpy*) outcomes, assessed by ANOVA F-values (inset - close up on persistence/genetic factors). Factors: Persistence *CrR* & *RlR*; Host genetics h^2 & σ^2; Infection process *IR* & *ElR*.

In all ANOVA models, *IR* was a significant factor ($p<0.01$). *ELR* was significant with $p<0.05$ for *hepy* and *hldpy* and $p<0.01$ for all other outcome variables. No other factors were significant in any model. Disease outcomes varied mainly due to differences in infection process parameters while variation in these parameters contributed only a small amount to variation in heritability estimates. Variation in host genetic parameters influenced genetic outcomes more than disease outcomes. Genetic and disease outcomes form distinct clusters when examining factors and their influence on outcomes, as assessed by F-values (Figure 2). Persistence processes did not significantly affect any outcomes.

DISCUSSION

A stochastic, individual-based model was constructed to simulate the epidemiology of footrot in a sheep flock which included genetic (heritable) processes. The model includes four core areas that contribute to disease presentation and spread – population dynamics, host genetics, transmission of infection and bacterial dynamics in the environment. Footrot is a complex disease and there are still many unknown variables that contribute to its epidemiology. The aims in producing this model were to investigate the interactions between genetics and epidemiology in the presentation of footrot in sheep flocks, and to determine the influence of parameters for which data are not available.

Prevalence seen in the model is higher than seen in field data but this may be explained by the fact that no treatment or control measures are currently included in the model. Peaks in prevalence occur following the birth of lambs, which suggests that at least some of the seasonal dynamics seen in footrot may be caused by increases in the susceptible population and density of sheep when lambs are born. The prevalence of infection increases

asymptotically with increasing *IR* (data not shown), so that the three lowest values used in the sensitivity analysis will create lower flock prevalences.

During the development of this model four areas were identified where parameter values are not available (carrier sheep, survival time of viable bacteria in the environment, host genetics and infection rate). Distributions of probable values were assigned to parameters controlling these four areas and used to perform sensitivity analysis. There is a possibility, because only the most probable values are represented in the model, that the true values lie outwith those used in the sensitivity analysis. However, the values have been assigned using, where possible, data from published sources (Table 2) or estimates from field data (Wassink *et al.,* 2010), and it is likely that the true values lie within the ranges used.

The model is stochastic so it would be desirable to obtain many replications of the model with a wide range of parameter values to account for both fluctuations between runs and the wide range of possible values for unknown parameters. ANOVA was used to compensate for the limited range of values used, and in this approach the model stochasticity is contained in the residual variance. This approach reduces the need for multiple runs because effects of all parameters are considered at the same time instead of individually, whilst still accounting for variation in all parameters (Saltelli *et al,* 2008).

Disease outcomes are more sensitive than estimated heritabilities to variation in input parameters, and estimated heritability values are affected more by infection rate and bacterial survival time ($p<0.05$) than by variation in true heritability and genetic variance. This suggests that the infection rate and the death rate of the bacteria not only combine to drive the system but they also effectively mask the genetic components it is desirable to measure. The dominance of infection parameters in determining outcomes means that it may be difficult to use observed outcomes from field data to infer the strength of genetic control of underlying traits describing the infection process i.e. resistance.

One of the aims in constructing this model was to investigate the use of selection strategies to reduce the incidence and prevalence of footrot. However, currently the measure on which such a selection scheme would be based is the observed heritability (e.g. Nieuwhof *et al*, 2008; Conington *et al,* 2008) and our current results suggest this to be largely inaccurate in representing the true heritability. It should be noted however that the method used to calculate heritability in each individual simulation was simplified due to software constraints (accounting only for sire and dam variance components, without other factors or repeated measures) and it may be that using a fuller mixed statistical model would give results that more accurately mirror the underlying heritability values. We are pursuing this.

Under certain model conditions heritabilities close to unity were observed, which would suggest that in the right conditions selection could be extremely efficient. However, under other conditions heritability was very low or not possible to estimate which would make selection difficult. With field data it may be hard to infer the true strength of genetic effects as heritability estimates can vary greatly with disease prevalence, but it may still be possible to determine the sheep that show the optimal reaction to bacterial exposure, i.e. those with a high resistance phenotype. If correlations between ranked estimated breeding values calculated under different infection pressures are high then highly resistant sheep may be identified, however if animals' ranks are very different with different infection rates then genotype by environment interactions would need to be considered. This would make selection more difficult.

Infection rate and death rate of the bacteria in the environment are clearly important values because they control a large proportion of the variance seen in disease outcomes. However, these are both difficult to estimate from field data, and will likely vary significantly over time and space. With respect to the bacterial death rate, soil must be examined for live bacteria and both survival and viability must be considered. In order to get values for this parameter it would be necessary to conduct infection trials to determine for how long the bacteria remained capable of causing new infections when transmitted in a natural way through contact with infected pasture, continuing the work by Whittington (2008). Infection rate is also difficult to determine experimentally because bacteria are not transmitted directly between sheep but via contaminated pasture. This raises further questions including whether all sheep have been equally exposed to bacteria, what area of the pasture is contaminated, what the infectious dose is and which sheep are susceptible at any one time, all of which would require highly controlled conditions to answer with any accuracy.

These results indicate that the infection rate and the rate at which bacteria die in the environment are the most significant factors in the incidence and prevalence of footrot seen in sheep flocks. They also suggest that for footrot, and perhaps for other similar persistent, infections with environment and host reservoirs, the observed/estimated heritability is not a reliable measure of the extent of genetic control of the underlying resistance traits. Further work will allow us to address the potential for genetic selection under these circumstances and how this potential can be assessed in the short term.

ACKNOWLEDGEMENTS

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CONTROL EVALUATION

FISH FARM SIZE AND SURROUNDING WATER CURRENT SPEEDS DICTATE THE SEPARATION DISTANCE REQUIRED TO AVOID TRANSMISSION OF DISEASE AGENTS BETWEEN PRODUCTION SITES.

N.K.G. SALAMA* AND A.G. MURRAY

SUMMARY

Scotland is the largest Atlantic salmon (*Salmo salar*) producer in the EU with an output of over 150,000 t, contributing £500 million annually towards the economy. Production continues to increase across Scotland, predominantly due to the increase in output per farm and reduction in losses to infectious diseases. Farms are grouped within disease management areas (DMAs) whose boundaries are defined as being where the closest pair of farms is separated by more than twice the tidal excursion distance (TE) (TE is defined as 7.2 km, or 3.6 km in Shetland). The majority of salmon farms are located within relatively sheltered inshore areas where non-tidal advective current speed is minimal. However there is an aspiration for offshore production where current speeds will be greater and so TE models may break down, furthermore it may be possible to increase stocking levels. It has previously been demonstrated that the number of stocked fish alters the disease dynamics within a farm by altering transmission. However, an assessment has not been undertaken to assess how farm size impacts on the transmission of pathogenic agents between farms.

A discrete-time *SEIR* model was developed representing fish farms. The model incorporates transmission, expression and recovery parameters as well as pathogen shedding and decay. An expression is derived representing the time required for shed particles to decay below the minimum infective dose. The critical time expression is used to assess the distance travelled by a cohort of pathogen particles transmitted by a simplified hydrodynamic that incorporates residual advection, tidal advection and turbulent diffusion elements.

Applying characteristics for a robust pathogen, infectious pancreatic necrosis virus type (IPNVt), and less robust pathogens such as infectious salmon anaemia virus type (ISAVt), and *Aeromonas salmonicida* type (ASt) pathogens it is possible to obtain separation distances whereby farms avoid infection. Simulation outputs indicate that separation distances increase to avoid disease as farm size and current speed increase. The more conserved IPNVt pathogen requires separation distances of hundreds of km, ASt tens of km, whilst the distances for ISAVt are within the scale of the current DMAs, which were developed for ISAV control. However, should production be moved in to areas of faster moving currents and increased farm production the current disease management area principles may need readdressing.

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Should a farm be located where it is exposed to a dose above the minimum threshold, the farm is infected with a response proportional to the minimum infective dose per individual unit in the farm. This is used to test the response of allocating production to many small farms, clustered together compared to few larger farms spread apart. Although larger farms require greater separation distances to avoid infection, they decrease the level of infection across the production system compared to many smaller farms located closer together. Furthermore, larger farms might have improved biosecurity measures and disease detection.

INTRODUCTION

Farmed Atlantic salmon (*Salmo salar*) is Scotland's largest food export, contributing an estimated £500 million (SSPO, 2010) annually towards the economy making it the largest salmon aquaculture producer in the EU. Since the late 1980s production has increased from 30,000 t to an estimated total production of over 150,000 t in 2010 (Walker, 2010), and there is an aspiration for the industry to grow by 50% by 2020 (Baxter et al. 2011). However, the number of farms across Scotland has reduced (Walker, 2010) indicating that the average biomass produced per farm has increased. Farms in Scotland are granted production consents by the Scottish Environmental Protection Agency (SEPA) of up to 2500 t, whilst in Norway, farms stock at over double this value (Fiskeridirektoratet, 2011) suggesting that Scottish farms could grow to this level increasing total overall production. Generally, farms are located in sheltered areas where currents are relatively low velocity however, due to potential space constraints and to minimise any environmental impact farms may become located further offshore in higher velocity current areas in order for the industry to expand.

A further method of increasing output is minimising the loss of stock to pathogenic disease (Murray and Peeler, 2005) For example a recent outbreak in Chile of the notifiable disease, infectious salmon anaemia (ISA) caused by a pathogenic virus, caused losses of around 2 billion USD (Aldrin et al., 2011). The importance of pathogenic disease control is highlighted by the costs incurred in disease management which globally are in the region of 3 billion USD (Subasingh, 2001), with sea lice management of salmonids costing over €305 million (Costello, 2009).

Herd size has been demonstrated to be a risk factor in terrestrial agriculture (e.g. Humblet et al., 2009; Zhang & Woolhouse, 2011) and population size can determine the transmission and persistence of an epidemic (Anderson & May, 1979; May & Anderson, 1979; Grenfell & Dobson, 1995). This is also the case in aquaculture (Ögüt, 2001; Krkošek, 2010). Increased numbers of infectious fish may lead to increased pathogen concentration in the surrounding environment (Murray, 2009), which in turn could lead to greater risk of pathogen transmission. This ability for pathogens to be transmitted through the environment by water movements enables farms to become connected by a hydrodynamic transmission route (Amundrud & Murray, 2009, Foreman et al., 2009). This can cause infection to occur on farms exposed to a transmitted pathogen agent without the presence of an infected host. This has been considered to be a risk factor in the spread of ISA (Aldrin et al., 2011; Aldrin et al., 2010; Gustafson et al., 2007; McClure, 2005), pancreas disease (PD) (Aldrin et al., 2010; Kristoffersen et al., 2009; Viljugrein et al., 2009) and sea lice (Amundrud & Murray, 2009; Foreman et al., 2009). Recent outbreaks of ISA in Norway (Lyngstad et al., 2008), Chile (Mardones et al., 2009) and Scotland (Murray et al., 2010) have reported disease occurrence on farms located near initially infected sites, demonstrating the importance of farm location

in disease transmission. Disease management areas (DMAs) in Scotland of 7.2 km for the mainland and 3.6 km for the Shetland Islands have been developed based on simplified, yet robust, hydrodynamic modelling in order to help prevent the spread of ISA (Scottish Executive, 2000). Farms are classed as being in the same DMA if they are positioned less than a tidal excursion away from another farm. Modelling is also used to describe regions over which enhanced surveillance and movement controls are imposed in order to minimise the risks of transmitting ISAV in the event of an outbreak. Ideally management and fallowing are co-ordinated over these areas in the absence of disease as this reduces risk of emergence of diseases (Werkman et al., 2011).

Pathogenic agents decay in the environment over time (e.g. Rose et al., 1990; Toranzo & Hetrick, 1982), therefore as they are transported the effective dose decreases. A farm must be exposed to a pathogen level above the minimum infective dose for disease to occur (Ward & Akin, 1984). Below this threshold the individual will not demonstrate symptoms of disease. Minimum infectious doses have been presented for pathogens of interest to fish farming causing ISA (Raynard et al., 2001; Gregory et al., 2009), IPN (Urquhart et al., 2008) and amoebic gill disease (Morrison et al., 2004).

Here a discrete-time epidemiological model representing fish farms is presented. The model incorporates transmission, expression and recovery parameters as well as pathogen shedding and decay. An expression is derived representing the time required for shed particles to decay below the minimum infective dose. The critical time expression is used to assess the distance travelled by a cohort of pathogen particles. This is calculated by a simplified hydrodynamic model that incorporates residual advection, tidal advection and turbulent diffusion elements. This framework is used to assess the required separation distances for farms to avoid developing ISA, IPN and furunculosis. The separation distances developed using this framework were compared to the distances produced using the particle tracking model approach of Murray et al. (2005) to assess how a simplified analytical approach compares to a more computationally demanding method.

MATERIALS AND METHODS

Figure 1 is a schematic representation of the model system showing two farms represented by an *SEIR* model connected by a waterborne pathogen phase which is transported between farms by hydrodynamic movements. The description and values of the parameters used are shown in Table 1. The model represents change in biomass compartments within a farm over time intervals of 15 minutes, with the described transition rates.

This schematic demonstrates the transition of biomass between compartments within a farm, the shedding of infectious particles into the aquatic environment, the transport and decay of these agents, their subsequent infection of a naïve farm and transitional changes in the exposed farm. Details of the model system are described in Salama & Murray (2011).

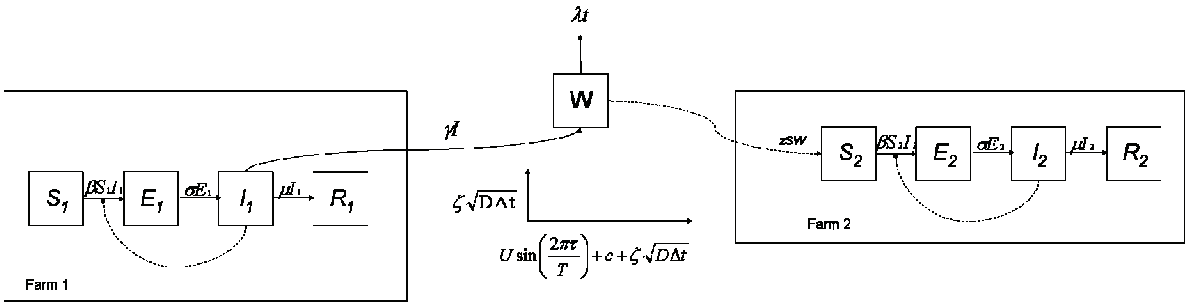

Figure 1. A schematic representation of the SEIR farm models connected by a waterborne phase (W) that transports the shed pathogen particles in a two-dimensional plane.

The exposure risk is conditional on the minimal infective dose for disease expression. This enables an expression for the maximum amount of time a shed pathogen cohort can survive in order to cause an infection Eq.(1).

$$t_c = \frac{-\ln\left[\dfrac{\mu\phi}{S_2\gamma I_1 z\beta}\right]}{\lambda} \tag{1}$$

To assess the distance a cohort can be transmitted during t_c, a simplified hydrodynamic particle tracking model described by Murray et al. (2005) is altered to produce a discrete expression of distance travelled in both the X and Y planes Eq.(2 & 3). The physical movements involved are residual advection currents and tidal advection currents which act in the horizontal plane, and turbulent diffusion which acts on the vertical plane.

$$\bar{x}_t = \bar{x}_{t-1} + \frac{s}{t}\sqrt{\frac{\frac{s}{t}D}{T}} + s\sqrt{3} \tag{2}$$

$$\bar{y}_t = \bar{y}_{t-1} + \frac{s}{t}\sqrt{\frac{\frac{s}{t}D}{T}} \tag{3}$$

With standard deviations $\pm s\sqrt{t}$

Both the analytical model and the particle tracking model of Murray et al. (2005) were compared using *R* 2.8.1 (R development core team 2008) in order to assess whether the analytical expression produced results comparable to the simulation model. The reason for this is that a particle tracking model is computationally intensive and it is only possible to track a subset of the total number of pathogen particles. As a result, it potentially loses some representation of the system. The models use the same physical parameter values as Murray et al. (2005). To reduce complication of an epidemiological model representing a source farm that produces many varying cohorts of pathogen particles, a worst-case scenario of peak-shed number of infectious salmon anaemia virus type (ISAVt) pathogen particles being released is used. The initially infected farm is determined as having a biomass of 1000 t and prevalence of 30% (McClure et al., 2004; M. Hall *pers. comm.*). Analytical model simulations were also conducted for IPNV type pathogens and *Aeromonas salmonicida* (the causative agent of

furunculosis). The prevalence for IPN is 12.5% for Scottish salmon (Bruno, 2004) and 75% for *A. salmonicida* from experimental infection of Chinook salmon (*Oncorhynchus tshawytscha*) (Ögüt & Reno, 2005).

Table 1. Parameter descriptions and values. Note that subscripts relate to farm position in a sequence.

Pathogen parameter	Description	ISAV-t	IPNV-t	AS-t
β (z)	Pathogen transmission probability	0.015 d^{-1} (Gregory et al. 2009)	0.013 d^{-1} (Smith et al. 2000)	0.0214 d^{-1} (Ögüt & Bishop 2007)
σ	Infectious individual expression probability	0.14 d^{-1} (Gregory et al. 2009)	0.36 d^{-1} (Smith et al. 2000)	0.29 d^{-1} (Ögüt & Bishop 2007)
μ	Infected individual epidemic removal probability	0.04 d^{-1} (Gregory et al. 2009)	0.062 d^{-1} (Smith et al. 2000)	0.33 d^{-1} (Ögüt & Bishop 2007)
γ	Pathogen particle shedding rate	$7.2x10^{-1}$ $mL^{-1}h^{-1}kg^{-1}$ (Gregory et al. 2009)	$6.8x10^{-2}$ $mL^{-1}h^{-1}kg^{-1}$ (Urquhart et al. 2008)	$1.75x10^{6}$ cfu mL^{-1} h^{-1} (Rose et al. 1990)
λ	Pathogen decay rate	0.12 h^{-1} (Løvdal & Enger 2002)	0.016 h^{-1} (Toranzo & Henrick 1982)	0.12 h^{-1} (Rose et al. 1989
φ	Minimum infectious dose	10^{-1} $TCID_{50}$ mL^{-1}-kg^{-1} (Gregory et al. 2009)	10^{-4} $TCID_{50}$ mL^{-1}-kg^{-1} (Urquhart et al. 2008)	10^{8}cfu mL^{-1} (Perez et al. 1996)

Population Parameter	Description	Transport parameter	Description
N	Total number of individuals on a farm 10^{2} - 10^{5} (SEPA Fiskeridirektorat)	A	Minimum transport step-length
S	Number of susceptible individuals on a farm	X	Maximum transport step-length
E	Number of exposed individuals on a farm	S	Mean transport step-length
I	Number of infectious individuals on a farm	U	Tidal current amplitude 51cm s^{-1} (Murray et al. 2005)
R	Number of individuals removed from an epidemic on a farm	T	Tidal Period 12.42 h (Murray et al. 2005)
W	Number of particles in the environment	C	Constant residual advection current 1 – 8cm s^{-1} (Murray et al. 2005)
t	Time-step	D	Diffusion Coefficient $10^{4}cm^{2}s^{-1}$ (Murray et al. 2005)
ε	Dose related between farm infection probability	X	X coordinate location
		Y	Y coordinate location

Shed particles are moved by persistent currents at speeds experienced in Scottish inshore waters, ranging from 1 – 8 cm s^{-1} (Lee & Ramster, 1981) at 1 cm s^{-1} intervals. The production units used represent the smaller farms (10^2t) in Scotland, increasing to moderate sized farms ($10^{2.5}$t) to larger farms (10^3t). In recent years production has been administered in farm management areas (FMAs) defined by industry (Code of Good Practice Management Group, 2011). Several farms together in a FMA can be considered as one production unit because they follow coordinated management procedures. These units of greater than single farms are considered to have biomass from $10^{3.5}$t up to 10^5t.

The model structure is used to obtain example separation distances for farms of varying sizes downstream from an infected farm in varying tidal conditions. The model is populated with parameters representing ISAV-type, IPNV-type and *A. salmonicida* type pathogens. The model is used to assess two production strategies in order to produce 5 x 10^3 t fish: 1) having a large farm of 5 x 10^3 t separated 10 km from an IASV-type infected site, and 2) two farms of 2.5 x 10^3 t located 5 km from an infection source. Farms in sequence are also considered, where trajectories are observed for ISA-type outbreaks on farms of increasing size and separation.

RESULTS

There is limited movement in the y-dimensions for each of the initial conditions, whereas the particle tracking model predicts movements for a range of outcomes in the range of ±3m. When comparing the model estimations, Fig. 2 demonstrates there is considerable parity ($F_{(1,43)}$=1.663x10^5). The standard deviations are underestimated by the analytical model. However in terms of the dispersal distances predicted by the model the differences in the standard deviations of a few hundred meters are likely to be minimal in predicting the transmission distances between sources and susceptible farm sites

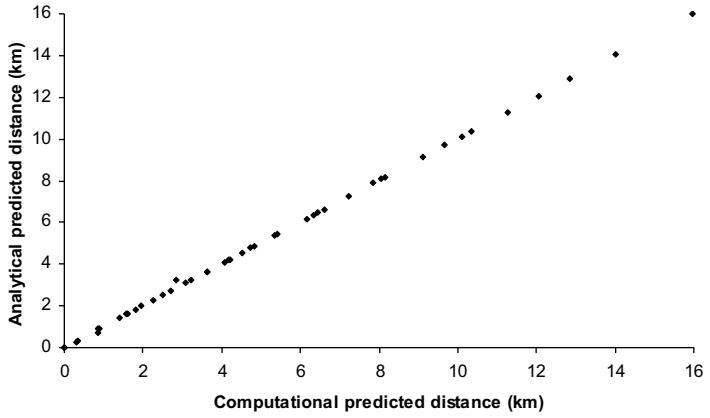

Fig. 2 A comparison between the computational and analytical approaches to predicting the threshold distances under increasing farm size and residual currents.

An example simulation demonstrating that mean simulated safe distances to avoid persistent infection for farms exposed to the peak, half-peak and one quarter-peak shed of IPNV-t, ISAV-t and AS-t pathogens from an infected 1000t farm are shown in Fig. 3. As secondary farm size, shedding dose from a source farm and residual tidal current speed

increases for each of the pathogen types, the separation distance required to avoid infection also increases.

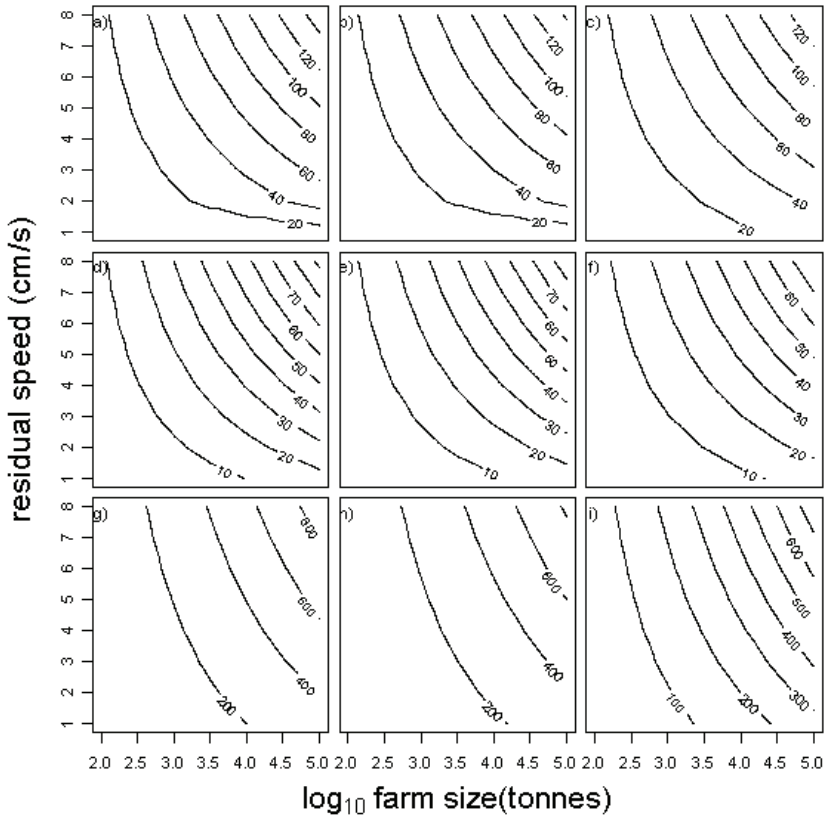

Fig.3. The mean distances (km) whereby a farm of a given size in varying current speeds avoids having a persisting epidemic when exposed to a 1000 t farm infected with reported prevalence of AS-t at: a) peak shed, b) half peak shed, c) a quarter peak shed; infected with ISAV-t at: d) peak shed, e) half peak shed, f) a quarter peak shed; infected with IPNV-t at: g) peak shed, h) half peak shed, i) a quarter peak shed.

One large farm separated from a source by 10 km experienced fewer infections than two smaller farms located closer to the outbreak site which suffered complete infection (Fig. 4),

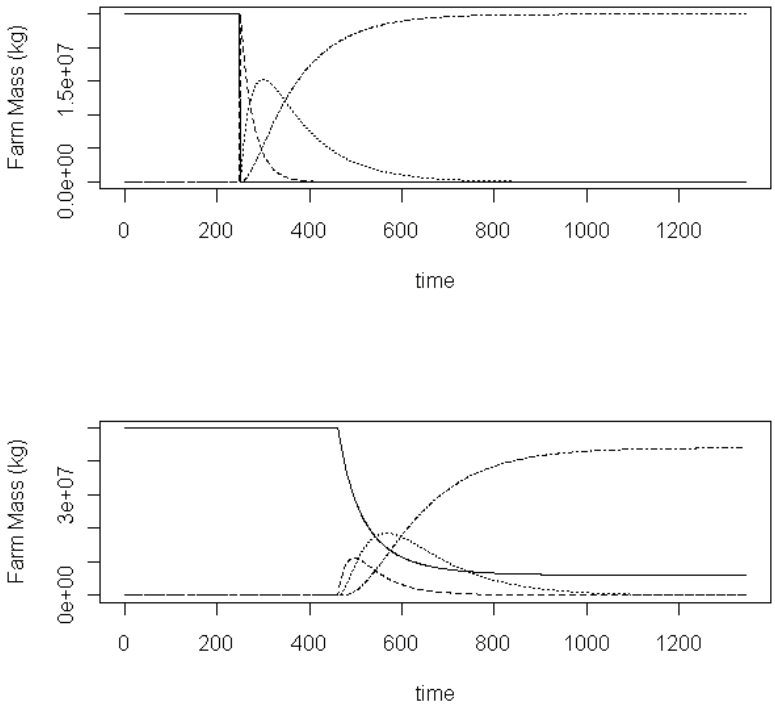

Fig 4. The epidemic trajectory of a) a 2.5 x10^3 t farm separated by 5 km from a same size farm with 5% ISAV-t pathogen. Infection causing complete infection and b) a 5x10^3 t farm separated by 10 km from another farm of the same size with 5% infection of an ISAV-t pathogen causing ~88% infection (solid: *S*, dashed: *E*, dotted: *I* and dot-dash: *R*).

When considering a sequence of four equal sized farms separated by equal distances allowing for pathogens to be transmitted from a source farm to the fourth farm via intermediate farms (Fig. 5), more of the total production becomes infected when smaller farms are located close together compared to when larger farms are separated at a greater distance.

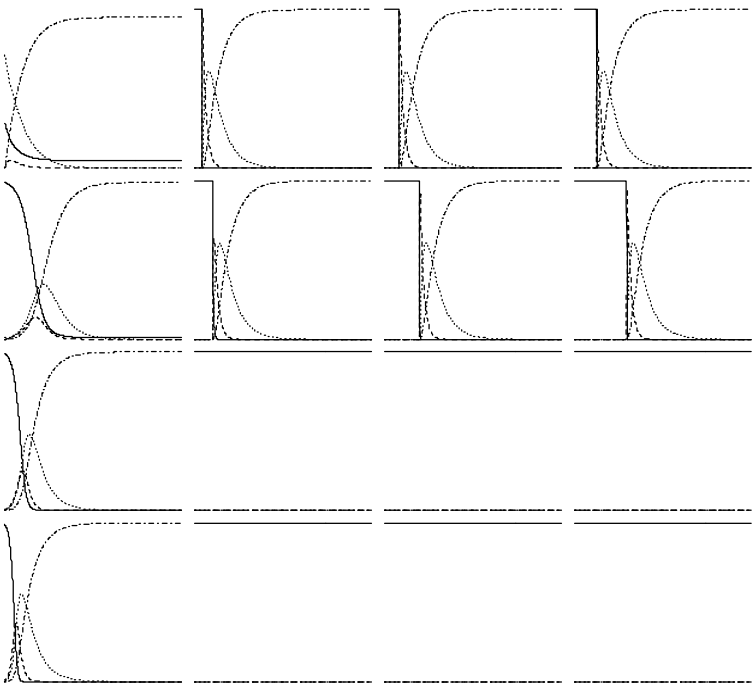

Fig. 5. Disease progression over a 2 wk period from a 1% ISAV-t initially infected site between farms in sequence: First row - $5x10^6$t at 1km intervals, Second row - $1.25x10^7$t at 2.5km intervals, Third row: $2.5x10^7$t at 5km intervals, Fourth row - $5x10^7$t at 10km intervals. The axis scale is removed so that comparison in total disease incidence across sites can be compared. (solid: *S*, dashed: *E*, dotted: *I* and dot-dash: *R*).

DISCUSSION

This study aimed to develop a model framework that could be used to assess how the size of the stock biomass on a fish farm may alter the pathogen transmission and disease persistence. The model was adapted and compared to the particle tracking model approach of Murray et al. (2005). Although the analytical model is simplified and underestimates the variation in potential transmission, it produces comparable infection threshold distances to the more process consuming model of Murray et al. (2005). This alternative modelling approach can be used to attain possible worst-case transmission distances for farms of different size located in varying current speeds.

By populating the model with parameters found in literature it is possible to estimate the transmission distances of IPNVt, ISAVt, and ASt pathogens which lead to disease persistence in initially disease-free farms connected to infected farms by hydrodynamic pathways. It is shown that as the size of the initially disease-free farm increases, then the further it is required to be separated from infected farms in order to avoid disease acquisition. Likewise, as farms are located in faster moving water, such as in offshore environments, the further pathogens are able to be transported within their survival time frame, therefore separation distances need to be increased. This could be useful for farm managers as part of their planning process. To obtain site consents, managers record the physical environment surrounding farms. These readings are entered into spreadsheets containing formula expressions for environmental impact. A similar approach could be used to assess the

required distance farms need to be in order to minimise farm-to-farm transmission of pathogens.

As production is concentrated in larger farms there are fewer of these and therefore separation distances can be increased. In order to assess the disease dynamics of differing production systems the model is considered with farms producing $5x10^3$t in four production methods: 1) Ten farms stocked with 5 x 10^2t, located 1km from an initial source, 2) Four farms stocked with 1.25 x 10^3t located 2.5km from an initial source, 3) Two farms stocked with 2.5 x 10^3t located 5 km from an initial source, 4) One farm stocked with $5x10^3$t located 10km from the initial source. The closer the farms are together, the higher the percentage infection per farm to a pathogen such as ISAVt, however when exposed to highly conserved pathogens a complete infection of the farm occurs. This demonstrates that the optimal aquaculture production strategy to avoid individuals on a farm becoming infected is to have larger separation distances and larger farms, compared to many small farms in close proximity.

When considering the influence of disease spread between many small farms situated close together versus few larger farms situated farther apart on additional farms downstream, simulations indicate that two farms separated by less distance and stocked with less fish have more infected individuals compared to larger $5x10^3$t farms farther apart. Additionally the rate of infection within the larger, more separated farms is lower, whereas in smaller farms the spread of infection throughout the individuals within the farm is instantaneous. This is an important for monitoring and mitigation. With lower rates of infection, the farm operators might be able to act before the epidemic peaks, thus preventing spread to downstream farms. Whereas infection in a smaller farm system requires immediate response thatwill still lead to substantial spread of infection to downstream farms. For farms in sequence; smaller farms clustered together experience disease occurrence, whereas larger farms separated farther apart do not. For smaller farms in sequence, there is a time lag before disease occurrence based on distance from source, with those farther away becoming infected later than those nearby. This is a similar pattern to the recent ISA outbreak in Shetland where incidence reporting occurred later with increased distance from the initially infected site (Murray et al., 2010).

Production unit size varies representing moderate and large sized farms to area management scale. Current ISAV DMA's are based on TE of 7.2 km for mainland Scotland and 3.6 km for the Shetland Islands. Disease management areas are continuous over the area in which adjacent farms overlap, so a separation distance of greater than 14.4 km (or 7.2 km in Shetland) is required for a DMA boundary. Figure 2 shows these DMA's are likely to be appropriate for current production site size in mainland Scotland and the Shetland Islands for ISAV-type pathogens and for farms located in low residual current areas. Such distances may become unsuitable for more robust pathogens. Should farms be increased in size or be situated in faster current locations, such as offshore, it is possible that the DMA's will need to be reconsidered. However, longer distance spread requires strong advection currents persisting in a single direction without excessive turbulent dispersal. Risk will be highest in the immediate neighbourhood of farms, as even DMA's with imperfect boundaries can be useful for the management of disease (Werkman et al., 2011) the separation distance need not exclude all trans-boundary transmission.

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MULTI-CRITERIA DECISION ANALYSIS FOR EVALUATING CONTROL OPTIONS DURING FMD OUTBREAKS

K. MINTIENS* AND D. VOSE

SUMMARY

Multi-criteria decision analysis (MCDA) helps to provide a structure for decision-making that involves stakeholder groups with conflicting criteria. It combines hard data with informed judgement and distinguishes between facts and value judgements. Outbreaks of highly virulent diseases, such as foot and mouth disease (FMD), require prompt decisions on control options that often receive criticism by stakeholders with conflicting objectives. The aim of this study was to evaluate the value of MCDA in providing decision makers with better-informed decision alternatives that can be better supported by all stakeholders when controlling animal disease epidemics.

When controlling an FMD epidemic, preventive depopulation, vaccination-to-live, or vaccination-to-dead can be used for reducing the population at risk. The optimal option should minimize economic losses, maximize social values and maximize political values. All these objectives can be expressed in terms of attributes. The decision options will have different impacts on the attribute values. All attribute values are brought to the same scale between 0 and 100 for comparison.

Stakeholders were asked to weight the importance of changing from the worst to the best value for an attribute. Simple Multi-Attribute Rating Technique (SMART) was used to obtain multi-attribute values for the different decision alternatives using the scaled attribute values and the weights. The decision option with the lowest value was the one that was best supported by all the stakeholders.

INTRODUCTION

Multi-criteria decision analysis (MCDA) is a sub-discipline of operational research that explicitly considers multiple criteria in decision-making environments (Wikipedia). Multi-criteria decision analysis provides a structure for decision-making that involves different stakeholder groups with conflicting criteria. It combines hard data with informed judgement and distinguishes between facts and value judgements. The earliest known reference to MCDA can be traced to Benjamin Franklin (1706-1790) and it has been used in many disciplines, e.g. natural resource management, health management, environmental interventions, etc. Still, traceable applications of MCDA in veterinary epidemiology remain limited, as conflicting criteria might often be ignored in animal health management. Multi-

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criteria decision analysis is used to support animal welfare policy (Hellstrom and Bicknell, 2010), and it is in that context that objectives might become conflicting when optimising animal health.

MCDA methodology comprises three major parts: problem structuring, value trade-off and sensitivity analysis. Problem structuring starts with the description of the decision context and the definition of the objectives that have to be achieved with the decision. Each of these objectives will then be valued in terms of attributes, whereas the decision alternatives can have different impacts on the values of the attributes. To make that impact comparable, all the values have to be brought to the same scale.

When performing the value trade-off, stakeholders are asked to weight the importance of changing from the worst to the best value for an attribute. All these weights are then averaged out and standardised for all stakeholders. The Simple Multi-Attribute Rating Technique (SMART) is a compensatory method of multiple criteria decision analysis developed by Edwards in 1971 (Edwards and Barton, 1994). It uses an additive value model in which the standardized weights are multiplied with the scaled values for each of the attributes and summed for each of the decision alternatives. The decision alternative with the lowest value is the one that is best supported by all the stakeholders. Finally, in a sensitivity analysis, the robustness of the total attribute values to changes of the input weight is evaluated and critical points of weights where decisions change are identified.

Emerging diseases are those that appear in a population for the first time, or that may have existed previously but are rapidly increasing in incidence or geographic range. Globalisation and climate changes have increased the risk of emergence of animal diseases (e.g. African horse sickness, epizootic haemorrhagic disease) and zoonoses (e.g. West Nile virus, Rift Valley fever) in the world. A 2004 study found that emerging animal diseases were 1.93 times more likely to be zoonotic than non-zoonotic (Brown, 2004). Outbreaks of emerging animal diseases, particularly zoonoses, can cause considerable economic and social upheaval. Epidemics of bovine spongiform encephalopathy, classical swine fever, foot and mouth disease, avian influenza and bluetongue which occurred in Europe in recent decades illustrate this trend. Because of their possible devastating consequences, emerging disease outbreaks require prompt decisions on control options. This can force decision makers to make urgent 'gut' decisions, because scientific information is insufficient or not available in time. These fast decisions often receive a lot of criticism especially for stakeholders with conflicting values, such as, for example, farmers or animal welfare organisations.

In the 2001 Foot and Mouth Disease (FMD) outbreak in the UK, 2,030 cases of FMD were reported between February and September 2001(Defra 2011). Thousands of healthy animals were culled as a preventative measure. Many farmers were economically devastated and the public was bombarded with images of the mass slaughter of animals. The European Commission non-vaccination policy for eradicating emerging diseases received a lot of criticism (CEC 2001) and was revised. Emergency vaccination during FMD outbreaks became possible (CEC 2003) under strict conditions, e.g. using differential diagnostics, but also with considerable economical consequences. The favourable animal welfare conditions of applying emergency vaccination were opposed by unfavourable economic consequences, which made the control strategy for FMD outbreaks a hard decision.

The aim of this study was to evaluate the value of MCDA in providing decision makers with better-informed decision alternatives, that can be better supported by all the stakeholders when controlling animal disease epidemics. The eradication of FMD was used a case study.

MATERIALS AND METHODS

Problem structuring

We consider a hypothetical outbreak of FMD in a densely populated livestock area where four, twelve and four infected premises are confirmed in cattle, small ruminants, and pigs respectively. The veterinary authorities will eliminate the infection according to EC directives (CEC, 2003) by depopulation of the infected premises, installing restriction zones and reducing the population at risk. For the latter, they have to decide on one of three alternatives that are available:

1. Preventive depopulation of all susceptible animals within a zone of 1km radius around the infected premises (preventive slaughter).

2. Vaccination of all susceptible animals within a zone of 10km radius around the infected premises. Vaccinated animals will be marketed after restrictive measures are lifted (vaccination-to-live).

3. Vaccination of all susceptible animals within a zone of 10km radius around the infected premises. Vaccinated animals will be destroyed according to the destruction capacity of the rendering plant (vaccination-to-death).

Different additional measures and criteria are imposed for each of the three alternatives (CEC, 2003).

The end objectives of the decision are minimising economic losses, maximising social values, and maximising political values. The end objectives are further detailed in mean objectives, which are summarised in Table 1.

Table 1. End and mean objectives of controlling a foot and mouth disease outbreak

END OBJECTIVES	MEAN OBJECTIVES	ATTRIBUTES
Maximising social values	Maximising farmers welfare	Loss of farm heritage Stress
	Maximising animal welfare	Number of animals killed
Minimising economical losses	Minimising direct costs	Eradication costs Surveillance costs
	Minimising indirect costs	Losses due to trade restrictions
Maximising political values	Maximising reputation of the decision maker	Time to lifting of restriction zones Time to obtain free status

Table 1 also presents the attributes that were identified for valuation of each of the mean objectives. Some of the attribute values (e.g. eradication costs) were estimated using hard data whereas others (e.g. farmers' stress) were assessed based on expert judgements.

- Loss of farm heritage was judged on a scale between 0 and 100, where 100 was allocated to the alternative with the largest loss.

- Stress was judged on a scale between 0 and 100, where 100 was allocated to the alternative giving the most stress to the farmers.

- Number of animals killed was obtained from the livestock Identification and Registration system and calculated for the different restriction zones.

- Eradications costs were estimated by summarising all costs at herd and animal level to depopulate premises and depended for the different decision alternatives on the number of animals to be culled and premises to be depopulated. Following costs were included:

    - Animal value appraisal,

    - Cleaning and disinfection of premises,

    - Euthanasia of animals,

    - Indemnification of animals,

    - Disposal/destruction of animals.

- Surveillance costs were estimated by summarising all costs at herd and animal level related to sampling and testing for absence of FMD virus. The use of SPC-ELISA was considered for detecting FMD–virus in non-vaccinated animals whereas the NSP-ELISA was considered for detecting FMD–virus in vaccinated animals. The following costs were included:

    - Sampling

    - SPC-ELISA diagnosis

    - NSP-ELISA diagnosis

- Losses due to trade restrictions were calculated for the different decision alternatives, by multiplying the number of animals under restriction with the length of the restriction periods (in days) and a unit cost per animal-day under restriction.

- Time to lifting of restriction zones accounted for the time needed to achieve the requirements as stated in the EC directive (CEC, 2003). The time varied for different decisions based on the capacity for performing certain actions (daily capacity for culling, vaccination, sampling and diagnostic testing).

- Time to obtain free status was obtained ina way similar to the time to lifting of restriction zones but the EC directive prescribed time for obtaining free status was included.

All attribute values x_{ij} were brought to the same scale between 0 and 100 using the following equation:

$$s_{ij} = \frac{x_{ij} - Min(x_i)}{Max(x_i) - Min(x_i)} * 100$$, for *i* attributes and *j* decision alternatives (1)

Value trade-off

The 'swing' weights w_i express the importance to the different stakeholders of changing from the worst to the best value for an attribute. Animal welfare organisations might give high swing weights to reducing the number of animals killed, whereas farmer organisations might allocate high swing weights to reducing direct economic losses. The individual swing weights were obtained through stakeholder elicitation on a scale from 0 to 100 (lowest to highest importance) and averaged for each attribute. The averaged weights were normalised for all attributes in order to meet the following condition:

$$s_{ij} = \sum_{i=1}^{n} \bar{w}_i = 100$$, for *i* attributes (2)

The SMART was used to evaluate the multi-attribute values S_j for the different decision alternatives:

$$S_j = \sum_{i=1}^{n} \bar{w}_i s_{ij} = \bar{w}_1 s_{1j} + \bar{w}_2 s_{2j} + \cdots + \bar{w}_n s_{nj}$$, for *n attributes, j* decision alternatives (3)

The decision alternative with the lowest S_j value was considered as the most favourable according to the different objectives and best supported by all the stakeholders.

Sensitivity analysis

The change in multi-attribute value induced by changing swing weight values was evaluated in the sensitivity analysis. The swing weights for all individual attributes were altered from 0 to 100 in steps of 10 and included in Eq. (3). The changing S_j values were mapped on a graph and the swing weight value at which the decision alternative with the lowest value might change is identified.

RESULTS

For the simulated FMD epidemic, separate MCDA analyses were performed for cattle, pigs and small ruminants, because different decisions can be expected for these species. The authors, an animal disease crisis manager, and a representative of a farmer organisation provided the data for the MCDA exercise, based on their expertise and background in animal disease epidemics. The data were provided to illustrate the methodology and may deviate

from the true parameter values. The data that were used for the MCDA are presented in Table 2.

Table 2. Input values for the multi-criteria decision analysis, at animal and herd level

	CATTLE		SMALL RUMINANTS		PIGS	
	ANIMAL	HERD	ANIMAL	HERD	ANIMAL	HERD
Numbers affected at onset		4		12		4
Population @risk in culling zone	1,558	148	792	92	61,431	72
Population @risk in protection zone	3,709	350	1,885	218	146,265	172
Population @risk in surveillance zone	144,872	2,188	11,780	1,365	914,155	1,075
Population @ risk in vaccination zone	144,872	2,188	11,780	1,365	914,155	1,075
Population @ risk in buffer zone	85,007	1,372	6,771	885	540,289	677
Culling capacity (per day)	1,250	2	1,250	2	2,500	2
Appraisal costs (€)	1	30	1	30	1	30
Cleaning and disinfection costs (€)	1	30	1	30	1	30
Euthanasia costs (€)	1	30	1	30	1	30
Indemnification costs (€)	1,000	0	300	0	600	0
Carcass disposal costs (€)	1	30	1	30	1	30
Vaccination capacity (per day)	400		400		800	
Vaccination costs (€)	1	30	1	30	1	30
Sampling capacity (per day)	8,000		8,000		8,000	
Sampling costs (€)	1	30	1	30	1	30
SPC test capacity (per day)	400		400		400	
SPC ELISA costs (€)	30		30		30	
NSP test capacity (per day)	400		400		400	
NSP ELISA costs (€)	30		30		30	
Cost of trade restriction (per day, €)	0.5		0.5		0.5	
Sample size culling zone	46,964	796	2,676	310	31,978	542
Sample size vaccination zone	150,139	2,686	14,457	1,675	1,121,851	1,319
Sample size vaccination+buffer zone	261,649	2,984	22,095	2,560	1,161,794	1,996

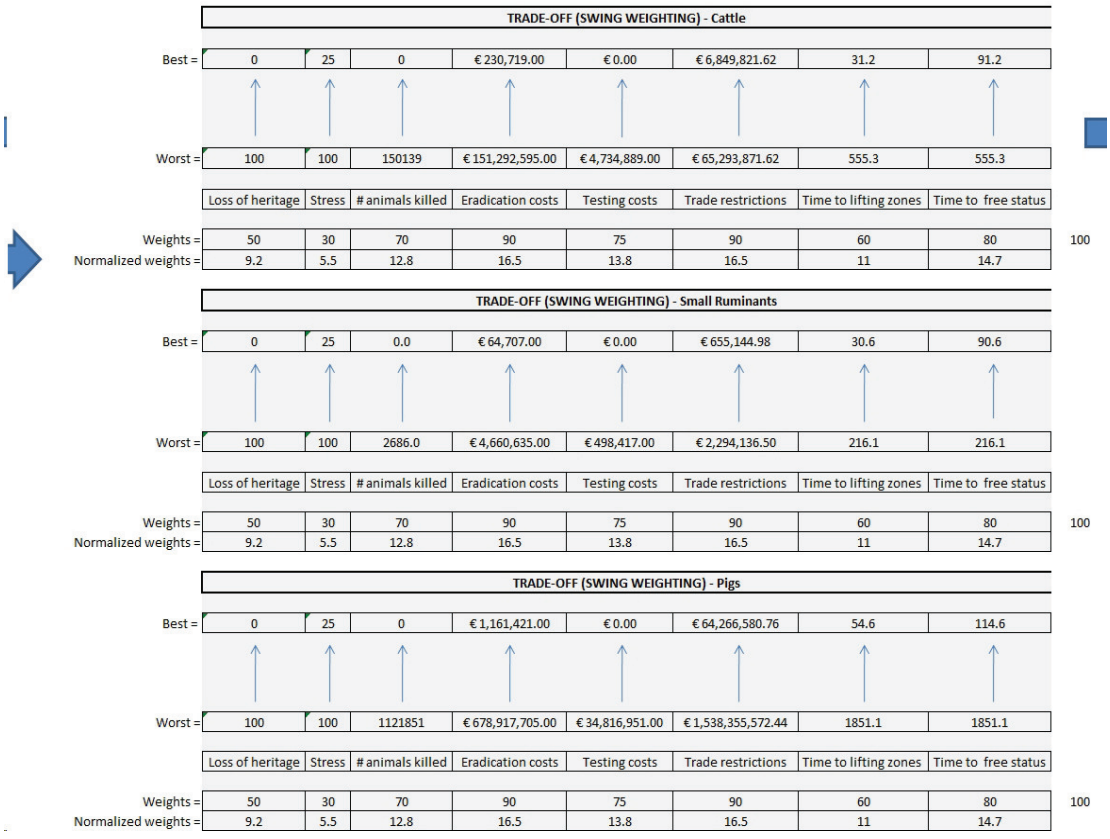
Table 3. Attribute values for the different decision alternatives and animal species

	CATTLE			SMALL RUMINANTS			PIGS		
	PREVENTIVE SLAUGHTER	VACCINATION TO LIVE	VACCINATION TO DEATH	PREVENTIVE SLAUGHTER	VACCINATION TO LIVE	VACCINATION TO DEATH	PREVENTIVE SLAUGHTER	VACCINATION TO LIVE	VACCINATION TO DEATH
Social Values									
Loss of heritage	50	0	100	50	0	100	50	0	100
Stress	75	25	100	75	25	100	75	25	100
# animals killed	1,558	0	150,139	792	0	2,686	61,431	0	1,121,851
Economical Values									
Eradication costs (€)	1,581,992	230,719	151,292,595	251,808	64,707	4,660,635	37,112,964	1,161,421	678,917,705
Testing costs (€)	1,479,764	4,734,889	0	92,244	498,417	0	1,007,578	34,816,951	0
Trade restrictions (€)	6,849,822	65,293,872	65,293,872	655,145	2,294,136	2,294,136	64,266,581	1,315,013,488	1,538,355,572
Political Values									
Time to lifting restriction zones (days)	31	405	555	31	66	216	55	1,543	1,851
Time to free status (days)	91	555	555	91	216	216	115	1,582	1,851

The attribute values for the decision alternatives and animal species are presented in Table 3. The judgement values for the two first social values, 'loss of heritage' and farmers' stress were kept equal for the three animal species. For loss of heritage, it was judged that there was no loss in case of vaccination-to-live as no animals were killed. It was judged to be maximal for vaccination-to-death as more animals were killed compared to preventive slaughter, where the loss of heritage was expected to be intermediate. Farmers' stress was judged to be low but not absent in case of vaccination–to-live and again judged to be maximal for vaccination-to-death but also for preventive slaughter farmers' stress was judged to be high. All the other attribute data were based on hard data and show distinct differences between the decision alternatives. For all of these attributes, preventive slaughter appears to be the best option.

The authors and the experts who provided the input data also provided the swing weights for the different attributes. Again, the weights' values were provided purely to illustrate the methodology and therefore they were kept the same for the different animal species. The actual and normalised weights are presented in Figure 1. For the illustration, it was decided to give most weight to eradication costs and economic losses due to trade restrictions, followed by the time to obtain free status for the country and number of animals killed.

Figure 1. Swing weights for different attributes and animal species



TRADE-OFF (SWING WEIGHTING) - Cattle

	Loss of heritage	Stress	# animals killed	Eradication costs	Testing costs	Trade restrictions	Time to lifting zones	Time to free status	
Best =	0	25	0	€ 230,719.00	€ 0.00	€ 6,849,821.62	31.2	91.2	
Worst =	100	100	150139	€ 151,292,595.00	€ 4,734,889.00	€ 65,293,871.62	555.3	555.3	
Weights =	50	30	70	90	75	90	60	80	100
Normalized weights =	9.2	5.5	12.8	16.5	13.8	16.5	11	14.7	

TRADE-OFF (SWING WEIGHTING) - Small Ruminants

	Loss of heritage	Stress	# animals killed	Eradication costs	Testing costs	Trade restrictions	Time to lifting zones	Time to free status	
Best =	0	25	0.0	€ 64,707.00	€ 0.00	€ 655,144.98	30.6	90.6	
Worst =	100	100	2686.0	€ 4,660,635.00	€ 498,417.00	€ 2,294,136.50	216.1	216.1	
Weights =	50	30	70	90	75	90	60	80	100
Normalized weights =	9.2	5.5	12.8	16.5	13.8	16.5	11	14.7	

TRADE-OFF (SWING WEIGHTING) - Pigs

	Loss of heritage	Stress	# animals killed	Eradication costs	Testing costs	Trade restrictions	Time to lifting zones	Time to free status	
Best =	0	25	0	€ 1,161,421.00	€ 0.00	€ 64,266,580.76	54.6	114.6	
Worst =	100	100	1121851	€ 678,917,705.00	€ 34,816,951.00	€ 1,538,355,572.44	1851.1	1851.1	
Weights =	50	30	70	90	75	90	60	80	100
Normalized weights =	9.2	5.5	12.8	16.5	13.8	16.5	11	14.7	

The results of the SMART model for the different decision alternatives and different species are presented in Table 4. Using preventive slaughter to reduce the population at risk for the simulated FMD outbreak was the option that was supported by the stakeholders, given the attributes and the allocated swing weights.

Table 4. Multi-attribute values for the different decision alternatives and different species

SPECIES	DECISION ALTERNATIVE	MULTI-ATTRIBUTE VALUE	PREFERENCE (CHOOSE ALTERNATIVE WITH LOWEST VALUE)
Cattle	Preventive slaughter	1,286	X
	Vaccination-to-live	5,285	
	Vaccination-to-death	8,990	
Pigs	Preventive slaughter	1,527	X
	Vaccination-to-live	4,711	
	Vaccination-to-death	8,620	
Small ruminants	Preventive slaughter	1,024	X
	Vaccination-to-live	4,934	
	Vaccination-to-death	8,620	

Sensitivity analysis

The influence on the multi-attribute values of changing the swing weights for the different attributes between 0 and 100 is illustrated for pigs. Figure 2 shows that increasing the swing weights for the 'farmers' stress' attribute will increase the multi-attribute values for 'preventive slaughter' and 'vaccination-to-death'. Still, 'preventive slaughter' remains the best option.

Figure 2. Influence of changing swing weights for 'farmers' stress' on the multi-attribute values for pigs

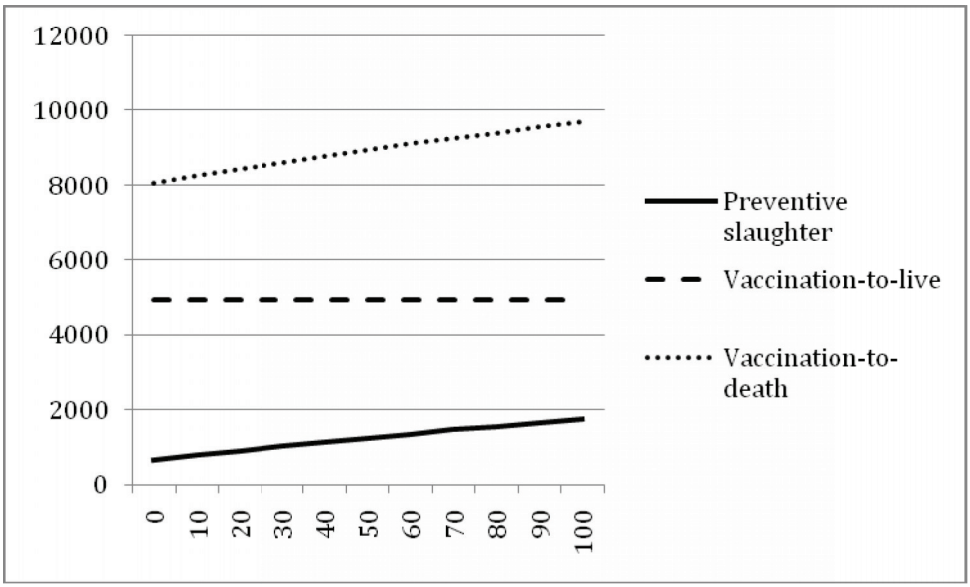
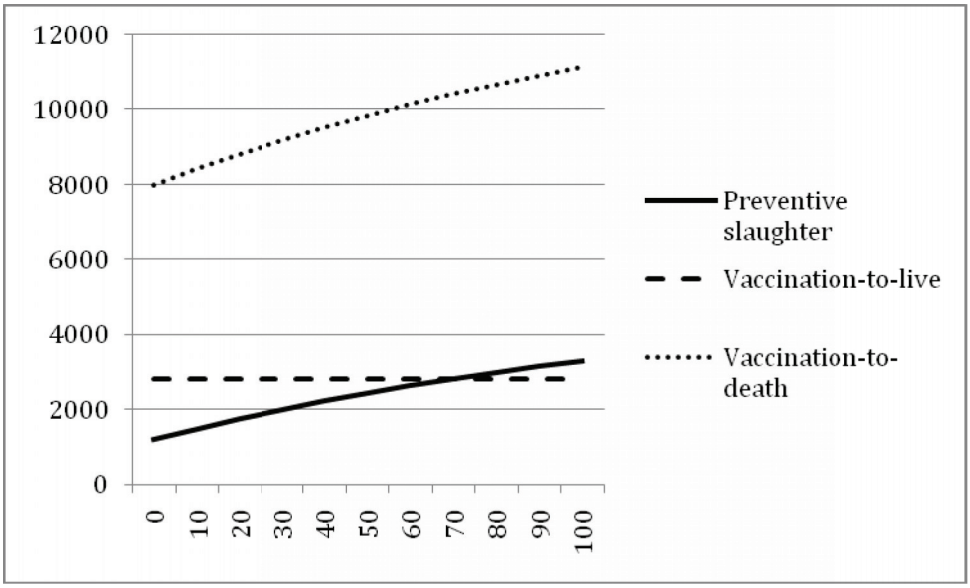


Figure 3 shows the preferred decision alternative changing to vaccination-to-live when all social value attributes are given a swing weight of 70 and the swing weights for the remaining attributes are reduced to 20.

Figure 3. Influence of changing swing weights for all social value attributes on the multi-attribute values for pigs, when reducing all remaining swing weights to 20.



DISCUSSION

Several exercises of extensive data analysis and mathematical modelling used data from the 2001 FMD epidemic in the UK as guidance to the development of control policies (Ferguson *et al.,* 2001; Kao, 2001; Keeling *et al.,* 2001; Morris *et al.,* 2001). In all cases rigorous statistical analysis was necessary to validate parameters, which is critical if these models are to be used as tactical tools (Kao, 2002). Complex statistical models often result in large confidence and prediction intervals. Most analyses of animal disease epidemic data relate to estimation of between-herd transmission ratio's (Stegeman *et al.* 1999b; Stegeman *et al.,* 2004) or incubation period distribution (Laevens *et al.,* 1998; Stegeman *et al.,* 1999a). These parameters can be useful to assess the efficacy of control measures once they are implemented but are not able to predict which measures would be best to use at the onset of an epidemic, given its specific characteristics. Moreover, decision makers are reluctant to use results from complex statistical models because they appear to them as 'black boxes', which they don't understand.

Data analysis tools used during disease epidemics should in the first place be informative and descriptive. During the 2006 bluetongue epidemic in Northern Europe, the European Food Safety Authority provided a number of descriptive data analyses which were very useful to the veterinary authorities involved in the epidemic. (Elbers *et al.,* 2008; Gerbier *et al.,* 2008; Hendrickx *et al.,* 2008; Mintiens *et al.,* 2008; Rodeia *et al.,* 2008). In the first instance, the MCDA for selecting the best alternative for reducing the population at risk during the FMD outbreak was very informative as it highlights the difference in numbers between the different alternatives. It becomes clear that selecting 'vaccination-to-death' will involve huge costs and large numbers of animals slaughtered, in contrast to selecting 'preventive slaughter' or

'vaccination-to-live'. This information on its own, without using SMART, is already of great help to decision makers.

The MCDA also adds transparency to decision making because it focuses on inputs that make a difference and highlights areas of dispute. The SMART is a robust approach to deal with the different attribute values, but it explicitly accounts for the values of the stakeholders. Moreover, all stakeholders become involved in the decision making process, which takes away the opportunity for them to give conflicting criticism. It is important that uncertainty is addressed when performing MCDA and is done by a sensitivity analysis.

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IMPACT ASSESSMENT

EFFECTIVENESS OF MEDICAL STRATEGIES COMBINING ANTIBIOTICS AND VACCINATION TO PREVENT *COXIELLA BURNETII* SHEDDING IN INFECTED DAIRY COWS

A.-F. TAUREL*, R. GUATTEO, A. JOLY AND F. BEAUDEAU

SUMMARY

The effectiveness of strategies combining vaccination and/or antibiotherapy to prevent vaginal shedding of *Coxiella burnetii* at parturition in dairy cows was assessed through a 13 months follow-up in 22 dairy herds. Four medical strategies were compared: vaccination, antibiotherapy, both, and none. Two hierarchical, logistic regression analyses were performed, considering vaccination in 2 (yes/no) or 3 modalities (no/before insemination/after insemination), respectively. Antibiotherapy (*i.e.* oxytetracycline, 20 mg/kg at each injection) was considered in 3 modalities (no/once at drying off/twice at drying off). The models were adjusted for serologic status at inclusion time, age at calving, and herd was included as a random effect. Among the 883 cows, 18.3% were detected as shedders. Antibiotherapy and initial serologic status were associated with shedding ($P<0.05$), whereas vaccination and age at calving were not ($P>0.05$). Two injections at drying off did not provide any additional benefit to one. This study provides first results for rational use of antibiotics in *Coxiella burnetii* infected herds.

INTRODUCTION

Coxiella burnetii is the infectious agent responsible for Q fever, a world wide spread zoonosis. The apparent prevalence of *C. burnetii* infection is quite important in domestic ruminants, with estimated mean value at animal and herd level of 20% and 37.7% in cattle and 15% and 25% in sheep and goat (Guatteo et al., 2011). In those species, Q fever can induce abortion and metritis (Tainturier, 1987). Moreover, domestic ruminants are recognized as the main source of human infection (Marrie and Raoult, 1997), which occurred after inhalation of infectious aerosols. Infected animals shed the bacteria mainly through birth products (e.g., placenta), but also through semen (Kruszewska and Tylewska-Wierzbanowska, 1997), vaginal mucus, urine, milk and faeces (Arricau-Bouvery and Rodolakis, 2005; Guatteo et al., 2007). Additionally, parturition period is considered as a period at risk, as ruminants have been reported to shed the highest bacterial load at that time (Berri et al., 2002). Therefore, any

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measure which aims to control *Coxiella burnetii* shedding in ruminants will improve animal health status and result in reducing the zoonotic risk.

Vaccination using a phase 1 vaccine was recognized to be effective in goats in an experimental study (Arricau-Bouvery et al., 2005) and in cattle when applied to susceptible (*i.e.* seronegative and non-shedder) animals before service (Guatteo et al., 2008). In cattle, nulliparous heifers, which were reported as quite systematically non infected even in infected herds (Taurel et al., 2011), are considered as a target population for vaccination. In contrast, a wide distribution of within-herd prevalence of antibody-carrier cows is observed in infected herds, making the use of vaccination questionable in adults, as vaccination does not prevent shedding when applied to infected animals.

In routine practice, antibiotics (mainly tetracycline) are classically used by practitioners to prevent shedding in infected animals. They are used at different regimens, at calving time to limit the shedding peak (Berri et al., 2001; Arricau-Bouvery et al., 2003) and/or at drying off to prevent late abortion. Few studies aiming at assessing its effectiveness have been performed up until now, in cows (Behymer et al., 1977) and in sheep (Astobiza et al., 2010a). These studies, which both included few numbers of animals subjected to a unique regimen, reported contradictory results.

The aim of this study was to compare the effectiveness of different medical strategies combining vaccination using a phase 1 vaccine and/or antibiotics (tetracycline) at drying off, to prevent *Coxiella burnetii* vaginal shedding at calving time in cows from commercial dairy herds clinically affected with *Coxiella burnetii*.

MATERIALS AND METHODS

Herds and animals

This work was conducted in compliance with the STROBE statement for cohort studies (von Elm et al., 2007). From February 2008 to May 2010, 22 dairy herds clinically affected by *Coxiella burnetii* were recruited. All dairy herds that fulfilled the following requirements were included in the study: detection of *Coxiella burnetii* by PCR on the placenta or in a vaginal swab of at least one aborting cow within the month before the possible inclusion and 50% of seropositive results in ELISA of at least 6 sampled cows (in line with the EFSA report (EFSA Panel on Animal Health and Welfare (AHAW), 2010); no implementation of vaccination directed against *Coxiella burnetii* within the previous five years; no tetracycline treatment for reproductive tract disorders, such as metritis or abortion, within the previous six months. Farmers had to sign a consent form and to give their agreement for cows to be vaccinated during gestation.

All dairy females older than 12 months expected to experience a calving in the 13 months following inclusion time were included in the follow-up.

Experimental design

Nature and allocation of treatments: At inclusion time, medical strategies were randomly allocated to each herd. Due to ethics concerns, and given the effectiveness of vaccination on

susceptible animals (Guatteo et al., 2008) and the susceptible status of nulliparous heifers (Taurel et al., 2011), the vaccination of nulliparous heifers was systematically performed regardless of the strategy for cows. Dairy cows within each of the selected herds were randomly assigned to 4 different strategies: (i) vaccination, (ii) vaccination and antibiotherapy, (iii) antibiotherapy without vaccination, or (iv) no intervention. Vaccination consisted in 2 injections 3 weeks apart and an annual booster injection, clustered in time with the vaccination of new eligible animals after one year follow-up (heifers of 12 months and more at booster time injection). The vaccine used was the phase 1 inactivated vaccine (Coxevac®, CEVA Santé Animale, ZI de la Ballastière, Libourne, France). The antibiotic used was oxytetracycline (Ténaline LA, CEVA Santé Animale, ZI de la Ballastière, Libourne, France). Within herds allocated to medical strategies including antibiotics, cows were randomly assigned to 5 different individual antibiotic regimens (20 mg/kg at each injection): antibiotic at (i) drying off, at (ii) calving, at (iii) drying off and calving, at (iv) drying off and 15 days later, or (v) drying off and 15 days later and calving.

In order to help farmers improving the compliance of the antibiotic strategies, the farmers received a reminder text message on their cell phone on the expected day of intervention (based on estimated date of drying off and calving provided by the milk recording scheme).

Sample and laboratory analysis: At inclusion time, in each selected herd, blood samples were collected by the practitioner from all animals older than 12 months, to determine their antibody carrier status. Samples were immediately sent to the laboratory (Institut Départemental d'Analyses et de Conseil, Nantes, France) to be tested using the Q fever LSI ELISA kit (LSI, Lissieu, France) according to the manufacturer's instructions. The results were expressed in an optical density Sample/Positive control (S/P) ratio. A serum sample was considered to be positive when the S/P ratio in serum was >40, and seronegative otherwise.

A vaginal swab was performed within 4 days after each calving on each included animals by the practitioner. The vaginal swabs were immediately sent to the laboratory (IDHESA Bretagne Océane, Quimper) to be tested using the real time PCR LSI TAQVET *Coxiella burnetii* kit (LSI, Lissieu, France) to detect putative *Coxiella burnetii* shedding. The results were expressed in number of bacteria per mL of vaginal mucus.

Strategy of analysis

The statistical unit was the cow. In a first model, vaccination was considered in 2 modalities, not vaccinated and vaccinated. As pregnancy status of animals have been reported to impact the vaccination effectiveness (Guatteo et al., 2008), in a second model the variable describing vaccination took into account the time of injections with regards to the presumed conceiving artificial insemination (AI), with 3 modalities: not vaccinated, vaccinated after AI, vaccinated before AI. In both models, only antibiotic injections at drying off were considered. It was assumed that there was not enough time between injection at calving and vaginal mucus sampling to observe a putative effect of antibiotics at calving on shedding. Antibiotic strategies were described through one variable with 3 modalities taking into account the number of injections: received antibiotic at drying off: no vs. at drying off vs. at drying off and 15 days later. Cows receiving antibiotic at calving were then considered as not treated, those receiving antibiotic at drying off and calving were considered as treated at drying off only.

The data structure was hierarchical (with cows clustered within herds). To assess the effectiveness of medical strategies to prevent *Coxiella burnetii* shedding at calving, we modelled

the probability of being shedder for a *j* cow from an *i* herd through a hierarchical, logistic model regression (Proc Glimmix, SAS® v. 9.2) with herd as the random factor, and taking account of individual adjustment variables (i.e. initial serologic status and age) (Eq. (1).

$$P(Y=0/1)_{ij} = \beta 1 * vaccination + \beta 2 * AB + \beta 3 * \text{age} + \beta 4 * serostatus + \mu * \text{herd} + \varepsilon \quad (1)$$

where $P(Y=0/1)_{ij}$ is the probability for a *j* cow of being shedder or not (yes =1, no=0) in an *i* herd, βs are parameter estimates of the fixed part of the model, μ the random herd effect, and ε the error term. The term *vaccination* represents the vaccination strategy (in 2 or 3 modalities depending of the model, 1 or 2 respectively), *AB* represents the antibiotic strategy, and *serostatus* represents the cow serological status at inclusion time. A binomial distribution and a logit link were used for the model. Two-order interaction terms among the variables describing the medical strategies (vaccination, antibiotics at drying-off) and with the initial serological status were tested. All variables with a *P*-value<0.05 were considered significantly associated with the shedding status at calving.

RESULTS

A sample of 883 calving cows with complete demographic data from 22 herds was considered for analyses. There were 57.8% of seronegative cows at inclusion time and 18.3% were detected shedders at calving time through the follow up. Herd characteristics are displayed in Table 1.

The risk of being detected shedder at calving time associated with the medical strategies, considering vaccination in 2 modalities (not vaccinated vs. vaccinated) is displayed in Table 2. Neither vaccination, nor age at calving was significantly associated with shedding status in cows. Antibiotics, especially once at drying off (OR 0.40, CI 95% [0.21-0.75]), and seronegative initial status were associated with a lower risk of being detected shedder at calving. No interactions between medical strategies, and between medical strategies and serological status at inclusion time, were found to be significant (P>0.05).

When considering vaccination in 3 modalities (not vaccinated vs. vaccinated after artificial insemination vs. vaccinated before artificial insemination), vaccination was once again not significantly associated with the risk of being detected shedder at calving time (vaccinated after AI OR 1.03, IC 95% 0.59-1.82; vaccinated before AI OR 0.63, IC 95% 0.28-1.40). Direction and magnitude of effects associated with the other variables did not change.

Table 1. Characteristics of the 22 herds clinically affected by *Coxiella burnetii* according to the implemented herd medical strategy

Variable	Herd medical strategy				Total
	Vaccination	Vaccination and antibiotherapy	Antibiotherapy	No treatment	
N. of herds	6	5	5	6	22
No. of cows/herd					
1st quartile	32	39	28	27	28
2nd quartile	40	51	34	31	35
3rd quartile	67	58	35	39	51
% of initially seropositive cows / herd					
1st quartile	32.3	17.9	21.4	46.4	21.7
2nd quartile	50.4	48.3	42.4	48.6	48.6
3rd quartile	54.3	71.4	39.4	50.0	55.6
% of shedder cows / herd					
1st quartile	13.4	5.1	14.3	5.7	5.7
2nd quartile	25.8	10.3	32.0	9.9	16.6
3rd quartile	29.6	19.0	35.7	21.4	29.6

Table 2. Risk of being detected shedder at calving time associated with cow characteristics and the medical strategy the cow received (883 dairy cows located in 22 herds clinically affected by *Coxiella burnetii*, mixed logistic regression)

Variable	N. of cows	% shedder cows	Risk of shedding		
			OR	CI 95%	*P*-value
Total	883	18.3			
Vaccination					0.83
No	448	17.0	1		
Yes	435	19.8	0.94	0.53-1.67	
Antibiotic					0.02
No	687	18.9	1		
At drying off	129	16.3	0.40	0.21-0.75	
At drying off and 15 days later	67	16.4	0.51	0.22-1.15	
Initial serologic status					<0.0001
Seropositive	373	26.0	1		
Seronegative	510	12.7	0.39	0.26-0.59	
Age at calving			0.93	0.84-1.04	0.21

DISCUSSION

To our knowledge, this is the first study that aimed at comparing the effectiveness of medical strategies, using vaccination and/or antibiotics at different regimens, to prevent *Coxiella burnetii* shedding at calving in dairy cows from clinically affected herds.

The originality of this study lies on its ambitious design. This study was performed under field conditions on a large number of herds and animals contrary to previous studies (Behymer et al., 1977; Woernle et al., 1985; Arricau-Bouvery et al., 2005; Guatteo et al., 2008; Rousset et al., 2009; Astobiza et al., 2010b; Cremoux et al., 2011). Additionally, the evaluated strategies (including up to 3 different antibiotic regimens) were applied within each herd to the whole population of cows, in contrast to recent studies based on split design and unique regimen (Guatteo et al., 2008), possibly leading to bias in estimating of medical strategies effectiveness. Lastly, contrary to previous studies conducted in cattle aiming to assess the effectiveness of antibiotics (Behymer et al., 1977; Woernle et al., 1985), the choice made here to sample vaginal mucus at calving time (instead of milk) and to use real time PCR (instead of serological methods), led to a more accurate description of the dynamics of *Coxiella burnetii* infection.

In the present study, in contrast to previous recent findings in goats and cattle (Guatteo et al., 2008; Hogerwerf et al., 2011) vaccination does not appear to prevent significantly individual shedding at calving time, although the estimated ORs were lower than unity when the cows were vaccinated before AI (Table 2). A possible explanation for this dissimilarity could rely on the less strict definition of the infectious status of cows in our study. In Guatteo et al (2008), given the putative *Coxiella burnetii* shedding patterns previously reported (Guatteo et al., 2007) (*i.e.*, no concomitancy in shedding routes, intermittent shedding), and the existence of seronegative shedders, the infectious status of cows was determined based on PCR results on 3 shedding routes (individual milk, vaginal mucus and faeces) explored concomitantly and ELISA responses on individual blood, all samplings being performed twice 15-days apart. In our study, the shedding status of cows was not tested, possibly leading to the existence of seronegative cows (considered as non-infected) but in fact shedders. A lack of sensitivity of ELISA result cannot be also excluded, although we used an ELISA based on ovine antigen recognized as the most sensitive ELISA (Horigan et al., 2011). Additionally, the unknown delay between the exposition to potential infectious aerosols and the determination of initial serological status, together with the delay (2 to 5 weeks) between the determination of the initial serological status and effective immunization of animals by vaccination, can have allowed the occurrence of undetected infection. This hypothesis about the lack of accuracy in the definition of the infectious status of cows was strengthened by the fact that we did not evidence any significant interaction between initial serologic status and vaccination, whereas it evidenced in previous studies (Guatteo et al., 2008; Cremoux et al., 2011). Thus the accurate determination of the infectious status of animals (towards shedding and antibody carriage) seems to be important to assess the effectiveness of vaccination. Moreover, a cow was considered to be shedder at calving time when the PCR was positive regardless the estimated bacterial load, while the reproducibility and the biological significance (infectiousness and viability) of very high Ct value (corresponding to <100 bacteria) are still debated (EFSA Panel on Animal Health and Welfare (AHAW), 2010).

Antibiotic, when used once at drying off (Table 1), was found to significantly prevent shedding at calving time, as reported in other study (Behymer et al., 1977). A second injection done 15 days later did not improve this preventive effect. In sheep (Astobiza et al., 2010a), antibiotic was reported to have no effect neither on prevention or duration of shedding. In this

latter study, only one antibiotic regimen was applied, and few animals were tested (50-60 sheep per sampling time). Given the need for a decreased use of antibiotics to limit the risk of antibioresistance, our results provide evidence for a reasoned use to avoid massive implementation of repeated and useless injection of tetracycline at drying off. Moreover, slight differences were observed in the overall proportions of animal detected shedders among the 3 antibiotic modalities: 18.9% in cows without antibiotics, and respectively 16.3% and 16.4% in cows with 1 or 2 injections at drying off. However, despite this slight difference on average, large variability existed between farms: the proportions of cows detected as shedders ranged from 3% to 38% depending on the herds. Thus, herd characteristics should be further investigated to identify and target herds where use of antibiotic could be of interest.

Moreover, as Q fever in ruminants is also described to be associated with clinical signs, such as metritis and abortion, studies are needed to highlight the impact of antibiotherapy on those clinical signs.

In this study, the focus is on shedding occurrence at calving as it is known as the period of higher risk of shedding. As *Coxiella burnetii* can be shed over the whole lactation period through different shedding routes (Berri et al., 2000; Guatteo et al., 2006), further field studies would be relevant to evaluate the effectiveness of medical strategies under study to control shedding over a longer period, especially through routes (faeces, urine and vaginal mucus) which mainly contribute to generate infective aerosols. Moreover, as a wide range can be observed in bacterial load shed (Guatteo et al., 2007), with possible implications in terms of environmental contamination and human and animal exposure (Courcoul et al., 2011), it would be interesting to assess the impact of medical strategies on shedding reduction, as it has been reported in small ruminants whatever the infectious status (Rousset et al., 2009; Cremoux et al., 2011; Hogerwerf et al., 2011). The identification of animals in which vaccination reduce shedding could reinforce the interest of vaccination in infected herds.

This study, conducted under field condition in naturally and clinically affected dairy herds, is the first aiming at assessing the efficacy of vaccination combined with antibiotherapy to prevent *Coxiella burnetii* shedding at calving. This study provides first results for the rational use of antibiotic in infected herds.

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ESTIMATION OF THE RELATIVE IMPACT OF TREATMENT AND MANAGEMENT FACTORS ON THE PREVENTION OF DIGITAL DERMATITIS BY SURVIVAL ANALYSIS

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SUMMARY

An interventional longitudinal study was conducted in 52 French dairy farms endemically affected by digital dermatitis (DD) to concurrently estimate the effect of treatment and management practices on DD incidence. Farms were allocated in one of four treatment regimens and followed 7 times every 4 weeks. Cox frailty survival models including time-varying covariates were used to estimate the effect of potential risk factors and treatment practices on the time until the first case of DD. These models identified that high initial DD prevalence strongly increased the hazard for DD occurrence, as well as absence of hoof-trimming, and poor leg cleanliness. "Prim'Holstein" and high-productive cows were found to be most likely to develop DD. Collective treatments tended to decrease the hazard for DD occurrence only when applied at least every fortnight, compared to only individual topical treatments. These results confirm the multi-factorial character of DD and provide useful data to design prevention programmes.

INTRODUCTION

While unknown before the 1970s, digital dermatitis (DD) is currently a major cause of lameness in dairy cows, reported as endemic in most of dairy herds in North America and Europe (Offer et al., 2000, Somers et al., 2003). It manifests by circumscribed erosive to ulcerative lesions around the coronary band, mostly observed between the heels on the hind feet (Read and Walker, 1998). This disease represents a real challenge for dairy farmers, as once introduced in a herd, it leads to recurrent and explosive outbreaks, with only anecdotal reports of eradication. This results in intensive labor to detect and treat affected animals, thus impairing herd productivity, increasing consumption of antibiotics and compromising welfare of affected animals (Bruijnis et al., 2010).

As DD is a multifactorial infectious disease, control strategies can be based on both herd management and medical approaches, which usually consists in applying collective treatments through walk-in footbaths (Laven and Logue, 2006). Despite several research studies to identify risk factors for DD (Holzhauer et al., 2006, Somers et al., 2005, Wells et al., 1999), and the

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development of commercial disinfectants for footbaths (Laven and Logue, 2006), there are two important limitations for improvements in the control of DD. First, there is a lack of knowledge of the effectiveness of farm control strategies derived from studies of risk factors, with a need to test whether some herd management practices associated with DD are causal and not only associated. Indeed, most of risk factors related to herd management have been identified through cross-sectional studies, which do not permit distinction between cause and effect. Second, despite their wide use in the field, there is a lack of knowledge of the effectiveness of collective topical treatments to prevent DD, including optimum regimen or influence of herd management practices on their effectiveness (Laven and Logue, 2006). This results in a huge variety of treatment practices in the field, often with unsatisfactory results (Laven and Logue, 2006, Relun, 2011). These practices may influence the spread of DD, and thus confound estimates of risk factors measured through observational field studies. Longitudinal studies, with intervention on treatment regimen, could therefore improve our understanding of the role that various risk factors and collective treatments play in occurrence and spread of DD.

There are several limitations associated with the use of common regression models for estimating relationship between herd management, treatment practices and occurrence of DD. First, the dynamic nature of some putative risk or protective factors, such as access to pasture or lactation stage, may change the risk of occurrence of DD over time. Typical regression models do not allow incorporation of time-varying exposure to these factors, which may lead to biased estimates. Another important limitation relates to the difficulty in incorporating censored observations, or data missing due to cows that are culled over the study period or have not yet experienced DD by the end of the study period. Survival models make use of information from both censored and non-censored cases to produce consistent parameter estimates (Cox, 1972). Additionally, the Cox model has the flexibility to include time-dependent explanatory variables (Beaudeau et al., 1995, Ducrocq et al., 2010).

This paper presents the use of a Cox's proportional hazard model with time-dependent covariates to estimate the relative influence of cow characteristics, farm facilities, herd management practices and treatment practices on the incidence of DD in endemically affected dairy herds.

MATERIALS AND METHODS

The study was designed to be a quasi-randomised, multi-arm, multi-site, controlled but not blind, field trial. All procedures were carried out under the agreement of the Ethics Committee for Animal Experimentation of Pays de la Loire (CEEA, France).

Study population

Fifty-two dairy farms located in west part of France were included in the study. This cohort was recruited through professional hoof-trimmers, veterinarians and technicians from the Animal Health Service (GDS: “Groupement de Défense Sanitaire”) and milk recording scheme (MRS). The number of farms included was determined by convenience criteria as the maximum number that could be followed, in order to implement the trial on farms with management practices as variable as possible. Farms had to meet with the following requirements: (i) experienced DD for over one year (endemic situation), (ii) milk their cows in a milking-parlor (location for DD scoring and DD treatments) and (iii) no plan to merge with another farm during

the study period (to avoid the massive introduction of cows). As far as possible, farmers had to participate in the national MRS, but one farm did not have any milk records. All cows in lactation during the trial were included in the study.

Most farms housed their cows in cubicles (45 farms) with solid or grooved concrete floors that were automatically or tractor scraped, with access to pasture in the spring season (46 farms). On average, the 305-day cow milk production was 8,937 kg (range: 5,357 to 12,636 kg) for "Prim'Holstein" cows, which represents more than 80% of the cows and 6,457 kg for "Normandy" cows (range: 3,369 to 9,513 kg). Cows were milked twice daily. In many farms, cows were trimmed preventively by a professional hoof-trimmer or by their veterinarian once (n=19) or twice a year (n=14). Before the trial, 50% of the farmers used collective topical treatments to control DD, either once a month (n=14), once every fortnight (n=4), once a week (n=4), daily for 3 weeks per month (n=1), or 'occasionally, when needed' (n=6). Only 3 farmers did not use any individual treatments against DD. At the pre-study visit, the median within-farm prevalence of DD was 10% (range from 0% to 37% of hind feet with an active DD lesion).

Treatment regimens and solutions

After one month applying only individual topical treatments, farms were quasi-randomly allocated in one of four treatment regimens and applied treatments over a 6-months period. In all the farms, for welfare and ethical reasons, farmers were expected to individually treat all active DD lesions that they detected, regardless of the treatment regimens to which they had been assigned, with a specific protocol. Regimen "Control" was the control regimen, without any collective treatment (17 farms). Other regimens comprised collective treatments differing by the way of application, *i.e.* footbath or collective spraying, with two frequencies of application for footbath regimens. Thus, under footbath regimens, cows walked through a split foot-bath after 4 consecutive milkings every 4 weeks ("FB/4W", 11 farms) or every fortnight ("FB/2W", 11 farms). In regimen "CS/2W", the front and the back of the hind feet of all lactating cows were sprayed with the disinfectant solution after 2 milkings 4 days apart every fortnight (13 farms). In the footbath regimens, 2 sub-groups were constituted to assess the effectiveness of a more aggressive initial treatment (footbath applied after 6 consecutive milkings every week during the 4 first weeks). For all treatments, farmers had to wash the hind feet with a water-hose at medium pressure before treating them.

The product used for collective topical treatments was a disinfectant solution, in which active ingredients are copper and zinc chelates (Hoof-Fit Bath® and Hoof-Fit Liquid®, Intracare, Veghel, Pays-Bas). With the recommended dilutions, namely 5% for the footbath solution and 50% for the spraying solution, dilution of copper and zinc were 3.5g/l and 0.5g/l respectively in the footbath solution and 20g/l for both ingredients in the spraying solution. The product used for individual topical treatment was oxytetracycline (OTC) (oxytetracycline chlorhydrate, 3 mg/ml, Oxytetrin P®, MSD Animal Health/Intervet, Beaucouzé, France) with a standard protocol: 2 applications on washed feet 2 days apart, one application consisting of 2 sprayings 15 seconds apart. This treatment could be repeated or the farmers could change the product, if the lesion was still active 10 days after the first application.

Treatments were allocated quasi-randomly on the basis of the proportion of hind feet detected with an active DD lesion (M1or M2, *c.f.* follow-up section) during the pre-study visit. They were assigned by minimising the imbalance between treatment for initial DD prevalence, with deviation from a random selection due to the implementation of footbaths being unfeasible on some farms and the unwillingness of some farmers to participate in some of the treatment

regimens. The median proportion and range of hind feet with an active DD lesion at randomization and before implementation of collective treatments are described in Table 1.

Table 1. Median proportion of hind feet with an active digital dermatitis (DD) lesion per farm at randomization and 4 weeks later before implementation of collective treatments

		At randomization		Before implementation of collective treatments	
Treatments[a]	No. of farms	No. of cows	Median farm prevalence[b]	No. of cows	Median farm prevalence[b]
Control	17	1,028	0.09 (0.03-0.37)	1,034	0.06 (0.02-0.16)
FB/4W$_i$	5	408	0.12 (0.07-0.23)	405	0.13 (0.05-0.22)
FB/4W$_{ni}$	6	562	0.09 (0.03-0.18)	579	0.13 (0.08-0.20)
FB/2W$_i$	6	377	0.07 (0.02-0.13)	381	0.10 (0.09-0.23)
FB/2W$_{ni}$	5	394	0.07 (0.00-0.18)	400	0.08 (0.03-0.28)
CS/2W	13	837	0.09 (0.04-0.24)	846	0.13 (0.00-0.21)

[a] i: submitted to initial aggressive treatment (after 6 milkings once a weeks over the 4 first weeks); ni: not submitted to the initial aggressive treatment

[b] Proportion of hind feet with an active DD lesion (the M1 and M2 stages with erosive to ulcerative circumscribed lesions) per farm; range in brackets

Follow-up and data collection

The farms were visited from November 2009 to October 2010 by 14 investigators, either veterinarians (n=8; 36 farms followed) or technicians specialized in health advising or hoof-trimming (n=6; 16 farms followed). As investigators had varied experience in DD detection and scoring, they were trained by the first author to score DD and leg hygiene during a training session held prior to the study. A second session was held halfway through the study (April 2010) to ensure the homogeneity of scorings. If an investigator's scorings were discordant from those of the other investigators during this session, i.e. if the mean kappa value of his scorings with those of other investigators was under 0.6, data of the farms that investigator followed, were removed from the entire analysis. Throughout the trial, all investigators had two cards to which they could refer when necessary, one for DD and one for leg cleanliness scorings, with concise definitions of each possible score and their representative pictures. As investigators were in charge of explaining the study to the farmers and of checking whether the protocol was observed, they could not be blinded to the treatment assigned to the farm they followed.

After the pre-study visit, each farm was visited 7 times, approximately 4 weeks apart, with one visit just before the implementation of collective treatments and 6 follow-up visits. Each visit was based on four steps: (1) scoring of the hind feet of all lactating cows for DD and leg hygiene during milking, (2) detection of moisture and dirtiness in housing and pastures in the pasture season, (3) checking any changes in management practices, and (4) checking compliance with the protocol.

DD status was assessed during milking using a telescopic mirror and a powerful headlamp as already described by Relun et al. (2011). The hind feet of all lactating cows were scored for DD using a 4-point scale based on the one first developed by Döpfer et al. (1997), which takes into account the stage of DD lesion development. M0 refers to normal skin, when no DD lesion was observed, M1 and M2 are the active stages with erosive to ulcerative circumscribed lesions

under (M1) or larger (M2) than 2 cm in diameter, and M4 is the chronic stage characterized by dyskeratosic or hyperkeratosic epithelium. In cases with more than one lesion on a hind foot, the most active prominent stage of DD lesion was recorded.

Leg cleanliness was assessed by scoring the hind legs during milking using a 4-point ordinal scale, varying from clean (score 1) to very dirty (score 4), as described by Cook (2006) and Schreiner and Ruegg (2002).

Detailed information about housing characteristics and management practices were obtained using a questionnaire filled in by the investigator and farmer together during the pre-study visit. Changes in these practices were checked during each visit. Dates of collective and individual treatments were recorded by the farmers throughout the study. Compliance with the protocol was checked by completing a questionnaire with the farmer during each visit. Data on health disorders, hoof care, movements of cows from the lactating herd and pasture management also were documented by the farmers, with a record of the identification number and the feet affected when necessary. These data were collected by the investigator on each visit. The farmers had to report any adverse effects observed to the corresponding author. Within 15 days after each visit, they were informed about the proportion of affected feet, but were not told which cows were affected so as not to interfere with their own detection of DD lesions. They had the option of withdrawing from the study at any time, if the situation deteriorated.

Data on breed, 305-day milk production, parity and lactation stage were obtained from the MRS.

Data analysis

All data were initially entered in a Microsoft Access database (Microsoft Corp., Redmont, WA). New variables were built from the raw data using SAS 9.1.3® (SAS Institute Inc., Cary, NC). Farm leg cleanliness was calculated at the farm level as the proportion of feet scored ≥3 at each visit with 3 levels (good: < 25%; fair: 25-50%; poor: > 50%) based on the levels defined by Cook (2006). Interventionism of the farmer for DD individual treatment was calculated at the farm level as the mean proportion of feet scored with an active DD lesion (M1 or M2) at each visit that had been treated individually by the farmer between this visit and the next visit. This variable was used as categorical variable with 3 levels (low: ≤ 10%; medium: 11-50%; high: > 50%) based on the distribution of the variable.

The preventive effectiveness of treatments and management practices was evaluated on the first occurrence of active DD lesions through survival analysis with a hind foot as the statistical unit. Survival analysis was carried out using the Survival kit® v6.0 (Ducrocq et al., 2010). For the purpose of analysis, an active DD lesion was defined as an early or acute DD stage (M1 or M2) on a hind foot. Feet included in the analysis were those considered to be with no active lesion the first time they were observed, i.e. with no DD lesion (M0) or with a chronic DD lesion (M4). The outcome variable tested in the model was time to occurrence in days. The occurrence date was determined as the first date a foot was observed with an active DD lesion. If the foot was still healthy at the end of the follow-up period or if a cow was culled over the study period, the record was considered to be censored at this date. Feet observed with consecutive visits spaced more than 45 days were removed from analysis.

For survival analysis, we used a Cox proportional hazard model (Cox, 1972) that included a joined farm and investigator frailty effect to adjust for clustering within farms and investigators.

All factors considered as potential effect modifiers of the cure rate were included as covariates in the Cox model. They are listed in Table 2.

Table 2. Overview of farm, cow and foot related potential effect modifiers tested as covariates in the Cox proportional hazard frailty model

Level	Factors
Farm	DD prevalence at 1st visit, grazing system, herd size, housing system, farm leg cleanliness[a,b], purchase of dairy cows, proportion of heifers, interventionism of farmer for DD individual treatment[c]
Cow	Breed, parity[b], stage of lactation[b], grazing[b], 305-day milk production[d], leg hygiene score[c]
Foot	Initial stage of DD, hoof-trimming, active DD lesion on controlateral foot

[a] Proportion of feet scored ≥3 at each visit

[b] Time-dependant covariates

[c] Mean proportion of feet scored with an active DD lesion at a visit n and treated individually by the farmer between visit n and n+1

[d] Adjusted for breed and parity

The model was written as described by Eq. 1

$$\boldsymbol{\lambda(t, X(t)) = \lambda_0(t) \times \exp\left\{\sum_{i=1}^{p1} \beta_i X_i + \sum_{j=1}^{p2} \delta_j X_j(t) + U_{kl}\right\}} \quad (1)$$

where the term $\lambda(t,X(t))$ is the hazard function at time t, i.e. the probability for a foot to be observed with an active DD lesion at t, given that it is "healthy" prior to t. The term $\lambda_0(t)$ is the unspecified hazard function, i.e. the baseline hazard function, in the absence of covariates. $\beta_i X_i$ describes the effect of the ith time-independent covariate, $\delta_j X_j(t)$ describes the effect of jth covariate which may vary over time and U_{kl} denotes the joined kth farm and lth investigator random effect, which is normally distributed. Five factors were included as time-varying predictors: leg cleanliness at farm and cow levels, parity, stage of lactation and grazing at cow level. Leg cleanliness could change on each visit and other factors could change at any day of the follow-up. An adjusted relative hazard ratio (HR) was estimated for each covariate from the hazard function by taking the exponent of the estimates of effects. This HR measures the instantaneous risk for a foot to become ill when exposed (e.g. to treatment regimen FB4W) versus being unexposed (e.g. control regimen).

The survival analysis was planned in 3 steps: in a first step, only subjects without missing data were included. If some variables did not contribute to the final model, a second analysis was performed including subjects with missing data for these variables. If the sub-groups of treatment regimens did not significantly differ ($FB/4W_i$ *vs*. $FB/4W_{ni}$, $FB/2W_i$ *vs*. $FB/4W_{ni}$), a third analysis was performed considering only four treatment regimens: Control, FB/4W, FB/2W and CS/2W. For each analysis, treatment regimens, all covariates and biological relevant

selection, all models were checked for confounding, which was assumed to occur when estimates changed by more than 20% when a variable was removed from the model. In such a case, the incriminated variable was forced into the model even if it did not significantly contribute to it. The proportional hazards assumption and the goodness-of-fit of the final model were checked by graphic procedures. The proportional hazards assumption was evaluated by plotting log−log survival functions against time (Kalbfleisch and Prentice, 2002). Final model fit was assessed by evaluating the distribution of the Cox-Snell residuals (Cox and Snell, 1966).

RESULTS

Over the follow-up period, the hind feet of 4,677 cows have been inspected between 1 to 7 times. Four farmers asked to change treatment in the middle of the trial (huge increase of DD in their herds that were assigned to FB/4W). The frequency of footbath application was increased and the farms were followed until the end of the trial but data from their cows were truncated from analysis from the date of discontinuation. After exclusion of data that did not fit inclusion criteria (*i.e.* more than 45 days between visits; foot scored by one investigator, whose scorings were divergent from others; change in treatment regimen), 5,598 hind feet were free of active DD lesion at their first observation. Among these feet, 948 (17%) experienced at least once an active DD lesion, with a median time before first case of DD of 146 days (Q1: 60 d.; Q3: 169 d.), and a mean incidence rate of 4 cases for 100 feet-months. Main characteristics of incidence of DD per treatment regimen are presented in Table 3.

Table 3. Characteristics of occurrence of active DD lesions over the 24 weeks follow-up period in the 52 farms of the study for each treatment regimen

Treatment[a]	Monthly incidence rate[b]	% of new DD lesions[c]	Time before 1st occurrence of an active DD lesion (days)		
			Median	1st quartile	3rd quartile
Control (n=1,917)	4	18	84	53	125
FB/4W$_i$ (n=656)	6	22	57	28	77
FB/4W$_{ni}$ (n=778)	7	21	28	28	84
FB/2W$_i$ (n=491)	3	12	147	83	167
FB/2W$_{ni}$ (n=572)	3	13	60	33	119
CS/2W (n=1,184)	3	14	59	28	107

[a] i: submitted to initial aggressive treatment (after 6 milkings once a weeks over the 4 first weeks); ni: not submitted to the initial aggressive treatment
[b] Number of new active DD lesions (M1 or M2) per 100 foot-months
[c] Over 24 weeks of follow-up

As there was no significant differences in the Cox analysis between sub-groups (FB/4Wi vs. FB/4Wni and FB/2Wi vs. FB/2Wni), only results of the Cox analysis with the four treatment regimens, i.e. Control, FB/4W, FB/2W and CS/2W, are presented hereafter. Eleven factors were associated with a risk for DD at a 20% significance level in the univariate analysis, 4 at farm level (treatment regimen, leg cleanliness, initial DD prevalence, proportion of calving heifers), 4 at cow level (breed, lactation stage, milk yield level, access to pasture) and 3 at foot level (DD

initial stage [M0 or M4], presence of an active lesion on controlateral foot and hoof-trimming). After adjustment, 7 factors remained significantly associated with a risk of DD (Table 4).

Table 4. Multivariate Cox regression analysis to time to 1st occurrence of active DD lesion on hind feet

Item	No. of feet	Multivariate analysis	
		HR (95% CI)	*P*-value
Treatment regimen			0.009
Control	1,917	Reference	
FB/4W	1,434	1.70 (0.97-2.96)	
FB/2W	1,063	0.75 (0.42-1.33)	
CS/2W	1,184	0.63 (0.35-1.13)	
Farm leg cleanliness[a,b]			0.001
Good	3,451	Reference	
Fair	1,515	1.54 (1.27-1.88)	
Poor	631	2.42 (1.82-3.21)	
Initial farm DD prevalence			0.001
M1+M2 < 0.10	3,154	Reference	
M1+M2 ≥ 0.10	2,444	2.09 (1.34-3.23)	
Breed			0.01
Prim'Holstein	5,115	Reference	
Normande	352	0.51 (0.27-0.94)	
Others	131	0.43 (0.18-1.01)	
Milk yield level[c]			0.05
Low	1,175	Reference	
Medium	2,945	1.24 (1.03-1.49)	
High	1,478	1.26 (1.01-1.56)	
DD initial stage			<0.0001
No DD lesion (M0)	3,641	Reference	
Chronic DD lesion (M4)	1,957	2.69 (2.34-3.10)	
Active DD on controlateral foot			<0.0001
Yes	1,131	1.72 (1.50-1.97)	
No	4,467	Reference	
Hoof-trimming			<0.0001
Yes	563	Reference	
No	5,035	1.76 (1.36-2.27)	

[a] Proportion of hind feet scored ≥ 3 (good: < 25%; fair: 25-49%; poor: ≥50%)
[b] Time-dependant covariates; number of feet at inclusion in the analysis
[c] Milk yield level estimated on 305 days production, levels having been determined on terciles per breed and lactation range

High initial DD prevalence strongly increased the incidence risk of DD (Fig. 1), as well as poor leg cleanliness and absence of hoof-trimming. Collective treatments tended to delay DD occurrence only if they were applied for 2 days every fortnight, whatever the way of application (Fig. 2). At cow level, Prim'Holstein breed and high-producing cows were more likely to develop DD compared to Normandy or other breeds, and low-producing cows. A cow with an active DD lesion on controlateral foot was also more likely to develop a DD lesion compared to cows free of DD on both feet. At foot level, a foot initially observed with a chronic DD lesion

(M4) was more likely to develop DD than a foot initially free of DD (M0). None of the interactions analyzed (treatment regimen x initial DD prevalence; treatment regimen x leg cleanliness; treatment regimen x interventionism of the farmer) was statistically significant in the multivariable model.

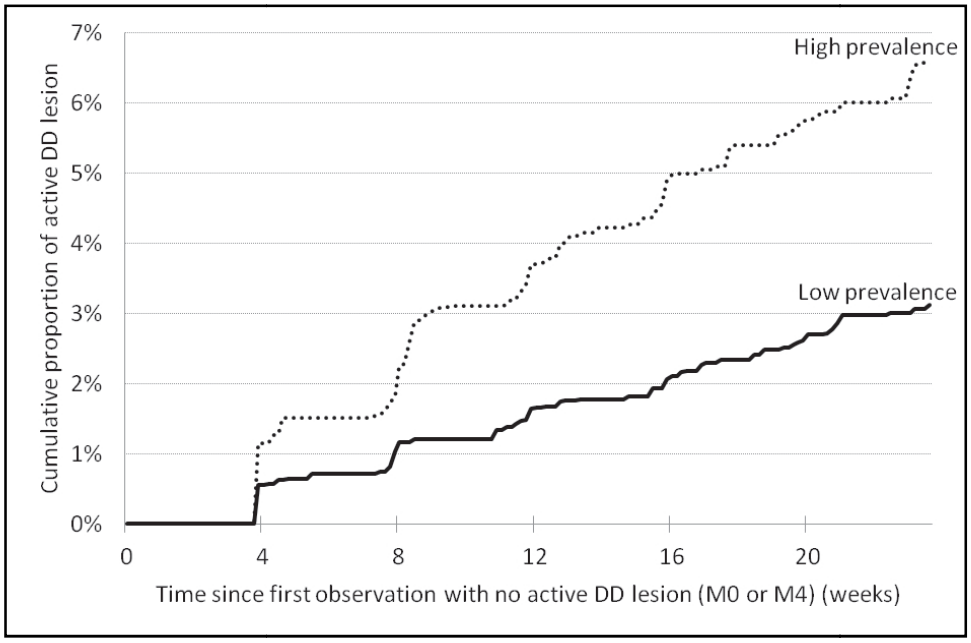

Figure 1. Cumulative proportion of DD occurrence on hind feet initially free of active digital dermatitis for farms with high (≥ 10% of hind feet with an active DD lesion) or low (<10%) initial DD prevalence over the 24 weeks follow-up period

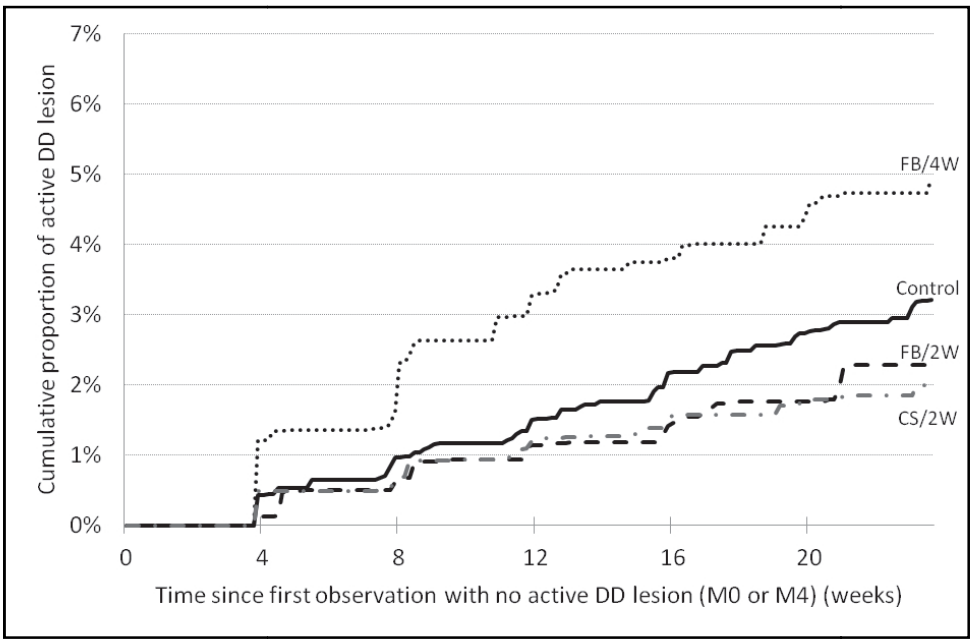

Figure 2. Cumulative proportion of DD occurrence on hind feet initially free of active digital dermatitis for each treatment regimen over the 24 weeks follow-up period

DISCUSSION

The purpose of this study was to concurrently determine the relationship between several risk factors, collective treatment practices and first case of DD using a Cox proportional hazard frailty model. Results confirm the multi-factorial character of DD, in which initial prevalence, environmental conditions, cow characteristics, management and treatment practices were found significantly associated with DD incidence.

Until now, effects of herd management and treatment practices on DD incidence were assessed separately, through observational studies and clinical trials respectively. The originality of this study was to combine a longitudinal study and a quasi-randomized controlled field trial conducted on a large scale. This allowed having reliable data on both effectiveness of different collective treatment practices and risk factors for occurrence of DD. In return, a DD scoring method which could be used frequently without disturbing farmers work had to be applied. This method was less accurate than DD scoring in a trimming chute (Relun et al., 2011). The resulting non-differential misclassifications may thus have underestimated the estimates for all variables (Dohoo et al., 2003).

Results of this study highlighted that one of the most influential factor on DD incidence was leg cleanliness. Wet and unhygienic environment had already been suggested as a key factor in the etiopathogenesis of DD through cross-sectional and experimental infection studies (Gomez et al., 2011, Wells et al., 1999). Measuring cleanliness directly on animals was a good way to measure the factor that directly impact occurrence of lesions of DD, namely cleanliness of the feet, rather than attempting to identify farm practices that are responsible for this cleanliness. Indeed, leg cleanliness depends on many factors, including housing design, scraping procedures, bedding comfort and grazing management, which effects may be interconnected and difficult to individualize (Cook, 2002). Previous studies gave some indication on factors that may increase risk of DD through unhygienic conditions, such as cubicles, grooved concrete floor and limited access to pasture (Somers et al., 2005, Wells et al., 1999). In the present study, impact of housing type on DD incidence was not observed, but this can be due to a lack of statistical power, the vast majority of animals being housed in cubicles. Despite the precision of exposure to pasture permitted using the variable ‘access to pasture’ as time-dependent, we did also not identify access to pasture as influencing DD incidence. This effect may partly have been correlated with the cleanliness of feet at herd level and thus could not be demonstrated. However, when the factor ‘leg hygiene’ was removed from the model, the impact of access to pasture not yet emerged as significant in the multivariable model (data not shown). This effect may exist, but it may have been too small, too variable, or need quite a long time before its effect on DD occurrence would become evident.

The second factor impacting the most DD incidence was initial prevalence of DD measured by the proportion of feet with an active DD lesion before the implementation of collective treatments. This observation was not too surprising, since DD lesions are currently considered the main source of *Treponema*, the main infectious agent involved in DD. However, no interaction was found between this prevalence and treatment regimen, limiting the recommendations of a regimen adapted to the observed prevalence.

Among herd management factors, hoof-trimming was found to reduce risk of DD. In a cross-sectional study, Somers et al. (2005) reported that long intervals between hoof-trimming were associated with increased DD prevalence. In the present study, we observed that hoof-trimming reduced the risk of DD occurrence. Two hypotheses can be put forward: either the

animals have actually been affected by DD between two observations, then treated against DD at the time of trimming and recovered quickly so that the lesion was no longer active at next visit, or trimming may have improved the conformation of the feet, which increased the resistance of animals to DD (Nuss, 2006).

Among cows' characteristics, three factors were found to limit risk of DD incidence: Normandy breed, absence of active DD lesion on the controlateral foot and low milk yield level. Other studies already found a decreased risk of DD for local breed and for cows at the end of lactation (Holzhauer et al., 2006, Somers et al., 2005). This suggests differences in individual susceptibility to DD, which may be related to hoof conformation, level of immunity or integrity of the skin. Even if heritability of DD seems to be quite low (Onyiro et al., 2008), it could be interesting to identify genetic determinants for DD in order to improve natural resistance against DD.

Although usually considered a cured lesion (Döpfer et al., 1997, Holzhauer et al., 2008), it was found that feet with chronic DD lesions (M4) were more likely to develop DD than feet free from DD (M0). This may be due to the fact that interdigital space cannot be inspected when the feet are not lifted. Thus, feet which seems to have a chronic DD lesion may also have an acute lesion in the inter-digital space, conducting to resurgence of active DD lesions from the inter-digital space (Relun et al., 2011). This observation could also support findings of previous studies that suggested that lesions that appear clinically cured may not be bacteriologically cured since *Treponema* were detected by PCR in the deep layer of the dermis of such lesions (Döpfer et al., 1997). It would be interesting to know whether *Treponema* identified in such lesions are still alive or not, in order to clarify whether a lesion whose clinical appearance suggests a recovery is really bacteriologically cured.

In the present study, collective treatments tended to limit DD occurrence when applied for 2 days every fortnight, whatever the way of application used, *i.e.* footbath or collective spraying in the milking parlor. Treatment regimens had been chosen to be compatible with farmers' organization. Indeed a recent survey (Relun, 2011) revealed that many French farmers were reluctant to use a footbath and when they use it, they tended to use it once a month or even less frequently due to the time needed to implement such treatments and the cost of the products. Even if not significant, these results are better than those of previous studies which found no effectiveness of collective treatments without formalin or copper sulphate on DD prevention, the treatment being mainly applied 2 days a week for 4 to 8 weeks (Thomsen et al., 2008). However, in these studies, either there was no negative control group (Teixeira et al., 2010) or the control group was one of the two hind feet, which may have decreased the potential effectiveness of tested treatments (Thomsen et al., 2008). Indeed, when assessing effect of an intervention on an infectious disease, cluster-interventions are preferred to individual interventions in order to estimate the true effect of the intervention (Vaucher, 2009). Holzhauer et al. (2008) reported that while a weekly application of a 4% formalin solution through footbath (after 2 consecutive milkings) failed to completely prevent the occurrence of new lesions, this treatment regimen had greatly limited DD incidence compared to other treatment regimens using collective treatments with other active compounds or lowest frequencies. Additional studies should therefore be conducted to assess if more frequent applications or application of other disinfectants could improve preventive effectiveness of collective treatments.

Using Cox proportional hazard model proved to be a powerful tool to identify risk factors for DD and assess the effectiveness of different treatment regimens. Such models have been often used in biomedical research to assess effect of treatments on fatal or incapacitating

diseases, or of vaccines in case of infectious diseases (Fleming and Lin, 2000, Guatteo et al., 2008). In addition, in veterinary research, such models have been used to assess effect of diseases on reproductive performances or culling (Beaudeau et al., 1995, Nusinovici et al., 2011). This study showed that survival analysis can also be useful in epidemiological research to identify risk factors for diseases for which delaying time to occurrence is an important outcome (*e.g.* infectious or incapacitating diseases), particularly when exposure to these factors may vary over time. Thanks to recent advances in software, Cox models can be easily programmed, even with large data sets, frailty effects and time-dependant covariates (Ducrocq et al., 2010). The main difficulty is to correctly estimate the exposure time-window for those time-dependant covariates. This choice should be based on scientific data, the lower bound being determined on time between exposure and the first measurable effect and the upper bound on the duration of this effect (Dohoo et al., 2003).

In conclusion, implementing an interventional study on a large scale and analyzing the data with a Cox model appeared to be a relevant approach to estimate the concurrent effects of treatment and management practices on DD incidence. It was found that, in addition to initial prevalence, some management factors strongly influenced DD incidence, particularly leg cleanliness, and, in a lower extent, hoof-trimming. Some cows' characteristics, such as Normandy breed and low-productive cows, were identified to be less likely to develop DD compared to Prim'Holstein and high-productive cows, respectively, suggesting differences in host susceptibility. Applying collective treatments using a disinfectant solution of chelated minerals tended to delay DD occurrence, but they may require more frequent applications than those tested in this trial to really limit DD incidence.

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DISTRIBUTION OF MEAT INSPECTION LESIONS IN FINISHER PIGS BEFORE AND AFTER THE INTRODUCTION OF THE YELLOW CARD ANTIMICROBIAL SCHEME IN DENMARK

L. ALBAN[*], J. DAHL, M. ANDREASEN, J.V. PETERSEN AND M. SANDBERG

SUMMARY

Potential associations were investigated between lesions found at meat inspection and changes in antimicrobial consumption and vaccine use due to the yellow card scheme recently introduced in Denmark. Data originated from Vetstat. Moreover, data from meat inspection of finisher pigs from before and after introduction of the scheme were compared, including 1.7 million finisher pigs. Logistic regression models were used to identify whether the prevalence of selected lesions changed from 2010 to 2011. For osteomyelitis, pleuritis, chronic arthritis and condemnation, no differences were observed. Chronic enteritis, umbilical hernia and chronic peritonitis were statistically more frequent in 2011 compared to 2010 ($p<0.01$), whereas tail infection, chronic pneumonia and chronic pericarditis were less frequent ($p<0.01$). The higher prevalence of specific lesions found during meat inspection might be related to the marked reduction in use of antimicrobials, and higher coverage of vaccines might have had efficacy against development of chronic pneumonia.

INTRODUCTION

There is concern about the development of bacteria that are resistant to the antimicrobials commonly used to cure infections in both humans and livestock. However, it might be speculated that animal health might deteriorate if diseased animals are not treated, hereby jeopardizing animal welfare. Antimicrobials are in general used to treat ill animals or humans, but they might also be used as prophylactic agents. Flock treatment of pigs is an example of a situation where both ill pigs as well as apparently non-affected pen mates which are at risk of developing disease are treated. A third way of using antimicrobials is as growth promoters, because several types of antimicrobials are known for resulting in increased average daily weight gain and a higher feed conversion ratio. However, long-term use of low doses of antimicrobials in a large proportion of the animals in a herd might result in the development of antimicrobial resistance.

In Denmark, much attention is on the consumption of antimicrobials in pigs, because pigs account for 79% of the total consumption of antimicrobials in livestock (DANMAP, 2010). This reflects that pig production is the predominant animal production in Denmark involving production of 28 million finisher pigs annually, while the total population of cattle is less than

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two million. The amounts of antimicrobials used per livestock unit are low in Denmark compared to other countries with a similar pig production (European Medicines Agency, 2011). This is the result of actions taken over the years to mitigate the risk related to antimicrobial resistance. Among others, use of antimicrobials for livestock is by prescription only, and all prescriptions are filed into a database called Vetstat (http://www.uk.foedevarestyrelsen.dk/AnimalHealth/Veterinary_drugs/forside.htm).
The veterinary practitioner makes the prescriptions at the level of the species and the age group, and this is how it is recorded into the database. For pigs, the age groups are 1) sows and piglets, 2) weaners, and 3) finisher pigs). Moreover, the indication is given (e.g. gastrointestinal or respiratory disease) and the type of antimicrobial substance.

In July 2010, further reductive actions were implemented due to an increase in the consumption of antimicrobials in the Danish pig production. The actions consisted of a voluntary ban on use of cephalosporins in Danish swine herds for a 2-year period initiated by the Danish pig industry, as well as a so-called "yellow card scheme" from the Danish government. The voluntary ban on use of cephalosporins in pigs is unique in the world. As long as there other effective antimicrobials for treatment of infections are on the market, banning cephalosporins should in principle not reduce the possibility to treat diseased pigs.

According to the yellow card scheme, restrictions are put on farmers who use more than twice the average consumption of antimicrobials in one of the age groups. By the end of 2010, this corresponded to approximately 10% of the pig farmers. The consumption in pigs is evaluated as animal daily doses (ADD) per 100 animals reported to Vetstat over the last 9 months by age group. Permit limits by December 2011 for a yellow card in ADD/100 animal days depend on the age group. It is 5.2 ADD for sows and piglets, 28 ADD for weaners, and 8 ADD for finishers. An example of a yellow card evaluation (in Danish) can be found here: http://www.foedevarestyrelsen.dk/SiteCollectionDocuments/25_PDF_word_filer%20til%20download/05kontor/Kort_Laesevejledning_VetstatGultkort.pdf.

In July 2010, farmers with an antimicrobial use close to these limits received a letter from the Danish Veterinary and Food Administration which stated that unless actions were taken to reduce the antimicrobial use, the producer would receive a yellow card by December 2010. A yellow card would result in requirements for various measures like restrictions on oral medication usage and supervision from the authorities, all at the expense of the farmer. The warning resulted in a decrease in the national antimicrobial consumption in pigs of 13 % during the last half-year of 2010 compared to the same half-year in 2009. Furthermore, the consumption in January-February 2011 was 25% lower compared to January-February 2010 (Andreasen et al., 2011).

In Sweden and Denmark the use of growth promoters have been banned for years (Wierup, 2001; Aarestrup et al., 2001). In the European Union, a prohibition of use of antimicrobials as growth promoters came into force on January 1, 2006 (EU Commission, 2005). In countries outside the European Union it is being debated whether similar actions should be taken, and what the implications would be of restrictions on the use of antimicrobials, see for example the US Government Accountability Office (2011). The health of the pigs is a cause of concern, and it has been suggested that pig health might deteriorate if too many severe restriction are put on use of antimicrobials, and this might be seen during meat inspection as an increase in the prevalence of selected lesions.

Vaccines are often used to maintain health in livestock. They are costly in particular when they are applied routinely and therefore frequently only used if they are found to be cost-effective for the farmer. In some instances vaccines can be used as alternatives to use of (prophylactic) antimicrobial treatment, in other instances not. In pigs, vaccines are commonly used e.g. against erysipelas, *Mycoplasma* lung infections and parvo virus infection.

The recent introduction of the yellow card scheme in Denmark might be viewed as a large experiment - and from this intervention it might be possible to learn what impact a marked decrease in use of antimicrobials will have on pig health. Therefore, the aim of this study was to look closer into the use of antimicrobials and vaccines against different diseases in pigs in Denmark in 2010-2011 and compare data from meat inspection of finisher pigs from before and after the introduction of the scheme to elucidate the potential impact of a decrease in use of antimicrobials. Meat inspection data have their inherent weaknesses (like variation in meat inspectors' ways of recording), but were the best available at the time for the purpose of our study.

MATERIALS AND METHODS

Data describing use of antimicrobials and vaccines in Danish pigs

Data describing the monthly use of antimicrobials used for Danish pigs covering the time period between January 2010 and July 2011 were obtained from Vetstat. Consumption was described in kg and not in ADD because it is easier to relate to and compare between countries. Data were divided according to the three age groups: 1) sows and piglets, 2) weaners, and 3) finisher pigs).

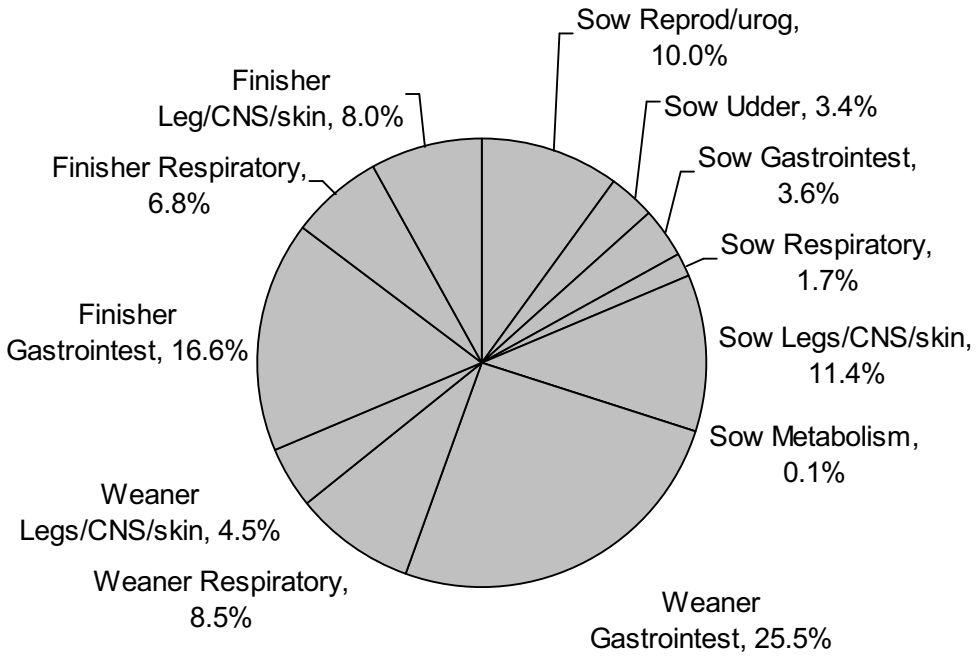

Fig. 1 Relative distribution of antimicrobials used in Danish pig production divided according to age group and indication, January 2010 – July 2011. Source: Vetstat, the Danish Veterinary and Food Administration.

Figure 1 presents the relative distribution of prescribed antimicrobials during the chosen time period. Prescriptions for treatment of gastrointestinal and respiratory disorders accounted for 62% of the antimicrobials used. Only these two indications were included in the further analyses.

Moreover, data on vaccine usage were provided by the Vetstat administration covering the time period from the third quarter of 2009 to the second quarter of 2011. These data are not routinely summarised in Vetstat, and they were therefore evaluated by 3-month periods. The vaccines were divided into groups according their presumed effect; and here a list developed by the main Danish supplier of vaccines, Dianova, was used (Dianova, 2011). The numbers of doses of vaccines sold were summarised and divided according to effect on 1) gastrointestinal infections, 2) respiratory infections, 3) Porcine circovirus type 2 (PCV2) related infections, or 4) other infections.

Meat inspection data

In Denmark, lesions found during meat inspection are routinely recorded into a database belonging to the abattoir. A pre-defined set of codes are used, and any abnormality observed will be recorded by the meat inspector and filed into the database. More than one lesion might be identified on a carcase, and based on the lesions found it will be determined whether the outcome will be local or total condemnation. The determination is based on the current meat inspection circular issued by the Danish Veterinary and Food Administration.

The data used for the present study included 1.7 million finisher pigs slaughtered on one large Danish abattoir. This covered all finisher pigs - implying a weight of less than 110 kg - slaughtered at that abattoir between the first and the ninth week in year 2010 and 2011, respectively. Hereby, variation between abattoirs as well as seasonal variation was ruled out. Only lesions of assumed chronic nature and infectious origin were included in the analyses.

The following nine lesions which can be found at meat inspection were judged as having relevance for pig health: chronic pleuritis, chronic pericarditis, chronic pneumonia, tail bite infection, osteomyelitis, umbilical hernia, chronic peritonitis, chronic arthritis, and chronic enteritis. Each of these lesions was included as the dependent variable in a separate statistical model. Moreover, one separate model was made with “condemned” vs. “not condemned” as the dependent variable. This included any reason for total condemnation of the carcase. Hence, a total of 10 “lesions” were included in the analyses.

Statistical analysis

The decrease in consumption of antimicrobials for each combination of the three age groups and the two indications treatment of gastrointestinal and respiratory disorders was assessed; first by plotting and visually inspecting the data over the entire time period from January 2010 to July 2011. Next, a straight line was fitted to the data by use of Microsoft Excel®, and the R^2 was calculated. Based on the equation for the straight line, the relative decrease in consumption was calculated for a 12 month period. A similar exercise was made for the vaccine data, where data covering the time period July 2009 to July 2011 were available.

The prevalence of each of the selected meat inspection lesions was calculated. Logistic regression analysis was conducted in order to investigate whether the prevalence changed from 2010 to 2011 in SAS® version 9.2 by use of the GENMOD procedure. The dependent variable was the number (y) of a specific lesion divided by the total number of carcases (n) slaughtered

per week in the nine week period in 2010 and 2011, respectively. Hence, the dependent variable describes the weekly prevalence (y/n) of a lesion in carcases from a herd. Year and week were included as explanatory variables in the models. Week was inserted to account for the variation that is known to occur between weekly deliveries of finisher pigs. In order to assess linearity week was plotted against the prevalence of each of the lesions. If a visual inspection revealed a linear association, then week was included as a continuous variable in the regression analysis, if not it was included as a class variable if statistically significant. Animals being raised by the same producer were expected to be more similar than animals originating from different producers. To take this into account, a REPEATED statement was used, where producer was specified as the subject. A compound symmetry (CS) correlation structure was used when modelling the correlation of the responses within the herds. This implies that the correlation between observations is assumed to be constant. A first order autoregressive correlation structure was also tried and used if it provided a better fit than CS. In datasets that are very large, statistical significance is easily obtained. Moreover, there were 10 different models, which would make it easier to obtain statistical significance by chance compared to only having one model. Therefore, a significance level of 1% was applied.

For the carcases that were condemned, a specific analysis was conducted to assess whether the specific lesions involved differed statistically between the 2 years. Here, a χ^2-test was conducted for each of the selected lesions. For these analyses, the biological importance of an association was evaluated on top of the statistical significance test by use of the Odds Ratio (OR). Only associations, where OR> 2 or OR< 0.5, and where more than 20 carcases were condemned in total over the two years were judged as having a biological significance.

Finally, it was assessed whether an identified increase or decrease in prevalence plausibly could be biologically ascribed to the yellow card initiative or not. This assessment was based on current knowledge about pig diseases as well as discussions with meat inspection experts and veterinary practitioners working with pig herds.

RESULTS

Decline in use of antimicrobials in Danish pigs between 2010 and 2011

Viewed over a smoothened 12-month period, the use antimicrobials for treatment of gastrointestinal disorders decreased by 25% in weaners (R^2=0.83) and by 24% in finisher pigs (R^2=0.82), whereas the use remained constant in sows and piglets (R^2=0.79) (Fig. 2).

A decline in use was also seen for treatment of respiratory disorders, and here the decline occurred in all three age groups: for weaners (R^2=0.65) and finishers (R^2=0.46), each by 21%, and for sows and piglets by 35% (R^2=0.56) (Fig. 3). Other treatments that plummeted were against skin, leg and CNS diseases (data not shown).

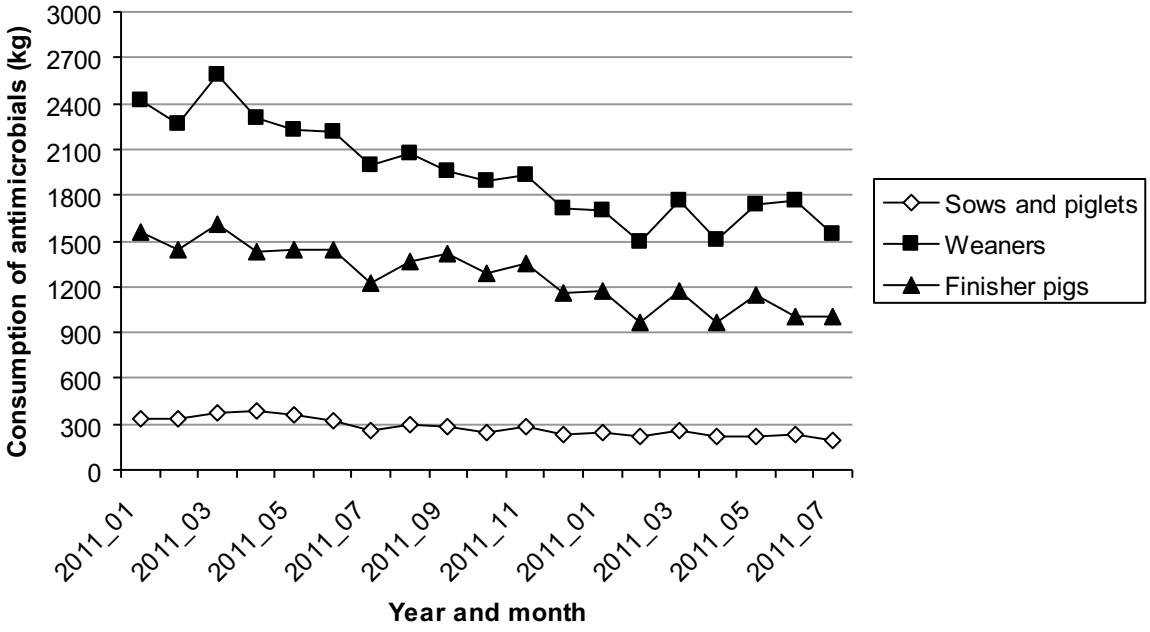

Fig. 2 Development in monthly consumption of antimicrobials for Danish pigs for treatment of gastrointestinal disorders, divided according to age group, January 2010 to July 2011. Source: Vetstat, the Danish Veterinary and Food Administration.

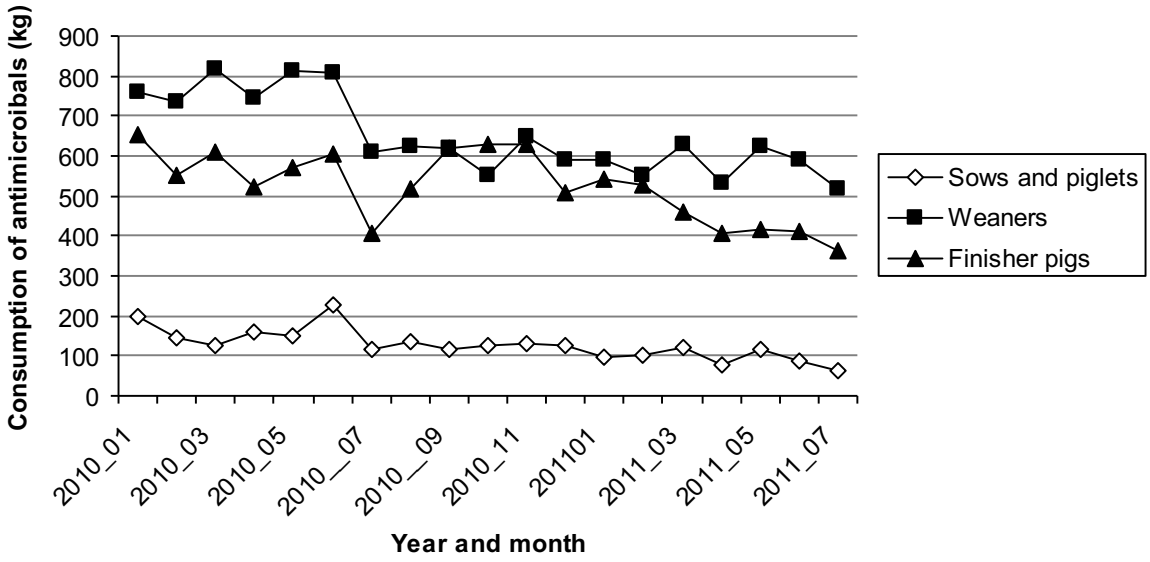

Fig. 3 Development in monthly consumption of antimicrobials for Danish pigs for treatment of respiratory disorders, divided according to age group, January 2010 to July 2011. Source: Vetstat, the Danish Veterinary and Food Administration.

General increase in use of vaccines in Danish pigs between 2010 and 2011

An increase was seen in the use of vaccines for prevention of respiratory infections, gastrointestinal disorders as well as PCV2-related infections. Viewed over a smoothened 12-month period, the use of vaccines against PCV2-related infections increased by 90% from the third quarter of 2009 to the second quarter of 2011 (R^2=0.82), for gastrointestinal disorders by 43% (R^2=0.80) and for respiratory disorders by 21% (R^2=0.92) (Fig. 4). The use of vaccines against other infections (e.g. erysipelas) declined slightly over the time period (-7%) (R^2=0.15).

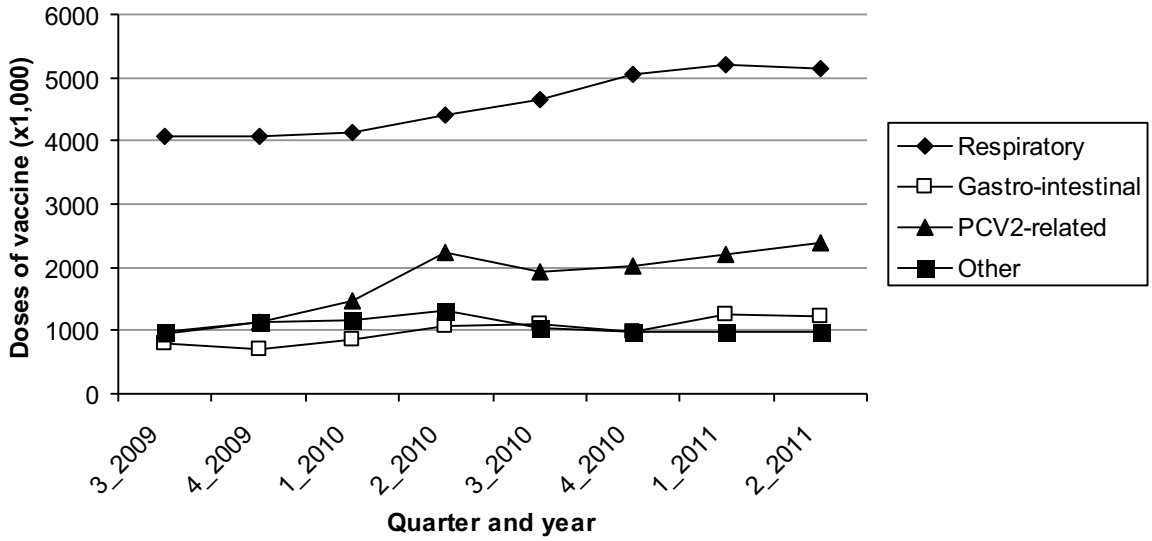

Fig. 4 Development in number of vaccines doses sold, divided according to indication and used in Danish pig production between third quarter of 2009 and second quarter of 2011. Source: Vetstat, the Danish Veterinary and Food Administration

Description of meat inspection data and univariable associations

A total of 2,765 farmers delivered finisher pigs to the abattoir in the two time periods. A total of 85% of the pigs included in the analyses originated from herds that delivered finisher pigs to the abattoir in both years. The lowest number of pigs delivered per producer to the abattoir was one and the highest number was 6,475 finisher pigs – when evaluating both years in one.

The most common lesion recorded was chronic pleuritis (23.9% in 2010 and 23.2% in 2011). The other selected lesions occurred less commonly; all below 1% prevalence. The univariable analyses revealed strong statistical associations between the probability of each of the lesions and year (P<0.01), except from the lesion chronic arthritis (P=0.49) (Table 1).

Table 1. Univariable associations between selected lesions recorded at meat inspection during slaughter of 1.7 million Danish finisher pigs at one large abattoir in the first 9 weeks of the year 2010 and 2011, respectively, presented as number of cases and prevalence (%)[a]

Lesion	No. of cases and prevalence (%) in		Unadjusted RR	*P*-value for association
	Winter 2010	Winter 2011		
Tail bite infection	2,390 (0.29)	1,530 (0.16)	1.81	<0.0001
Chronic pericarditis	1,172 (0.14)	785 (0.08)	1.75	<0.0001
Chronic arthritis	1,576 (0.29)	1,739 (0.19)	1.53	0.49
Chronic pneumonia	2,337 (0.28)	1,871 (0.20)	1.40	<0.0001
Chronic pleuritis	197,508 (23.90)	216,401 (23.16)	1.03	<0.0001
Osteomyelitis	2,631 (0.32)	3,244 (0.35)	0.91	0.0009
Condemnation	1,350 (0.16)	1,704 (0.18)	0.89	0.0025
Chronic enteritis	2,616 (0.32)	3,501 (0.37)	0.86	<0.0001
Umbilical hernia	2,185 (0.26)	3,008 (0.32)	0.81	<0.0001
Chronic peritonitis	1,719 (0.21)	3,014 (0.31)	0.68	<0.0001

[a] The prevalence was calculated as number of cases recorded divided by the total number of pigs slaughtered (N) in each 9-week time period, where N in 2010 = 826,334, and N in 2011 = 934,191

The proportion of condemned carcases varied between 0.16% and 0.18% in the two time periods. In 2010, the most common lesions found on condemned carcases were osteomyelitis (22.2%), tail bite infection (17.8%) and chronic pneumonia (13.6%). Chronic pneumonia, plummeted as a lesion found in condemned carcases in 2011 compared to 2010 (1.1% versus 13.6%, OR=0.07). Other lesions that decreased significantly were: pleuritis (OR=0.28), enteritis (OR=0.45), whereas umbilical hernia increased significantly (OR=2.94) as a reason for condemnation.

Multivariable associations

The results of the multivariable statistical analyses are presented in Table 2. For the following lesions a statistical significantly higher prevalence was seen in 2011 compared to 2010: chronic enteritis (OR=1.2), umbilical hernia (OR=1.2) and chronic peritonitis (OR=1.6). Likewise, a significantly lower prevalence was observed in 2011 compared to 2010 for: tail bite infection (OR=0.5), chronic pneumonia (OR=0.7) and chronic pericarditis (OR=0.6). For osteomyelitis (OR=1.09), pleuritis (OR=0.97), chronic arthritis (OR=0.98) and condemnation (OR=0.97), the prevalence remained constant between the two periods.

In the models describing chronic peritonitis, chronic pericarditis, and tail bite infection, week was included as a class variable ($P<0.01$).

All models were re-run by use of a subset of data consisting of the 85% of the finisher pigs that originated from herds that delivered pigs in both years. The difference in parameter estimates are minor and the conclusions remained the same.

Table 2 Results of analyses of effect of year in 10 hierarchical logistic regression models* describing association between specific lesions found at meat inspection of 1.7 million Danish finisher pigs at one large abattoir during the first 9 weeks of year 2010 and 2011, respectively – sorted by Odds Ratio.

Lesion	Evaluation of meat inspection findings in 2011 compared to 2010				
	Parameter estimate	Odds Ratio	Standard Error	95% Confidence limits	*P*-value (Wald's test)
Chronic peritonitis	0.4381	1.55	0.0364	1.44-1.66	<0.0001
Umbilical hernia	0.2036	1.23	0.0360	1.14-1.32	<0.0001
Chronic enteritis	0.1765	1.19	0.0381	1.11-1.29	<0.0001
Condemnation	0.1138	1.12	0.0582	1.00-1.26	0.047
Osteomyelitis	0.0878	1.09	0.0387	1.01-1.18	0.020
Chronic arthritis	-0.0179	0.98	0.0468	0.90-1.08	0.702
Chronic pleuritis	-0.0336	0.97	0.0309	0.91-1.03	0.266
Chronic pneumonia	-0.3604	0.70	0.0853	0.59-0.82	<0.0001
Chronic pericarditis	-0.5196	0.59	0.0747	0.51-0.69	<0.0001
Tail bite infection	-0.6070	0.54	0.0607	0.48-0.61	<0.0001

*: The dependent variable consisted of the number of finisher pigs (y) in which a specific lesion was recorded at meat inspection divided by the number of pigs (n) sent for slaughter from that producer in a week. A REPEATED statement was used, where producer was specified as the subject. In the models describing chronic peritonitis, chronic pericarditis, and tail bite infection, week was included as a class variable ($P<0.01$).

DISCUSSION

This study compared data from large official registers on use of antimicrobials and vaccines and meat inspection recordings. However, use of antimicrobials or vaccines in individual herds was not compared with the prevalence of lesions found at meat inspection at herd-level. Therefore, only indications of biological associations can be derived. In order to investigate these further, cohort studies should be performed, e.g. where antimicrobial and vaccine data are available at the level of the individual farm or from cohorts of pigs during a period prior to slaughter. Moreover, other variables such as SPF-status and herd size might be included.

Nevertheless, the results were based on a large number of slaughtered pigs. Denmark is a relatively small country with an indoor pig production which is very homogenous and covers around 25 million pigs of which around 7 million are exported as 30 kg pigs. This study included all pigs slaughtered on one of the large abattoirs, which is located in Jutland where the majority of Danish pigs are raised. This abattoir accounts for 25% of the finisher pigs slaughtered in Denmark, and it only slaughters finisher pigs from integrated production system where the animals are raised under controlled housing conditions.

The antimicrobial consumption and vaccine use data were summarised for Denmark as a whole. Antimicrobials and vaccines are used intermittently and individually during the 6-month life of a finisher pig, making it difficult to relate findings at meat inspection to time of treatment. Despite these limitations, these data are of higher quality than similar data from most other countries, because they are collected monthly for each of three age groups compared to a total sales data over a year for the entire animal population; see e.g. Anon. (2010). In line, the European Medicine Agency estimates the average use of antimicrobials in a country by dividing

the annual sales figure by the estimated weight at treatment of livestock and of slaughter animals in the corresponding year (European Medicines Agency, 2011).

Other factors might confound the association between use of antimicrobials and vaccines and lesions recorded at meat inspection; this includes type of feed, indoor and outdoor climate, herd size and presence of different diseases within the herds, and these factors vary between the farms and to some extent between the years. Such factors could not be included in this study. In summer 2010, the yellow card scheme became known to all pig farmers: 20% of the pig farmers received a warning letter from the Danish Veterinary and Food Administration by July 2010 saying that unless the individual farmer changed his management/housing he would receive a yellow card by December 2010 (DANMAP, 2010).

According to the analyses, a significantly higher prevalence of chronic enteritis, umbilical hernia and chronic peritonitis was seen in 2011 compared to 2010. However, the increase was not large in absolute terms, because the odds ratios are between 1.2 and 1.6 and the prevalence of each of these lesions was below 1% in both years. The increase in prevalence of chronic enteritis might be explained by a reduction in the use of antimicrobials due to the yellow card scheme. During the same period, the use of vaccines for prevention of gastrointestinal disease increased; apparently not with a fully protective effect on the population, but most likely with a possible effect in individual farms. The increase in the prevalence of umbilical hernia and chronic peritonitis might be explained by a reduction in metaphylactic treatments of neonatal umbilical infections, which were previously commonly conducted in Denmark.

A significantly lower prevalence of chronic pneumonia and chronic pericarditis was observed in 2011 compared to 2010. Mild cases of chronic pneumonia might not be recorded during meat inspection. This probably explains the general low prevalence found in both years. It is, however, remarkable that the prevalence decreased in 2011 compared to 2010, and that the lesion chronic pneumonia was found in a lower proportion of the condemned carcases in 2011 compared to 2010. This occurred at the same time as, the use of antibiotics went down and the use of vaccines with an effect on pneumonia increased, so it is possible that the yellow card scheme might have had an indirect positive effect on the health of pigs by encouraging more focus on prevention of pneumonia.

The lower prevalence of chronic pericarditis might also be explained by the observed increase in use of vaccines against respiratory infections as well as against PCV2-related infections.

There was an increase in the use of vaccines with effect on respiratory infection. However, chronic pleuritis occurred equally common in both years. Only one vaccine with effect on *Actinobacillus pleuropneumoniae* is registered officially in Denmark. Full protection against *Actinobacillus pleuropneumoniae* cannot be guaranteed with the vaccines presently available. Housing conditions, immunity levels among the sows, virus infections and vaccination procedures are elements that influence on the effect or lack of effect seen (S.E. Jorsal, Personal comment).

A statistical significant lower prevalence of tail bite infection was observed in 2011 compared to 2010. Tail bite infection is a complex issue where several factors play a role, and no attempts shall be made here to explain the lower prevalence seen. The interested reader is kindly referred to review papers about tail biting e.g. Schrøder-Petersen & Simonsen (2001).

It was assumed that the ten selected lesions - because of their chronic nature - could be used to recognize lack of treatment of the associated diseases. For each lesion, a simple model was made consisting of week and year as explanatory variables. Moreover, herd was included in a REPEATED statement to take into account that animals produced by the same producer are more alike than animals produced by different producers. PROC GENMOD in SAS was used because it is able to deal with both REPEATED statements and responses that are proportions (y/n). However, model validation is not possible in such a model. A Homer-Lemeshow test was considered, however, this was impossible when a REPEATED statement is used. Therefore, no model control was conducted.

The current permit limits for antimicrobial use for the yellow card scheme were set by Danish Veterinary and Food Administration based on an intention to focus on the herds with the highest use. Naturally when the consumption decreases the permit limit of twice the average consumption will decrease. Therefore, when considering the future permit limits, a thorough assessment should be conducted in order to find a suitable permit limit where the animal health and welfare is not jeopardized. The amounts of antimicrobials used per pig are already low in Denmark compared to other countries with a similar pig production (European Medicines Agency, 2011), and so is the prevalence of resistant zoonotic bacteria in both human and animal isolates (DANMAP, 2010). In line, the food safety risk related to use of antimicrobials in Danish pigs is very low – if resistant strains of zoonotic bacteria on the meat surface are killed by heat treatment or other treatment as effectively as their sensitive counterparts. As long as the existing hygienic measures are kept in place, both in primary production and at slaughterhouse level – the food safety risk related to antimicrobial resistant strains of zoonotic bacteria on meat will remain negligible. Therefore, a further decrease in use of antimicrobials in production animals in Denmark might have limited impact on food safety. The existing low use of antimicrobials in Denmark has an effect on the total release of resistant strains of zoonotic bacteria into the whole environment as such, reflected in the general low prevalence of zoonotic bacteria in meat and humans (DANMAP, 2010). However, strict measures in one country's animal production have a limited impact on the general release of resistant strains, when animals, humans and meat are moved freely between European Member states as well as in and out of the European Union. Initiatives regarding prudent use of antimicrobials in humans and livestock on a European or global level are therefore welcomed.

No estimates of the effect of the yellow card scheme on the productivity of the pig production have been made so far, because the necessary data have not been collected yet. It is possible that the food conversion ratio has gone up and the average daily weight gain has gone down. This was e.g. seen after the ban on antimicrobial growth promoters in 1999 in Denmark (Maribo et al., 2006).

The Danish and Swedish experience is that banning antimicrobial growth promoters required an increased focus on production conditions - including quality of feeding - to counteract a negative effect on productivity (Wierup, 2001, Vigre et al., 2008). This will probably also be the lessons learnt with respect to the yellow card scheme. In line, the slight increase in the prevalence of chronic gastroenteritis observed in the present study might be a short-term reaction to the legislative changes in ways of using antimicrobials. Such an effect might disappear when farmers and veterinarians get used to the new conditions that they are working and producing under e.g. by applying other risk-mitigating measures. Aarestrup et al. (2010) have argued that long-term productivity in Danish pig production was not affected negatively by the ban on use of antimicrobials as growth promoters. However, more than 80% of the Danish pig farmers stopped producing over the time period which was included in the study

by Aarestrup et al. (2010). It is most likely that farms with low productivity are more at risk of ceasing production; hence, the development in productivity over several years cannot alone be used for evaluating the effect of an intervention.

Care should be taken before extrapolating the effect observed in our study to other pig-producing countries; both because of the limitations of the study related to the type of data included and because the majority of the Danish pig production is taking place in a SPF-system with generally high health status.

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RISK ANALYSIS

A STOCHASTIC MODEL TO IDENTIFY BREAK-EVEN COMPENSATION VALUES FOR INDIVIDUAL FARMERS COMPLYING WITH A TEST AND SLAUGHTER PROGRAM FOR BRUCELLOSIS IN EGYPT

W. BEAUVAIS*, Y. HEGAZY AND J. GUITIAN

SUMMARY

Compliance is critical to the success of control programs, particularly for zoonotic or chronic diseases, as farmers may not perceive a direct benefit in vaccinating or culling animals. The effects of test and slaughter schemes on individual farmers are subject to stochasticity and this is more profound with smaller herds. Using the Egyptian brucellosis test-and-slaughter program as a case study, we developed a stochastic simulation model to determine break-even compensation values for individual farmers, using a partial analysis framework. According to the model assumptions, current compensation would be sufficient for approximately 45% of farmers to break even, but would need to be increased by a factor of approximately 26 for 90% of farmers to break even. The results indicate that break-even compensation for individual households is highly variable and uncertain and there is likely to be a significant degree of financial risk for individual smallholders participating in test-and-slaughter programs.

INTRODUCTION

Compensation in animal disease control programs should be sufficient to cover direct, indirect and intangible net-costs (Yanhong Jin, 2006). Inadequate compensation was identified as a key factor in under-reporting of disease during the Egyptian avian influenza outbreak (May, 2007) and increased reporting of scrapie was noted following an increase in compensation offered in the UK (Bohning & Del Rio Vilas, 2009). If compensation is too high, there may be no incentive for farmers to use preventative measures; farmers may purposefully procure infected animals or falsify infection status (Yanhong Jin, 2006). Variability and uncertainty in the costs and benefits of disease control, for individual farmers, are therefore key challenges for policy makers. There is scant information available on the financial impact of disease control programmes on smallholders in the Middle East and elsewhere. Understanding this impact and setting appropriate incentives could be critical to the control of diseases such as brucellosis in Egypt and further afield.

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The World Health Organisation (WHO) has described brucellosis as a neglected zoonosis (World Health Organization, 2011). Human brucellosis can be incapacitating, causing chronic fever and arthralgia. It is frequently undiagnosed or misdiagnosed, and difficult to treat (Jennings et al., 2007, FAO, 2009). The main routes of transmission are contact with the fluids of infected animals, and ingestion of unpasteurized milk and milk products (Gillespie, 1988). The economic impacts of brucellosis on the agricultural sector are insidious - subfertility, abortion, reduced milk production and neonatal mortality in domestic species are the direct effects (Gillespie, 1988). However, the disease also restricts access to international markets and causes sub-optimal herd structures by reducing availability of replacement animals (Rushton, 2008, Roth et al., 2009). Less tangible costs are the emotional value, social status and financial security attached to domestic animals that may be culled as a result of control measures or due to infertility (Behnke, 1987).

Brucellosis is often a particular problem in rural communities of endemic countries. Households reliant on small numbers of animals for income and food supply are particularly vulnerable to the unpredictable nature of disease events such as abortion and infertility, and to official test and slaughter programs (Rushton, 2008).

Vaccination and / or test and slaughter of livestock are feasible means of brucellosis control, despite some limitations (European Commission, 2001). Eradication has been achieved in several countries, although in general *Brucella (B.) abortus* was the main species and commercial farms were predominant, whereas in the Egypt *Brucella melitensis* is considered to be the predominant species, and most livestock are owned by smallholders (Hugh-Jones et al., 1975, Radunz, 2006). An economic model predicted that mass vaccination of livestock in Mongolia, where *B. melitensis* predominates, would be cost-effective, taking into account agricultural and public health sectors, with a benefit cost ratio of 3.2 (Roth et al., 2003). Furthermore, by sharing the costs between agricultural and public health sectors, a cost of US$19.1 per disability-adjusted life year (DALY) averted was estimated (Roth et al., 2003). These estimations do not take account of the indirect and intangible effects of disease. In addition, control measures themselves have indirect and intangible effects: the time taken by farmers is often valued at zero, and the stress and emotional costs involved, particularly in slaughtering animals, should also not be underestimated (Roth et al., 2003, Peck, 2005).

The majority of Egyptian livestock are owned by individual households. Cattle and buffalo are usually kept in small numbers and moved daily between the household and plots of grazing land (Holt et al., 2011). Small ruminants mix freely within the villages (personal communication, H. Holt). Some larger mobile flocks are also kept, and there are a small number of government and privately-owned commercial dairy units (Hegazy et al., 2005, personal communication, Y. Hegazy).

Brucellosis is endemic at high levels in Egypt; the main isolate is *B. melitensis* biovar 3. Although representative estimates are scarce, a recent survey found 11-12% of small ruminants were seropositive (Hegazy et al., 2011). The current Egyptian brucellosis control strategy involves compulsory six-monthly testing of all cattle, sheep, buffalo and goats over six-months-old using the Rose Bengal and complement fixation tests, in series. Positive animals are slaughtered, and yield a reduced price at the slaughterhouse; positive holdings should be disinfected, quarantined and re-tested every 21 days until three consecutive negative tests are declared. Vaccination is voluntary (Hegazy et al., 2005, personal communication Y. Hegazy).

The apparent poor success of this programme is, at least in part, explained by poor compliance. In a recent study less than 7% of female livestock were tested in one year and less than 66% of positive herds were re-tested in the subsequent 2 months. A transmission model showed current testing frequency is insufficient to reduce prevalence in sheep and goats below 8% (Hegazy et al., 2005). Furthermore, selling animals which recently aborted to markets or butchers appears to be common (Holt et al., 2011). Anecdotal reports suggest quarantine and disinfection are rarely practised (personal communication, M. El Tholth).

Inadequate compensation is cited as a key reason for poor compliance, and may be a source of conflict of interest for government veterinarians since they are responsible for carrying out the program in their local communities but procure the majority of their income providing private veterinary services to the same communities. In addition, there is a lack of resources to carry out testing on such a large scale every six months (Holt et al., 2011, personal communication, Y. Hegazy).

The aims of this study were to describe the uncertainty and variability in break-even compensation for individual households in Lower Egypt participating in a test-and-slaughter programme for brucellosis and to identify critical factors affecting its value.

MATERIALS AND METHODS

For the purposes of this study, a hypothetical village was considered. Two scenarios were simulated:

- Scenario 1: No testing takes place in the village in which the household resides.
- Scenario 2: All animals in the village are tested every 18 months.

Discrete 18-month time intervals were used and testing occurred at the end of each time interval, during which sero-status was assumed not to change. A partial analysis framework was used: *NPV* was calculated by subtracting the discounted net benefits under Scenario 1 from the discounted net benefits under Scenario 2, for individual households.

Uncertainty and variability in the parameters were incorporated simultaneously, by inputting distributions, using @RISK 5.7 for Excel (Palisade Corporation Inc., Newfield, NY, USA). Input parameters were based on i) a recently conducted survey of Egyptian households, ii) the outputs of a transmission model of brucellosis in Lower Egypt, iii) published literature, iv) interviews conducted with two final-year Ph.D. students, both with experience of veterinary work in Egypt and brucellosis research and v) a self-administered questionnaire sent to a veterinarian working in Lower Egypt (Hegazy et al., 2009, Holt, et al., 2011).

Module 1: Village prevalence

Initial village prevalence took three different values: 0.05, 0.1 or 0.15. For scenario 1, seroprevalence was assumed to remain constant over time. For scenario 2, seroprevalence was obtained from a previous stochastic disease transmission model (Hegazy et al., 2005).

Module 2: Individual-level production parameters

Seronegative cattle and buffalo: A negative binomial distribution with parameters (s, p) was used to model the number of abortions that occur prior to a live calf in seronegatives Na^n, where s is the number of live calvings (equal to one), and p is the probability of a live calving in pregnant seronegative dams Pc^n. The distribution was truncated at the maximum number of consecutive abortions per animal before it is culled, M (Table 1). Pc^n was calculated as follows:

$$Pc^n = 1\text{-}Pa^n \quad (1)$$

where Pa^n is the probability of an abortion in pregnant seronegative dams (Table 1).

The calving interval for seronegatives I^n, was then calculated as follows:

$$I^n = Na^n \times Ta + Tg \quad (2)$$

where Ta is the time taken for a cow to conceive and then abort, and Tg is the time taken for a cow to conceive plus gestation length (Table 1).

The mean rate at which calves are born per 18 months to seronegatives R^n, was calculated as follows:

$$R^n = d / I^n \quad (3)$$

where d is the number of days in 18 months.

The number of calves per animal was then modelled separately for each individual as a Poisson distribution with parameter λ equal to R^n. The distribution was truncated at the maximum number of calves per time interval (for buffalo, one, and for cattle, two). It was assumed that all large animal births were to single offspring. The milk produced per lactation in seronegative animals L^n, was modelled as a betapert distribution (Table 1).

Seropositive cattle and buffalo: For *Brucella* positive cattle and buffalo the rate of offspring production and milk production per lactation were calculated in the same way as for *Brucella* negative animals, except they were multiplied by $(1\text{-}D_o)$ and $(1\text{-}D_l)$ respectively, where D_o is the proportional decrease in live offspring attributable to *Brucella* seropositivity (incorporating subfertility, abortion and neonatal mortality) and D_l is the proportional decrease in milk production per lactation attributable to *Brucella* seropositivity (Table 1).

Table 1. Parameter values for Module 2

Parameter	Symbol	Species	Value [a]
Number of consecutive abortions before a dam is replaced.	M	Cattle, Buffalo	Betapert (1,2,3) (rounded to a whole number)
Proportion of conceptions which abort (seronegatives)	Pa^n	All species	Betapert (0.02, 0.05, 0.08)
Time (in days) from abortion or calving to conception (seronegatives) and from conception to abortion	Ta	Cattle	Betapert (45,90,365) + Uniform (56, 283)
		Buffalo	Betapert (335,427,730) + Uniform (90, 320)
Time (in days) from abortion or calving to conception (seronegatives) and from conception to parturition	Tg	Cattle	Betapert (45,90,365) + 283
		Buffalo	Betapert (335,427,730) + 320
Proportional reduction in offspring production attributable to seropositivity	D_o	All species	Betapert (0.1;0.15;0.5) (Bernuès et al., 1997, Roth, et al., 2003)
Proportional reduction in milk production attributable to Brucella seropositivity	D_l	Cattle, buffalo	Betapert (0.1;0.15;0.25) (Bernuès et al., 1997, Roth, et al., 2003)
Milk produced per lactation in seronegative animals / 1000 litres	L^n	Holstein cattle	Betapert (5,6,7)
		Native Cattle	Betapert (2.5, 3, 3.5)
		Buffalo	Betapert (1.5, 2, 2.25)

[a.] Unless references are given, the values are from the questionnaire or interviews.

Module 3: Household-Level Production Parameters

Within each iteration, the household level production parameters were modelled twice: once for Scenario 1 and once for Scenario 2, as follows.

Cull animals: The total number of cull animals for Scenario 1 was equal to zero. For Scenario 2, the number of cull animals of each species (x) at each time interval (t) $Nc_{x,t}$, was equal to the sum of the number of true positive animals T_pD_p, and the number of false positive animals T_pD_n, of each species in each time interval.

T_pD_p was modelled as a binomial distribution with parameters (n, p) where n is the number of true brucellosis positive animals in a household N_p, and p is the test sensitivity Se (Table 2). N_p was modelled as a binomial distribution with parameters (n, p) where n is the total number of animals per household N (Table 2) and p is seroprevalence (output from module 1). T_pD_n was modelled as a binomial distribution with parameters (n, p) where n is the number of brucellosis negative animals N^n, and p is $(1\text{-}Sp)$ where Sp is specificity (Table 2).

Offspring and milk production: Household offspring and milk production was modelled in the same way for Scenarios 1 and 2, as follows.

Large ruminants: The number of household offspring produced by each species (x) in each time interval (t), $No_{x,t}$ was the sum of the offspring born to each positive and negative individual (Module 2). The household milk produced by each species in each time interval $Nm_{x,t}$, was the sum of the milk produced by each individual that produced live offspring (Module 2).

Small ruminants: The proportion of seronegative small ruminants which produce live offspring each reproductive cycle Po^n, was calculated as follows:

$$Po^n = Pc^n \times (1-Pa^n) \quad (4)$$

where Pc^n is the proportion of seronegatives that conceive in one reproductive cycle (Table 2). Pa^n is the probability of abortion in seronegatives (Table 1).

The number of successful pregnancies per species (x) per time interval (t) $Pr_{x,t,}$ was modelled as a binomial distribution with parameters (n, p) where n is the number of opportunities for an animal to go through a lambing / kidding season in one time interval Nr, and p is the proportion of animals that give birth to live offspring in each reproductive cycle Po.

The number of single births $Ns_{x,t}$, was modelled as a binomial distribution with parameters (n, p) where n is the number of pregnancies $Pr_{t,x}$ (see above), and p is proportion of single births Ps (Table 2). The remaining successful pregnancies were assumed to result in twin births.

Table 2. Parameter values for Module 3

Parameter	Symbol	Species	Value [a]
Sensitivity of Complement Fixation Test and Rose Bengal test used in Series	*Se*	All species	Uniform (0.7;1)
Number of animals per household	*N*	Holstein cows	Betapert (0;2;3) rounded to a whole number
		Native cows	Betapert (0;2;3) rounded to a whole number
		Buffalo	Betapert (0;1;2) rounded to a whole number
		Sheep	Betapert (1;2;5) rounded to a whole number
		Goats	Betapert (0;1;5) rounded to a whole number
Specificity of Complement Fixation Test and Rose Bengal test used in series	*Sp*	All species	Uniform (0.7; 1)
Proportion of seronegatives which conceive in one reproductive cycle	Pc^n	Sheep, Goats	Betapert (60,65,70)
Number of lambing / kidding seasons per 18 months	R_x	Sheep	3
		Goats	In 1st time interval: Discrete (p = 0.5, n=1; p = 0.5, n = 2) In subsequent time intervals: alternates between 1 and 2.
Proportion of births that are singles	*Ps*	Sheep, Goats	0.5

[a.] All the values are from the questionnaire or interviews.

Module 4: Break-Even Analysis

Net Present Value: The net benefit for each scenario time interval (t) and species (x) $B_{t,x}$, was equal to the sum of the total benefits: compensation $C_{t,x}$, slaughterhouse cull prices $S_{t,x}$, the value attributed to new offspring $O_{t,x}$ and the value attributed to milk production $M_{t,x}$, minus the total cost of replacing culled animals $R_{t,x}$, for each time interval t, and species x. These parameters were calculated as follows:

$$C_{t,x} = Nc_{t,x} \, x \, Vc_x \tag{5}$$

$$S_{t,x} = Nc_{t,x} \, x \, Vs_{x,t} \tag{6}$$

$$O_{t,x} = (No_{t,x}^{scenario2} - No_{t,x}^{scenario1}) \; x \; Vo_{x,t} \tag{7}$$

$$M_{t,x} = (Nm_{t,x}^{scenario\ 2} - Nm_{t,x}^{scenario\ 1}) \; x \; Vm_{x,t} \tag{8}$$

$$R_{t,x} = Nc_{t,x} \, x \, Vr_{x,t} \tag{9}$$

where Vc_x is the compensation value per cull animal, $Vs_{x,t}$ is the slaughterhouse value of each cull animal, $Vo_{x,t}$ is the value of each offspring produced, $Vm_{x,t}$ is the value of each litre of milk produced and $Vr_{x,t}$ is the value of each replacement animal, for each species and at each time interval (Table 3).

The NPV was then calculated as follows:

$$NPV = \sum_{t,x} (B_{t,x} \; / \; (1+ADR)^{1.5t}) \tag{10}$$

Break-even compensation: Initially, the compensation per animal was set to zero. Thus, the break-even compensation per household (discounted) was equal to $-NPV$. To calculate the break-even compensation per cull animal for each species, firstly Vc_x was programmed to be relative to the compensation for each native breed cow Vc_N, according to the relative differences in replacement value. The total number of cull animals was then standardized to the equivalent number of native cows, after discounting Nc_{st}, as follows:

$$Nc_{st} = \sum_{t,x} (Nc_{t,x} \, x \, F \; / \; (1+ADR)^{1.5t}) \tag{11}$$

where F is a compensation factor.

The breakeven compensation per native cow BEC, was then calculated as follows:

$$BEC = -NPV \; / \; Nc_{st} \tag{12}$$

Table 3. Parameter values for Module 4

Parameter	Symbol	Species	Value [a]
Price per newborn	$Vo_{x,t}$	Native calf	Betapert (1000; 1500;2000)
		Holstein calf	Betapert (2000;2500;3000)
		Lamb	Betapert (100; 150; 200)
		Buffalo	Betapert (1000; 1500; 2000)
		Kid	Betapert (50; 100; 150)
Price per adult (> 6 months)	$Vr_{x,t}$	Native cow	Betapert (3000; 3500; 4000)
		Holstein cow	Betapert (8000; 9000; 10000)
		Sheep	Betapert (300; 400; 500)
		Buffalo	Betapert (2500, 3000, 3500)
		Goat	Betapert (250, 300, 450)
Slaughterhouse Price per cull animal	$Vs_{x,t}$	Cow	Betapert (1000; 1500; 2000)
		Sheep	Betapert (200; 250; 300)
		Buffalo	Betapert (1000; 1500; 2000)
		Goat	Betapert (150; 200; 250)
Price of milk/litre	$Vm_{x,t}$	Cow	Betapert (2.5; 3; 4)
		Buffalo	Betapert (3.5; 4; 5)
Current compensation offered	*None*	Native cow	700
		Holstein cow	1800
		Sheep	600
		Buffalo	80
		Goat	57

[a.] All the values are from the questionnaire or interviews.

The compensation for the other breeds were automatically calculated within the spreadsheet, as described above.

The model was run using Latin Hypercube sampling, for 10 000 iterations. The simulation was repeated to check empirically that the results were stable. To calculate compensation values for which 90% or 95% of household make a profit, the Ridder's method, using Monte Carlo simulation, was used to find values of Vc_N for which the 5th or 10th percentile of NPV was equal to 0 ± 100 Egyptian Pounds.

Sensitivity analysis was done for all inputs which varied at iteration level, using Spearman's rank correlation coefficients. The effect of uncertainty versus stochasticity was investigated by replacing all uncertainty distributions with the most likely value and leaving only distributions that represented stochasticity i.e. the number of offspring per animal and the number of test positive animals. This model was deterministic in every other respect.

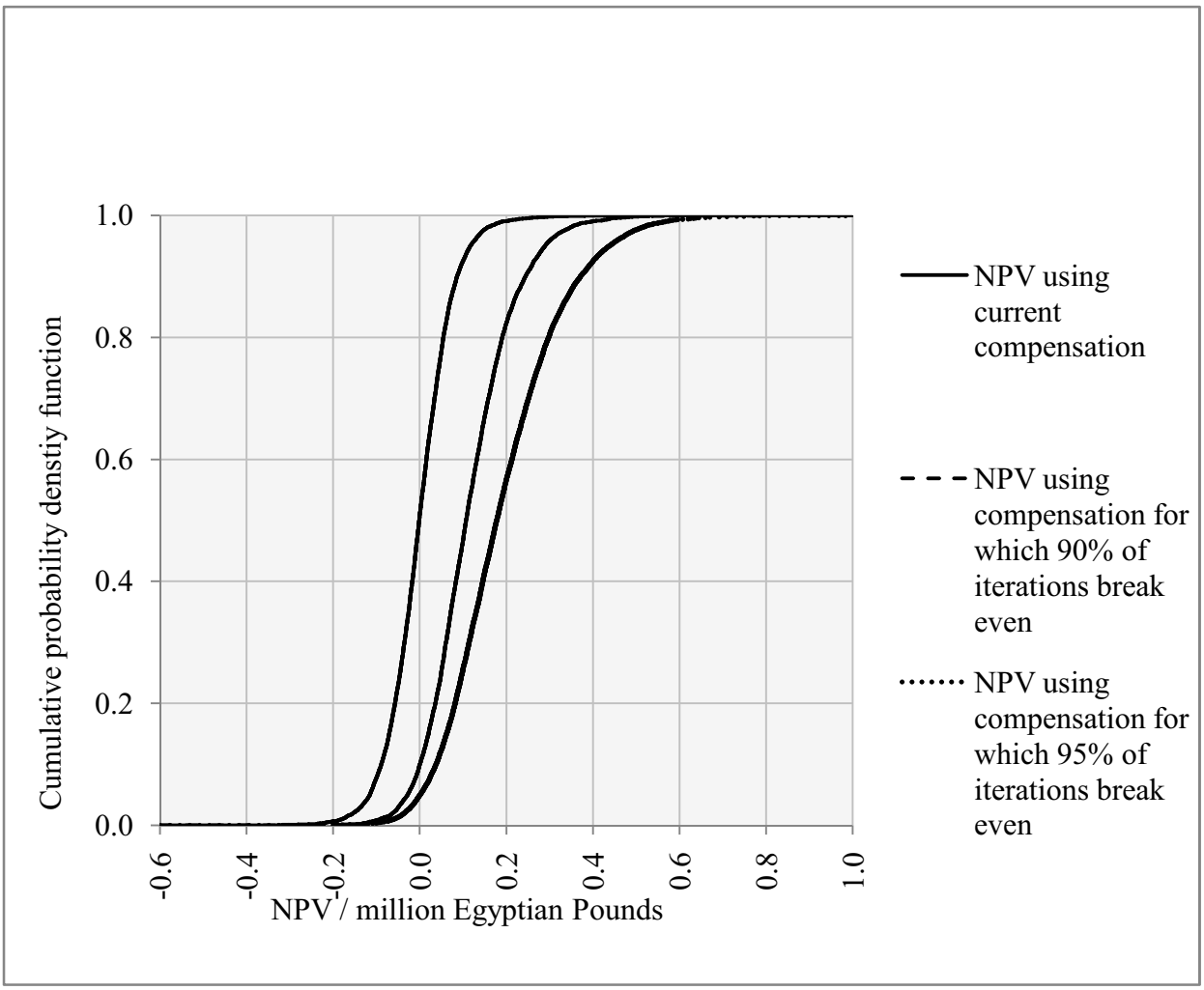

Fig. 1 Cumulative Net Present Value per household with current compensation (700 LE per native breed cow), compensation for which 90% of households break even (26572 LE per native breed cow) and compensation for which 95% of households break even (30372 LE per native breed cow). (9 year time horizon, 5% discount rate and 10% initial village prevalence)

RESULTS

The break-even compensation per household over the first nine years of test and slaughter ranged between - 416809 and 528805 Egyptian Pounds (LE), with a most likely value of -16625 LE (10% initial village prevalence, 5% ADR). The median break-even compensation per native breed cow was approximately zero, and the 5^{th} and 95^{th} percentiles were -30000 and 30000 Egyptian pounds, respectively.

Approximately 45% of household iterations broke even using current compensation values. Compensation values had to be increased by a factor of approximately 26 for 90% of household iterations to break even and a factor of 43 for 95% of household iterations to break even (Figure 1).

Initial village prevalence, *ADR* and time horizon each had a relatively minor impact on the most likely break-even compensation value (Figure 2). The most sensitive input parameters were

the decrease in bovine fertility attributable to *Brucella*-status, specificity and conception and abortion rates in seropositive and seronegative small ruminants. Break-even compensation per native cow remained highly variable after replacing all uncertainty distributions with the most likely value and leaving only distributions that represented stochasticity i.e. the number of offspring per animal and the number of test positive animals (Figure 3).

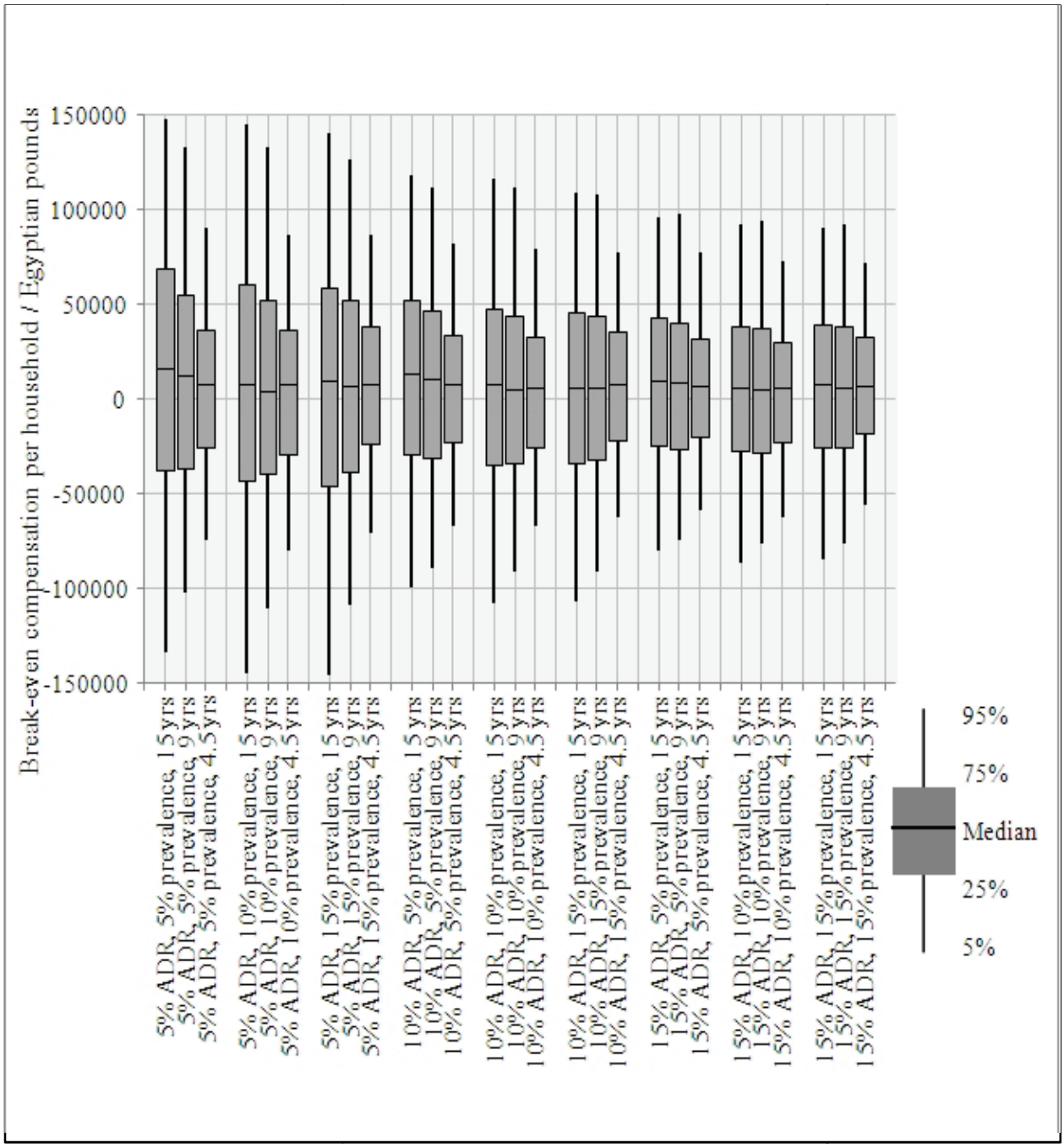

Fig. 2 Break-even household compensation at 4.5, 9 and 15 year time horizons, with 5%, 10% and 15% *ADR*, and initial village prevalence of 5%, 10% and 15%

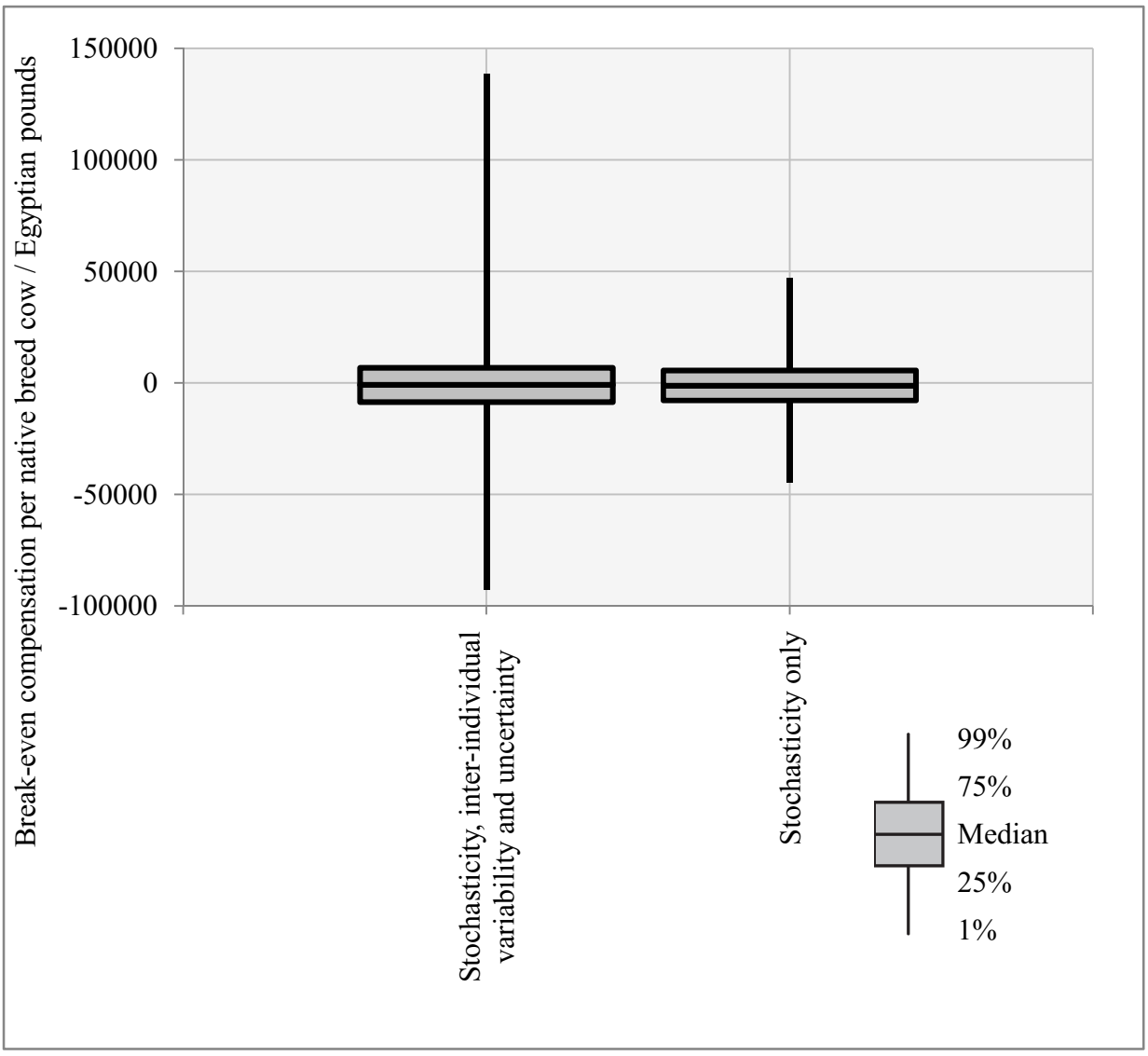

Fig. 3 Probability density function of break-even compensation per native cow taking into account stochasticity in production parameters and test accuracy, but no other sources of uncertainty or variability. (9 year time horizon, 5% *ADR* and 10% initial village prevalence)

DISCUSSION

Inadequate and delayed compensation has been suggested as a cause of low compliance with several disease control programmes, including the current brucellosis test and slaughter programme in Egypt (May, 2007, Bohning & Del Rio Vilas, 2009, Holt et al., 2011). The effects of control programmes on individual farmers can be highly variable (Bennett & Cooke, 2009). Understanding the impacts of control measures and incentives on individuals is critical to achieving compliance.

According to the model assumptions, the *NPV* for households in Lower Egypt over the first nine years of the Brucellosis test and slaughter programme is highly variable and uncertain, with many households losing–and others gaining–over a hundred thousand Egyptian Pounds (irrespective of compensation value). Uncertainty in test accuracy and uncertainty and variability in reproductive parameters contributed most to the variability in *NPV*.

Current compensation –approximately 20% of an animal's value— would enable approximately 45% of households to break even; however for 90% of households to break even, compensation would need to be 26 times the current value.

The model that described only stochastic effects, and not the effects of uncertainty, inter-individual or inter-household variability, shows that even if uncertainty could be reduced, the stochastic effects are such that there would still be a wide range of break-even compensation values. It is obviously unrealistic to assume that every cow produces the same amount of milk per lactation, that the effect of disease on production is the same for all animals, and that the price for products remains constant and certain— all of these factors in reality would add to the uncertainty in break-even compensation.

Best available data were in most cases estimations, frequently based on the opinion of one veterinarian, which may have caused bias in the values and increased the uncertainty range. However, this uncertainty is probably a reasonable representation of the uncertainty that an individual smallholder would face when estimating the likely effects of test and slaughter.

The model was highly simplistic – animals are all female and of breeding age. Sero-status is assumed to confer disease status and therefore effects on production. The assumption of random mixing within a village is reasonable for small ruminants, however less so for large ruminants, since they are generally not allowed to wander (personal communication, H. Holt). There is also an implicit assumption in this analysis that the variation in NPV is explained entirely by the intervention, which is not true. However we feel that this model is sufficient to explore the variability and uncertainty in the perceived effects of test and slaughter, and it would seem reasonable to assume that test and slaughter introduces additional risks to individual smallholders who operate in a financial system that is already highly uncertain – the test positive animal may not be truly diseased, and even if it is, it could be more productive than its replacement animal of unknown status. In addition, the farmer has no control over when the animal is removed in relation to its stage in the production cycle, and current prices.

One hundred per cent compliance, assumed for Scenario 2, is perhaps unrealistic and may have caused some over-estimation of the benefit of the intervention, and therefore underestimate of the break-even compensation required. In reality, an individual farmer cannot be sure that others in the village will comply. High levels of externalities can lead to "free-riders." This adds to the uncertainty of the benefit of disease control for the individual. This is particularly important in Upper Egypt, where the population density and production systems make effective bio-security challenging. However, because a large number of government veterinarians live and conduct private practice in villages, and therefore have close contact with the smallholders, village-wide interventions are possible. In fact, large sections of villages are sometimes tested over the course of a few days (personal communication, Y. Hegazy).

The model assumes seroprevalence amongst replacement animals is the same as in village surveys. Since farmers commonly sell animals which aborted, at markets (Holt et al., 2011) it is possible prevalence could be higher amongst replacements, so break-even compensation may have been under-estimated.

More frequent testing may improve the efficacy of the intervention, and therefore the benefits to farmers, reducing the relative compensation per animal. However there would be a pay-off since the number of culled animals and the less tangible effects of more regular testing, such as inconvenience to the farmer, would need to be considered.

Intangible benefits of removing infected stock are also omitted from this analysis, such as reduced zoonotic risk to the household itself and the emotional stress caused by the disease and its control. How Egyptian farmers would value these effects is not known.

In many disease control scenarios, including the case of brucellosis in Egypt, the supply of funds for compensation is unpredictable (personal communication, Y. Hegazy) and increasing compensation in order to improve compliance is unlikely to be feasible. In addition, due to the wide distribution of *NPV* for individual households, compensation would need to be increased substantially to increase adherence, and this may be inefficient. It may also encourage counter-productive behaviour by some farmers who may profit considerably from procuring infected animals.

Alternative strategies should therefore be sought. Consideration should be given not only to adequate compensation, but also to improving the efficacy of control so that there is perceived benefit and reduced risk of loss for individual farmers, and to incentivising disease prevention and control other ways.

There are considerable externalities for individual farmers controlling brucellosis—improved public health, possible international trade in the future, decrease in risk of catastrophic loss freeing farmers to invest in more profitable breeds and improved supply of replacements, for example. To increase compliance, some of these could be internalized, for example an assurance scheme could guarantee a higher price for Brucella-free milk, or penalties could be introduced for commercial dairies selling contaminated milk. There is currently no incentive for producing Brucella-free milk in Egypt (personal communication, Y. Hegazy). This would also benefit the public health sector. An assurance scheme for Brucella-free livestock, or enforced pre-movement testing, may incentivize prevention and control for buyers as well as sellers – vaccination would be more cost-effective if the animals are known to be disease-free.

A survey suggested farmers frequently report abortions to their local veterinarian, and this has been highlighted as an opportunity for surveillance (Holt et al., 2011). In addition, recently aborted animals are commonly sold on at markets or to butchers (Holt et al., 2011). Compulsory testing of animals which abort could have several advantages. The positive predictive value would be relatively high since seropositive animals commonly abort, and have high titres around time of abortion (Gillespie, 1988). Culling would occur at a time of high risk for human and animal transmission - due to increased infectiousness and the practice of selling at markets and to butchers – and at a time when the farmer would want to sell the animal anyway. Anecdotal reports suggest this would be acceptable to veterinarians and would not impinge on private practice (personal communication, Y. Hegazy).

In conclusion, although this model is highly simplistic, it demonstrates the importance of taking into account the variability and uncertainty in the net effect of disease control on individual farmers, when setting compensation. Rather than attempting to compensate farmers for routine test-and-slaughter programmes, compliance may be improved by using control measures which have more inherent benefits, and less risk and uncertainty in their effects, for individual farmers.

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A STOCHASTIC SCENARIO TREE MODEL FOR THE EVALUATION OF RISK-BASED MEAT INSPECTION FOR BOVINE CYSTICERCOSIS

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SUMMARY

The current surveillance system for bovine cysticercosis (BC) is time-consuming, costly, and might be of limited value in countries with low prevalence. The aim of this study was to develop a stochastic simulation model for analysis of tentative risk-based meat inspection systems for BC in Danish cattle with regard to system sensitivity (*SSCSe*), specificity and potential monetary benefits compared to the current *SSCSe* = 15%. The relevant risk factors used to construct three alternative scenario trees were identified from previous Danish risk factor studies, and included: 1) gender, 2) grazing and 3) access to risky water sources. The scenario using gender to differentiate high and low risk groups had the highest cost-effectiveness ratio (*CE*) = €28.3 million and *SSCSe*=12%, compared to *CE* = €20.3 and *SSCSe*=10% when using grazing, and *CE* = €12.1 million and *SSCSe*=4% when using access to risky water sources to differentiate risk groups. The use of gender to differentiate high and low-risk groups might be most feasible, because that information is readily available at the slaughter line.

INTRODUCTION

The term bovine cysticercosis (BC) refers to a foodborne zoonotic infection with the larval stage of the tapeworm *Taenia saginata*. The larvae are found as so-called cysticerci in beef. Cattle become infected by accidental ingestion of *Taenia saginata*-eggs containing larvae that later develop into cysticerci. The adult stage develops in the intestine of the obligate human host upon ingestion of cysticerci when consuming raw or undercooked meat (Murrell et al., 2005).

The current European Union (EU) meat inspection Regulation (EC) No 854/2004 (European Community, 2004a) requests that every single carcass of bovines above 6 weeks of age is examined for BC. The tongue, diaphragm and heart are inspected visually and the external masseters are examined by two parallel incisions in the mandible and the pterygoid masseter muscles, which must be incised along one plane. This is time-consuming, costly, and might be considered of limited value in countries with low prevalence. However, from a consumer's point of view it is important to identify and handle infected carcasses. BC positive carcasses are condemned if heavily infected, and frozen if lightly infected to inactivate cysticerci before consumption (Dorny et al., 2010).

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In Denmark, the prevalence of BC in cattle was estimated to range between 0.1 – 0.7 % in 1990 (Cabaret et al., 2002). In a recent register-based cross-sectional study of all cattle herds in Denmark (unpublished data) the true animal level prevalence of BC-positive cattle slaughtered in Denmark between 2006 and 2010 was estimated to 0.04%, indicating an even lower prevalence in recent years. In that study it was also concluded that 65% of BC-positive animals can be attributed to female gender, which might be explained by differences in grazing practices between the genders. In previous studies from Belgium and Denmark, cattle having access to risky water sources with sewage treatment plant effluent in proximity was found to be a major risk factor for BC (Boone et al., 2007; Kyvsgaard et al., 1991). In line with this, in an observational case-control study (unpublished data) it was concluded that herds having all or most of their animals grazing, independent on whether they were organic or conventional herds, was a risk factor for BC in Denmark.

Food Chain Information (FCI) is a concept that involves mandatory collection of relevant information about housing and management of food producing animals. FCI is required by EU (European Community, 2004b) to be made available to the abattoir by the farmer prior to slaughter. If risk factors for BC, such as the above mentioned, were included in FCI, a more cost-effective meat inspection system could be established by targeting high-risk herds or animals. However, the design of risk-based surveillance systems must be both evidence-based and feasible. Advantages, disadvantages and consequences must be evaluated and taken into account when designing an alternative system in order for it to become widely accepted. Thus, there is a need for a model in which different hypothetical scenarios can be tested and compared to the current system before being implemented.

The aim of this study was to incorporate known risk factors into a stochastic simulation model for analysis of the performance of tentative risk-based meat inspection systems for BC in Danish cattle with regard to system sensitivity, specificity and potential monetary benefits for different alternative systems compared to the current system.

MATERIALS AND METHODS

Scenario tree model

A stochastic scenario tree simulation model was developed to evaluate the performance (i.e. sensitivity (*Se*) and specificity (*Sp*)) of a surveillance system component (SSC) for detection of BC at meat inspection at the estimated true animal-level prevalence for BC in Denmark. The population of cattle slaughtered in Denmark during a one-year period (*SPop*) was divided into subpopulations, in which each unit (i.e. animal) had equal probabilities of inspection outcomes in the basic scenarios. Probabilities for the possible outcomes, test-positive (T+) or test-negative (T-) were estimated for each tree branch. Three trees were constructed with the risk factors that were assumed to influence the probability of infection and detection of the surveillance unit (Fig. 1) (Martin et al., 2007). They each represent different feasible risk-based meat inspection scenarios based on known risk factors for BC in Denmark as described below.

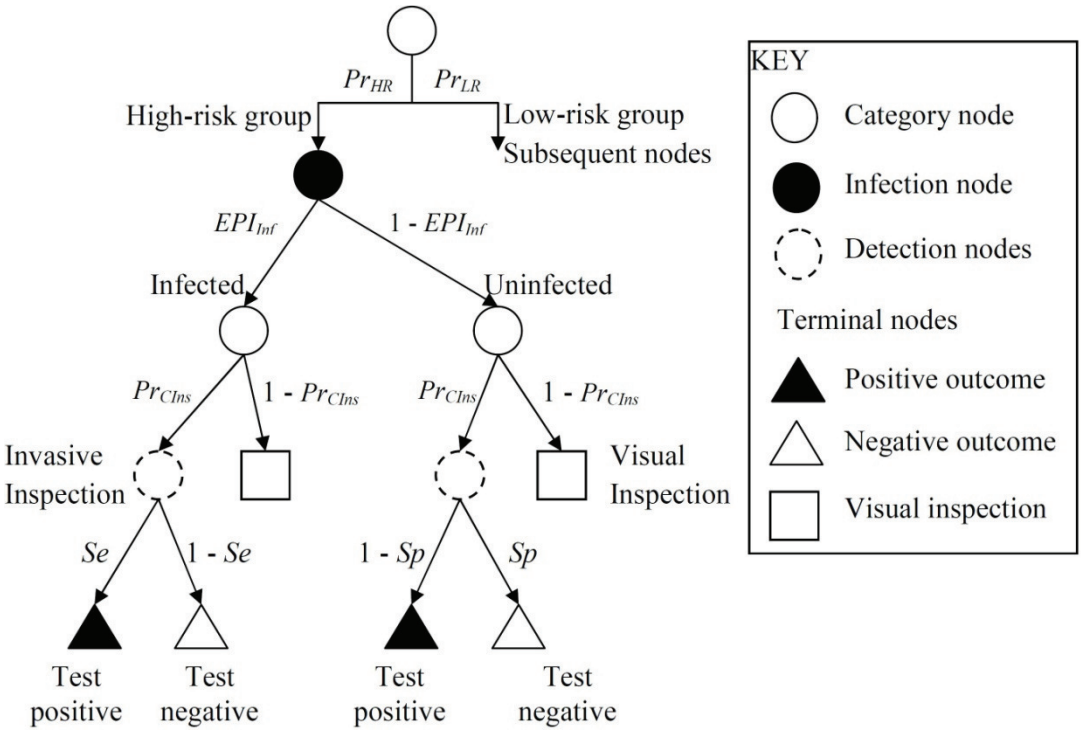

Fig 1. Conceptual scenario tree developed to evaluate the performance (i.e. sensitivity (*Se*) and specificity (*Sp*)) of a surveillance system component (SSC) for detection of bovine cysticercosis (BC) at meat inspection at a given true animal level prevalence for BC in Denmark. Pr_{HR} and Pr_{LR} are proportions of animals in the high-risk and low-risk groups, respectively. EPI_{inf} is the effective probability of infection in the given risk group. Pr_{CIn} is the proportion of animals inspected with the current invasive meat inspection procedures for BC.

Population and risk factors for BC

Register data were obtained from the Danish Cattle Database (DCD) for the period from 2004 to 2010 of all recorded cattle slaughtered in Denmark, including the proportion of T+ and T- animals. The total number of cattle slaughtered per year was then calculated.

A list of relevant risk factors for BC was generated (Table 1) based on risk factor studies carried out in Denmark (Kyvsgaard et al., 1991). The relevant risk factors used to develop the different scenario trees were 1) gender, 2) grazing and 3) access to risky water sources (e.g. lakes, streams, rivers, etc.), with sewage treatment plants in the proximity. The first node in the tree was a category node that divided all slaughtered cattle into proportions of high-risk (Pr_{HR}) and low-risk (Pr_{LR}) for each risk factor group.

Probability of infection

The next node in the tree was an infection node, in which the estimated true animal-level BC prevalence (P_A) of 0.04% was used in the calculations to obtain effective probabilities of infection (EPI_{Inf}) within each subpopulation defined by the previous category node. The P_A was modelled using a pert distribution, where the 95% confidence limits of the original estimates were used as the minimum and maximum values, and the most likely value was the P_A. First, the relative risks between the high-risk (HR) and low-risk (LR) groups were obtained from an analysis of data from the DCD or based on previous risk factor studies. Then, the relative risks

(*RR*) for the high-risk group compared to the low-risk group (Table 1), and the proportion of animals in the high-risk group were used for calculation of the adjusted risk (AR) for the low-risk group (AR_{LR}) as shown in Eq. (1), and multiplied by the *RR* to obtain the AR of the high-risk group (AR_{HR}). Finally, the ARs were multiplied by P_A to calculate the EPI_{Inf} for each risk group, and the probability of not being infected (EPI_{NInf}) was calculated as (1- EPI_{Inf}) (Martin et al., 2007).

$$AR_{LR} = \frac{1}{RR * Pr_{HR} + (1 - Pr_{HR})} \quad (1)$$

Where: AR_{LR} is the adjusted risk for the low-risk group, *RR* is the relative risk in the high-risk compared to the low-risk group and Pr_{HR} is the proportion of all slaughtered cattle in the high-risk group.

Sub-populations for differentiated meat inspection

The next node in the scenario tree was a category node determining what type of meat inspection the animals in the different branches would be subjected to. One scenario represented the current surveillance system where every single animal goes through the current invasive meat inspection procedures and hence has the same probability of being tested. Alternative risk-based scenarios were constructed in which all cattle in the high-risk group were subjected to the current invasive meat inspection (Pr_{CInH}), while all of the cattle in the low-risk group were only inspected visually (Pr_{CInL}) (Table 2).

Sensitivity and specificity of meat inspection procedures

According to Kyvsgaard et al. (1990) the *Se* of the invasive meat inspection procedures is around 15% in lightly-infected animals. Most BC-infected cattle in Denmark are lightly infected. Dorny and Praet (2007) suggested that the *Se* of the current meat inspection might be influenced by the motivation and skills of the meat inspector, and the degeneration stage of the cyst. Therefore, the last step in the scenario trees was the detection node in which the meat inspection *Se* in the basic scenarios was modelled as a pert distribution, where the minimum, most likely and maximum values were modified from Kyvsgaard et al. (1990). In 2006, the Danish meat inspection recording system was improved enabling an easier registration of lesions found. Therefore, we set the upper limit of the *Se* estimate to 0.25, instead of the 0.18 estimated by Kyvsgaard et al. (1990). The *Sp* of the meat inspection was included in the model as a pert distribution to allow for false positive BC-recordings to occur. The values for this distribution were obtained through a calibration exercise to ensure that we obtained realistic numbers of T+ cattle per year in the current scenario.

Model outputs

The conditional probabilities were calculated for each branch in the tree by multiplying all proportions and probabilities for each branch. The probability of being test-positive P(T+) was calculated as the sum of all branch probabilities with a T+ outcome. The probability of being test-negative P(T-) was the sum of all probabilities from branches with T- outcomes. The total expected numbers of cattle being T+ and T- were calculated by multiplying P(T+) and P(T-) with the total number of cattle slaughtered per year, respectively.

The probability of a false-positive test result, P(T+|D-) was calculated as the sum of the probabilities of the branches with T+ outcomes in the non-infected groups of animals in the tree. The total number of cattle being false-positive (*nFP*) was calculated multiplying the P(T+|D-) by the total number of cattle slaughtered per year. In the same way, the probability of a T+ result given that the animal was infected, P(T+|D+), was calculated as the sum of the probabilities of the branches with T+ outcomes in the infected groups of animals in the tree. The total number of detected cases (*nDC*) was calculated by multiplying the P(T+|D+) by the total number of cattle slaughtered per year (*SPop*).

The expected number of infected cattle (*CInf*) was calculated as the sum of the expected number of infected cattle in each risk group using the following equation:

$$CInf = (SPop * Pr_{LR}) * EPI_{InfL} + (SPop * Pr_{HR}) * EPI_{InfH} \quad (2)$$

Where Pr_{LR} is the proportion of slaughtered cattle in the low-risk group, Pr_{HR} is the proportion of slaughtered cattle in the high-risk group, and EPI_{InfL} and EPI_{InfH} are the probabilities of being infected for the low-risk and high-risk group, respectively

To get the number of undetected cases (*nUC*) for each scenario the total number of detected cases, *nDC*, was subtracted from *CInf*.

The total number of cattle that would undergo visual meat inspection (C_{Insp}), to be used in a cost-benefit analysis, was calculated in the following way:

$$C_{Insp} = (SPop * Pr_{HR} * Pr_{CInH}) + (SPop * Pr_{LR} * Pr_{CInL}) \quad (3)$$

Where *SPop* is the total cattle slaughtered population per year, Pr_{LR} is the proportion of all slaughtered cattle in the low-risk group, Pr_{HR} is the proportion of all slaughter cattle in the high-risk group, Pr_{CInH} is the proportion of slaughtered cattle in the high-risk group subjected to visual inspection, and Pr_{CInL} is the proportion of slaughtered cattle in the low-risk group subjected to visual inspection.

The SSC sensitivity (*SSCSe*) for each scenario was calculated by dividing the *nDC* by the *CInf*.

Simulations

The models were set up in @Risk 5.7 (Palisade Corporation) and run with 10000 iterations. The input parameters were described by use of distributions to account for the uncertainty in the parameter estimates. The input parameters for P_A, *Se* and *Sp* are provided in Table 3 together with the source of information used. Sensitivity analysis was performed by regression analysis for each scenario, identifying significant outputs, displayed as “tornado” type charts.

Economic analysis

The economic analysis was carried out by calculating the total amount of money that would be saved by performing visual inspection of all of the animals in the low-risk group, rather than the current invasive inspection, and the total amount gained on increased price of masseter

muscles meat not incised for each scenario. Both input parameters were described using a distribution to account for uncertainty in the parameter estimates (Table 3).

Assuming that a slaughterhouse would be able to reorganise the work procedures and remove a person from the inspection line in a way that would allow one of the other meat inspectors to inspect the head visually instead of cutting the masseter muscles, the slaughterhouse would save approximately €0.94 per animal (min. €0.54, max. €1.34). In line with this, the increase in the price of meat by not cutting the masseter muscles was estimated by use of expert opinion to be approximately €1.8 per animal (min. €1.6, max. €1.9) corresponding to 1.2 kg of meat per animal at current market prices. These inputs were then multiplied with the total number of animals that would undergo visual inspection and summed up to obtain the monetary net gain (*NG*) for each surveillance scenario.

Variation in *NG* and *SSCSe* was expected between each scenario tree. Therefore, cost-effectiveness analysis was performed to enable comparison of the different scenarios. A "cost-effectiveness" ratio of each scenario (*CE*) was calculated by dividing *NG* with the change in the *SSCSe* (*ΔSSCSe*) estimates. The *CE* can be interpreted as the net gain in Euro per unit of decrease in *Se*. The *ΔSSCSe* was expressed relative to the *SSCSe* of the current surveillance system.

RESULTS

The results for the *SSCSe*, *nDC*, *nUC*, *nFP* and number of animals subjected to invasive meat inspection for each scenario are shown in Table 2. The current surveillance system had the highest *SSCSe*, 15% under the current estimated sensitivity (*Se*) of the meat inspection, which was expected as this was the number entered in the distributions for this input.

Table 1. Relevant risk factors used to develop three scenario trees to evaluate the performance of current and alternative risk-based surveillance systems for detection of bovine cysticercosis (BC) at meat inspection in Denmark; the relative risk (*RR*), proportion of animals in the Danish cattle population (Pr) and adjusted risk (AR) for the high-risk group compared to the low-risk group

Risk factor	Risk group	RR	Pr	AR
Gender	Female [a]	4.7	0.5	1.7
	Male [b]	1	0.5	0.3
Grazing	Grazing [a]	3.6	0.4	1.8
	Not grazing [b]	1	0.6	0.5
Access to drink from risky water source	Drink from risky water source [a]	3.1	0.1	2.6
	No access to risky water source [b]	1	0.9	0.8

[a] High-risk group
[b] Low-risk group

Out of the three alternative scenarios, the scenario using gender to differentiate high and low-risk groups had the highest *SSCSe* at 12%, compare to 10% and 4% when using grazing and access to risky water source-scenarios, respectively. In line with this, the *nDC* under these two alternative scenarios, was lower (31 and 11, respectively) compared to 36 *nDC* when using gender. However, the three alternative scenarios had lower *nDC* compare to the current surveillance programme which detected 44 cases. The *nDC* should be seen in relation to the *nUC* which was on average 252 under the current meat inspection scenario, and 259, 265 and 284 under the gender, grazing and access to risky water source-source scenarios, respectively.

Table 2. Results of simulation with 10000 iterations and scenarios describing the type of meat inspection slaughtered cattle in the different risk factor groups would be subjected to. Results are shown for the current meat inspection system (i.e. all cattle subjected to invasive meat inspection) and alternative meat inspection systems (i.e. all cattle in the high-risk (HR) group subjected to invasive meat inspection and all cattle in the low-risk (LR) group subjected to visual meat inspection) for bovine cysticercosis in Denmark over a one-year period

Risk factor and scenarios	Number of detected cases (*nDC*) (95% CI)[a]	Number of undetected cases (*nUC*) (95% CI)	Number of false positive animals (*nFP*) (95% CI)	Number of animals subjected to invasive inspection	Sensitivity of the SSC *(SSCSe)* (95% CI)
Current surveillance	44 (15, 95)	252 (113, 476)	25 (7, 43)	498,927	0.15 (0.07, 0.22)
Gender	36 (12, 78)	259 (117, 490)	12 (4, 21)	247,630	0.12 (0.06, 0.18)
Grazing	31 (10, 67)	265 (120, 500)	10 (3, 17)	199,583	0.10 (0.05, 0.16)
Access to drink risky water source	11 (4, 24)	284 (129, 536)	2 (1, 4)	49,896	0.04 (0.02, 0.06)

[a] 95% Credibility interval

The results of the economical analysis assuming that all abattoirs would be able to reorganise the work at the slaughter line to save money on the inspection of the head of the carcase are shown in Table 4. The scenario using gender to differentiate high and low-risk groups had the highest *CE* (€28.3 million), compared to €20.3 and €12.1 million when using grazing and access to risky water source-scenarios, respectively.

On the other hand, the scenario using access to risky water sources to differentiate high and low-risk animals had the highest *NG* at € 1.2 million, whereas for the gender and grazing scenarios the *NG* were €0.7 and €0.8 million respectively.

The most influential input parameters for the *CE*, *SSCSe*, *NG* and *nUC* outputs were identified by investigation of tornado plots obtained from the sensitivity analyses in @Risk. For *CE* the most influential inputs were the *Se*, euro saved on meat inspection and euro gained on sold meat; for *SSCSe* the most influential input was the *Se*; for *NG* euro saved on meat

inspection and euro gained on sold meat were most influential, and for *nUC* the *EPI* (either from *HR* or *LR*) and *Se* were most influential.

Table 3. Pert distribution input parameters for money saved by performing visual inspection rather than invasive inspection, money gained on increased price of masseter muscles meat not cut, true animal prevalence for bovine cysticercosis (P_A), sensitivity (*Se*), improved sensitivity (*Se+*) and specificity (*Sp*) of the meat inspection procedures for detection of bovine cysticercosis used in the scenario tree models

Parameter	Minimum	Most Likely	Maximum	Source
P_A	0.00023	0.00043	0.0016	Calculated based on prevalence estimates from a registered-based cross-sectional study (unpublished data) and *Se* estimates modified from Kyvsgaard *et al.* (1990)
Se	0.04	0.15	0.25	Modified from Kyvsgaard *et al.* (1990)
Sp	0.9999	0.99995	1.0	Calibrated to obtain realistic numbers of BC-positive cattle
Money saved on meat inspection	0.54	0.94	1.34	Expert opinion
Money gained on sold meat	1.6	1.8	1.9	Expert opinion

DISCUSSION

A stochastic scenario tree model was developed for analysing the performance of alternative risk-based meat inspection systems for BC in Danish cattle with regard to system sensitivity, specificity and cost-effectiveness for different alternative system scenarios compared to the current system, incorporating known risk factors. The alternative surveillance scenarios were evaluated against the current surveillance system by calculating the *CE* and the *SSCSe* for each scenario. This method has been used by others to compare hypothetical disease control scenarios (Bergevoet et al., 2009). The results presented here depend on epidemiological and economical assumptions, and on the assumption that all abattoirs would be able to reorganise the work on the slaughter line allowing them to save the person that inspects the head of carcases from low risk animals.

Currently, information about indoor/outdoor production in pigs is being used as part of FCI in Denmark to modify the traditional meat inspection (Alban et al., 2008a). Accordingly, a risk-based surveillance programme was designed for *Trichinella* spp. in Denmark, targeting pigs housed outdoors as those having risk of getting *Trichinella*. As a result the status of "negligible risk" of this pathogen was granted by the European Commission, to Danish pigs and pork (Alban et al., 2008b). A similar system would be desirable for BC in Danish cattle, and this

study shows that this might be feasible with very little change in food safety. Some of the risk factors used for differentiating risk of BC in the presented models are readily available through the DCD such as gender, whereas others are only accessible through farmers´ reporting of FCI.

Table 4. Results of the economical simulations for comparison of scenarios where all Danish cattle in the low-risk (LR) group are visually inspected. The number of animals visually inspected, money saved by performing visual inspection rather than invasive inspection, money gained on increased price of masseter muscles meat not cut, the total amount of money gained and the cost-effectiveness

Risk factor and scenarios	Number of cattle visually inspected [a]	Euro saved on meat inspection Million €/year [b] (95% CI)[c]	Euro gained on sold meat Million €/year (95% CI)	Net gain (*NG*) Million €/year [b] (95% CI)	Cost-effectiveness ratio (*CE*) Million €/year (95% CI)
Gender	251327	0.24 (0.16, 0.31)	0.45 (0.42, 0.47)	0.7 (0.6, 0.8)	28.3 (17.1, 52.7)
Grazing	299374	0.28 (0.20, 0.37)	0.53 (0.50, 0.56)	0.8 (0.7, 0.9)	20.3 (12.3, 37.9)
Access to risky water source	449061	0.42 (0.29, 0.55)	0.80 (0.75, 0.84)	1.2 (1.1, 1.3)	12.1 (7.3, 22.5)

[a] Number of cattle visually inspected out of the total cattle population slaughtered per year n = 498,927

[b] The result are relevant for abattoirs that are able to reorganise the slaughter line, so that the meat inspection could save one person on the line and have one of the other meat inspectors, from the slaughter line, to inspect the head visually

[c] 95% Credibility interval

The input parameters of the model were based on data obtained from the DCD, previous risk factor studies for BC in Denmark and on distributions derived from expert opinion. The proportions of cattle in the HR and LR groups (Table 1) for the scenario using gender were obtained from raw data from the DCD while the proportions for the same groups in the scenarios using grazing and access to risky water source were obtained from an observational risk factor study (unpublished data). Therefore, there is a risk that the cattle proportions could be biased in the scenarios using grazing and access to risky water source since they may differ from reality. However, the proportion of cattle in the HR or LR groups was not one of the most influential inputs for the outputs in the model by investigation of tornado plots obtained from the sensitivity analyses in @Risk.

It could be speculated that not inspecting any of the low risk animals invasively would compromise food safety. Different scenarios evaluating 100%, 90% and 75% of the cattle in the LR group for each of the three risk factors that would only be visually inspected were run (data

not shown). There were only minor differences in the results between each of these scenarios. Therefore, we have only shown the results for the scenarios in which 100% cattle in the LR group would undergo visual inspection for each risk factor. It is good that the differences were small between these scenarios, because it would complicate the meat inspection system, if for instance 10% of the LR group was to be inspected invasively.

The economical results are based on the assumption that the abattoirs are able to reorganise work at the slaughter line, so that the meat inspection could save one person on the line and have one of the other meat inspectors, from the slaughter line, to inspect the head visually. However, these results do not apply for those abattoirs where this kind of reorganization is not possible. In those the *NG* would be lower because they would not be able to save money on the work reorganization of the slaughter line. It is not likely that all abattoirs would be able to or willing to reorganise the work, so the total *NG* in the cattle sector is not likely to become as high as suggested in Table 4. This should be further investigated after discussing the procedures with the abattoirs.

The scenario for access to risky water source had the highest potential *NG* (€1.2 million) out of the alternative surveillance scenarios, but had the lowest *CE* and *SSCSe* (4%). Furthermore, using this scenario as an alternative surveillance system would result in the lowest *nDC* (11) and highest *nUC* (284). The feasibility of using this risk factor to differentiate low risk and high risk cattle is also poorer compared to using gender, which has a higher *CE* and *SSCSe* (12%) but lower potential *NG* (€0.7 million), but with much higher *nDC* (36) and a lower *nUC* (259). The current surveillance system performed slightly better than the alternative surveillance system scenarios with regard to *nDC* (44), *nUC* (252) and *SSCSe* (15%), but these numbers do not differ greatly from those obtain under the scenario using gender.

It has been concluded that the scenario using gender to differentiate high and low-risk groups has a greater advantage over the other risk factors, due to the fact that the information is readily available from the DCD, which makes it easier and more accurate when categorizing the cattle in HR and LR groups. In line with this, the *nDC* and *nUC* outputs when using gender are closer to the same outputs under the current surveillance system, which means that we can maintain a similar level of detection while having a potential *NG* of €0.7 million. The use of grazing and access to risky water source might become more feasible, if the farmer could certify that the herd is free of BC infection for instance by testing using serology, or that the cattle has no access to risky water sources through a good farming practices programme. Furthermore, sensitivity analyses are warranted to clarify the importance of the proportions of cattle in the risk categories. Finally, investigation of possibilities to improve sensitivity of the meat inspection procedures is recommended.

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STOCHASTIC SPATIO-TEMPORAL MODEL FOR AFRICAN SWINE FEVER SPREAD IN THE EU DURING THE HIGH RISK PERIOD

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SUMMARY

This project aimed to develop a model for the spread of ASF within and between the EU Member States (MS) during the high risk period (HRP) and to identify MS that most likely contribute to ASF spread ("super-spreaders") or MS that receive cases from other MS ("super-receivers")

A stochastic spatio-temporal state-transition model with 3.5 million individual farm records was developed to simulate ASFV spread. Seven MS caused between-MS spread due to intra-Community trade during the first ten days after seeding infection. For a HRP of 60 days, movements of infected pigs will originate at least once from 16 MS. Two thirds of all intra-Community spread was linked to six trade links only. Denmark, the Netherlands, Lithuania and Latvia were identified as "super-spreaders"; Germany and Poland as "super-receivers". The total number of premises per country involved in intra-Community trade was found to be a key determinant for the between-MS spread dynamic.

INTRODUCTION

The European Union (EU) has experienced several outbreaks of exotic diseases during the last few years, and, despite preventive measures, future epidemics are likely to occur. Following its eastward enlargement, the EU now shares more borders with countries with problematic disease status. Since the introduction of African swine fever (ASF) into Georgia in 2007, probably from Africa, ASF has spread to several countries close to the external frontiers of the Community with cases reported in Russia, Armenia and Azerbaijan (OIE, 2011).

The recently published scientific opinion of the European Food Safety Authority (EFSA, 2010) assessed the risk of ASF being released from the Russian Federation and Trans-Caucasia into the EU as moderate or low, depending on whether domestic pigs or wild boar are considered. Due to unrestricted trade within the EU, silent spread during the High Risk Period (HRP), which is defined as the time from first infection until first detection of disease (De Vos et al., 2005; Staubach et al., 2008), was estimated to be moderate to high for the domestic pig population, and with considerable consequences for certain infected Member States (MS) (EFSA, 2010). The duration of the HRP is a critical determinant of outbreak size (Horst et al., 1998). For example for classical swine fever (CSF) the HRP of the last epidemics experienced in

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Europe was estimated to be three to eight weeks (Staubach et al., 2008) and resulted in outbreaks in several MS.

ASF is a notifiable viral pig disease with high mortality that affects all members of the family Suidae (Wilkinson, 1984; Plowright et al., 1994; Penrith et al., 2004). It is one of the most important pig diseases due its severe socio-economic consequences for affected countries, the difficulty of preventing spread across country boundaries, and the lack of vaccine and therapeutic control measures (FAO, 2009).

ASF has a complex epidemiology, including a sylvatic cycle between wild boar (*Sus scrofa*) and soft ticks (Argasidae) (Bastos et al., 2004; Penrith et al., 2004), and it can be transmitted via insufficiently cooked pork products (swill) (Wilkinson, 1984, 1989; Mannelli et al., 1997; Penrith & Vosloo, 2009). Direct transmission between pigs is considered to be the most efficient transmission route (Sánchez-Vizcaíno, 2006). Indirect contact through people, vehicles and fomites can play a role, but mainly in the presence of a high virus load, in blood for example (Wilkinson, 1989; Sánchez-Vizcaíno, 2006). The virus has a high tenacity in the environment and can survive for several months, up to years, in pork products (Adkin et al., 2004). Swill feeding is believed to play a major role in the spread of ASF over long distances and was responsible for most outbreaks in Europe in the past (Plowright et al., 1994; Seifert, 1996; Lyra, 2006; Sánchez-Vizcaíno et al., 2009; Blome et al., 2011).

Factors influencing virus transmission include the intensity of pig-to-pig contact, poor biosecurity, management problems, and lack of awareness amongst farmers, hunters and veterinarians (Böhle & Depner, 2010). There are quite a large number of differential diagnoses, resulting in late detection in countries that have not experienced ASF before.

Pig farming in the EU shows considerable variation between MS. Major intensive production regions are located in Denmark, Germany, the Netherlands, Belgium, France, Spain, Italy and Poland. Small pig producers are mostly found in the new MS (EUROSTAT, 2010). The pig industry is structured into pyramidal production cycles where specialised breeding units supply fattening units with weaned piglets. The functions of breeding and fattening may be combined on farrow-to-finisher farms. This specialisation is also noticeable across borders with Germany being the main importer and Denmark the main exporter of piglets and breeding animals (EUROSTAT, 2010).

The production type and size greatly influence the contact pattern between holdings (Boklund et al., 2008; Ribbens et al., 2009; Nöremark et al., 2011). Furthermore, a correlation between farm size and biosecurity standard has been described: large units have been found to be more likely to implement preventive measures such as quarantine and to have disinfection facilities, and thus to have a lower risk of introducing pathogens (Boklund et al., 2003; Boklund et al., 2004).

The objective of this project is to develop a simulation model for the spread of ASF within a country and between MS during the HRP, with a focus on the identification of “super-spreaders” (MS that are most likely to spread disease to other countries) and “super-receivers” (MS that are most at risk of introduction of ASF once the virus circulates in any of the EU MS) for different lengths of HRP.

MATERIALS AND METHODS

The model for ASF spread was implemented in the InterSpreadPlus® software (version 2.001.14, www.interspreadplus.com), a stochastic, individual-based, discrete-time, spatio-temporal state-transition simulation model (Stern, 2003). Within- and between-country spread was assessed using network analysis of intra-Community live pig trade data, national contact patterns and selected attributes of pig holdings. In the model, intra-Community trade was referred to as export (outbound movement of one or more live pigs as a single consignment) or import (inbound movements of one or more live pigs from other EU countries).

Model structure

The model consists of two parts, within-country and between-country spread. For modelling spread within a country, transmission through movement of live pigs between farms, transport contacts and professional person contacts were considered.

For the spread of ASF between countries, four steps were followed: 1) based on farm attributes, farms were eligible or not for intra-Community trade; 2) the frequency of outgoing movements from an eligible farm to a fictitious export location was calculated using TRACES data; 3) the probability for a movement to take place from this export location to a fictitious import location in another MS; and 4) the survival probability of infected pigs during transport to take account of the delay in arrival at the destination farm due to transport of pigs over longer distances (e.g. piglets sent from Denmark to Spain) and the progressive nature of the disease. For these four steps in the model the following results were recorded: step 1) in the MS with the index case, the number of infected premises as an indicator of the magnitude of the epidemic during the HRP; step 2) the number of live pig movements during the HRP from infectious premises in the country of origin to its export location (countries with the greatest number were defined as “super-spreaders”); step 3) the sum of incoming movements from the other 26 countries to the import locations of each MS (countries with the greatest number were defined as “super-receivers”); step 4) the number of infected premises in a “secondary case MS” as an indicator of how often imported infected pigs may come into effective contact with the domestic pig population. Only part of the movements arriving at the import location (step 3) will actually be forwarded to step 4 as infected pigs may die during transport. Thus, movements between export and import locations are referred to as “targeted” imports, whereas movements with surviving pigs are “completed” imports.

Further spread within the importing country was not modelled due to computational limits. However, by comparing the period between potential introduction into the secondary case MS and detection of the index case in the index case MS with the same number of days in the initial phase of the respective within-country spread model, the outbreak size in a secondary case MS can be estimated (example: MS “A” infects MS “B” on day 20. The index case is detected in MS “A” on day 30. The potential spread in MS “B” between day 20-30 corresponds to the spread occurring in the model with MS “B” as index case MS between day 0-10). In case a movement terminates at step 3 (i.e. infected pigs die before they infect the destination holding), the possibility of indirect spread via fomites was not considered in the spread model.

The duration of one time step is one day. All farms were considered susceptible at day 0. In every country model the state “Infected” was set on day 1 for one randomly selected index farm. It was assumed that once the first animal became infectious, the herd was infectious until disease was detected. Individual animals may recover or, in the context of ASF, more likely will die, but

the farm itself will remain infectious as disease spreads through the herd. As the model was terminated after a specified duration of the HRP, no farm was assumed to be able to recover.

Model parameterisation

Study population: The population at risk comprised all pig keeping premises in the EU27 and resulted in ca. 3.5 million individual simulated records (source: EUROSTAT, latest numbers available for 2007). The farm input file consisted of a list with Cartesian coordinates of all farms and attributes specifying the production type and size associated with each location (Stern, 2003). As exact coordinates of premises were not available EU-wide, they were generated using ArcGIS (ArcMap Version 9.3, www.esri.com) and randomly located into the respective region based on its known total number of pig holdings in the Nomenclature of Territorial Units for Statistics (NUTS) 1 and NUTS2 regions. The French overseas departments and Sardinia were excluded; the latter as the disease is already present on this Italian island and legal trade restrictions currently in place (European Commission, 2011). When generating the random coordinates, the minimum permitted distance between any two farms was set as 100 metres.

Each farm location generated in this way was randomly assigned a production type and size category (based on the predefined EUROSTAT categories) taking in account the known structure of the pig industry of the MS (source: EUROSTAT). The production type determined the mixing structure ("with whom"), whereas farm size triggered the frequency of contacts ("how often"), the probability of becoming infected (biosecurity) and the probability of being eligible to export.

The resulting seven contact groups were i) large breeder (>100 breeding sows and <10 fattening pigs), ii) large fattener (>400 fattening pigs and no breeding sows), iii) large farrow-to-finisher (>100 breeding sows and/or >400 fattening pigs not covered under another large production type class), iv) medium-sized breeder (10-100 breeding sows and <10 fattening pigs), v) medium-sized fattener (10-400 fattening pigs and no breeding sows), vi) medium-sized farrow-to-finisher (farms with >100 breeding sows and >400 fattening pigs not covered under another production type class), and vii) backyard farms (<9 fattening pigs and no breeding sows). The rationale for this categorization was: breeders are considered to be mainly piglet producers for fatteners; in farrow-to-finishers all age groups are present and these farms do not generally purchase animals but may supply fatteners with weaner piglets; backyard farms concentrate on producing pork for own consumption and make a minimal contribution to regional level trade. Classification into size categories was performed to be able to distinguish between farms operating mainly at a regional level and highly specialised, industrial-type pig producers.

Contact structure: To model the within-country contact structure, a simplified, generic contact pattern between farms was developed for the EU MS (detailed description in Tables 1-2). Each movement was characterized by a set of parameters obtained from the attributes of the source farm (location, size and production type), frequency of movement, movement distance, probability of transmission and destination probability (dependent on the attributes of the destination farm).

Data: The model was parameterised with data obtained from (i) EUROSTAT (EUROSTAT, 2007), TRACES (requested from the European Commission for the period Sept. 2010 – Aug. 2011) and national databases, (ii) the scientific literature, (iii) a questionnaire sent to all EU

Table 1. Contact frequency within country: Number of live pig movements per time period, λ, and approximate number of days between two off-farm movements. Source: Several scientific papers from eleven countries, see "Data"

Contact group	Distribution and λ of the number of movements per day[a]	
Large breeder	Poisson 0.125	(every 8 days)
Large fattener	Poisson 0.0165	(every 60 days)
Large farrow-to-finisher	Poisson 0.0103	(every 96 days)
Medium-sized breeder	Poisson 0.0625	(every 16 days)
Medium-sized fattener	Poisson 0.0083	(every 120 days)
Medium-sized farrow-to-finisher	Poisson 0.0052	(every 192 days)
Backyard	Poisson 0.0028	(every 365 days)

[a]Transport of slaughter animals is covered under movements of transport lorries, therefore λ is considerably lower for fatteners and farrow-to-finishers than for breeders.

Table 2. Distributions and values used in InterSpread Plus® to describe the contact structure within- and between countries. The last column gives additional explanations, comments and, in brackets, the data source. MS = Member States

Parameter	Distribution & Values	Rationale & Comments (Source)
Contact structure, within-country		
Movement distance of pig movements	Movement probabilities in three distance bands (0 – 10 km, >10 – 100 km, >100 – 3.000 km)	MS specific values (collected with a Questionnaire)
Movement distance of lorries	0 – 15 km: 5 % >15 – 55 km: 45 % >55 – 110 km: 45 % >110 – 150 km: 5 %	Probabilities of movement in four distance bands (EUROSTAT: [tran_r_veh_jour], [food_pd_aatran27])
Movement distance of professional contacts	0 – 10 km: 72 % >10-20 km: 8 % >20-30 km: 8 % >30-100 km: 12 %	Probabilities of movement in four distance bands (Boklund et al., 2008)
Contact structure, between countries		
Number of export movements per time period ($\lambda_{ti,Export}$)	Poisson distribution	MS specific values (TRACES: requested for the period Sept. 2010 to Aug. 2011)
Destination probabilities of exporting movements	Tabular form with probabilities of movement	MS specific values (EUROSTAT: EU27 Trade Since 1988 By CN8 [DS-016890] for the years 2008 - 2010)

chief veterinary officers and national pig associations and (iv) expert opinion (one virologist interviewed by telephone). Data to describe the contact pattern was generated by combining published data from eleven European countries (Stärk et al., 2006; Bigras-Poulin et al., 2007; Boklund et al., 2008; Lindström et al., 2009; Martinez-Lopez et al., 2009; Nigsch, 2009; Ribbens et al., 2009; Lurette et al., 2011; Nöremark et al., 2011) and Marques, personal comm. (Tables 1-2). Data related to infectivity are summarized in Table 3. Assumptions made due to lack of data were tested in the sensitivity analysis. Table 4 summarises parameters used in the model and outlines major assumptions. A detailed description of the full model is provided in Nigsch et al. (2012).

Table 3. Distributions and values used in InterSpread Plus® to describe infectivity settings. The last column gives additional explanations, comments and, in brackets, the data source

Parameter	Distributions & Values	Rationale & Comments (Source)
Time to clinical signs	BetaPERT (3; 5; 13)	Depner, personal comm.; (FAO, 2009)
Infectivity	2 days before clinical signs: 50 % 1 day before clinical signs until end of simulation: 100 %	Pre-infectious period: 24-48 h before clinical signs appear, infected domestic pigs may shed infective amounts of virus. (FAO, 2009)
Probability of transmission , general setting		Relative to clinical signs
Probability of transmission due to pig movements (PoT_{Pig})	Uniform 0.0647	Values based on CSF, halved to account for less infectious ASFV. Adapted from (Stegeman et al., 2002)
Probability of transmission due to transport lorries (PoT_{Lorry})	Uniform 0.011	Values based on CSF, halved to account for less infectious ASFV. Adapted from (Stegeman et al., 2002)
Probability of transmission due to professional person contacts (PoT_{Prof})	Uniform 0.03	(Mangen et al., 2002)
Adjustment in the transmission probability due to better biosecurity on large farms ($Adjust_{Bio}$)	Uniform 0.5	Large farms have a 50 % lower probability of becoming infected due to better biosecurity status. Based on (Boklund et al., 2003; Boklund et al., 2004)
Survival probability of exported infected pigs	BetaPERT (0.05; 0.2; 0.8)	Due to progressive nature of disease and long transport distances infected pigs may not survive until arrival at the destination location (Mur et al., 2011)

Scenarios

A separate model was run for every MS (27 country models), with the seed of the outbreak set in a densely populated pig area selected according to the regions indicated for each MS in the questionnaire or, if information was missing, the NUTS1 regions with the highest number of

pigs/km^2 (EUROSTAT, 2011). The index case location was randomly chosen and allocated, one-by-one, to any of the seven possible combinations of production type and herd size categories. For all seven contact group scenarios six HRP scenarios (10, 20, 30, 40, 50 and 60 days) were simulated, resulting in a total of 1,134 scenarios (27*7*6). Every scenario was run with 300 iterations to allow estimation of the likely variability in the outcome variables. 300 iterations provided a good balance between computational run time and the stability of the distribution of outbreak size with the mean number of infected premises varying by <2.5 % with a higher number of iterations.

The number of infected farms and exports leading to infection were summarized (mean, most likely, 95 % interpercentile range) and compared between countries. To identify "super-spreaders" and "super-receivers" the frequency of export and import of potentially infected pigs (proportion of iterations with exporting movements) was determined. Additionally, the number of trade partners of "super-spreaders" and "–receivers" was derived.

Table 4. Major model assumptions and simplifications and their likely impact on the results, if incorrect

Assumption / simplification	Consequences / Impact on the results if incorrect → justifications
Infectivity settings	
Transmission probabilities for ASF are 50 % lower than values presented for CSF (Stegeman et al., 2002)	Under- or overestimation of probability of transmission → tested in sensitivity analysis
Large farms have a better biosecurity and therefore 50 % less the probability of becoming infected via incoming infected live pigs, professional contacts and transport lorries (better implementation of quarantine, etc.)	Under- or overestimation of probability of transmission → tested in sensitivity analysis
Contact structure within the country	
Every farm has the same probability of being a contact of an infected farm if basic requirements are fulfilled (e.g. distance and contact group). No fixed routes due to business contracts between farms, etc. exist.	Under- or overestimation of disease spread within a country, not modelled explicitly
Contact structure between countries	
80 % of all exports of a MS originate from large farms, 20 % from medium-sized farms. No pigs are exported from backyard farms.	Under- or overestimation of likely spread via export → tested in sensitivity analysis
Exported slaughter pigs and cull sows do not come in contact with the domestic pig population in the MS of destination.	Underestimation of role of abattoirs→ tested in sensitivity analysis

Sensitivity analysis

Due to the large number of models only a selected number of scenarios were included in the sensitivity analysis. A crude sensitivity analysis was performed to identify the key input variables by increasing parameter values by 25 % and 100 %. In an advanced sensitivity analysis (Vose, 2008) the impact of key variables identified in the crude sensitivity analysis was further investigated by running the model with a wider range of parameter values.

RESULTS

Different contact groups as seed for a given set of parameter values led to very similar outbreaks. Thus, detailed results are only presented for the "worst-case" scenarios with large breeders as index cases. This contact group consistently resulted in most infected premises and export activities.

The longer the HRP, the more premises became infected both due to within- and between-country spread, this was reflected in an increasing percentage of iterations in which exporting movements take place. However, in most scenarios the index premise would not result in spread. During the first ten days of an outbreak the model predicts the export of potentially infected pigs for seven MS. With a HRP of 60 days, movement of infected pigs will occur in at least one out of 300 iterations from 16 MS, with six MS spreading ASF in >10 % of iterations and will target 18 MS in total. Only six MS neither spread nor received movements (Table 5).

"Super-spreaders"

By comparing the number of movements from infectious premises for export, Denmark, the Netherlands, Lithuania and Latvia were identified as "super-spreaders". With a 60-day HRP all four countries provided from two to eleven trade partners with infected piglets or breeding animals, but had only one main trade link for which export occurred in more than 10% of iterations (Table 5). With eleven trade partners, the Netherlands had the most trade partners among the "super spreaders".

"Super-receivers"

Germany and Poland were the countries that most often became the target destination for export of infected pigs, followed by Romania and the United Kingdom. With seven trade partners from which potentially infected pigs were imported during a 60-day HRP, Poland and Romania were the MS with the highest number of different trade partners (Table 5). When considering "completed" imports only (infected pigs arrive at the destination farm alive), Germany (19.3 %) and Poland (14.7 %) still had a high risk of becoming infected (results not presented).

Sensitivity analysis

The sensitivity analysis was performed for Denmark and Lithuania, which were two "super-spreaders" with different pig production system structures. Amongst all parameters tested in a crude analysis, for both MS the number of infected premises within the country was most

Table 5. Overview: Number of trading partners and percentage of iterations in which trade with live pigs from infectious premises took place between the Member State (MS) of origin (row) and the MS of destination (column). Iterations: in light grey: 5 – 10 %; in medium grey: 10 – 20 %; in dark grey: > 20 %. MS that are not listed[a] did not produce and/or were not targeted by a movement of infected pigs. Scenario: High Risk Period = 60 days; index premise = large breeder; 300 iterations.

MS of origin	MS of destination (%)																		**Total**
	AT	BE	CZ	DE	DK	ES	FR	GR	HU	IT	LU	LV	NL	PL	RO	SI	SK	UK	**(%)**
AT	-	-	-	0.7	-	-	-	-	0.3	-	-	-	-	-	-	4.3	-	-	**5.3**
BE	-	-	-	-	-	-	0.7	-	-	0.3	-	-	0.7	-	-	-	-	-	**1.7**
CZ	-	-	-	-	-	-	-	-	0.7	-	-	0.3	-	0.3	-	-	0.7	-	**2.0**
DE	-	-	-	-	-	0.3	-	-	-	0.3	-	-	0.7	-	0.3	-	-	-	**1.7**
DK	0.3	-	1.0	38.7	-	-	0.3	-	-	2.7	-	-	1.0	5.3	-	-	0.7	-	**50.0**
FR	-	-	-	0.3	-	0.3	-	-	0.3	-	0.3	-	-	-	0.3	-	-	-	**1.7**
HU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6.0	-	0.7	-	**6.7**
IE	-	-	-	-	-	0.3	-	0.3	-	0.7	-	-	-	0.3	-	-	-	12.3	**14.0**
LT	-	-	-	-	-	-	-	-	1.0	-	-	-	-	23.7	3.3	-	1.3	-	**29.3**
LU	-	-	-	13.7	-	0.7	1.0	-	-	-	-	-	-	-	2.7	-	-	-	**18.0**
LV	-	-	-	-	1.0	-	-	-	-	-	-	-	-	26.3	-	-	-	-	**27.3**
NL	-	2.7	0.3	14.7	-	2.0	0.3	-	2.7	4.0	-	-	-	1.3	3.3	0.3	0.7	-	**32.3**
PT	-	-	-	-	-	0.3	-	-	-	-	-	-	-	-	-	-	-	-	**0.3**
SI	-	-	-	-	-	-	-	-	-	-	-	-	0.3	-	-	-	-	-	**0.3**
SK	-	-	0.3	-	-	-	-	-	1.7	-	-	-	-	1.0	0.7	-	-	-	**3.7**
UK	-	-	-	-	-	-	-	-	-	-	-	-	0.3	-	-	-	-	-	**0.3**
Total (%)	**0.3**	**2.7**	**1.7**	**68.0**	**1.0**	**4.0**	**2.3**	**0.3**	**6.7**	**8.0**	**0.3**	**0.3**	**3.0**	**58.3**	**16.7**	**4.7**	**4.0**	**12.3**	

[a] Bulgaria, Cyprus, Estonia, Finland, Malta and Sweden.

sensitive to changes in the transport frequency ($NpTP_{Lorry}$) and transmission probability (PoT_{Lorry}), see Fig. 1. The export frequency ($_{Export}$) had no effect on the within-country spread but showed the highest impact on the final number of export movements and was therefore further investigated in the advanced sensitivity analysis.

Sensitivity analysis

The sensitivity analysis was performed for Denmark and Lithuania, which were two "super-spreaders" with different pig production system structures. Amongst all parameters tested in a crude analysis, for both MS the number of infected premises within the country was most sensitive to changes in the transport frequency ($NpTP_{Lorry}$) and transmission probability (PoT_{Lorry}), see Fig. 1. The export frequency ($_{Export}$) had no effect on the within-country spread but showed the highest impact on the final number of export movements and was therefore further investigated in the advanced sensitivity analysis.

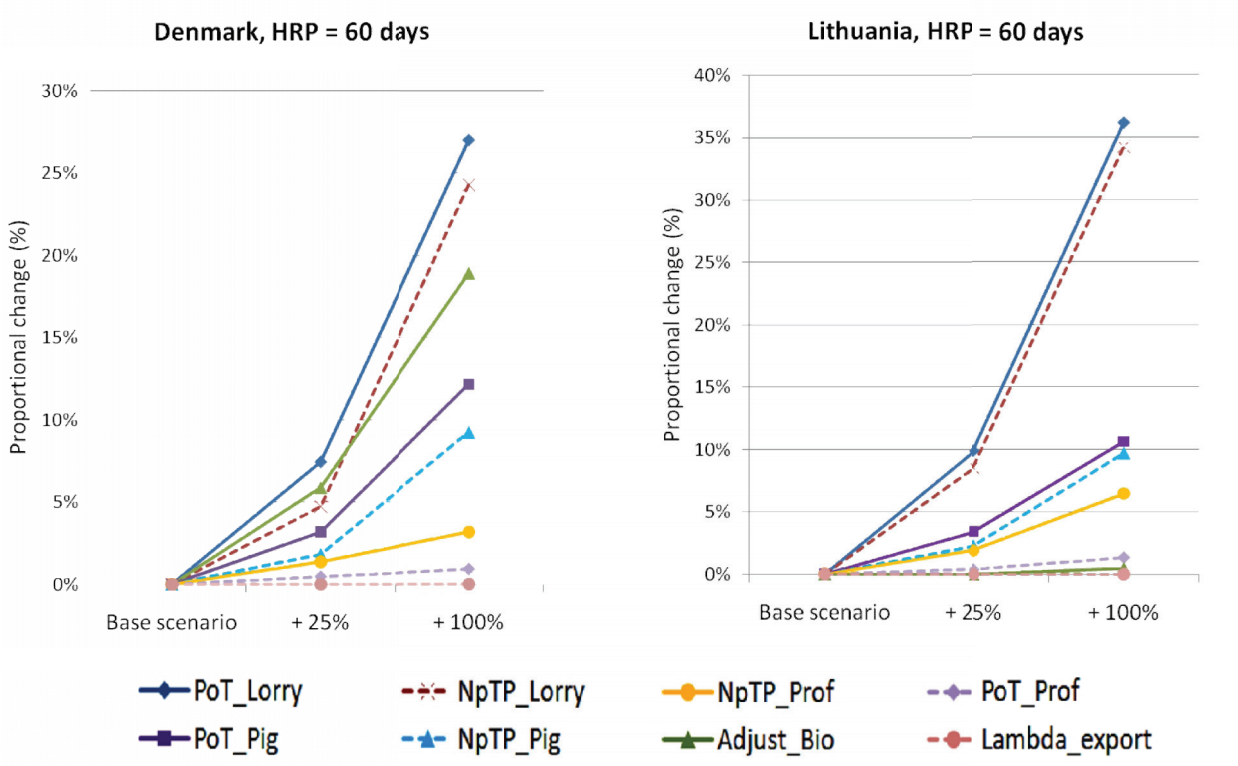

Fig. 1 Results of the crude sensitivity analysis: Proportional change (%) of the number of infected premises during a HRP of 60 days if one of eight selected input parameters is increased at a time by 25 % and by 100 %. All other input parameters were kept constant. Scenario: seed countries = Denmark (left) and Lithuania (right); index premise = large breeder; 300 iterations. Pot_Pig / _Prof / _Lorry = Probability of Transmission due to pig movements / professional contacts / transport lorries; NpTP_Pig / _Prof / _Lorry = Number per Time Period of movements of pigs / professional contacts / transport lorries; Lambda_export = of export movements per premise; Adjust_Bio = Adjustment in the transmission probability due to better biosecurity standards

In the baseline scenarios all large and medium-sized premises were eligible for export. This assumption was tested by changing this proportion to 75, 50, 25, 5 and 1 %. The model suggests

that the number of exports from infectious premises is strongly influenced by (i) a decreasing proportion of eligible premises, with a critical threshold of 25 % for Denmark, and (ii) the index premises being eligible, if the total annual number of exports per MS is kept constant (Fig. 2). Other MS show a similar trend, but with different thresholds.

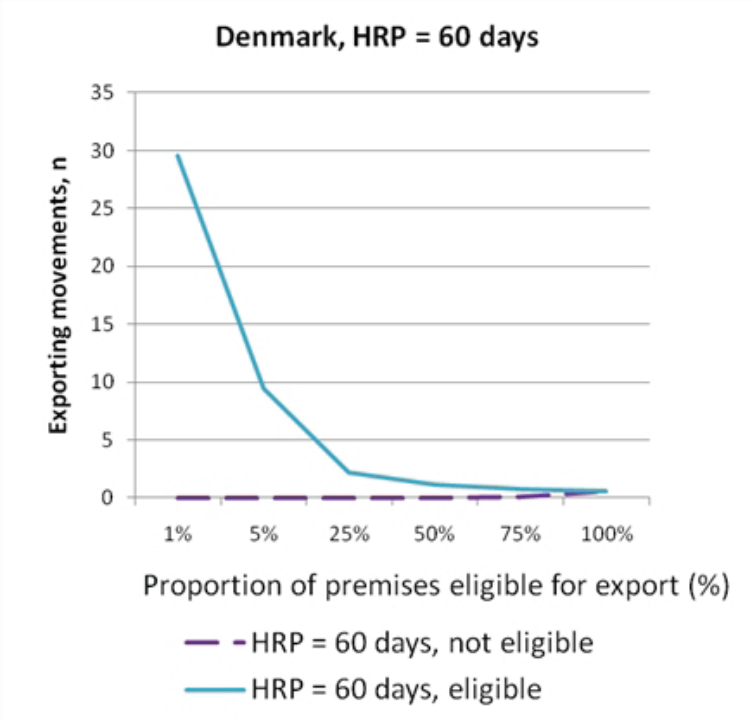

Fig. 2 Advanced sensitivity analysis: Proportion of large and medium-sized premises participating in intra-Community trade plotted against the number of simulated exporting movements of infected pigs occurring during a HRP of 60 days with the index farm being once eligible (solid line: the index farm is among the exporting farms) and once not eligible for export (dashed line: the index farm is part of the non-exporting proportion of large and medium-sized farms. In this case, exporting movements of infected pigs can only occur if infection spreads from the index farm to a farm eligible for export). Note: because the total number of exports per year per country stayed constant in all scenarios (based on TRACES data) while the proportion of eligible farms was decreased, the of export movements per eligible premise increased. Therefore the number of infectious exporting movements is highest when a small proportion of farms, including the index farm, participates in export. Scenario: seed country = Denmark; index premise = large breeder; 300 iterations

DISCUSSION

In this study an individual-farm-based model approach was used to predict ASF spread within the EU. Similar models have been used in animal health to model within-country spread (Taylor, 2003; Harvey et al., 2007), but, to the authors' knowledge, never for the whole EU. In the current study, the use of this modelling approach was considered appropriate due to the presence of a fully susceptible population within the EU, the recent occurrence in Russia and Trans-Caucasia of a highly virulent virus strain (EFSA, 2010) and the transboundary

connectedness of the pyramidally structured pig production sector. At the same time, it is recognised that individual farm data is incomplete, and that the understanding of the relative importance of different transmission pathways in the European context is limited.

Generally, data quality was good for countries where pig production and international trade play an important role, and for those that experienced outbreaks of notifiable pig diseases in the past. However, apart from the answers to the MS questionnaire, little data was available for the twelve new MS, except for Romania and Bulgaria where detailed epidemiological data had been collected during the recent CSF outbreaks. Language certainly was an additional hurdle for the literature search. Nevertheless it seems unlikely that differences in data availability and quality between MS resulted in biased model outcomes regarding between-country spread, since intra-Community trade of live pigs is of minor importance for some of these countries (according to EUROSTAT and TRACES data).

The EUROSTAT database served as a key information source. It is based on MS reports and the latest data on agricultural demographics date back to 2007 (EUROSTAT, 2007). However, the pig industry is a rapidly changing sector. Europe-wide the number of pig farms has decreased between 7-12 % annually since 2000, with a trend towards larger farms (EUROSTAT, 2010). Extrapolating this trend to 2011 would have resulted in 30 % less farms, and thus the size of the study population in the model, based on data from 2007, would be overestimated. On the other hand, MS apply different criteria for counting pig farms, e.g. some governments will only include holdings that have more than a certain number of livestock (Statistisches Bundesamt, 2009) whereas elsewhere all small-scale farms are reported (Statistik Austria, 2011). It is not possible to assess whether these two biases cancel each other out, but for the purposes of this study it was decided that the official data would be used. Scientific studies that combine data from several EU countries would strongly benefit from harmonized data collection.

This project particularly aimed to investigate the transmission of ASF through legal cross-border movement of live pigs during the HRP. In order to account for differences between countries, within-country spread was also simulated to reflect a realistic infection reservoir that would be a source of infected live pigs for export. Backyard farms generally appear to be excluded from studies involving pig contact network data as these farms often are not covered by production organisations or national databases. Importantly, the amount of small-scale farms differs considerably between the (first) fifteen EU MS and the twelve new MS. The role of backyard farms in disease spread is difficult to estimate as these farms often keep several livestock species (EUROSTAT, 2011). The literature suggests that mixed farms have more contacts than farms with a single species (Nöremark et al., 2011). Thus, the importance of small farms in disease dynamics may be underestimated in this model because little contact with other contact groups was assumed. On the other hand, indirect contact is described to be of lesser importance for ASF than direct contact as long as no blood products or swill are involved (Sánchez-Vizcaíno, 2006). For CSF, Alexandrov and co-workers (2011) conclude that backyard pigs are important for sustaining an endemic situation but play a minor role in the spread of disease over wider distances and therefore are of less importance during the HRP. Similar observations have been made for ASF in Russia (OIE, 2011; Rosselkhoznadzor, 2011).

According to EUROSTAT, Denmark supplied 23 MS with live pigs between 2008 and 2010, but has only three main trading partners (each receiving >5 % of the total Danish trade volume of live breeding animals and piglets). The results of this study highlight the impact of these "strong" trade links during the HRP: two thirds of all intra-Community spread was due to six specific trade links between two countries (Denmark → Germany, Latvia → Poland,

Lithuania → Poland, the Netherlands → Germany, Luxembourg → Germany and Ireland → UK, see Table 5).

Cross-border spread of disease is likely to be faster if exporting and importing farms are closely connected through traders or as members of large cooperatives, or even if the same animals are moved several times. Prominent examples are the 2001 FMD outbreak (Gibbens et al., 2001) or the introduction of equine infectious anaemia from Romania via France and the Netherlands into the UK in 2010 (Standing Committee on the Food Chain and Animal Health, 2010). However, pigs are less likely to be re-sold compared with horses or cattle (Statistik Austria, 2011) due to their usually shorter life span and low profit margins. The spread of CSF from Germany to the Netherlands in 1997 supposedly was linked to indirect transmission via a lorry during the HRP. It is worth noting that the spread from the Netherlands to Belgium, Spain and Italy during the same epidemic occurred when the Dutch index farm had already been identified as a suspect case and potentially even after it had been confirmed (Elbers et al., 1999).

In the current study, most importantly, none of the "super-receivers" was identified as a "super-spreader" and *vice versa*. This finding corresponds with the EU-wide trend towards specialisation of the pig industry at a national level and also between MS. Furthermore, the model showed that predictions are sensitive to the proportion of exporting premises. These numbers were unfortunately not available for all countries, and also may be strongly influenced by a MS's disease status. Live pig trade from Romania and Bulgaria was restricted during the time period used for this study due to their CSF history and hence these two countries were not found to contribute to disease spread in the present model. It is important to continuously monitor changes in intra-Community trade patterns so that valid predictions can be generated.

A range of known transmission pathways have not been included in the current model. For wild boar, soft ticks and pork products, it was assumed that these pathways play a minor role during the HRP. Inclusion of illegal pig transports would have added large uncertainty and would have made it difficult to interpret the validity of any other results. The model showed though, that the number of exporting premises and the total number of exports are most important and it is plausible that the same would be true for illegal transport.

Due to lack of data, a simplified model of virus transmission and a generic contact matrix that was applied to all countries were used. The contact network could easily be extended to a more detailed contact network for future modelling experiments so that within-country spread could be modelled more accurately as additional data becomes available. Because quantitative data on ASF transmission were not available, data published for CSF were used. It is acknowledged that CSF is easily transmissible between animals through direct and indirect contact (Moennig et al., 2003) whereas ASF requires close contact (EFSA, 2010), thereby leading to an overestimation of the transmission risk for ASF in the current model. It is likely that the relative and absolute importance of different transmission routes differs between the two diseases. However, the results of the sensitivity analysis indicated that varying the parameters linked to transmissibility resulted in only minor changes in the main modelling outcomes.

Validation of the model itself is important to assess the value of the results (Kopec et al., 2010). The stochastic nature of Interspread Plus® allowed the modelling of the initial stages of an outbreak, when small numbers are involved and individual-to-individual transmission dominates (Germann et al., 2006), and the software demonstrated the required flexibility for the needs of the project. Validation for this project is difficult because no outbreaks have occurred in a region that is comparable to Europe, and no other ASF spread models for the EU exist.

However, external comparison of Interspread Plus® with other models has been undertaken for FMD (Dubé et al., 2006) and it was shown that it performs similarly to other simulation models. Even though this gives the software and its underlying algorithms credibility, it does not provide proof that the modelling results are suitable for decision making (Schley, 2007). However the results of this ASF spread model are epidemiologically plausible as EUROSTAT data already indicated Denmark and the Netherlands as prime exporters and Germany and Poland as the main importers of piglets and breeding animals. In addition, whilst the important role of Lithuania and Latvia in viral spread may be surprising, it is necessary to keep in mind that in this study it was assumed that only medium-sized and large farms were eligible for export. Both Baltic countries may have low numbers of exports, but also a low number of eligible farms; accordingly, the probability of spread across borders was relatively high once a large or medium-sized farm became infected.

The results of the model suggest that spread during the HRP is likely to be limited, especially if the HRP is short, which highlights the importance of having good disease awareness in all MS so that intra-Community spread can be prevented and a large scale ASF outbreak avoided if the disease were to be introduced into the EU. The role of the twelve new MS in ASF spread should not be underestimated and close co-operation should be sought to improve data quality for future research. In particular, the contact patterns of backyard farms need to be better understood. Up-to-date data on the production system structure and contact networks needs to be obtained from all MS to be able to map the country-specific contact networks more explicitly. Monitoring trends in intra-Community trade is crucial for informing the between-country spread part of such models.

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EPIDEMIOLOGICAL METHODS 2

INFLUENCE OF CONTACT STRUCTURE BETWEEN HOST ANIMALS ON THE DYNAMICS OF INFECTIOUS DISEASES

C.R. DIETRICH* AND H.H. THULKE

SUMMARY

This paper assesses the differences between the stochastic variant of the SEIR model with 'mass-action mixing' (homogeneous mixing; MAMM) and models with heterogeneously structured contacts of hosts. Individual-based models (IBM) of different hypothetical contact structures were realized by using adjacency matrices to construct heterogeneous networks. Four heterogeneous contact structures were produced:

1) The 'Hierarchy' structure: Only individuals close to each other by hierarchy have relevant contacts.

2) The 'Leadership' structure: A central individual is characterized by a very high contact rate (a 'superspreader').

3) The 'Mother /Child' structure: The close and frequent contacts between a mother and its offspring are simulated.

4) The 'Social' structure: Developed manually, it dictates for each individual of the herd a specific contact behaviour.

On these networks the spread of an infection was simulated and analysed. The main focus was placed on the evaluation of the comparison of major epidemiological variables that characterize an infectious disease and therefore play an important role for epidemiologists and clinicians. It was found that a heterogeneous contact structure has great influence on the spread dynamics of infectious diseases. The stability of the impact of structural properties on the dynamics of spreading infection was found sensitive to randomisations in the contact pattern over time to deal with limited amount of data. The usefulness of an approximation of epidemiological systems by a homogeneous contact structure was qualified. The results can be applied to other epidemiological scales e.g. contact networks between livestock farms or wildlife metapopulations.

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INTRODUCTION

Modelling transmission of infectious diseases ubiquitously is based on the assumption of mass–action mixing (e.g. Anderson & May, 1991). The undoubted reason for the manifold application of the assumption is the technical elegance and easy to solve epidemiological models with mass-action mixing. Despite its widespread use, the appropriateness of mass–action mixing is doubted within limited or spatially structured populations (McCallum et al., 2001; Eisinger&Thulke 2008).

The limited number of studies which have searched evidence for mass-action mixing within animal populations report varied results. For example, mass-action mixing has been found unsuitable for modelling virus spread in juvenile stage of gypsy moth (D' Amico et al., 1996) and the African army–worm (Reeson et al., 2000). Also transmission of bovine tuberculosis in bush tail possums (Barlow, 2000) and pseudorabies in domestic pigs (Bouma et al., 1995) are suggested to be inadequately modelled using the assumption of mass–action mixing. On the other hand, mass-action mixing was found sufficient for the purposeful modelling of brucellosis within herds of bison (Dobson & Meagher, 1996).

Modern techniques allow to scale down the epidemiology of infectious disease transmission. First the application of proximity data loggers allow measurement of contact structures on the level of individuals (e.g. Swain & Bishop-Hurley, 2007) allowing detailed deviation from random and homogeneous contact probability. Second, advanced computer technology enables epidemiologists to use dynamic disease spread models representing detailed contact patterns in time and space with full explicitness. With the shift of epidemiological paradigm from population level towards heterogeneous individual-based transmission processes the debate of sufficiency of mass-action assumption motivated the question under what conditions the assumption of mass-mixing might still be a purposeful choice or whether the individual-based contact structure has to be considered during epidemiological modelling.

The first steps were undertaken theoretically through comparing models with more complex mixing structures involving explicit contact networks. It has been shown that simple homogeneous mixing can successfully model disease transmission in populations where each individual is continuously moving on a straight line path or when the duration of the contacts are much shorter that the period of infection of the disease (Rhodes & Anderson, 2008; Volz& Meyers, 2007). However, mass–action mixing needs modification to model disease spread through networks where the contacts are clustered (Keeling, 2005) and is unsuitable for modelling the transmission of some sexually transmitted infections (May & Anderson, 1987).

Another motivation to study the sufficiency of mass-action mixing when modelling animal units of limited size is related to limited data structures as generated in the measurement of contact structures from field populations. For example Turner et al. (2008) studied the transmission of E. coli in a dairy herd and constructed contact network models based on a two hour observation period of the animals involved. This very short observation period is common with empirical investigation of contact structures due to technical restrictions although today contact data of animal groups exist that exceed the monthly time scale. Epidemiological analysis of network structure with regards to their support of infectious diseases may result in more robust findings if more extensive data sets could be used. Therefore available data segments may be collated with randomised reproduction or concatenation of shuffled contact data. Hypothetically, an existing structure in the contact network thus might be permuted and eventually suggesting appropriateness of the mass–action mixing assumption due to a technical

artefact rather than the sufficiency of the model approximation by homogeneous contact structures.

The issue raised with epidemiological modelling of disease transmission within livestock or wildlife systems remains unassessed. We therefore evaluate the deviation of epidemiological processes on alternative contact structures with regards to duration and magnitude of exposure of susceptible hosts resulting from spread of an infection through an animal cohort but assuming different typical structures in the contacts of the animals. Moreover, we provide insight into whether or not shuffling the contact structure may converge to mass-acting mixing in the fate of the epidemiological measures.

MATERIALS AND METHODS

The adjacency matrix

Contact structures are always constructed according to certain rules, which are formulated in the form of adjacency matrices. The adjacency matrix A is a symmetric ($a_{ij} = a_{ji}$),n-dimensional square matrix, at which$n$corresponds to the number of individuals. Each entry $a_{ij}$of the matrix is governed by the model-specific construction rules and defines the quality of the contact intensity between two different individualsi and j. The greater a_{ij} is, the greater is the likelihood of a contact between individuali and j. For each of the fivestructures 2000 matrices were prepared, standardized and analysed with respect to structuralparameters and output measures. The sum S_{std} of all matrix entries of a standardized matrix is always the same.

The structure construction

The goal of all considerations of the models is to identify differences between structural approaches. So it is essential to construct a basic model (the mass-action mixing model; MAMM), which serves as a reference to other models and allows comparability. In the adjacency matrix A all entries are equal to 1 with the exception of the main diagonal.According to that all contact frequencies between the individuals of the population are the same. The formalization of the matrix construction is

$$a_{ij} = \begin{cases} 1, & i,j \in \{1,\dots,n\}, i \neq j \\ 0, & i,j \in \{1,\dots,n\}, i = j \end{cases}$$

The structure ‘Hierarchy’ is based on the assumption that there are certain rankings of individuals within a population. Two individuals interact with each other regularly only if their ranking is similar. The hierarchical levelis represented by the index of the individual. The larger the difference of the indices, the greater is the hierarchical difference. Different functions were applied to generate this hierarchical difference.The matrix design is now implemented on the basis of

$$a_{ij} = \begin{cases} X \cdot \dfrac{1}{|i-j|^{\alpha}}, & i,j \in \{1,\dots,n\}, i \neq j, X \sim U(0,1), \alpha \in \{0, \frac{1}{2}, 1, 2\} \\ 0, & i,j \in \{1,\dots,n\}, i = j \end{cases}$$

The exponent α of the function $f = \frac{1}{|i-j|^{\alpha}}$ may take different values. The default value $\alpha = 2$ is used for the analysis of the general spread of infection.

The structure ‘Leadership’ gives one central individual i_c the ability to have more contacts to all individuals than the other individuals have to each other. The adjacency matrix is formed according to the specification

$$a_{ij} = \begin{cases} X \cdot CC, & i,j \in \{1,\dots,n\}, i = i_c, i \neq j, X \sim U(0,1) \\ X, & i,j \in \{1,\dots,n\}, i \neq i_c, i \neq j, X \sim U(0,1) \\ 0, & i,j \in \{1,\dots,n\}, i = j \end{cases}$$

The centrality coefficient CC may take different values. The default value $CC = 128$is used for the analysis of the general spread of infection.

The ‘Mother/Child’ structureis based on a mother-child relationship that is often extremely close.

$$a_{ij} = \begin{cases} X \cdot MCC, & i \in N_{odd} = \{1,3,5,\dots,n-1\}, j = i+1, X \sim U(0,1) \\ X, & i,j \in \{1,\dots,n\}, j \neq i+1, i \neq j, X \sim U(0,1) \\ 0, & i,j \in \{1,\dots,n\}, i = j \end{cases}$$

The mother-child-coefficient MCC may take different values. The default value $MCC = 128$ is used for the analysis of the general spread of infection.

The structure ‘Social’ is a manually developed structure. So the matrix entries a_{ij} are set by hand, but it is also $a_{ij} = a_{ji}$. The goal was to establish contact barriers between several groups of individuals. These barriers are reinforced by social relationships and geographical reasons.

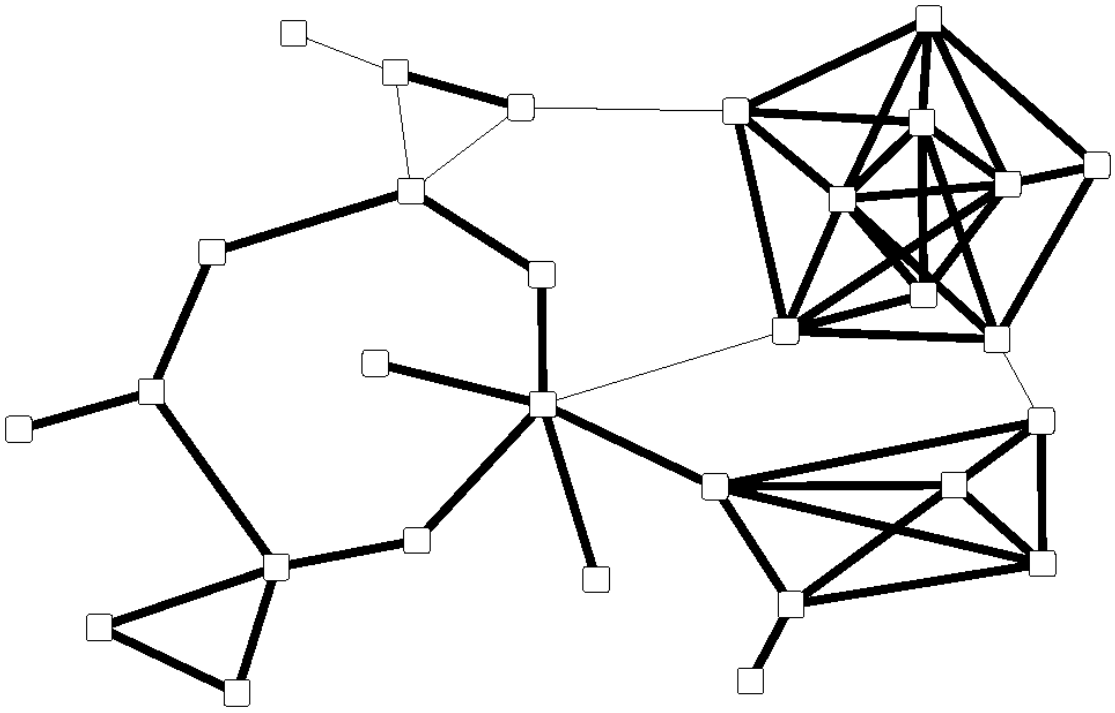

Fig. 1 Visualization of the social structure. Nodes represent individuals and edges represent contact intensity. The weight of the thicker edges is larger by a factor of 100 than the weight of the thinner edges.

The disease spread

The course of the SEIR model is deterministic: $S \to E \to I \to R$. At the beginning of an epidemic, almost all individuals are in the susceptible state*S*. Only one individual is in the exposed state*E*. It goes to the infectious state *I* after a certain time of incubation. Each individual has its own times of incubation and infectiousness which are normally distributed. The waiting time for the next contact event is exponentially distributed.If a contact event happens between a susceptible individual and an infectious individual, there is a chance *p* to infect the susceptible one.

The evaluation values

The evaluation value $\bar{d}$ is the mean number of contacts needed till the infection went extinct and measures how long the disease remains within the herd. $\bar{d}$determines the temporal risk of infection for all susceptible individuals.

The evaluation value $\bar{I}_{max}$ is the mean of the maximum number of infectious individuals and stands for the maximum risk of infection for the susceptible subpopulation.

For the heterogeneous structures ‘Hierarchy’, ‘Leadership’ and ‘Mother/Child’ a significance limit shall be determined by asking: for which intensity of the structural properties the heterogeneous structures differ by less than 5 % from the homogeneous approach with reference to $\bar{d}$? To find the significance limitthe appropriate parameters α, CC and MCC are

varied. The resulting $\bar{d}$ of simulations with different parameter values is regressed on the parameter value to determine the significance threshold for each structure.

The permutation coefficient PC indicates the frequency by which the individuals within the population are permuted, while maintaining the structure itself. $PC = 10$ would thus mean that after every 10 contacts, the population is permuted. The more often the structure is permuted, the more the structural identity vanishes. $PC_{crit.}$ is the critical permutation frequency beyond which the structured model did not show significant difference in $\bar{d}$ in comparison to the mass-action mixing model.

The set of standard parameters

For the analysis of the general spread of infection a set of standard parameters is applied (Table 1).

Table 1. Set of standard parameters.

PARAMETER	VALUE	MEANING
T	150	Simulation time units per simulation
λ	100	Parameter of the exponential distribution for the calculation of the waiting time to the next contact event
n	30	Number of individuals (matrix dimension)
p	0.6	Infection probability per contact
μ_E	4.5	Mean residence time in the exposed state
σ_E	0.75	Standard deviation of the residence time in the exposed state
μ_I	6.0	Mean residence time in the infectious state
σ_I	1.0	Standard deviation of the residence time in the infectious state
S_{std}	870	Sum of all entries in a standardized matrix
CC	128	Centrality coefficient
MCC	128	Mother/Child coefficient
PC	∞	Permutation coefficient

The implemented IBM

The stochastic, individual-based model was implemented in a C++-program. The flowchart of this program is shown in Fig. 2.

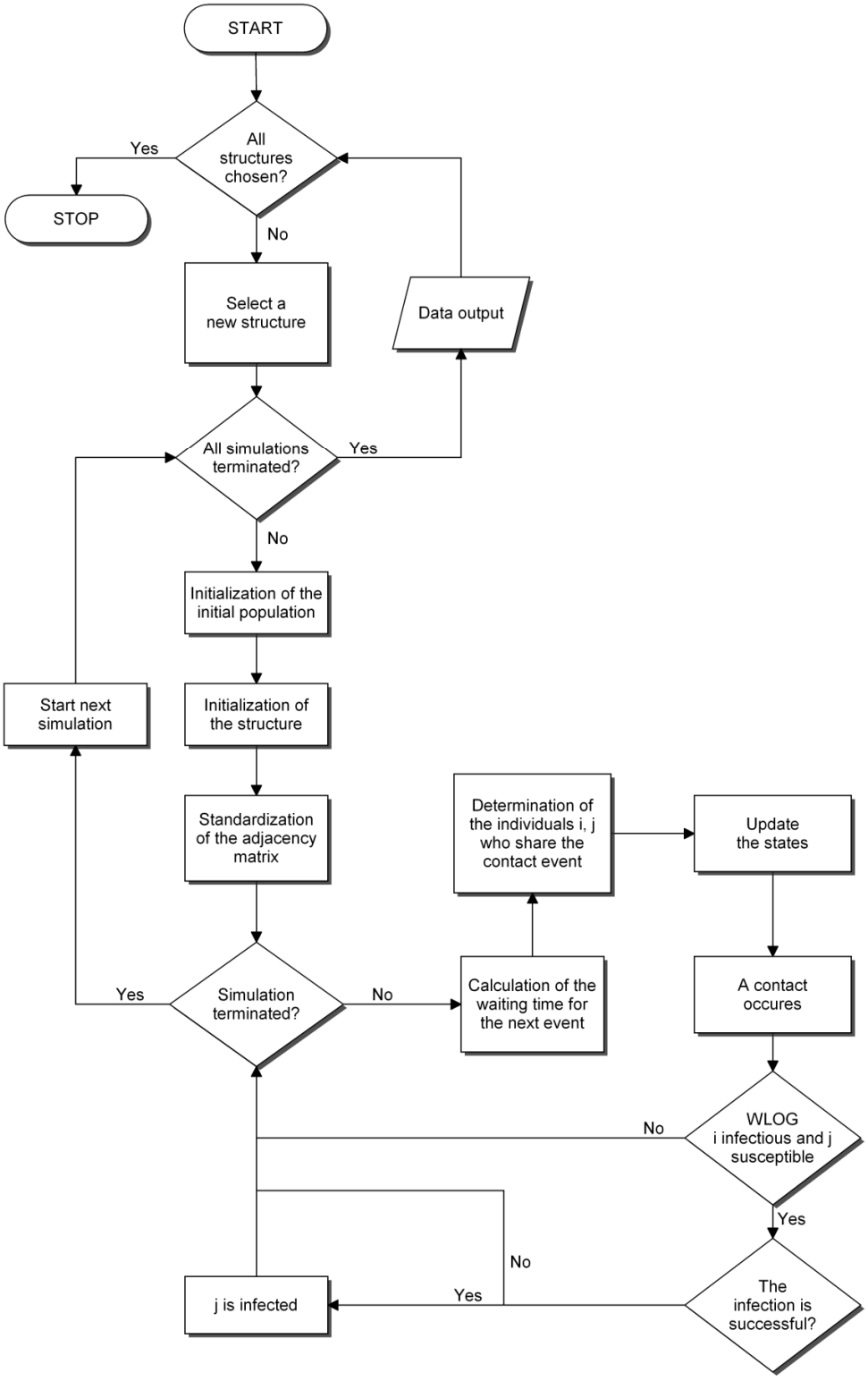

Fig. 2.Flowchart.

RESULTS

General spread of infection

The graphs (Fig. 3) of the general spread of infection give an overview of the diversity of the different structures. The thick lines represent the average cardinal numbers of the states S, E, I and R. The shaded area defines the approximated confidence interval $CI_{approx.}$ comprising 95 % of all simulation runs. The dotted lines indicate the minimum and maximum values of all simulation runs.

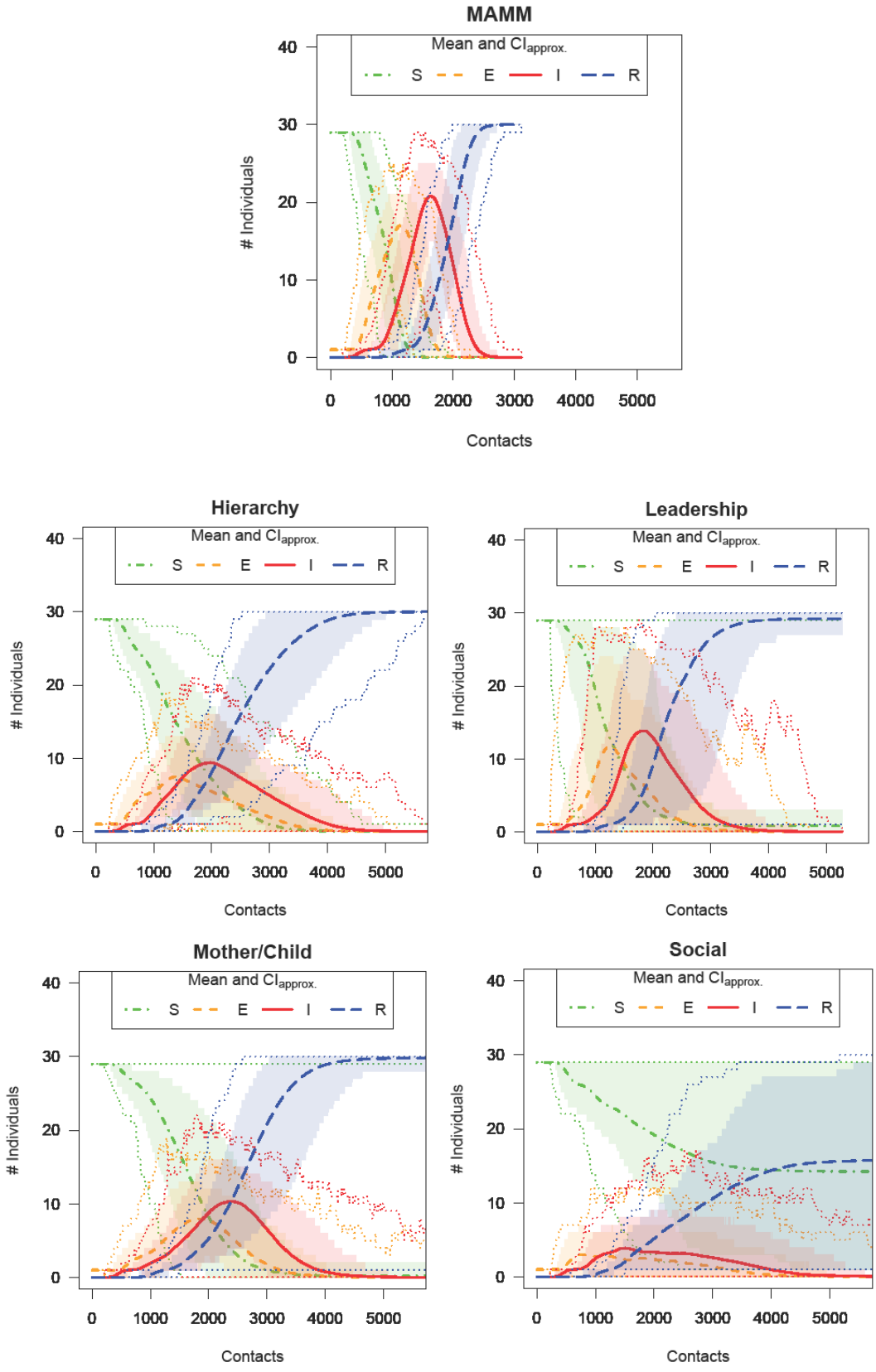

Fig. 3 General spread of infection.

Table 2.Differences of the structures with reference to the evaluation values$\bar{d}$and $\bar{I}_{max}$.

STRUCTURE	$\bar{d}$ [%]	$\bar{I}_{max}$ [%]
Mass-action mixing model	100.0	100.0
Hierarchy	160.1	52.3
Leadership	132.3	81.5
Mother/Child	159.3	58.7
Social	143.4	29.8

Table 2 shows the deviation of $\bar{d}$ and $\bar{I}_{max}$ of the structures 'Hierarchy', 'Leadership', 'Mother/Child' and 'Social' in comparison to the mass-action mixing model. It can be seen that the average duration a disease within a population can be up to 60 % longer, if a heterogeneous contact structure between the individuals is assumed. The average maximum number of infectious individuals shows large variation between contact structures. The $\bar{I}_{max}$ of the social structure reaches just 30 % of the $\bar{I}_{max}$ of the homogeneous model. The differences between the means, medians and standard deviations of d and I_{max} can be compared and assessed in Fig. 4.

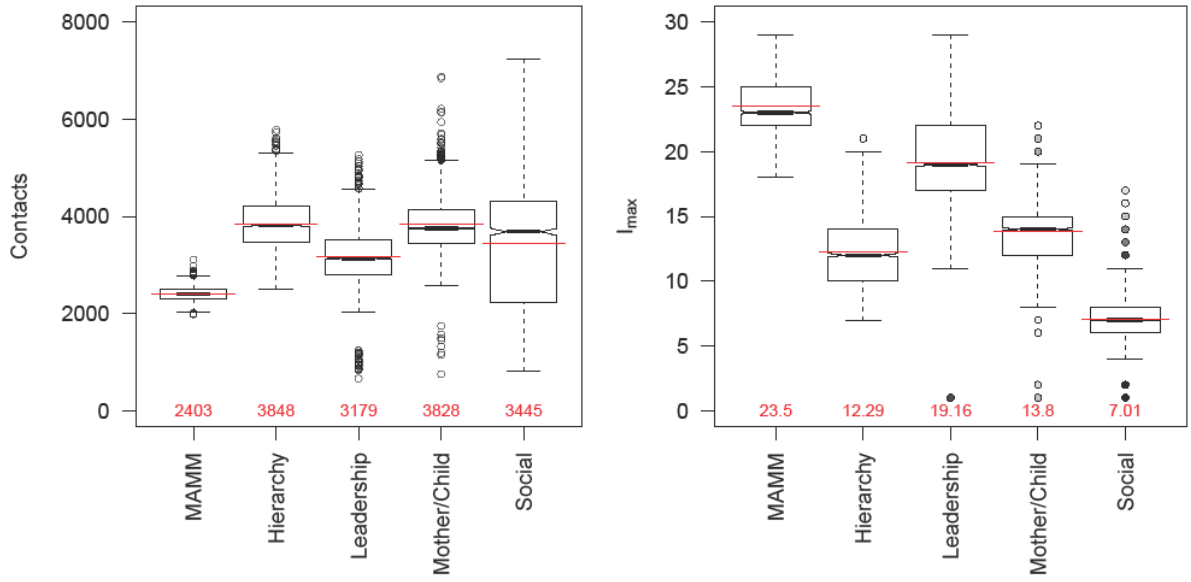

Fig. 4.Boxplots of the number of contacts d to freedom of infection (left) and of the maximum number of infectious individuals I_{max} (right). The means of these evaluation values are marked by the thin lines crossing the box-plots and are printed at the base line. Remarkable high differences between the five contact structures are seen.

Critical values and approximation

The significance limits of the structures 'Hierarchy', 'Leadership' and 'Mother/Child' were calculated with the aid of linear and square regressions. The critical hierarchic function is

$$f_{crit.} = \frac{1}{|i-j|^{0.635}}$$

If a leader has at least $CC_{crit.} = 17.54$ times more often contact to the individuals of the herd than the other individuals have to each other, the difference between a leadership structure and the mass-action mixing approach is significant with respect to $\bar{d}$.

The calculations have shown that the spread of an infectious disease within a population where dams have at least $MCC_{crit.} = 13.41$ times more often contact to their offspring than to other individuals differs significantly from a homogeneous mixed population with reference to the duration $\bar{d}$ of the infection.

Table 3. Differences and attributes of the structures with reference to the significance limit and the critical permutation coefficient $PC_{crit.}$.

STRUCTURE	SIGNIFICANCE LIMIT	$PC_{crit.}$
Mass-action mixing model	-	-
Hierarchy	$\alpha_{crit.} = 0.635$	90.73
Leadership	$CC_{crit.} = 17.54$	1
Mother/Child	$MCC_{crit.} = 13.41$	30.19
Social	-	53.20

The mean number of contacts $\bar{d}$till freedom of infection is shown in Table 4 for varied frequency of structure permutation, represented by thecoefficient PC. With decreasing PC the differences in the mean number of contacts $\bar{d}$ between heterogeneous and homogeneous models decreases. Only the ‘Leadership’ structure has for all PC values a deviation greater than 5 %.

To find the critical PC values of the structures ‘Hierarchy’, ‘Mother/Child’ and ‘Social’ a regression analysis about a part of the $\bar{d}$ values was made. The values $PC_{crit.}$ differ significantly from the homogeneous approach, i.e. they deviate by more than 5 % from the mass-action mixing model with reference to $\bar{d}$. The critical permutation coefficients are illustrated in Table 3.

Table 4. Frequency of structure destruction and the variation of the mean number of contacts $\bar{d}$ to freedom of infection related to $\bar{d}$ of the mass-action mixing model.

PC: FREQUENCY OF STRUCTURE DESTRUCTION	HIERARCHY $\bar{d}$[%]	LEADERSHIP $\bar{d}$[%]	MOTHER/CHILD $\bar{d}$[%]	SOCIAL $\bar{d}$[%]
1	100.8	105.7	101.5	102.7
2	101.1	105.7	101.6	102.4
4	101.2	105.9	101.8	102.7
8	101.1	107.4	102.5	102.7
16	101.7	110.7	103.5	103.1
32	102.7	114.0	105.2	104.1
64	103.8	117.3	108.1	105.4
128	106.3	120.7	111.7	108.1
256	108.4	123.6	115.3	111.4
512	111.6	124.0	119.8	116.6
1024	118.7	123.5	131.0	128.0
2048	145.8	130.9	148.2	156.2
4096	161.1	132.0	159.2	162.2
∞	160.1	132.3	159.3	143.4

DISCUSSION

Many of the evaluation parameters such as the mean duration $\bar{d}$to fade out of an infectious disease, or the mean maximum number of infectious individuals showed huge difference between the mass-action mixing model and heterogeneous model approaches. The differences are pronounced differently, but the conclusion can be drawn that homogeneous modelling should only be applied after analysing accurately the heterogeneity of the system.

With frequent permutation of the individuals involved in a contact structure the characteristics of an epidemiological process disappear. Moreover, the higher the frequency, the greater is the similarity of an infection spreading on a heterogeneous and homogeneous structure and the higher is the probability to mistake the approximation, without recognizing it.

Furthermore, it is clear that even a single permutation during an advanced course of infection leads to enormous variations in evaluation values like$\bar{I}_{max}$, or the overall spread dynamics. It is sufficientto set$PC = 2048$ (equivalent to about 59.45 % of the average duration of an infection process) to change the dynamics of the disease dramatically.

If there is a data set with insufficient recording time / contact number oneshould avoid the mistakeof reproducing a few results by permuting them and attachingthe extended data to the existing data. The effects of a social structure on the spread behaviorof diseases would disappear the more rapidly, the smaller the volume of existing data is.

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DEALING WITH MISSING VALUES: A COMPARISON OF TECHNIQUES

I.E.M. DEN UIJL*, W.A.J.M. SWART AND G. VAN SHAIK

SUMMARY

Ignoring unrecorded values may lead to biased results and lack of power. The aim of this study was to investigate the bias of five different techniques: complete case analysis (CC), missing indicator method (MIND), mean imputation (MEAN), single imputation (SI) and multiple imputation (MI)

Data from a previous study of 177 dairy farms was used as an example. The association between replacement and herd characteristics was modelled using a backwards linear regression (p_{in} =0.15, p_{out}=0.05). Missing values are rarely random. Therefore, missingness of mean age of dairy cows was simulated depending on a random number from a uniform distribution and the association with milk production and incidence of mastitis, which was repeated for an increasing percentage of missingness (5-30% with increments of 5%).

In CC analysis, only 123 out of 177 farms (70%) were available for the analysis, a considerable loss of information. Standard errors increased with increasing % of missingness because of the decreasing number of available cases. MEAN resulted in underestimation of the effect and increasingly smaller errors, because of a more narrow distribution due to imputing mean values for age of dairy cows. SI resulted in some imputations being very good and others way off.. With MI coefficients were closer to the original analyses because extreme imputations were averaged. Increasing percentage of missingness did not seem to affect the estimates nor standard errors of MI.

Multiple imputation seems to be the most appropriate method for dealing with missing values, even in relatively small samples.

INTRODUCTION

Every study suffers from missing values. Farmers quitting or animals dying of causes unrelated to the research are just a few examples of causes for missing values in animal health research. Almost every study suffers from missing values, high quality studies as well as studies of more dubious quality. The quality of a good study is not defined by the absence of missing values, but by the way one deals with missing values. Ignoring these unrecorded values may

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lead to biased results if the association between available records differ from that of missing records. (Dohoo et al., 2003)

Several different techniques are currently available for dealing with missing values. Commonly used are: complete case analysis (CC), or missing indicator method (IND). A more sophisticated method is imputation, where missing values are replaced with another value based on the remainder of the dataset. These values can be calculated in different ways. Mean imputation (MEAN) is the replacement of missing values with the mean of the variable containing missing values. Missing values can also be replaced by a value that is drawn from an estimate of the distribution of that variable (Donders et al., 2006). The distribution of a variable is then estimated by a multivariable regression model containing the non-missing variables. If every missing value is then replaced by a single prediction of that particular model it is called single imputation (SI).

The aim of this study was to investigate the possible bias of five different techniques available for dealing with missing values in a relatively small dataset. The five techniques tested were complete case analysis (CC), missing indicator method (IND), mean imputation (MEAN), single imputation (SI) and multiple imputation (MI). For illustrative purposes, the association between culling percentage and herd characteristics was analysed in a dataset previously constructed for another study, using all five techniques.

MATERIALS AND METHODS

Data on production, mortality, age, disease control, milk quality and udder health was available for 177 dairy farms from a previously conducted study. These farms were a non-random selection of the Dutch dairy farms: half were selected out of the 5% of farms with the lowest health scores based on the Continuous Cattle Health Monitor (henriette poster/artikel), the other half were randomly selected from the remaining 95% of farms.

To illustrate the different techniques for dealing with missing values, the association of culling on a dairy farm with herd and milk characteristics was analysed. This association was strictly used as an example and does not represent the actual reasons or associations for culling on dairy farms in the Netherlands.

Missing values

The dataset without missing values was named “the original dataset” and six other “simulated” datasets were constructed, each with an increasing percentage of missing values. Since missing values are rarely random (Donders et al., 2006; White et al., 2010), missing values in age of dairy cows were simulated depending on the association with two other variables, milk production and incidence of mastitis. A low production level and a high incidence of mastitis were associated with a high probability of being missing. Selected records were randomly transformed to include missing values. These simulations were repeated with an increasing percentage of missing values, 5-30% with increments of 5%, thus generating the six simulated datasets.

In CC all records containing missing values in age of dairy cows were deleted. In IND an extra variable, the indicator variable, was added which was coded 1 if age of dairy cows was missing and 0 if not, additionally age of dairy cows was standardised to (1-indicator variable)*

age of dairy cows. The recoded age and the indicator variable were added to the start model instead of the age of dairy cows which contained missing values. In MEAN, the mean of the remaining values for age of dairy cows replaced the missing values in that variable, e.g. in case of 20% missing values the age dairy cows was calculated based on the remaining 80%.

SI and MI were performed using multivariate linear regression models on the variables in the complete dataset, including the outcome (Moons et al., 2006). In SI, one value replaced a single missing value, while in MI five values were drawn for each missing value. Five complete datasets were constructed and analysed and the coefficients and errors were combined to single coefficients and errors adjusted for the variability between imputations.

Statistical analysis

A backwards linear regression (pin =0.15, pout=0.05) was performed on the original dataset. The final model was checked for the assumptions of linear regression. The coefficients and standard errors of the final model were used as the gold standard against which the five techniques were tested.

All five techniques for dealing with missing values were performed six times, once on each simulated dataset. Like the analysis on the original dataset, the same backwards linear regression was performed. The coefficients and standard errors were compared across techniques as well as the composition of significant variables in the final model.

RESULTS

The complete variable model consisted of 15 variables, which was reduced to three variables in the final model: age of dairy cows (mean age), milk fat and herd being closed or not. Coefficients and standard errors of the final model of the original data set are provided in Table 1. Table 1 shows the results of the original model compared to the models generated by the five techniques used on a simulated dataset. These results were consistent in all simulated datasets, therefore only the results from the simulated dataset with 30% missing values are shown.

Using CC, the dataset became smaller with increasing percentage of missing values, e.g in case of 30% missing, 123/177 records were available for analysis (Table 1). All other techniques had 177 records available. The mean age of dairy cows was 54 months (IQR 52-57 months) in the original dataset and was similar in all imputed datasets, despite missing values up to 30%. Using CC and IND, the coefficient for age of dairy cows was underestimated, as were the coefficients of the covariates. The standard errors of all variables were slightly higher in CC and IND compared to the original model.

With MEAN the coefficient of age of dairy cows was also underestimated, while the covariates were overestimated. The standard errors were slightly higher than the standard errors of the original model. With SI and MI the coefficient of age of dairy cows was close to the coefficient in the original model. Using SI, the coefficients of the covariates were slightly underestimated, while with MI these coefficients were also close to the coefficients of the original model. The standard errors were smaller in SI, leading to narrower confidence intervals, while in MI Standard errors were larger, resulting in wider confidence intervals compared to the original model.

Table 1. Results of backwards linear regression of the original dataset compared to 5 techniques for dealing with missing values in a dataset with 30% missing values of age of dairy cows

	Original dataset[a] *(n=177)*	*CC (n=123)*	*IND (n=177)*	*MEAN (n=177)*	*SI (n=177)*	*MI (n=177)*
Age of dairy cows (years)	-0.59 (0.09)	-0.67 (0.121)	-0.65 (0.12)	-0.65 (0.10)	-0.60 (0.08)	-0.59 (0.12)
Milk fat	-2.25(1.18)	-2.14 (1.48)[b]	-1.95 (1.21)[b]	-2.53 (1.22)	-1.71 (1.12)	-2.21 (1.22)
Closed herd	-1.98 (0.88)	-1.79 (1.11)[b]	-1.93 (0.91)[b]	-2.01 (0.90)	-2.04 (0.85)	-1.96 (0.92)

Values are coefficients (se)

[a] original dataset did not contain any missing values

[b] values were dropped from the final model and were forced in the final model for illustrative purposes.

The coefficient of age of dairy cows showed an escalating difference with the original coefficient with increasing percentages of missing values in CC, IND and MEAN (Fig. 1). Using SI the values for age of dairy cows were close to the original coefficient, however at 25% missing values, the coefficient of SI dropped compared to the original coefficient. Values of age of dairy cows using MI were similar to the original coefficient.

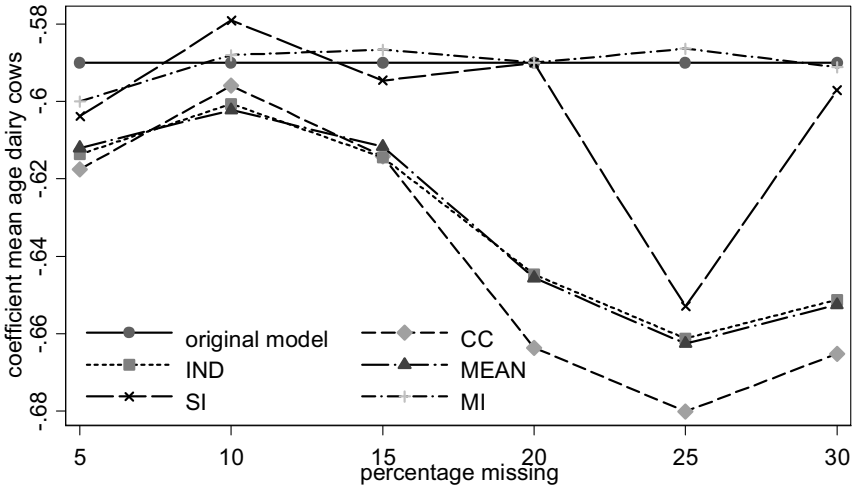

Fig. 1 Coefficients for age of dairy cows across techniques for handling missing values with increasing percentage of missing values

DISCUSSION

The aim of this study was to investigate the possible bias of five different techniques available for dealing with missing values, in a relatively small dataset consisting of 177 records.

MI consistently generated results closest to the original model in model composition as well as estimates of the effects.

Using CC and IND, model composition differed from the original model, and the bias in the coefficient estimates increased with increasing percentage of missing values. Bias in CC and IND is unpredictable, because it depends on the association of the variable containing missing values with the other variables and the outcome (Moons et al., 2006). In this case the effects were underestimated, which even led to the loss of variables in the final model, e.g. the effect of milk fat became insignificant compared to the original model.

Using MEAN, the model composition was similar to the original model, but the bias in the estimates increased with increasing percentage of missing values. Usually using MEAN, the standard errors will be smaller, because variation in the dataset is artificially tapered, because all missing values are imputed with the same value (Cleophas and Cleophas, 2011; Hardouin et al., 2011; Kadengye et al., 2011). In this case, however the standard errors were comparable to those of the original model. The distribution of age of dairy cows was relatively small to begin with and all simulated datasets had a mean and IQR similar to the original dataset.

Using SI, the model composition was similar to that of the original model, as were the estimates of the coefficient. Nevertheless, the coefficient can sometimes be very much off, as demonstrated in fig. 2. SI samples only once from the most likely distribution of the imputed value, by chance this could be an outlier.

Using MI, the model composition and estimates of coefficients were similar to the original model. The standard errors of those coefficients were larger, however, due to adjustment for the variability between imputations. The principle of MI is similar to SI. By performing multiple SI's and combining these results into one estimate the extreme values that can sometimes be imputed using SI are more or less averaged out (Janssen et al., 2010; Knol et al., 2010).

Imputation is not so much gaining information by replacing the missing values, as it is preventing the loss of information from the variables that were correctly collected. However it can never be an excuse to poorly design or execute any study. In this study MI seems to be the better technique, because the associations of all covariates as well as the association of the variable containing missing values were correctly estimated.

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COMPARING METHODS TO ESTIMATE THE REPRODUCTION RATIO OF BLUETONGUE

I.M.G.A. SANTMAN-BERENDS*, J.A. STEGEMAN, P. VELLEMA AND G.VAN SCHAIK

SUMMARY

Bluetongue virus serotype 8 (BTV-8) emerged in north-western Europe in 2006. In 2007, one of the affected countries (the Netherlands) implemented a sentinel network in dairy cattle. This data offered the opportunity to estimate transmission parameters. From our field data, the number of secondary infected cows that became infected by one infectious cow in a completely susceptible herd through the bites of infectious *Culicoides* was calculated. With that information, the R_0 of BTV-8 was estimated using an empirical SIR model. In 2007, the BTV-8 epidemic started in the south and spread northwards in the following months. R_0 could be estimated for 197 herds in which transmission occurred. The median R_0 was 2.3 and the mean R_0 was 3.7 (5^{th} percentile=1.8; 95^{th} percentile=11.0). In the northern region where BTV-8 transmission occurred later in the season with less favorable conditions for transmission, R_0 remained significantly lower than in the south. Our empirical model differed from published simulation models on BTV-8 transmission because we estimated transmission from serological field data while other models simulated transmission. Although there were many differences between our empirical model and the previously used simulation models, the results showed similar ranges of R_0 for BTV-8. The reasons for the similarity between the results may be that, although the part of the vector was not included with parameters in our empirical model, the transmission based on serological field data in cows represented both BTV-8 transmission influenced by cows and by its vector, *Culicoides*. Furthermore, in the simulation models the assumptions made on the vector part, although derived from literature, probably gave a good representation of the true behavior of the *Culicoides* species that were associated with BTV-8 transmission in north-western Europe.

INTRODUCTION

Bluetongue virus (BTV) is an arthropod-borne virus, which can cause clinical disease and mortality in ruminants. BTV is transmitted by certain species of *Culicoides* midges and was historically only present between latitudes 35°S and 50°N (Zhang et al., 1999; Mellor et al., 2008). Since 1998, BTV has been present in Europe and the Mediterranean basin (Lundervold et

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al., 2003) and in 2006, bluetongue virus serotype 8 (BTV-8) emerged in north-western Europe for the first time, and the Netherlands was one of the affected countries (Van Wuijckhuise et al., 2006; Elbers et al., 2008). In that year, the BTV-8 infection within the Netherlands remained restricted to the southern part. After this first introduction, several simulation studies were performed to estimate the basic reproduction ratio (R_0) of BTV-8 in north-western Europe using vector-borne transmission models (Gubbins et al., 2008; De Koeijer & Elbers, 2008; Hartemink et al., 2009; Szmaragd et al., 2010). The basic reproduction ratio (R_0) is an important parameter, which describes the transmission of an infection and is defined as the average number of newly infected individuals generated by one infectious individual e.g. a cow within a susceptible population. Information about the transmission of BTV-8 is important to quantify the risk of BTV-8 in an epidemic situation and with this knowledge decisions concerning monitoring, eradication or vaccination programs can be made in countries in which BTV-8 emerges for the first time.

In the formerly developed simulation models, actual data of host and vector densities were included, but other parameters were based on those published in literature. The advantage of these models is that transmission parameters can be calculated for both vectors and hosts without actually needing field data, which were not available at the time the simulation models were developed. However, many assumptions had to be included that were based on studies in other countries, and other *Culicoides* species.

Until this time, to our knowledge, transmission parameters based on empirical data of the seroprevalence of BTV-8 infected cattle have not been published.

In the winter of 2006-2007, the Dutch government decided to start a sentinel network of 275 dairy cattle herds to examine whether or not BTV-8 would reemerge and spread in 2007 (Santman-Berends et al., 2010a). Data from this network demonstrated the course of BTV-8 infected cattle in a dairy herd and therefore offered the possibility to estimate transmission parameters using an SIR model assuming that during the summer months in 2007, *Culicoides* were not a limiting factor for the transmission of BTV-8.

MATERIALS AND METHODS

Data collection

For disease control purposes, the Netherlands was divided in 20 compartments based on geographic boundaries as proposed in Commission Decision 2005/393/EC. Compartments 1 to 5 were located in the northern part, compartments 6 to 14 in the central part and compartments 15 to 20 in the southern part of the Netherlands (Fig. 1).

In May 2007, on average 13 sentinel herds per compartment were selected for the sentinel network (for a detailed description of the selection process see Santman-Berends et al. (2010a)). The inclusion criteria for sentinel herds were that they had to participate in four-weekly test-day recording of the Cattle Improvement Organization (CRV). In the sentinel herds, BTV-8 seronegative cows were selected at the start of the study and every four weeks, milk samples were collected from 16 randomly selected seronegative cows out of routinely collected milk samples. These samples were tested for antibodies against BTV-8 at the Dutch Animal Health service (GD) with a commercially available ELISA test (sELISA; ID.VET, Montpellier, France;

sensitivity = 98.1% and specificity = 99.0%). For a detailed description of the diagnostic test see Kramps et al. (2008).

Sampling was stopped in September 2007 in compartments 10 to 20, and in October in compartments 7 to 9, because the within-herd prevalence had increased up to almost 100% in most sentinel herds. Further sampling in these herds would not lead to more information. Sampling was stopped in December 2007 in compartments 1 to 6, because outside temperatures became too low for BTV-8 to spread (Fig. 1). In north-west Europe, BTV-8 only spreads during the late spring, summer and early autumn months when the outside temperatures are sufficiently high for *Culicoides* to incubate and transmit the virus. In the late autumn, winter and early spring, outside temperatures are too low for *Culicoides* to transmit the virus.

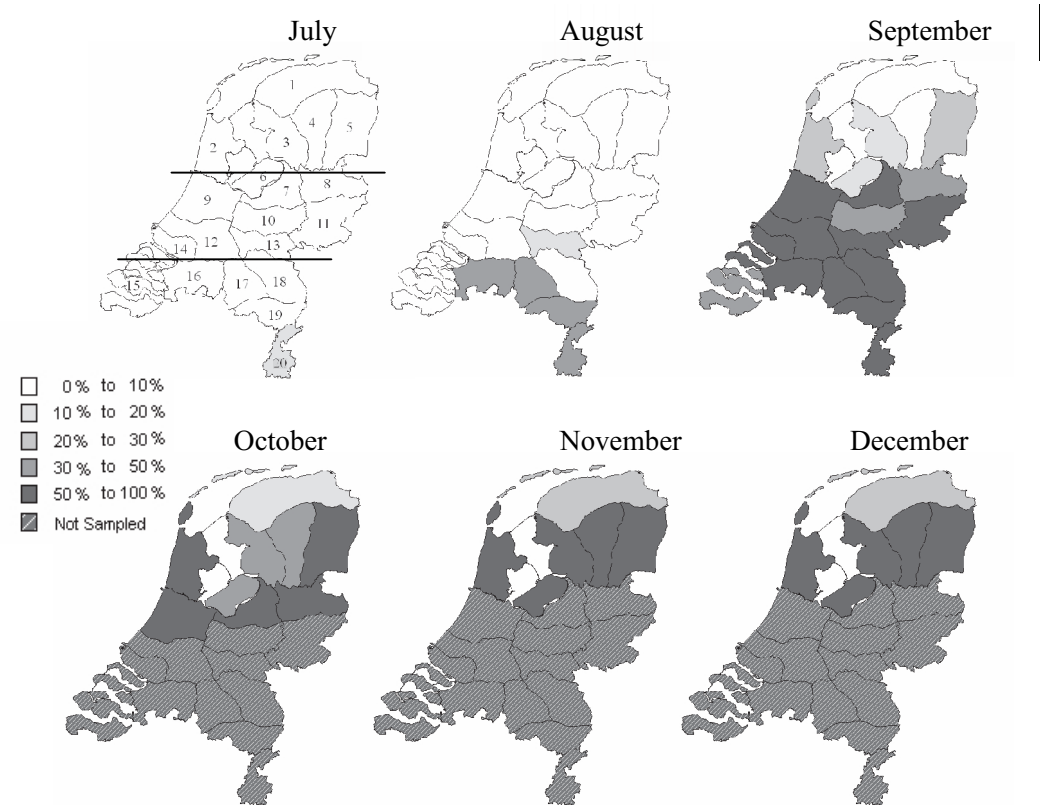

Fig. 1. Average within-herd BTV-8 seroprevalence of 275 dairy herds in the Netherlands per compartment per month from July until December 2007.

At the end of 2007, serological data of 275 sentinel herds was available with 356 time periods in which BTV-8 seroconversion occurred. The average number of days between two consecutive measurements in these herds was 29 days (minimum 14 days-maximum 37 days). In the model only the first month with transmission was included, because in those months most events were observed and 197 periods remained for the calculation of R_0.

Estimation of transmission parameters

For the calculation of R_0, a SIR model based on the empirical data was built in SAS version 9.1 (SAS, 2006). At the start of the measurements in June 2007 all cows that entered the study program were seronegative and were included in the susceptible class ($S(t)$). When in one of the

subsequent months a number of cows tested seropositive for BTV-8, transmission parameters were calculated. For the calculation of the transmission parameters, the number of seroconverted cows were included in the recovered (*R(t)*) class. Assuming that the recovery time for BTV-8 is constant at 25 days (Singer et al., 2001; Bonneau et al., 2002; Mellville et al., 2004), the recovery rate of BTV-8 in cattle (γ) was 0.04 per day (1/25=0.04).

With the available information about the number of days between subsequent measurements and the number of *R* cows at the test-day after introduction, the number of infected cows (*I(t)*) could be derived by using the following ordinal differential equations (ODE):

$$\frac{\Delta S(t)}{\Delta t} = -\beta * S(t) * I(t)$$
$$\frac{\Delta I(t)}{\Delta t} = \beta * S(t) * I(t) - \gamma * I(t)$$
$$\frac{\Delta R(t)}{\Delta t} = \gamma * I(t)$$

This model assumes that direct transmission of BTV-8 between cows occurs, or that other factors, like in our case the vector, do not limit transmission. In that case the chance to become infected is proportional to the number of infectious animals present. Especially in colder months, this was not the reality with BTV-8. Because measurements for BTV-8 transmission were only made in cows and not in the vector i.e. *Culicoides*, vector parameters were not included in the model.

In this study, the increase in the proportion of cattle that were infected with BTV-8 through the bites of *Culicoides*, and recovered was measured. The infection rate of new infections in time period *t* given the fact that the *Culicoides* infected the cows is represented by β. In addition, in accordance with a basic SIR model, all sentinel cows within the study herds were assumed to be randomly mixed and had equal probabilities to come in contact with the BTV-8 virus. The formula which reflects the dynamics of BTV-8 in cattle is then described by:

$$R_0 = \frac{\beta}{\gamma_h}$$

Where R_0 is the number of secondary infected cows that were infected by the first infectious cow within a herd.

RESULTS

In 2007, the BTV-8 epidemic started in the south and spread northwards in the following months. In the southern region, BTV-8 started to spread from June on, when the temperature was not a limiting factor and the within herd prevalence of BTV-8 increased up to almost 100% in most herds.

When only the first month in which transmission occurred was taken into account (197 observations), the median R_0 in the Netherlands in 2007 was 2.3 (5^{th} percentile=1.8; 95^{th} percentile=11.0) and the mean R_0 was 3.7. R_0 values varied from a minimum of 1.8 to a

maximum of 51.0 (increase in within-herd prevalence from 0% to 100% in 26 days). Most R_0 values had a value between two and three (58%) (Fig. 2).

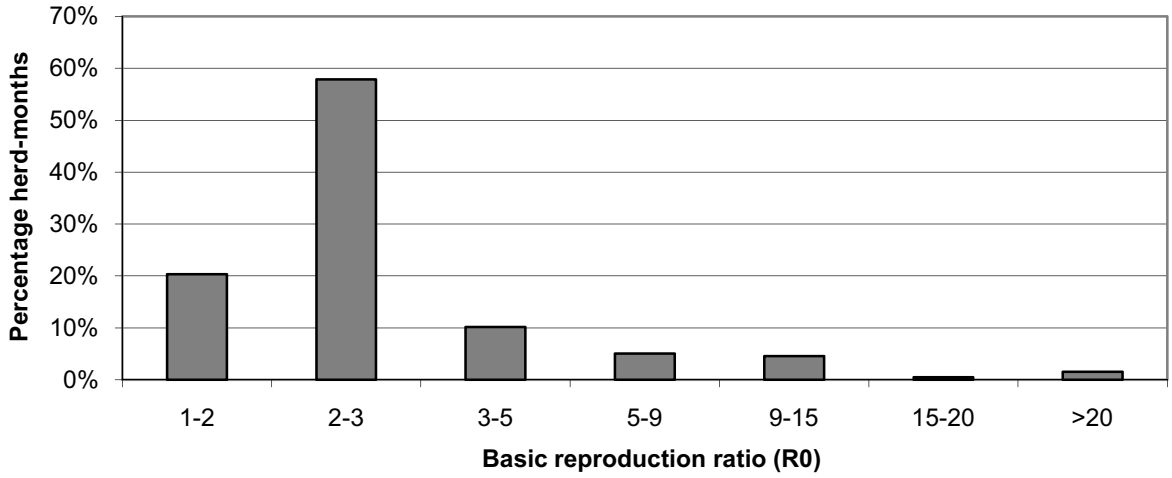

Fig. 2 The distribution of the basic reproduction rate (R_0) for BTV-8 within 275 Dutch dairy herds in 197 herd-months in which transmission occurred for the first time in 2007.

DISCUSSION

In our study, a simple SIR transmission model based on empirical data was used to estimate R_0 for BTV-8 within Dutch dairy herds. The empirical model estimated a median R_0 of 2.3 and a mean R_0 of 3.7.

Considerations of the model

For the calculation of R_0, formulae of a general SIR model were used that assumed that the modelled infection was directly transmittable, or was not limited by other factors. For BTV this is not the case, because it needs a vector to transmit the virus. Nevertheless, based on measurements on *Culicoides* densities in the Netherlands in 2006, it seems that in warmer months, in the Netherlands, the vector is not a limiting factor for the transmission of BTV-8. Nevertheless, when outside temperatures decline, vector biting rates decline as well (Paweska et al., 2002), resulting in lower values for R_0. In our study, this temperature dependence, although not included with a parameter in the empirical model, was also observed because the within herd transmission became lower when outside temperatures declined. The results of our model showed that the median R_0 value for BTV-8 in the northern region remained significantly lower compared to the southern region, which was assumed to be associated with the later start of the epidemic in the north and thus the less favorable conditions for spread of the virus by *Culicoides*. Furthermore, the temperature dependence of BTV-8 was also visible in the percentage of herds in which transmission occurred in each of the months included. In the central and northern region, the percentage of herds in which transmission occurred decreased in October and November, while parts of the herds were still susceptible.

Comparison with simulation models for BTV-8 transmission

In recent years, three vector borne SIR models have been developed that estimated the R_0 for BTV-8 in north-west Europe (Table 1). In all three simulation models, both cattle and sheep were included as host and *Culicoides* were included as the vector. Parameters such as probability of transmission between vector and host (and vice versa), biting rate, incubation period and mortality rate were included in all simulation models. The results of the three simulation models, although two of them only gave an indication of the R_0 (De Koeijer and Elbers, 2008; Hartemink et al., 2009) showed the same range of values for R_0 (Table 1).

The main differences between the earlier models and our estimations of R_0 was that all former models were simulations using density data of hosts and vectors and assumptions for the vector transmission parameters. While our R_0 estimates were based on actual serological data of BTV-8 within a cattle herd. Values for vector parameters such as biting rate (a), vector mortality rate (μ) and incubation period (τ) depended on temperature in all three simulation models and both Gubbins et al. (2008) and Hartemink et al. (2009) used the formulae that were published by Gerry and Mullens (2000) and Mullens et al. (2004). The probability of transmission from vector to host (p_1) was close to 1 in all studies and the probability of transmission from host to vector (p_2) was comparable between the studies of Gubbins et al. (2008) and Hartemink et al. (2009) and was not provided in the paper of De Koeijer and Elbers (2008). Differences between the models were that Gubbins et al. (2008) included a disease-induced mortality rate (d) (0 in cattle and between 0.1% and 1% in sheep) besides the time to recover (γ and T; $1/\gamma = T$), where De Koeijer and Elbers (2008) assumed no mortality in the host population. Hartemink et al. (2009) also included an increased dead rate in sheep as part of the recovery rate, but the exact death rate was not mentioned in their paper. Furthermore, Gubbins et al. (2008) included multiple infection stages in the host population and multiple latent stages in the vector population, whereas De Koeijer and Elbers (2008) and Hartemink et al. (2009) did not. Hartemink et al. (2009) and Gubbins et al. (2008) derived most information on the vectors from comparable literature, whereas the source of the estimations of the vector parameters in the model of De Koeijer and Elbers (2008) was not described. Finally, information of host and vector densities were based on information from the Netherlands in the studies of De Koeijer and Elbers (2008) and Hartemink et al. (2009) and on information in United Kingdom in the study of Gubbins et al. (2008). Nevertheless, all three models found the same range of values for R_0 (Table 1).

The three simulation models resulted in a range of secondary infected sheep or cattle which were caused by the introduction of one primary infection. In our study, instead of simulating the transmission, we used an empirical model to analyse the amount of secondary infections that were the result of one primary case and used that information to derive the R_0. Both our empirical model and the simulation models have advantages and disadvantages. The simulation models from De Koeijer and Elbers (2008), Gubbins et al. (2008) and Hartemink et al. (2009) have the advantage of modelling the dynamics of BTV-8 in both the host and the vector. On the other hand, these models have the disadvantage that they always had to include many literature based assumptions that may not be representative for the BTV-8 epidemic in north-west Europe. BTV serotype 8 differed from other BTV serotypes because it seemed more aggressive, causing more clinical signs (Elbers et al., 2006; Santman-Berends et al., 2011) and could even lead to vertical transmission (Santman-Berends et al., 2010b), which has not been observed in other BTV serotypes. Because we directly measured the transmission of BTV-8 in the cattle population, R_0 could be derived from the empirical SIR model with only the assumption that the recovery time of BTV-8 is 25 days, which was comparable to the recovery time Gubbins et al.

(2008) and Hartemink et al. (2009) assumed in their models. Nevertheless, by analysing transmission in this way, we also assumed that sheep played a minor role by the within herd transmission of BTV-8 in cattle herds and that *Culicoides* that were present in the herd were susceptible and only became infected through feeding on infectious cows within the herd in the first months of transmission.

Table 1. Published models for the estimation of the reproduction ratio (R_0) of bluetongue serotype 8 in north-west Europe

Source	Type of model	Model used	Results
De Koeijer and Elbers, 2008[a]	Deterministic	$R_0 = \sqrt{\frac{(p_1 p_2 T a e^{\mu\tau} v}{(\mu h_{c+s})}}$	20ºC: R_0≈2.4 25ºC: R_0≈4.0 30ºC: R_0≈3.5
Gubbins et al., 2008[a,b]	Stochastic	$R_0 = \sqrt{\frac{p_1 p_2 a^2}{\mu}\left(\frac{\tau}{\mu+\tau}\right)\left(\frac{h_c \phi}{\gamma_c + d_c} + \left(\frac{h_s (1-\phi)^2}{\gamma_s + d_s}\right)\right)}$	15-20ºC: median R_0≈2.0 20-25ºC: median R_0≈2.7 25-30ºC: median R_0≈1.5
Hartemink et al., 2009[a,b]	Deterministic	$R_0 = \sqrt{\frac{a^2 p_1 p_2 \tau v h_c}{y_c (h_c + h_s)^2 \mu(\tau+\mu)} + \frac{a^2 p_1 p_2 \tau v h_s}{y_s (h_c + h_s)^2 \mu(\tau+\mu)}}$	Ranging between 0 and 40. Most values between 2 and 5

[a] R_0 depending on temperature, [b] R_0 depending on *Culicoides* species
p_1=probability of BTV transmission when an infectious host is bitten by an susceptible vector, p_2= probability of BTV transmission when an susceptible host is bitten by an infectious vector, μ=mortality rate vector, v=vector density, h_i=host density (i=cattle (c), sheep (s)), a=biting rate, T= duration of infectious period, τ=incubation period vector, ϕ =proportion of bites on cattle, $y_i + d_i$=infectious period for the host species (i=cattle (c), sheep (s))
y_i=recovery rate, d_i=death rate , y_i=recovery rate (i=cattle (c), sheep (s))

The values for R_0 that were calculated in our study only described the number of secondary infected cows infected by one primary infected cow given that the infection is spread by *Culicoides*. It was not possible to differentiate the vector part from the host part. Therefore, these estimations can not be used to simulate eradication scenarios aimed at interventions on the vector. Nevertheless, our estimations were representative for the range of transmission of BTV-8, and showed the herd-to-herd variation and the effect of season within Dutch cattle herds.

The ideal situation would be to simulate BTV-8 like the studies of Gubbins et al. (2008), De Koeijer and Elbers (2008) and Hartemink et al., (2009) did and to validate their models to the transmission we observed in our serological field data. Until this moment this has not been done for BTV-8, but these kind of models have been developed in the past for, for example Paratuberculosis (Collins and Morgen, 1992).

The R_0 estimates obtained in this study are given the climate, grazing patterns and barn types used in the Netherlands. In an earlier study, we found that keeping cattle indoors during the summer and autumn, reduced the BTV-8 transmission in the herd (Santman-Berends et al., 2010a). When BTV-8 would emerge in a country where all cattle were kept inside or where the temperatures were lower compared to temperatures in the summer and autumn period in the Netherlands, R_0 can be somewhat lower. Nevertheless, the other studies that simulated the R_0 for

BTV-8 in north-west Europe, using vector borne transmission models, showed comparable ranges of R_0.

To conclude, this study presents the first estimates of the transmission rate (R_0) of BTV-8 within herds, based on serological field data from the 2007 epidemic in the Netherlands. Although, the simulation models that were published earlier, were completely different from our empirical SIR model based on serological field data of transmission in cows, the R_0 estimates from our model were in the same range as those from the simulation models. Even though, the simulation models included many assumptions based on other subspecies of *Culicoides* and other BTV serotypes, the results gave a fairly good indication of the transmission of BTV-8 in north-west Europe.

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SOCIAL SCIENCE

A STRUCTURAL EQUATION MODEL OF DETERMINANTS OF BIOSECURITY BEHAVIOUR OF CATTLE AND SHEEP FARMERS IN GREAT BRITAIN

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SUMMARY

This research uses behavioural economics methods to analyse the impact of determinants (socio-demographic, economic, informational and attitudinal) on the biosecurity behaviour of 900 cattle and sheep farmers in Great Britain. The results suggest that farmers' behaviour is significantly influenced by perceived importance of specific biosecurity strategies; organic certification of farm; knowledge about biosecurity measures; attitudes towards animal welfare; perceived usefulness of biosecurity information sources; perceived effect on business during the past five years of severe outbreaks of animal diseases; membership in a cattle/sheep health scheme; attitudes towards livestock biosecurity; influence on decision to apply biosecurity measures; experience; and economic factors (overall explaining 64% of the variance in behaviour).

INTRODUCTION

Despite strong evidence of considerable public and private net benefits from investment in biosecurity at farm level, uptake and implementation on UK cattle and sheep farms remains poor. Previous work has shown such farmers to be generally dismissive of biosecurity actions and focused more on attribution for the disease threats themselves. This has serious implications for policy. Understanding which determinants influence farmers' behaviour would assist policy makers to achieve behavioural change.

Biosecurity is an integral part, as well as legal requirement, of livestock production. There is a large number of biosecurity and animal health measures that can be taken along the supply chain from producers to processors; however, farmers are the ones who are generally considered to be the first line of defence in disease mitigation (Burrell 2002; Palmer et al., 2009). Farmers are provided with information, advice and regulation to learn and follow; however, not all of them have the same attitudes and/or behaviour towards biosecurity due to heterogeneous factors which affect their decision-making (Fairweather & Keating, 1994; Gasson, 1973; Maybery et al., 2005). These factors do not necessarily relate to business/profit aspects (Brodt et al., 2006; Garforth & Rehman, 2005; Gasson, 1973; Gasson & Errington, 1993; Maybery et al., 2005).

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The literature on farmer behaviour and decision-making has shown that there is a number of factors that potentially influence the decision-making process and hence farmer's behaviour, such as farms' physical and economic constraints, farmers' socio-demographics and their access to the information available, which will influence their understanding of the issues and the consequences of implementing, or not implementing, biosecurity measures.

The physical attributes of the farm will largely determine what biosecurity measures are required and the level of investment (be it financial or labour) needed. Farmers perceive the level of investment required to implement many biosecurity measures to be costly, either requiring an increase in management effort with a higher demand on labour and time (Dwyer et al., 2007; Gunn et al., 2008; Hubbard et al., 2007; Morgan-Davies et al., 2006) or requiring changes to the system, such as building improvements or maintenance of boundaries (Bewsell & Monaghan, 2007; Brennan et al., 2008). As well as the farm's physical constraints, the financial situation of the enterprise will have an impact on what measures the enterprise can afford to implement (Chilonda & Van Huylenbroeck, 2001; Stott et al., 2003; Tuyttens et al., 2007).

Farmers' socio-demographic characteristics and attitudes towards animal health/biosecurity measures also have a key role in the decision-making process. Age, education, experience, cognitive ability, household status, lifestyle attitude, goals and values of a farmer have all been shown to affect the decisions being made and the resultant behaviour seen (Blackstock et al., 2010; Fairweather & Keating, 1994; Gasson, 1973; Ostrom, 2003; Small et al., 2005; Zijp, 1998). Numerous studies have shown that farmers at different life stages make different management decisions. For example, younger farmers with large herds and few dependents are more likely to engage with an eradication programme whereas older farmers with no successors are less likely to implement changes in their management systems (BVA, 2005; Tuyttens et al., 2007). Education, experience and cognitive abilities are all variables which have a significant impact on the decision-making process and are often linked to the age of the decision-maker. Moreover, education and training have been shown to enhance and influence farmers' willingness to implement a change in management practices (Austin et al., 2001; Gasson, 1998; Kilpatrick, 1996). However, it is the farmers' ability to understand the problems, the risks and potential effects that will influence the farmers' behaviour and attitudes to animal health and biosecurity (Chilonda & Van Huylenbroeck, 2001; Mariner & Paskin, 2000; Palmer, 2006).

The ability of the farmer to assimilate and understand information on biosecurity issues affects the perceived risk of a disease outbreak. For example, exotic diseases which have not been present in a system for a prolonged time are considered to be low risk; as such, a farmer is more likely to behave in a 'risky' manner, whereas in an outbreak situation the perceived and potential risks are elevated and the likelihood of implementing biosecurity measures is greatly increased (Coleman et al., 1998; Delabbio, 2004; Ekboir, 1999; Lindberg et al., 2006).

A key factor influencing farmer behaviour is the available information on biosecurity measures and animal health issues. There are a number of variables that determine the type and use of the information available to farmers, one of these being the source of information (Bingham et al., 2008; Heffernan et al., 2008; Olmstead & Rhode, 2007; Palmer et al., 2009). Findings from the literature suggest that trusted advisers, such as veterinarians, are required to communicate biosecurity messages as farmers are more likely to act on the information given to them by their vets or by someone with whom they have built up a trusting relationship (Blackstock et al., 2010; Lindberg et al., 2006; Marshall et al., 2006; Mills et al., 2006). Farmers often perceive the requirements of regulation to be confusing as well as, in some cases, inappropriate to the needs of their farm system. This might have a negative effect on the uptake

of biosecurity measures if farmers are unable to interpret messages applicable to their enterprise (Gunn et al., 2005; Moore & Payne, 2007). Demonstrations of successful implementation of biosecurity measures and their benefits increase the level of uptake (Blackstock et al., 2010; Braun et al., 2006).

This paper analyses the impact of some of the aforementioned factors on the biosecurity behaviour of British livestock farmers.

MATERIALS AND METHODS

Data description

A survey took place between 25 March 2010 and 18 June 2010. Three thousand one hundred opt-out letters were sent to farmers in England, Scotland and Wales starting on 17 February 2010 and 84% of the farmers contacted by postal mail (opt-out letters stage) agreed to be contacted for a telephone interview. Overall, 900 farmers were contacted by telephone for the interview and 900 completed questionnaires were obtained. The survey was stratified by farm type, farm size and region and is representative of the sheep and cattle farmers in the Great Britain.

The questionnaire included questions on socio-demographic information about the farmer and economic information about her/his farm, access to information sources, opinions on herd biosecurity measures, influence on business of factors such as regulations, disease outbreaks, access to capital, labour availability, access to veterinary services, attitudes towards policies, regulations, responsibility, business, biosecurity behaviour, sources of influence on biosecurity behaviour such as family, business partners, advisors/consultants/veterinary surgeons, other local farmers, intentions to change farm size and intentions to change the way biosecurity measures are applied on farm.

As regards gender distribution, the sample consists of 6.8% male farmers and 23.2% female farmers. Age distribution shows 35.6% of farmers under 50 years old, 42.7% between 51-65 years and 21.8% over 65 years old. With respect to farm holding, most farmers (61.4%) own their farms, while 19.7% are partly tenants/partly owners and 17.6% are tenants. As regards educational level, 58.8% finished school, 31.2% college and 10% university. As regards labour, 90.2% have up to three full time people working on the farm, with 9.3% having between four and ten full time people working on the farm. While the majority (56.2%) do not hire part time labour, about a quarter (28.7%) of farmers hires one part time worker. About a quarter of farmers (24.1%) use casual relief stockmen on farm. A small majority (55.6%) use an animal health plan and 32.4% of farmers are members of cattle and/or sheep health schemes.

As regards biosecurity measures applied on farm, 38.2% apply quarantine of bought in livestock; 72.9% maintain farm boundaries to prevent nose to nose contact with animals from neighbouring farms; 49.6% maintain double fencing; 49.4% use scare wire; 58% avoid taking cattle/sheep to agricultural shows; 85.2% limit access to farm buildings to only those people deemed essential; 70.9% disinfect clothing, footwear, equipment and vehicles to avoid introduction of disease on farm; 23.1% use embryos transfer and/or artificial insemination rather than natural services; 95.6% control vermin and prevent access of vermin and wildlife to feed and bedding stores; 81.3% check whether available health/vaccination/testing records are in

order before buying/selling stock; 79.2% ensure as far as possible that bought-in stock are sourced from sources that they know carry out good biosecurity.

As regards stated intentions to change the way biosecurity measures are applied on farm in the next five to ten years, 10.9% of farmers stated intentions to increase biosecurity, 77.8% will maintain current levels of biosecurity, 0.8% will reduce biosecurity, 6.2% do not know, while 4.3% think they might leave farming business. Younger farmers with higher educational level are more likely to increase biosecurity.

As regards current biosecurity behaviour, most farmers (about 80%) stated to apply six to nine biosecurity measures. Farmers who stated to apply higher numbers of biosecurity measures are more likely to consider veterinary surgeons as the most important source of information on biosecurity, followed by media and government. They are also more likely to have membership in cattle/sheep health schemes.

Structural equation model

To test the influence of a priori determinants on biosecurity behaviour, the analysis employed structural equation models (SEM) with observed and latent variables. To the best of our knowledge, this is the first research paper to use SEM in this context on a Great Britain sample.

SEM is a statistical technique for testing and estimating causal relationships amongst variables, some of which may be latent (Ajzen et al., 1980; Bollen, 1989), using a combination of statistical data and qualitative causal assumptions. Latent variables are variables that are not directly observed but are inferred from other variables that are observed and directly measurable. SEM is derived from three primary analytical developments, namely path analysis, latent variable modelling, and general covariance estimation methods (Bollen, 1989).

SEM is not intended to discover causes (as the idea of causality may be controversial - see Mueller, 1996), but to test and assess the soundness of the causal relationships researchers formulate. SEM is most commonly used for confirmatory rather than exploratory modelling and thus, it is applied more to theory testing than theory development.

The model consists of two parts, namely the measurement model specifying the relationships between the latent variables and their constituent indicators, and the structural equation model designating the causal relationships between the latent variables. The model is defined by the following system of three equations in matrix terms (Jöreskog & Sörbom, 2007):

The structural equation model: $\eta = B\eta + \Gamma\xi + \zeta$

The measurement model for y: $y = \Lambda_y \eta + \varepsilon$

The measurement model for x: $x = \Lambda_x \xi + \delta$

Where: η is an mx1 random vector of endogenous latent variables; ξ is an nx1 random vector of exogenous latent variables; B is an mxm matrix of coefficients of the η variables in the structural model; Γ is an mxn matrix of coefficients of the ξ variables in the structural model; ζ is an mx1 vector of equation errors (random disturbances) in the structural model; y is

a px1 vector of endogenous variables; x is a qx1 vector of predictors or exogenous variables; Λ_y is a pxm matrix of coefficients of the regression of y on η; Λ_x is a qxn matrix of coefficients of the regression of x on ξ ; ε is a px1 vector of measurement errors in y; δ is a qx1 vector of measurement errors in x.

SEM takes into consideration both direct and indirect causal relationships between constructs, which means that one causal relation may be reinforced or counteracted by another. As there could be different ways to depict the relationships between the latent variables, running alternative models and comparing them will assist in choosing the model that best represents the data.

This research undertook SEM with categorical variables defined on ordinal scales (Likert scale) using the statistical package Lisrel 8.80 (Jöreskog & Sörbom, 2007). SEM estimation was performed by minimising the discrepancy between the covariance matrix of observed variables and the theoretical covariance matrix predicted by the model structure (Bollen, 1989). The estimation method used was the normal-theory maximum likelihood (MLE) method, which is consistent with the sample size (900 observations and, respectively, 400 observations for England and 250 observations each for Scotland and Wales; sample sizes which fall within standard limits for use within SEM) (Bollen, 1989).

RESULTS

The analysis consists of four SEM models, namely one model for the total sample (Great Britain) and three models for the countries (England, Scotland and Wales). This section will present first the statistical description of the latent variables and indicators included in the models and, second, the results for each of the four models.

Latent variables and indicators

Thirteen latent variables were identified and extracted, expressing the behaviour and the underlying determining factors. The variables are: experience (‘exper’); economic factors (‘econ’); organic certification of farm (‘organics’); membership in a cattle/sheep health scheme (‘member’); perceived usefulness of biosecurity information sources (‘infuse’); knowledge about biosecurity measures (‘knows’); perceived importance of specific biosecurity strategies (‘import’); perceived effect on business during the past five years of welfare and health regulation (‘effecta’); perceived effect on business during the past five years of severe outbreaks of animal diseases (‘effectb’); attitudes towards livestock biosecurity (‘attda’); attitudes towards animal welfare (‘attdc’); influence on decision to apply biosecurity measures (‘influen’); and biosecurity behaviour, namely the number of biosecurity measures applied on farm (‘behavs’).

The 13 latent variables are measured by 24 indicators (the constituent observed variables). Table 1 presents a series of descriptive statistics for the indicators of the latent variables included in the ‘total sample’ (Great Britain) model.

The behavioural variable ‘biosecurity behaviour’, namely biosecurity measures applied on farm (‘behavs’) is a single indicator latent variable with the indicator ‘biosecurity measures applied’ being a categorical variable taking value 0 for ‘no biosecurity measures applied’ to value 11 for ‘11 biosecurity measures applied’.

Table 1. Descriptive statistics of the observed variables included in the 'total sample' (Great Britain) model

	MEAN	STANDARD. DEVIATION
Age	2.82	.810
Number of years of having worked on the farm	3.69	1.390
Number of years of having been included in the decision making process of the business	3.01	1.249
Total number of finishing livestock on farm	2.53	1.242
Organic certification of farm	.09	.402
Membership in a cattle/sheep health scheme	.73	1.159
Perceived usefulness of biosecurity information sources - attending open days, monitor/demonstration activities	2.45	1.292
Perceived usefulness of biosecurity information sources - consulting government information sources (e.g. Defra)	2.50	1.306
Perceived usefulness of biosecurity information sources - consulting representatives of research & educational organisations	1.66	1.099
Knowledge about biosecurity measures - stating examples of biosecurity measures	.91	.280
Perceived importance of specific biosecurity strategies - keeping the number of purchased animals low	3.91	1.201
Perceived importance of specific biosecurity strategies - quarantine of purchased animals	4.05	1.098
Perceived effect on business during the past five years - animal health regulation	1.89	.781
Perceived effect on business during the past five years - animal welfare regulation	1.67	.732
Perceived effect on business during the past five years - severe outbreaks of animal diseases on your farm	1.32	.667
Perceived effect on business during the past five years - severe outbreaks of animal diseases on other people's farms	1.50	.730
'Biosecurity' attitudes - biosecurity measures are essential in maintaining a healthy herd	4.33	.692
'Biosecurity' attitudes - applying biosecurity measures could save farmers a lot of money	3.71	.929
'Biosecurity' attitudes - biosecurity regulations are a good thing for the health of the livestock	4.14	.724
'Welfare' attitudes - taking care of animal health improves animal's welfare	4.42	.606
'Welfare' attitudes - there is more to animal welfare than animal health	4.15	.792
'Welfare' attitudes - I am responsible for the welfare of my livestock	4.59	.525
Influence on decision to apply biosecurity measures - discussion with advisors/consultants/veterinary surgeons	2.10	.727
Biosecurity measures applied	7.03	1.868

The variables economic factors ('econ'), organic certification of farm ('organics'), and membership in a cattle/sheep health scheme ('member') are observed variables built into the model as single indicator latent variables, with indicators 'total number of finishing livestock on farm', 'organic certification of farm' and 'membership in a cattle/sheep health scheme' being respectively, a categorical variable taking value 1 if the farm has no finishing livestock to value 5 for 1001 or more number of finishing livestock; a categorical variable taking value 0 for farms without organic certification, value 1 for farms in conversion period to value 2 for farms with organic certification; and a categorical variable taking value 0 for farms without membership in a cattle/sheep health scheme, value 1 for membership in a cattle health scheme, value 2 for membership in a sheep health scheme and value 3 for farms with membership in both cattle and sheep health schemes.

The variables 'knowledge about biosecurity measures' ('knows') and 'influence on decision to apply biosecurity measures' ('influen') are single indicator latent variables with the indicators 'knowledge about biosecurity measures - stating examples of biosecurity measures' and 'influence on decision to apply biosecurity measures - discussion with advisors/consultants/veterinary surgeons' being a dichotomous variable taking value 1 for at least one correct example given and value 0 otherwise and, respectively, a categorical variable taking value 1 for no influence at all to value 2 for a little influence to value 3 for a lot of influence on decision to apply biosecurity measures from discussion with advisors/consultants/veterinary surgeons.

Variable ‘experience’ (‘exper’) is a latent variable measured by three indicators: ‘age’, ‘number of years of having worked on the farm’ and ‘number of years of having been included in the decision making process of the business’. The three indicators are categorical, with ‘age’ taking values from 1 for being between 18 – 35 years old to value 4 for over 65 years old; ‘number of years of having worked on the farm’ taking value 1 for ten or less years to value 6 for 51 or more years of having worked on the farm; ‘number of years of having been included in the decision making process of the business’ taking value 1 for ten or less years to value 5 for 41 or more years of having been included in the decision making process of the business.

The attitudinal variable ‘perceived usefulness of biosecurity information sources’ (‘infuse’) is a latent variable measured by three indicators: ‘perceived usefulness of biosecurity information sources - attending open days, monitor/demonstration activities’, ‘perceived usefulness of biosecurity information sources - consulting government information sources (e.g. Defra)’, ‘perceived usefulness of biosecurity information sources - consulting representatives of research & educational organisations’. The three variables are ordinal using a five-point Likert scale from ‘not at all useful’, ‘slightly useful’, ‘useful’, ‘very useful’ to ‘extremely useful’.

The attitudinal variable ‘perceived importance of specific biosecurity strategies’ (‘import’) is a latent variable measured by two indicators: ‘perceived importance of specific biosecurity strategies - keeping the number of purchased animals low’ and ‘perceived importance of specific biosecurity strategies - quarantine of purchased animals’. The two variables are ordinal using a five-point Likert scale from ‘not at all important’, ‘of little importance’, ‘moderately important’, ‘important’ to ‘very important’.

The attitudinal variable ‘perceived effect on business during the past five years of welfare and health regulation’ (‘effecta’) is a latent variable measured by two indicators: ‘perceived effect on business during the past five years - animal health regulation’ and ‘perceived effect on business during the past five years - animal welfare regulation’. The two variables are ordinal using a three-point Likert scale from ‘not affected’, ‘slightly affected’ to ‘much affected’.

The attitudinal variable ‘perceived effect on business during the past five years of severe outbreaks of animal diseases’ (‘effectb’) is a latent variable measured by two indicators: ‘perceived effect on business during the past five years - severe outbreaks of animal diseases on own farm’ and ‘perceived effect on business during the past five years - severe outbreaks of animal diseases on other people’s farms’. The two variables are ordinal using a five-point Likert scale from ‘strongly disagree’, ‘disagree’, ‘unsure’, ‘agree’ to ‘strongly agree’.

The attitudinal variable ‘attitudes towards livestock biosecurity’ (‘attda’) is a latent variable measured by three indicators: ‘biosecurity measures are essential in maintaining a healthy herd’, ‘applying biosecurity measures could save farmers a lot of money’, and ‘biosecurity regulations are a good thing for the health of the livestock’. The three variables are ordinal using a five-point Likert scale from ‘strongly disagree’, ‘disagree’, ‘unsure’, ‘agree’ to ‘strongly agree’.

The attitudinal variable ‘attitudes towards animal welfare’ (‘attdc’) is a latent variable measured by three indicators: ‘taking care of animal health improves animal’s welfare’, ‘there is more to animal welfare than animal health’, and ‘I am responsible for the welfare of my livestock’. The three variables are ordinal using a five-point Likert scale from ‘strongly disagree’, ‘disagree’, ‘unsure’, ‘agree’ to ‘strongly agree’.

Table 2. Factor analysis of the latent variables and their corresponding indicators. 'Total sample' (Great Britain) model

	COMPONENT												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Age	**.817**	.009	-.039	-.010	-.057	-.055	-.015	-.138	-.023	.044	-.040	-.075	-.072
Number of years of having worked on the farm	**.891**	-.023	-.026	.010	-.037	-.035	-.001	.071	.005	-.055	.024	.012	.014
Number of years of having been included in the decision making process of the business	**.903**	-.010	-.010	-.004	-.015	-.020	-.003	.035	-.013	-.041	-.039	.028	.020
Total number of finishing livestock on farm	-.020	-.012	-.027	.042	.025	.052	-.058	**.942**	.081	.000	-.014	-.014	.026
Organic certification of farm	-.049	-.045	.041	-.005	-.042	.000	-.002	-.003	-.050	**.943**	.008	.061	.101
Membership in a cattle/sheep health scheme	-.024	.001	.039	.015	.019	-.010	.024	.080	**.948**	-.048	.067	.046	.062
Perceived usefulness of biosecurity information sources - attending open days, monitor/demonstration activities	-.008	.033	.006	-.033	**.710**	-.009	.042	.160	-.169	-.076	.194	-.002	.180
Perceived usefulness of biosecurity information sources - consulting government information sources (e.g. Defra)	-.088	.131	.097	.097	**.712**	.042	-.101	-.167	.113	-.105	-.020	.128	.193
Perceived usefulness of biosecurity information sources - consulting representatives of research & educational organisations	-.062	.089	-.068	.083	**.691**	.098	.262	.050	.163	.246	.021	-.029	-.376
Knowledge about biosecurity measures - stating examples of biosecurity measures	-.028	.021	-.019	.077	.074	.053	.035	-.014	.047	.061	.017	**.966**	-.026
Perceived importance of specific biosecurity strategies - keeping the number of purchased animals low	.025	.121	.063	-.025	.007	-.056	**.872**	-.112	-.032	.024	.077	-.010	-.005
Perceived importance of specific biosecurity strategies - quarantine of purchased animals	-.096	.085	.207	-.028	.135	.038	**.592**	.128	.140	-.058	-.089	.117	.457
Perceived effect on business during the past five years - animal health regulation	-.039	-.044	.080	**.867**	.019	.189	-.011	.006	-.036	-.024	.078	.092	.004
Perceived effect on business during the past five years - animal welfare regulation	.035	-.019	-.015	**.910**	.064	.075	-.024	.034	.052	.020	-.031	-.007	.004
Perceived effect on business during the past five years - severe outbreaks of animal diseases on your farm	-.024	.082	-.037	.090	-.016	**.847**	-.023	-.006	.056	.039	.052	-.060	-.040
Perceived effect on business during the past five years - severe outbreaks of animal diseases on other people's farms	-.083	-.050	.063	.168	.091	**.776**	-.013	.063	-.070	-.039	-.033	.127	.090
'Biosecurity' attitudes - biosecurity measures are essential in maintaining a healthy herd	-.010	**.691**	.225	-.074	.108	-.036	.024	-.050	-.031	-.017	.034	.083	.089
'Biosecurity' attitudes - applying biosecurity measures could save farmers a lot of money	-.029	**.781**	-.011	-.008	-.021	.102	.179	.066	.006	-.015	.049	-.075	-.012
'Biosecurity' attitudes - biosecurity regulations are a good thing for the health of the livestock	.017	**.760**	.210	.014	.098	-.027	-.023	-.037	.030	-.011	.003	.021	.104
'Welfare' attitudes - taking care of animal health improves animal's welfare	-.086	.227	**.745**	.003	.101	-.014	.038	.052	.092	-.034	-.076	-.037	-.031
'Welfare' attitudes - there is more to animal welfare than animal health	.052	.083	**.708**	.078	-.033	.045	.071	-.195	.045	.114	-.087	-.002	.028
'Welfare' attitudes - I am responsible for the welfare of my livestock	-.056	.122	**.745**	-.013	-.018	-.008	.055	.116	-.104	-.045	.248	.025	-.122
Influence on decision to apply biosecurity measures - discussion with advisors/consultants/veterinary surgeons	-.049	.072	.028	.043	.144	.024	.039	-.017	.073	.012	**.931**	.016	.076
Biosecurity measures applied	-.023	.212	-.202	.026	.172	.056	.112	.011	.065	.179	.126	-.066	**.729**

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
Rotation converged in 12 iterations.
The loadings of indicators building the factors are in bold fonts.

As a test of the validity of the latent variables, we undertook factor analysis with varimax rotation. Each set of variables loaded onto a separate factor and only 13 factors were retained, such that these 13 factors could be taken to represent the relevant latent variables (Table 2).

Once it was established that the latent variables could be identified, separate factor analyses were undertaken for the multiple-indicator latent variables ('exper', 'infuse', 'import', 'effecta', 'effectb', 'attda' and 'attdc'). The individual factor analyses each extracted a single factor, with all variable loadings above the recommended value of 0.7 and total variance of the indicators explained by each of the latent variables was in most cases above 60 percent in each of the four models.

Great Britain model results

Based on the reviewed literature it was reasonable to assume a certain amount of underlying causality amongst the variables in the model. Hence the research tested the model described in Fig. 1, which presents the path diagram for the structural model.

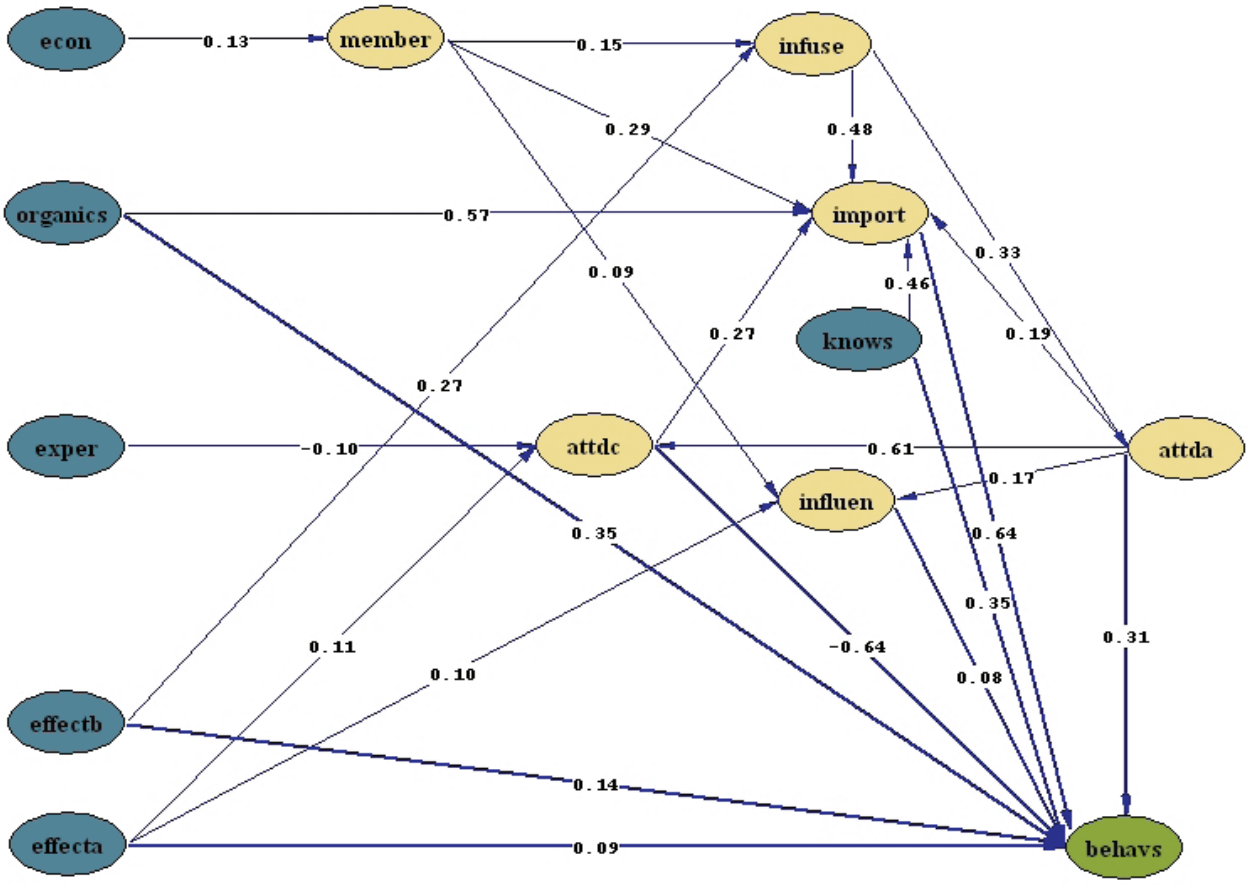

Fig. 1 Great Britain - structural model (standardised solution)

The model has an adequate fit according to the measures of absolute, incremental and parsimonious fit (Hair et al., 2006), with a root mean square error of approximation (RMSEA) value of 0.066, below the threshold maximum value of 0.10; standardised root mean residual (SRMR) value of 0.053, lower than the threshold of 0.08; comparative fit index (CFI) value of 0.84; incremental fit index (IFI) value of 0.84; goodness of fit index (GFI) value of 0.91;

adjusted goodness of fit index (AGFI) value of 0.87; and normed fit index (NFI) value of 0.81, all above or close to the recommended values for fit indices, the 'magic 0.90' (Hair et al., 2006).

Additional testing of the appropriateness of the models was achieved by comparing the estimated model with three other models that acted as alternative explanations to the proposed model, in a competing models strategy (using a nested model approach, in which the number of constructs and indicators remains constant, but the number of estimated relationships changes). The results across all types of goodness-of-fit measures favoured the estimated model in most cases, so the competing models were discarded.

After the goodness-of-fit tests, the validity of the model was assessed in a two-step procedure, the measurement model and the structural model. The measurement model results show that the sets of indicators for the three multiple-indicator constructs do not all have comparable indicators, however, all loadings are statistically significant. The significance tests for the structural model parameters represent the basis for accepting or rejecting the proposed relationships between exogenous and endogenous constructs. All variables, with the exception of effecta' (perceived effect on business during the past five years of welfare and health regulation), significantly influence behaviour. Table 3 presents the standardised total effects on the behavioural latent variable of all the other latent variables in the model. The model predicts 64% of the variance in behaviour.

Table 3. Standardised total effects on behavioural latent variable (t-values in parentheses)

DETERMINANTS	'BEHAVS'			
	TOTAL (DIRECT AND INDIRECT) EFFECTS GREAT BRITAIN MODEL	TOTAL (DIRECT AND INDIRECT) EFFECTS ENGLAND MODEL	TOTAL (DIRECT AND INDIRECT) EFFECTS SCOTLAND MODEL	TOTAL (DIRECT AND INDIRECT) EFFECTS WALES MODEL
'exper'	0.04 (2.56)	0.06 (2.17)	n.a.	n.a.
'econ'	0.03 (3.53)	n.a.	n.a.	n.a.
'organics'	0.61 (13.07)	n.a.	n.a.	n.a.
'member'	0.25 (8.34)	0.05 (3.10)	0.15 (3.25)	0.14 (2.82)
'infuse'	0.36 (8.03)	0.22 (4.81)	0.21 (3.57)	0.20 (3.05)
'knows'	0.55 (11.15)	n.a.	n.a.	n.a.
'import'	0.64 (7.61)	0.30 (3.53)	0.49 (4.00)	0.48 (3.14)
'effecta'	0.05 (1.20)	n.a.	n.a.	n.a.
'effectb'	0.24 (5.18)	n.a.	n.a.	n.a.
'attda'	0.17 (3.86)	0.21 (3.03)	0.25 (2.94)	0.03 (0.43)
'attdc'	-0.46 (-7.44)	-0.50 (-6.81)	-0.45 (-3.05)	-0.55 (-3.48)
'influen'	0.08 (2.41)	0.18 (3.18)	0.18 (2.53)	0.17 (1.97)

n.a. Variable not included in the model.
The latent variable scores and observational residuals depend on the unit of measurement in the observed variables. As some of these units are the result of subjective scaling of the observed variables the observational residuals were standardised (rescaled such that they have zero means and unit standard deviations in the sample) (Jöreskog & Sörbom, 2007). Total effects represent how much a one unit change in an independent variable will change the expected value of a dependent variable.

England model results

A smaller number of variables (eight) were included in this model to account for the smaller sample size (400 observations). The variables are: experience ('exper'); membership in a cattle/sheep health scheme ('member'); perceived usefulness of biosecurity information sources ('infuse'); perceived importance of specific biosecurity strategies ('import'); attitudes towards livestock biosecurity ('attda'); attitudes towards animal welfare ('attdc'); influence on decision to apply biosecurity measures ('influen'); and biosecurity behaviour, namely the number of biosecurity measures applied on farm ('behavs'). The model is presented in Fig. 2.

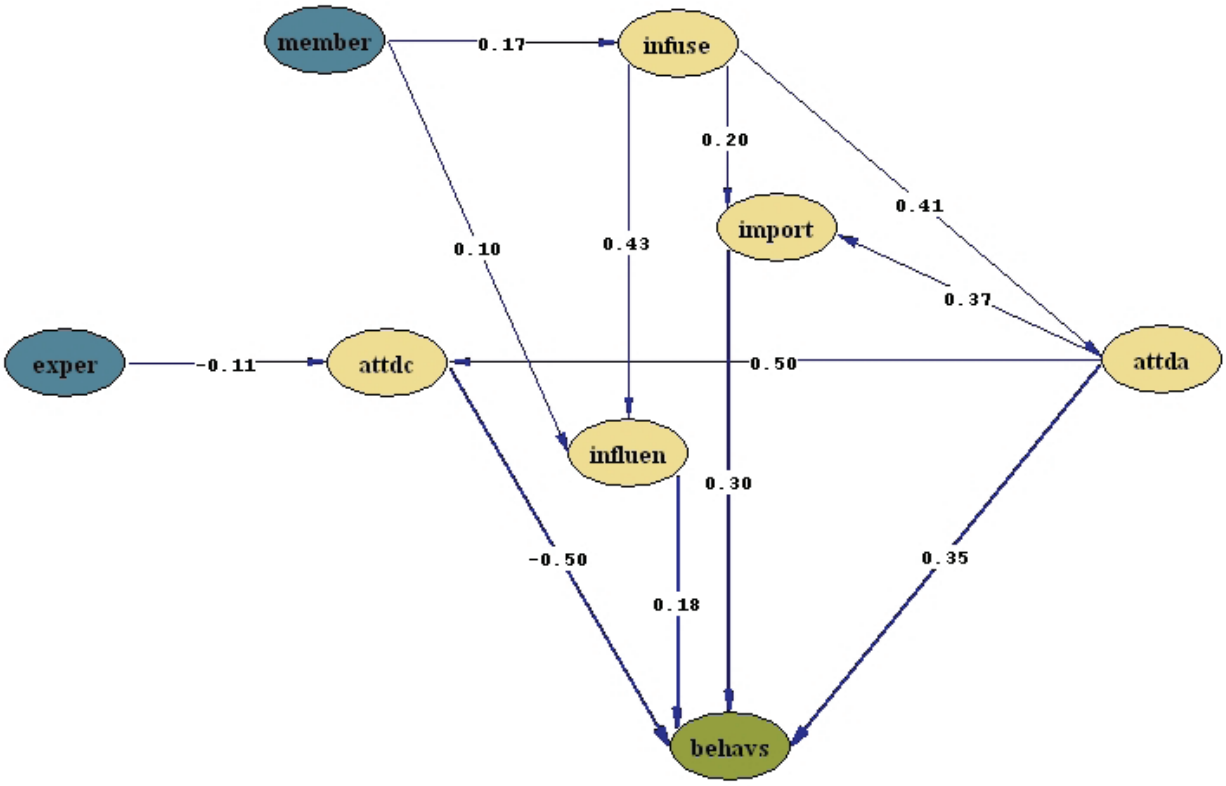

Fig. 2 England - structural model (standardised solution)

The model passed all the tests presented for the Great Britain model. Table 3 presents the standardised total effects on the behavioural latent variable of all the other latent variables in the model. All variables were found to significantly influence behaviour. The model predicts 37% of the variance in behaviour.

Scotland model results

A smaller number of variables (seven) were included in this model to account for the smaller sample size (250 observations). The variables are: membership in a cattle/sheep health scheme ('member'); perceived usefulness of biosecurity information sources ('infuse'); perceived importance of specific biosecurity strategies ('import'); attitudes towards livestock biosecurity ('attda'); attitudes towards animal welfare ('attdc'); influence on decision to apply biosecurity measures ('influen'); and biosecurity behaviour, namely the number of biosecurity measures applied on farm ('behavs'). The model is presented in Fig. 3.

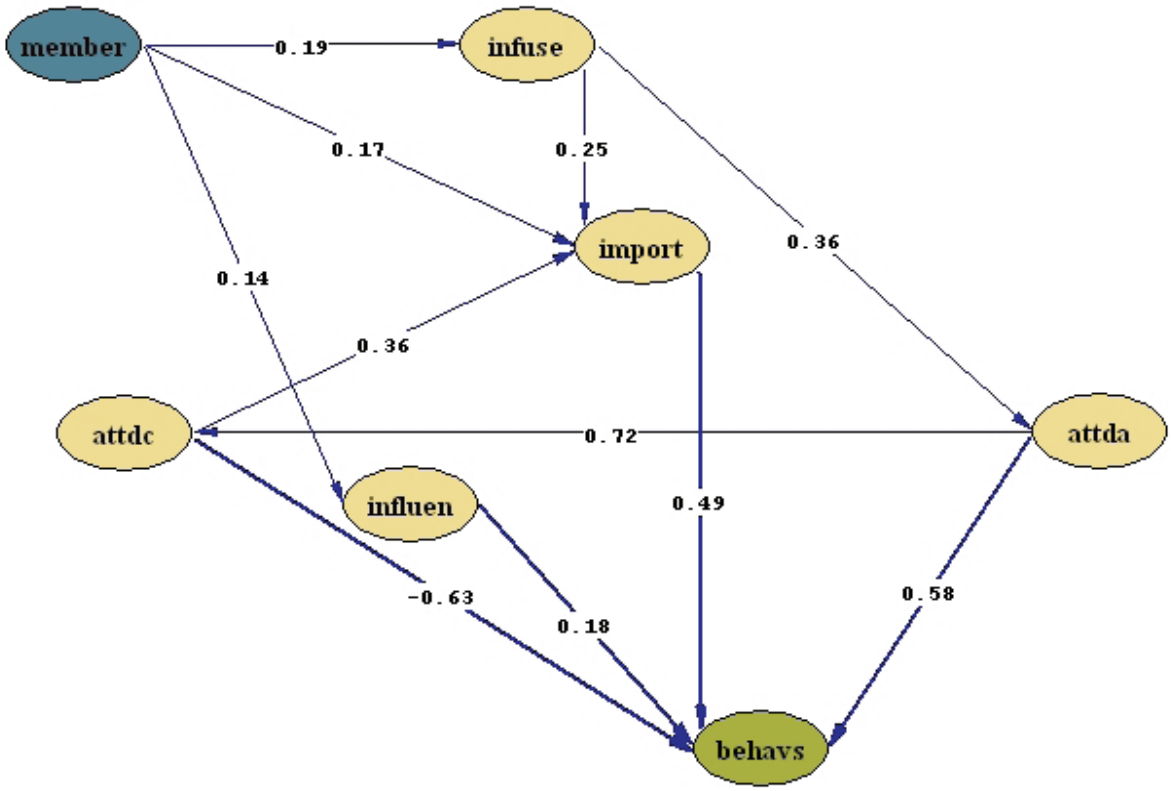

Fig. 3 Scotland - structural model (standardised solution)

The model passed all the tests presented for the Great Britain model. Table 3 presents the standardised total effects on the behavioural latent variable of all the other latent variables in the model. All variables were found to significantly influence behaviour. The model predicts 41% of the variance in behaviour.

Wales model results

A smaller number of variables (seven) were included in this model to account for the smaller sample size (250 observations). The variables are: membership in a cattle/sheep health scheme ('member'); perceived usefulness of biosecurity information sources ('infuse'); perceived importance of specific biosecurity strategies ('import'); attitudes towards livestock biosecurity ('attda'); attitudes towards animal welfare ('attdc'); influence on decision to apply biosecurity measures ('influen'); and biosecurity behaviour, namely the number of biosecurity measures applied on farm ('behavs'). The model is presented in Fig. 4.

The model passed all the tests presented for the Great Britain model. Table 3 presents the standardised total effects on the behavioural latent variable of all the other latent variables in the model. All variables, with the exception of 'attda' (attitudes towards livestock biosecurity) were found to significantly influence behaviour. The model predicts 40% of the variance in behaviour.

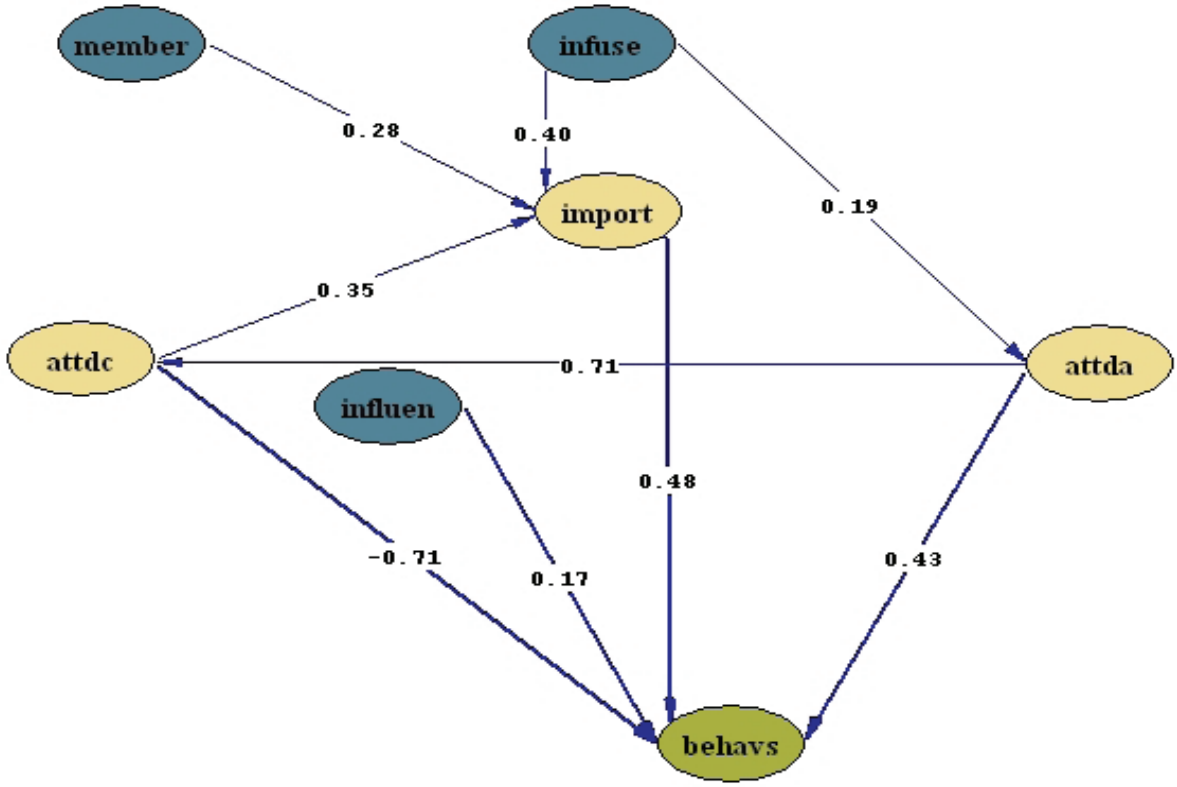

Fig. 4 Wales - structural model (standardised solution)

DISCUSSION

The results suggest that farmers' perceived importance of specific biosecurity strategies, organic certification of farm, knowledge about biosecurity measures, attitudes towards animal welfare, perceived usefulness of biosecurity information sources, perceived effect on business during the past five years of severe outbreaks of animal diseases, membership in a cattle/sheep health scheme, attitudes towards livestock biosecurity, influence on decision to apply biosecurity measures, experience and economic factors are significantly influencing behaviour. The results are comparable between the total sample (Great Britain) and the individual country (England, Scotland and Wales) models.

In terms of individual effects, farmers' perceived importance of specific biosecurity strategies has a very strong impact on their biosecurity behaviour (the variable explains between 30% and 64% of the variance in behaviour ceteris paribus in the four models). This means that the stronger the farmers' perceived importance of specific biosecurity strategies the stronger their biosecurity behaviour. Namely, farmers who consider quarantine of purchased animals and keeping the number of purchased animals low to be amongst the most important biosecurity strategies are more likely to apply a higher number of biosecurity measures on their farms. This is consistent with the literature as farmers will apply biosecurity measures if they consider them relevant/useful for their farms.

Organic certification of farm is another significant determinant of behaviour. The variable has a direct influence on behaviour and an indirect one through perceived importance of biosecurity measures and explains 61% of the variance in behaviour ceteris paribus. This might

mean that organic farmers are more likely to perceive keeping the number of purchased animals low and quarantine of purchased animals as important biosecurity measures and, implicitly, exhibit stronger biosecurity behaviour.

Knowledge about biosecurity measures is another strong determinant of biosecurity behaviour and explains 55% of the variance in behaviour ceteris paribus. This means that the better the farmers' knowledge about biosecurity measures the stronger their biosecurity behaviour. The literature supports these findings as knowledge is a main determinant of behaviour.

Attitudes towards animal welfare significantly influence behaviour (explaining between 45% and 55% of the variance in behaviour ceteris paribus in the four models), however the relationship is negative. It is not straightforward to explain the negative relationship and this issue might need further consideration. The 'statistical' explanation is related to the indicators corresponding to the welfare attitudes latent variable (taking care of animal health improves animal's welfare, there is more to animal welfare than animal health and I am responsible for the welfare of my livestock); namely most farmers (93%) strongly agree and agree with the welfare statements, which implicitly shows very strong welfare attitudes, while their actual biosecurity behaviour is less strong. One reason for the negative sign of the relationship might be related to farmers seeing themselves as guardians of the welfare of their animals and doing whatever they perceive is needed to ensure an acceptable level of welfare, which might not necessarily include the biosecurity measures analysed in this paper. Another explanation for the negative relationship between welfare and biosecurity attitudes might be the fact that some of the biosecurity measures (e.g., use of scare wire or use of embryos transfer and/or artificial insemination rather than natural services) might be perceived by farmers to reduce welfare.

Perceived usefulness of biosecurity information sources significantly influences behaviour and explains between 20% and 36% of the variance in behaviour ceteris paribus in the four models. Information has been frequently acknowledged in the literature as one of the main determinants of behaviour. Here it means that the more useful the farmers perceive sources of biosecurity information (i.e., attending open days, monitor/demonstration activities; consulting government information sources (e.g., Defra); consulting representatives of research & educational organisations) the more likely they are to exhibit stronger biosecurity behaviour.

Perceived effect on business during the past five years of severe outbreaks of animal diseases has a significant effect (explaining 24% of the variance in behaviour ceteris paribus). It influences behaviour directly and indirectly through perceived usefulness of information sources. This might suggest that farmers who experienced disease outbreaks in the past are more likely to use biosecurity sources of information and apply more biosecurity measures currently on their farms.

As expected, another strong influence on behaviour comes from membership in a cattle/sheep health scheme, which explains between 5% and 25% of the variance in behaviour ceteris paribus in the four models. This might suggest that farmers who are members in cattle and/or sheep health schemes are likely to apply more biosecurity measures on their farms.

As expected, attitudes towards livestock biosecurity will significantly influence behaviour, explaining between 17% and 25% of the variance in behaviour ceteris paribus. Biosecurity attitudes were found significant in three of the four models (Great Britain, England and Scotland). This confirms findings from the literature as regards the relationship between

attitudes and behaviour and means that the stronger the farmers' attitudes towards biosecurity (namely that biosecurity measures are essential in maintaining a healthy herd, that applying biosecurity measures could save farmers a lot of money and that biosecurity regulations are a good thing for the health of the livestock) the more likely they are to apply a higher number of biosecurity measures on their farms.

As expected, another significant determinant is the influence on decision to apply biosecurity measures (discussion with advisors/consultants/veterinary surgeons), which explains between 8% and 18% of the variance in behaviour ceteris paribus in the four models. This might suggest that farmers who discuss the application of biosecurity measures on their farms with advisors, consultants or veterinary surgeons are more likely to exhibit stronger biosecurity behaviour.

Other determinants with a small but significant influence on behaviour are experience and the number of finishing livestock on farm (explaining between 4% and 6% and, respectively, 3% of the variance in behaviour ceteris paribus), which suggests that more experienced farmers with a higher number of finishing livestock are more likely to apply more biosecurity measures on their farms. The small effect of the economic factors (herd size) might be explained by the fact that some of the biosecurity measures (e.g., double fencing and use of scare wire) might be more difficult to implement on very large farms.

CONCLUSIONS

The paper analysed the impact of a priori determinants of biosecurity behaviour of farmers in Great Britain. Results of the econometric analysis show that the models predict two thirds (64%) of the variance in behaviour for the total sample, with lower but still high levels of prediction for the individual models (about 40% for England, Scotland and Wales).

The results suggest that farmers' perceived importance of specific biosecurity strategies, organic certification of farm, knowledge about biosecurity measures, attitudes towards animal welfare, perceived usefulness of biosecurity information sources, perceived effect on business during the past five years of severe outbreaks of animal diseases, membership in a cattle/sheep health scheme, attitudes towards livestock biosecurity, influence on decision to apply biosecurity measures, experience and economic factors are significantly influencing biosecurity behaviour.

Besides the significant influence of socio-economic factors (experience, herd size, organic certification, membership in health schemes, and occurrence of disease outbreaks) and attitudes and perceptions on behaviour, which confirm findings from the literature, the analysis observed the high impact of information and advice on biosecurity behaviour. Namely, knowledge about biosecurity measures, perceived usefulness of biosecurity information sources (attending open days, monitor/ demonstration activities; consulting government information sources; and consulting representatives of research & educational organisations) and influence on decision to apply biosecurity measures (discussion with advisors, consultants or veterinary surgeons), all influence biosecurity behaviour to a large extent. This might suggest that ways to achieve behavioural change could include ensuring increased access of farmers to biosecurity information and advice sources.

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CLASSICAL EPIDEMIOLOGICAL APPROACH VERSUS PARTICIPATORY METHODS: A COMPARISON OF TWO RESEARCH METHODS APPLIED TO IDENTIFICATION OF KEY HEALTH ISSUES FOR WORKING HORSES IN LESOTHO

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SUMMARY

Few reported studies have simultaneously applied quantitative and qualitative methodologies for the purpose of direct comparison of their findings. This study compared issues identified via a cross-sectional study of individual horses and their owners, utilising structured owner questionnaires, clinical examination and standardised tack examination with those identified and prioritised via participatory resource mapping and matrix ranking techniques in owner workshops. Some issues identified were common to both approaches; others differed in terms of their specification and relative importance. The quantitative approach provided objective measurement of issue frequency for comparison with owners' perceptions of their importance. Participatory methods enabled researchers to gain detailed understanding of local issues and appreciate how owners defined and prioritised problems affecting them and their animals. Without the vital components of developing community ownership of the process and grounded researcher appreciation of local circumstances, interventions based solely on epidemiological measures of frequency are less likely to succeed.

INTRODUCTION

Veterinary epidemiology incorporates principles for the design, conduct and analysis of studies associated with animal health and welfare problems. Over the past quarter of a century it has developed an extensive body of evidence in support of animal health and welfare interventions and become a recognised component of veterinary training curricula worldwide; this has led to these principles being applied in a wide range of circumstances.

Veterinary epidemiology includes analysis of both direct animal-based measures and elicitation of information from individual animal keepers. Traditionally the latter has been undertaken using structured questionnaires. However, problems associated with elicitation and application of the findings arising from questionnaires in less developed countries, including cultural insensitivity, difficulties in achieving suitable design, time and expense in administration and propensity to produce erroneous or unsubstantiated results have been noted (Catley, 1999). In developing countries, field workers have recognised the challenges associated with applying the findings of large scale, classical approach research studies to community-

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based interventions and advocated community-based research methods and intervention design to address these (Chambers, 1983). In human health fields and, increasingly, in animal health-related work in these circumstances, potential for application of participatory techniques has been recognised (Catley et al., 2011; Oakley, 1991). However, there are few reports of these being directly compared with traditional techniques in a single research situation.

A UK-based equine welfare charity initiated a training intervention in Lesotho in mid-2007. Lesotho is a small, mountainous, landlocked country within eastern South Africa. It is one of the poorest countries in Africa, with an estimated horse population of 65,000 (FAOSTAT, 2009). In parallel with this intervention, the charity commissioned research to assess the impact of its work on the health and welfare of the working horses in target communities. The aim of the study described here was to compare the findings arising from a classical epidemiological approach to gathering data for this study with findings arising from a participatory methods-based approach to identify and prioritise key issues affecting working horses and their owners.

MATERIALS AND METHODS

Classical epidemiological surveys

Three cross-sectional surveys of horses and owners were conducted during April-June 2007, October-December 2008 and August-October 2009.

The reference population was working horses and owners residing in the area from which students attending the first training course were drawn in western Lesotho peri-urban and subsistence agricultural lowlands. Villages were randomly selected using a village listing which, in the absence of a formal national equine census, was created in consultation with knowledgeable local community workers. To maximise logistical efficiency, as many owners as possible were surveyed in each village visited; for owners with more than one horse a single animal was selected using a random number list with the exception of Lesotho Mounted Police Horse stabling centres, where approximately half of available horses were examined.

Each survey comprised three data collection components: owner questionnaire, clinical examination of horses by a veterinary-trained researcher including blood and faecal sample collection and structured tack examination. Details of methods used are described in Upjohn et al. (2011).

All field data were recorded by hand onto paper record sheets in the field. Faecal Egg Count data were recorded by hand into a laboratory book. Haematology and biochemistry parameters were printed out in hard copy from their respective processors and attached to the related clinical examination sheet. All blood results were recorded as both absolute values for each variable and whether they were within or outside the standard reference range as defined for the equipment used. FEC data were recorded as both absolute values and their categorisation according to a recognised scoring system (Soulsby, 1982).

The survey protocol, incorporating a process of feedback of the results of the clinical examination to each owner, was approved by the Royal Veterinary College's Ethics and Welfare Committee.

Upon return to the UK, all field and laboratory data for each survey were entered into a custom-designed Microsoft Access database (Microsoft Office 2007, Microsoft Corp). Analysis was undertaken using Stata v8.0 (Statacorp LP). Descriptive statistics and prevalence estimates with 95% confidence intervals (CI) were calculated for each horse-, owner- and tack-related variable for the aggregate sample.

Participatory methods-based approach

Drawing on Participatory Rural Appraisal (PRA) techniques (Chambers, 1997; Chambers, 1993; Chambers, 1983) one-day participatory workshops were arranged by local horsemen in three separate geographic locations (Mafeteng, Matsieng and Malealea) in March 2010. Each location was within one hour's drive of the project base camp.

A local facilitator, experienced in participatory methods, worked with owners to create a resource map of their areas, sketched out on the ground using locally available materials such as bottle tops, small stones and maize cobs. Features incorporated in the map included roads and tracks, rivers and water sources, location of villages, number of horses in each village and availability of horse-related services such as farrier, saddler and equine health advice and/or drugs. Each participant also identified the location of their own home on the constructed map. During the process of map creation, which lasted approximately two hours, the facilitator encouraged owners to identify and discuss issues associated with owning horses. These issues were written onto separate cards and then compared against each other using a pairwise ranking matrix, also drawn on the ground. Owners discussed the reasons why issues might be considered priority health concerns, reaching consensus through a process of comparing, assessing and defining key criteria. At the end of the ranking exercise, the number of times each issue had been selected as the more important one was summed to calculate an overall score for each issue and thereby create a summary ranking scheme.

Since each group of owners was encouraged to define their own issue categories, it was anticipated that variations in category definitions would arise between groups. For the purpose of aggregating results from the three groups, therefore, rankings from each of the three groups were converted to scores. Based on the number of categories ranked by each group, the lowest score was one and each incremental higher rank was allocated a score one greater until the highest ranked issue was scored.

Scoring protocols, adapted from a schema described by Tchangaï et al. (2010), were applied to aggregate the rankings, using two alternative approaches to account for categories being sub divided in different ways by different groups. In the first (protocol one), each of these sub-categories counted half a score toward the disease category. An alternative methodology (protocol two), whereby each score was 'normally' weighted, regardless of whether the owner definition represented the whole or part of the category, was also undertaken.

Issues identified and their rankings/scores according to both protocols were compared qualitatively with the issues identified during the cross-sectional surveys.

RESULTS

Classical epidemiological surveys

The first cross-sectional survey covered 312 horses and 287 owners. Detailed findings of the baseline survey were reported by Upjohn et al. (2011). The second survey included 295 horses and 285 owners. The final cross-sectional survey covered 245 horses and 237 owners. Prevalence estimates of selected key health-related parameters as recorded in the third survey are set out in Table 1.

Table 1. Prevalence and 95% confidence interval (CI) of selected horse health-related parameters identified from owner questionnaire, clinical examination and tack examination during cross-sectional survey of Lesotho working horses and their owners August-October 2009

Parameter	n	Prevalence (%)	95% CI (%)
Sharp enamel molar/premolar points	245	90	86-94
Positive Strongyle faecal egg count	233	89	85-93
"I do not check tack fit before use"	237	85	80-90
Saddle-associated injury on examination	245	78	73-83
Skin ticks on examination	245	76	71-81
Inadequate saddle fit on examination	113	72	67-77
Pain on spinal palpation	245	72	67-77
"I can't afford to shoe my horse"	166	65	59-71
Inadequate bridle fit on examination	149	56	50-62
Horses seen worked without saddle	245	54	48-60
Inadequate bit fit on examination	149	52	46-58
Overgrown hoof horn	245	42	36-48
Bit- and/or curb chain-related lesions	245	41	36-46
Horses seen worked without bridle	245	39	33-45
Hypoglycaemia	90	37	27-47
Poor fore limb mediolateral foot balance	245	34	28-40
Hyperglobulinaemia	90	26	17-35
"Swollen gums indicate mouth problem"	237	26	20-32
Lameness	245	17	12-22

The mean body condition score on a scale of 0-5 (Carroll & Huntington, 1988) was 2.1 (sd 0.7, range 0.5-4.5). Simultaneously, 73% of owners (95% CI 68-78) agreed with the statement "my horse's diet is sufficient for the work which it undertakes" although 41% (95% CI 35-47) stated that their horse's diet was "unbalanced". Most owners (94%, 95% CI 91-97) described their horse's mouth as 'healthy', 84% (95% CI 79-89) described their horse's feet as 'healthy' and when asked about endoparasites, 90% of owners (95% CI 86-94) reported that they treated their horse for worms. Over half of owners (56%, 95% CI 50-62) described their horse's general health as 'good' or 'excellent'.

Participatory methods-based approach

In Mafeteng, 26 owners attended discussions; in Matsieng, there were 14 owners involved and in Malelea, 16 owners participated in the discussions.

The criteria they selected for determining priorities were identical in every group, being the impact that each problem was perceived to have on the overall health of the horse and/or its ability to work, rather than on the frequency of occurrence of the problem alone. As anticipated, there were some differences in the issues raised as regards the exact definition of terms and the specific items or aspects of an issue which were included in each group's discussions.

Issues identified included:

- Nutrition: insufficient/no feeding due to lack of grazing, lack of supplementary feeding. Two of the three groups identified lack of knowledge regarding appropriate supplementary feeding to create a balanced diet.
- Diseases and parasites: infectious disease (e.g. Strangles, African Horse Sickness), endoparasites (bots), ectoparasites (ticks, bot eggs, lice, scabies) and colic. Each group included to varying extents disease identification, prevention and management once horse is infected.
- Feet and limb problems: each group mentioned a variable combination of swollen legs, windgalls, overgrown frogs, foot rot, bruised soles, lameness of unspecified origin.
- Mouth problems: every group described swollen palate and gums (cause unknown by owners) which they perceive result in a horse being unable to eat properly.
- Horse husbandry: primary horse care including housing and wound management.

Issues ranked by each discussion group and aggregated according to protocol 1 are shown in Table 2; issues ranked and scored according to protocol 2 are shown in Table 3.

Table 2. Aggregate ranking of priority issues relating to horse health as identified by Lesotho working horse owners March 2010 (protocol 1)

	DISCUSSION GROUP AND SCORING				
Category ranked	Mafeteng	Matsieng	Malealea	Total	Rank
Mouth problems	5	5	5	15	1
Nutrition/hunger	3.5	3	4	10.5	2
Disease, including infectious disease, parasites and colic	2.5	4	3	9.5	3
Feet and limbs	1.5	1	2	4.5	4
Horse husbandry	-	2	1	3	5

Table 3. Aggregate ranking of priority issues relating to horse health as identified by Lesotho working horse owners March 2010 (protocol 2)

DISCUSSION GROUP AND SCORING					
Category ranked	Mafeteng	Matsieng	Malealea	Total	Rank
Mouth problems	5	5	5	15	1
Disease, including infectious disease, parasites and colic	5	4	3	12	2
Nutrition/hunger	3.5	3	4	10.5	3
Feet and limbs	1.5	1	2	4.5	4
Horse husbandry	-	2	1	3	5

DISCUSSION

The aim of this study was to compare findings associated with different research methodologies. Although three cross-sectional surveys were undertaken during the impact assessment the comparison made in this study was only between the findings of the final survey and the participatory approach in order to minimise time elapsed between the use of the two methods and therefore maximise temporal comparability within the constraints of the field study design employed. Ideally, the two methods would both have been applied at the same time of year to avoid potential differences associated with seasonal effects.

The strict application of epidemiological principles to fieldwork was hindered by logistical constraints specific to the Lesotho environment. The lack of a formal sampling frame for villages, owners and horses meant that a best estimate had to be constructed in consultation with locally knowledgeable personnel. Logistical limitations on accessibility of villages and availability of owners and their horses when visiting villages meant that pure randomisation of sampling was impossible. Owner and horse demographic data collected during the three surveys were compared inter-survey for purposes of an internal check on similarities. It was impossible to ascertain how representative of the local horse owner population each participatory discussion group was; in a participatory context, in-depth questioning of individual participants and recording of their demographic characteristics was considered inappropriate. Although all three elements of the survey protocol were subject to a small-scale pilot test in Lesotho, the variables included in the clinical examination protocol and topics of questions asked were generally based on developed country variables. This contrasts with the approach followed by another equine welfare charity in Ethiopia, as described by Degefa et al. (2010) and Kumar et al. (2010), whereby animal-based indicators for use in an impact assessment were defined based on dialogue with community members, whereby owners also agreed on measurement indices to be employed.

The criteria which owners defined for assessing relative importance of the key issues identified were identical in every group, being the impact that each problem was perceived to have on the overall health of the horse and/or its ability to work, rather than on the frequency of occurrence of the problem alone. This is a key distinction which underlies the potential for differences between local and external perceptions of priorities in the absence of detailed, free format community discussions such as those encouraged by the participatory facilitator. It raises similar issues to those described by Hadrill and Haroon (1994) who reported, when investigating

ranking of livestock diseases in Somaliland, that owners considered common ailments which caused ongoing loss of production as less important than only occasionally seen, but potentially terminal, infectious diseases. Similarly, Heffernan (1994) noted the complexity of motivations underlying disease ranking by individuals and the potential influence of a wide range of cultural reasons for the priorities they propose. Stringer et al. (2009) also noted that the impact which owners perceive as a given problem on a horse's health or ability to work needs to be specifically identified and understood. Even though the survey questionnaire included questions asking owners for opinions regarding the importance of certain externally predefined topics, they were semi-closed questions which were asked in isolation of one another and generally resulted in all issues being described as important.

In Lesotho, the key horse-related issue for which the distinction between owners' and researchers' highlighted topics was notable by comparison of survey and participatory findings was mouth health. Local owners' perceptions of the major issue relating to mouth health was "swollen gums", which they perceived as impacting a horse's ability to eat and be worked. No horses participating in the impact assessment study were recorded as having "swollen gums" during clinical examination. It was therefore not recorded as a high frequency problem during the cross-sectional surveys, although owner references to making gum incisions to treat it meant the issue was identified as worthy of further investigation on welfare grounds. Subsequent desk-based research indicated that this is probably "lampas", a condition described during the Victorian era whereby the hard palate appears enlarged in younger horses as gingival tissue expands at times when permanent incisors are replacing deciduous precursors. Veterinary opinion views this as a cosmetic rather than pathological issue but this is clearly contrary to owners' views and their perceived need to take action to address it. By contrast, clinical examination identified high prevalence of sharp enamel points and bit- or curb chain-associated lesions, neither of which were mentioned by owners during the surveys and not referred to during participatory discussions.

The participatory discussions provided better opportunity for understanding the focus of owners' concerns in relation to mouth health and their understanding of the issues involved. As such, they provided a better foundation than the defined questions asked during cross-sectional surveys for the development of relevant learning materials for future owner workshops to ensure that a sufficient breadth and depth of relevant issues had been covered by external researchers. Creation of an intervention to achieve resolution on the owners' priority issue is likely to be complex, however, since it is based on long-established beliefs which cannot readily be dispelled.

A small scale local feed trial initiated by the charity during the course of the impact assessment study was anecdotally positively received by owners and indicated that a well-designed practical demonstration of a potential new approach can achieve recognition of benefits by owners. The participatory discussions in the field trial region indicated that the incremental knowledge relating to this trial had been retained although the behaviour change targeted had not been maintained. The positive reception to a local, purpose-designed intervention is in line with positive impact reported by Degefa et al. (2010) in connection with interventions to address feeding and wound management practices in Ethiopian working equines. It is also indicative, however, of the need to adopt sustained intervention programmes if progress towards sustained change in beliefs and behaviours is sought (Van Dijk et al., 2010).

Enactment of an intervention to address problems perceived by external parties is unlikely to be understood or highly valued in the absence of extensive discussions with owners to

highlight those problems and their impact on both horse and owner (Pradhan et al., 2010). Notable owner survey fatigue was evident by the third survey – they readily commented that they had seen little direct benefit from their previous survey involvement and perceived little or no direct contact with the charity's in-country activities. In Lesotho, the high prevalence recorded for visible tack-associated lesions is an example of an issue targeted by the charity's intervention but, when owners were specifically questioned regarding tack-associated wounds as a supplementary enquiry during the course of the structured questionnaire, there appeared to be only limited acknowledgement of this as a constraint on the horse being worked. There appeared little understanding of how to adjust tack, particularly saddles, to reduce the frequency of such problems. These findings likely explain the lower ranking of husbandry, including wounds, in the participatory prioritisation discussions. In addition, although it was not a topic on which specific questions were incorporated in the questionnaire, occasional details of inappropriate practices in respect of wound management, such as application of engine oil, were mentioned. Local belief systems, such as this, and lack of recognition of the value of addressing certain issues need to be taken into account and specifically addressed as part of any proposed intervention. The charity's saddlery training intervention is designed to reduce tack-associated lesions through improving the availability of skilled saddlers to supply well designed, well fitted tack. However, the survey findings in relation to uptake of the services of such trained people, and the issues highlighted by a parallel survey of trained students (Attwood et al., 2010) indicate only limited recognition of their skills in the local community. Without engaging the community in discussions to raise awareness of the full range of issues involved and improve understanding of why they need to be addressed for the benefit of both horses and owners, any such intervention is likely to be unrewarding and unsustainable.

In relation to diseases affecting the horses, there were two potential technical difficulties specific to the issue of diseases mentioned by owners in both the cross-sectional surveys and during the participatory discussions. The first was translation from Sesotho to English, both as regards the specificity of the Sesotho term by comparison with the English term and the accuracy of the English term selected by the interviewer. The specificity issue is similar to the difficulties acknowledged by Leyland (1994) when discussing translation of Pashtu terms for livestock illness in Afghanistan. The accuracy issue relates to the technical knowledge of the interviewer as well as the depth of their language skills. They are compounded by the lack of objective validation of the owners' diagnosis of disease. Although a subsequent study (Ling et al., 2011) used serology to assess population exposure to *Streptococcus equi*, the causative agent of strangles, no field-based diagnostic tools were available at the time of examining horses and talking to owners. In particular, there was uncertainty in respect of owners reporting an illness which was translated as African Horse Sickness; the clinical symptoms which they described did not appear to coincide with generally recognised presentations of this disease. Logistically feasible access to suitable diagnostic testing facilities was not available to undertake further investigations so this remains at best an interpretation rather than a definitive diagnosis. Without such corroboration, it is difficult to address creation of an intervention suitable to enhance owners' knowledge or ability to help themselves and their horses regarding prevention and/or management of such health problems.

One of the lowest priority topics identified by owners was that of feet- and limb-related problems. It should be noted that due to limitations in Sesotho vocabulary, the Sesotho word which is translated as 'feet' does not distinguish between feet and limbs, so problems relating to several anatomical structures were all incorporated within this category during participatory discussions, covering a wide range of issues including hoof-, limb-, soft tissue- and foot-associated lesions and lameness of unspecified origin. No mention was made by owners of

overgrown feet or poor foot balance. However, it was clear from discussions amongst the owners that few of the problems reported were viewed as of great concern in terms of morbidity or restriction of the animal from working. This attitude corroborated findings from the cross-sectional surveys which indicated that the frequently noted adverse clinical examination findings such as overgrown horn, medio-lateral foot imbalances and lameness were not associated with owners reporting their horse's feet to be in poor condition (Upjohn et al., 2011); the relatively low ranking ascribed during the participatory ranking process confirmed this impression. This attitude contrasts with the substantial proportion of the charity's intervention resources directed towards training in farriery skills. This mismatch has to raise doubts about potential owner appreciation of the value and therefore the sustainability of this intervention, in line with findings reported by a survey of charity-trained farriers (Attwood et al., 2010).

Overall, it is clear that whilst there was substantial similarity in the findings resulting from the two methodologies, key differences also emerged. The classical epidemiological approach provided objective measurement of the frequency of problems, subject to logistical country-specific limitations of the survey process and to the risk of overlooking locally-specific issues which had not been specifically incorporated into the predefined data collection protocols. These could be compared with the owners' perceptions of the frequency or importance of those issues. A participatory methods-based approach provided greater opportunity for the researchers to gain a detailed understanding of local issues and an appreciation of how owners defined and prioritised the problems affecting them and their animals. In the context of an impact assessment study of an intervention which was largely predefined, with a certain element of tailor-made localised activities, it was notable that without the vital components of developing community ownership of the process and grounded researcher appreciation of local circumstances, interventions defined solely on the basis of epidemiological measures of frequency are less likely to succeed. Ongoing education programmes could be devised, based on combining insights from participatory investigations with issues highlighted by classical techniques to define topics worthy of further discussion with owners.

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ARE THERE COUNTRY-WISE DIFFERENCES BETWEEN NORDIC DAIRY FARMERS IN THEIR THRESHOLD FOR CONTACTING A VETERINARIAN?

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SUMMARY

Disease incidence estimates based on the national central databases for dairy cattle vary between the Nordic countries, Denmark (DK), Finland (FI), Norway (NO) and Sweden (SE), which may be due to farmers' different thresholds for contacting a veterinarian when noting illness in their cows. This was quantitatively investigated for the disease mild clinical mastitis using a questionnaire based on the Theory of Planned Behaviour. The threshold behaviour in FI that would most commonly initiate the recording process for an episode of mild clinical mastitis was to take a milk sample, and hence this behaviour was compared with the behaviour of contacting the veterinarian in the other countries. The intent to perform the behaviour of interest was found to differ significantly between the countries, except for DK and NO. SE farmers had the lowest intent to contact the veterinarian, and FI farmers had the highest intent for their comparable behaviour.

INTRODUCTION

In the Nordic countries, Denmark (DK), Finland (FI), Norway (NO) and Sweden (SE), there are country-specific production and health recording schemes for dairy cows that include the reporting of disease events to a central database (Olsson et al., 2001). Based on these registrations between-country comparisons of disease incidence have been made to gain a better understanding of any country differences in the management and control of disease (Plym-Forshell et al., 1995; Valde et al., 2004). Differences have been found for several diseases, which has raised the question of data quality and if a comparison between countries is valid. Ideally, data in these central databases should capture all veterinary-attended disease events on dairy farms. However, this may not be the case as found in recent work by the authors (Espetvedt et al., 2012).

Differing thresholds for treatment and veterinary consultation could be one factor influencing differences in registered disease occurrence between countries. In the Nordic countries, an episode of clinical disease in a dairy cow is unlikely to be registered unless a veterinarian has been involved at some stage, whether the veterinarian directly diagnosed the case and initiated medical treatment, or was responsible for prescribing medicines after contact

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with the farmer. Different diseases may be expected to show more or less variation in this threshold between the four countries. Mild clinical mastitis (MCM) is a common disease where the threshold for the decision to consult the veterinarian may be expected to vary as it is influenced by a complex set of interacting factors, e.g. the yield of the cow, the somatic cell count situation of the herd and the farmer's experience with self-cure of MCM without prescription medicines (Vaarst et al., 2002).

Using a questionnaire based on a social psychology model, the Theory of Planned Behaviour (TPB) (Ajzen et al. 2007), it is possible to quantify the intent to perform a specific behaviour and, in this context, study any between-country variation in decision thresholds for contacting a veterinarian. This will, in turn, give information about how likely it is that a MCM case ends up as a registered disease event in each of the four countries' central databases. The specific objectives of this study were, firstly, to investigate whether farmers in DK, FI, NO and SE differ in their intent to contact a veterinarian on the same day as noting a cow with MCM, and secondly to look at behavioural factors underlying this intent. In FI the farmer's intent to take a milk sample for bacteriology from a cow with MCM was also studied. This is routine practice in FI (Saloniemi, 1980), and thus the first step in the process that could lead to a recording of mastitis in the FI central database.

MATERIALS AND METHODS

Creating the questionnaire

The methodology for constructing the TPB questionnaire was based on the manual by Francis et al. (2004). Below, the details of the TPB model (Fig. 1) will only be described briefly in relation to the components of the questionnaire, but further details are available from numerous sources (Ajzen, 2005; Ajzen et al., 2007; Rehman et al., 2007).

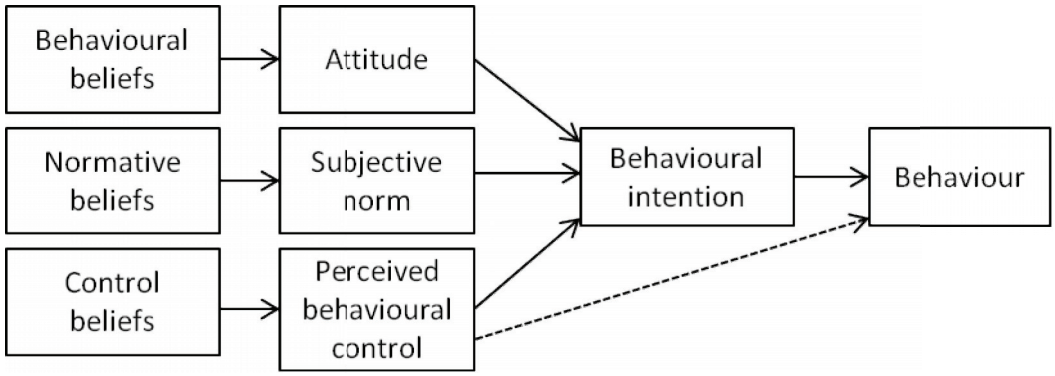

Fig. 1 The Theory of Planned Behaviour.

Elicitation interviews: In each of the four countries the responsible investigator conducted qualitative face-to-face interviews with eight to ten dairy farmers that were sampled based partly on convenience, partly on judgement. A common interview guide was used, with the particular aim to obtain saturated information about experience with MCM in dairy cows, but also demographic and general information about the farms. MCM was defined according to the International Dairy Federation's definition (IDF, 1999). In total, 38 interviews were voice recorded and transcribed. Interviews from FI and those to be read by the FI investigator were

translated to English. Each interview was read and analysed by two investigators. Statements relating to beliefs about MCM, possible outcomes of various treatment decisions, important referents and sources of information as well as any constraints for desired action, were identified. Commonly held beliefs were then used to construct questionnaire items in the TPB questionnaire.

The TPB questionnaire: The first step in creating the TPB questionnaire was to carefully define the behaviour of interest. This was important both to ensure that all statements included focused only on this behaviour and to inform the respondents about what situation they were to think of when answering the questionnaire. The behaviour was defined as: contacting the veterinarian for a visit the same day as detecting signs of MCM in a lactating dairy cow. The FI questionnaire had the additional definition: taking a milk sample for bacteriological analysis on the same day as detecting signs of MCM in a lactating dairy cow. The questionnaire was divided into five sections: (1) demographic questions, (2) assessment of intent to perform the behaviour (including eight case scenarios of MCM), (3) statements assessing attitude (ATT) towards the behaviour, (4) statements assessing social referents' (here called subjective norms) (SN) influence on the behaviour, and (5) statements assessing hindering factors, called perceived behavioural control (PBC), for performing the behaviour (Fig. 1). The demographic information will not be further presented in this paper.

Measuring behavioural intention in a TPB questionnaire is a proxy for measuring the actual performance of the behaviour. The eight case scenarios described common situations and included a variety of decision factors based on insight gained from elicitation interviews, and inspired by the work of Vaarst et al. (2002). For each scenario, the farmer could answer either yes or no (whether he/she would contact the veterinarian or not), and also for FI, yes or no with respect to taking a milk sample for bacteriology. All the statements used to assess ATT, SN and PBC had answer options on bipolar 7-point Likert scales, with end words being semantic opposites like 'important-unimportant', 'agree-disagree' (Osgood et al., 1957).

The questionnaire was translated from English into native languages, pilot-tested and revised twice. The final version was back-translated to English or Swedish by a person outside the project group, to detect any discrepancies between language versions.

Farmer population and data collection

The target population, and the sampling frame, was dairy farmers in DK, FI, NO and SE, enrolled in disease and milk recording, and with a herd size of at least 15 cows. A simple random sample of 400 farmers was drawn in each country. Approximately 10% of Danish dairy farmers participate in a herd health scheme where the herd owners themselves can initiate medical treatment; these herds were excluded from the Danish sampling frame.

Questionnaires were posted to farmers during April 2010, with a reminder three weeks after the initial distribution. In total 852 questionnaires were returned, and after removing herds that had ceased milk production (n=18), a response rate of 65%, 45%, 54% and 52% in DK, FI, NO and SE, respectively, was achieved.

Data management and analysis

Completed questionnaires were electronically scanned, and the resulting data files manually checked. The individual country files were then combined and a second data control performed by checking the distribution of answers. Poorly completed questionnaires were removed (n=18), and judged to be such if more than 25% of the questions were left blank, or more than 50% of the answers were neutral (i.e. '0' on the scale from -3 to 3). For all data management and analysis the software SAS, version 9.2 was used (SAS Institute, Inc., Cary, NC, USA).

The answers to the direct, general ATT and SN questions were combined to a mean value; the (positive or negative) direct ATT towards performing the behaviour, and the direct SN. One direct PBC question was included in the analysis. The more specific ATT and SN questions derived from the elicitation interviews were multiplied by a corresponding evaluation question that modified their importance. The products were summarised to the indirect ATT and indirect SN.

Intention score: For the case scenarios a behavioural intention score was calculated for each observation, a proportion based on the number of "yes" answers. To test the hypothesis of country differences in behavioural intention score, pair-wise Wilcoxon rank-sum tests were used.

Behavioural components underlying the intention scores: country-wise multivariable linear regression models with intention scores as the outcome variable were constructed The predictor variables evaluated were direct ATT, direct SN and direct PBC. The proportion of variability in intention that could be explained by the underlying behavioural components ATT, SN and PBS, alone or in combination, was estimated by the adjusted coefficient of determination (R^2_{adj}) for each model.

Determinants of ATT and SN: Spearman rank correlations were estimated between behavioural intention scores and each indirect ATT and indirect SN. This was to study the underlying behavioural beliefs and social referents (persons) with the strongest influence on the intention to contact the veterinarian and, for FI, to take a milk sample.

RESULTS AND DISCUSSION

Behavioural intention

Nordic farmers do vary in their intent to contact a veterinarian for a visit or, for FI, to take a milk sample and send for analysis on the same day as detecting a cow with MCM (Table 1). There were significant differences ($p<0.01$) between all countries, except DK and NO, where the highest intention scores were seen. This difference in intention for the action that initiates the recording process is likely to impact on the proportion of mastitis episodes on farms that eventually ends up as disease recordings in the national databases. Preceding this threshold for "action" is the farmers' ability to detect MCM cases, which is also likely to influence recorded disease incidences. However, this was not assessed in the current study.

As judged from the median intention score for FI (0.00), contacting a veterinarian was a very unlikely event when detecting a MCM case. On the other hand, FI farmers showed a high intent to take a milk sample and send for bacteriology, with a median intention score of 0.63 (Table 1). This finding is in agreement with a previous study (Saloniemi, 1980) that stated that

approximately 65% of clinical mastitis cases in FI were treated solely by the farmer after phone consultation and with a prescription sent to a pharmacy. A prescription for medical treatment should in this situation be based on known bacteriological results from a milk sample. The mastitis incidence in the Finnish national database is relatively low in comparison to the other Nordic countries (Østerås et al., 2002). This might be related to the practice of first taking a milk sample from an MCM case, meaning that only those with positive findings will receive medical treatment and become an event in the database, if the recording process is successful.

Table 1. Behavioural intention scores (IS), direct attitude (ATT), subjective norm (SN) and perceived behavioural control (PBC) (median with first and third quartiles) resulting from a questionnaire study, based on the Theory of Planned Behaviour. The studied behaviour was 'contacting the veterinarian for a visit the same day as detecting signs of MCM in a lactating dairy cow' (VET). In Finland, the behaviour 'taking a milk sample for bacteriological analysis the same day as detecting signs of MCM in a lactating dairy cow' (MILK) was also studied. The questionnaire was distributed to 400 dairy farmers per country in Spring 2010.

	POSSIBLE RANGE	DENMARK n=252	FINLAND n=174	NORWAY n=208	SWEDEN n=199
IS VET	0-1	0.50 (0.25, 0.63)	0.00 (0, 0.25)	0.50 (0.25, 0.63)	0.38 (0.13, 0.63)
IS MILK	0-1	-	0.63 (0.50, 0.88)	-	-
ATT	1-7	5.00 (3.33, 6.00)	6.00[a] (5.33, 6.67)	5.00 (3.50, 6.00)	4.33 (3.67, 5.67)
SN	1-7	4.50 (3.00, 6.00)	5.50[a] (5.00, 6.50)	4.00 (3.00, 5.50)	4.00 (3.00, 5.50)
PBC	1-7	6.00 (4.00, 7.00)	6.50[a] (6.00, 7.00)	7.00 (5.00, 7.00)	7.00 (5.00, 7.00)

n = number of observations

[a] In Finland the behaviour of interest was taking a milk sample (MILK).

Influence of underlying behavioural components

In all four countries, ATT explained most of the variation in the behavioural intention under study, i.e. to contact a veterinarian or, in FI, to take a milk sample when detecting a cow with MCM (Table 2). On a general level this means that underlying outcome beliefs about the behaviour, e.g. whether it is good or bad/useful or useless to perform, are the most important determinants for the intention to carry out the behaviour. The influence of ATT was highest in NO and SE, where the proportion of variation explained was 0.57 and 0.55, respectively.

When modelled on its own, the R^2_{adj} for SN was lower than for ATT in all four countries. This means that farmers are much less influenced by important others with respect to the intent to carry out the behaviour than by their own beliefs about its outcomes. In other words, it is not a social pressure that determines whether the farmer contacts the veterinarian or not or, for the FI situation, takes a milk sample or not.

Observations from elicitation interviews had indicated that PBC was not an issue for the behaviours studied here, i.e. there were no real hindrances to contact the veterinarian or to take a milk sample when detecting a cow with MCM. This was also supported by a high median score with a narrow range for the direct PBC in all countries (Table 1), and also by the low R^2_{adj} when PBC was the only predictor, and a minor contribution when combined with ATT or SN as predictors (Table 2).

Table 2. Proportion of variation, measured as the adjusted coefficient of determination (R^2_{adj}), in the behavioural intention that could be explained by the direct attitude (ATT), the direct subjective norm (SN) or the direct perceived behavioural control (PBC) in multivariable linear regression models. The studied behaviour was 'contacting the veterinarian for a visit the same day as detecting signs of MCM in a lactating dairy cow' or, in Finland, 'taking a milk sample for bacteriological analysis the same day as detecting signs of MCM in a lactating dairy cow'. The questionnaire based on the Theory of Planned Behaviour was distributed to 400 dairy farmers per country in Spring 2010.

PREDICTORS INCLUDED IN THE MODEL[a]	DENMARK n=225	FINLAND n=172	NORWAY n=193	SWEDEN n=188
ATT	0.50	0.24	0.57	0.55
SN	0.25	0.19	0.33	0.34
PBC [b]	0.09	0.15	0.23	0.10
ATT and SN	0.51	0.27	0.57	0.62
ATT and PBC	0.51	0.28	0.50	0.54
SN and PBC	0.23	0.24	0.40	0.38
ATT, SN and PBC	0.51	0.32	0.58	0.62

n = number of observations without missing values for ATT, SN and PBC.
[a] All the models had an F-test p-value <0.0001, unless otherwise indicated.
[b] The model for Sweden had an F-test p-value <0.03.

It is of interest to identify what behavioural components have the most influence on the behavioural intention under study in order to better understand the related behaviour, to modify it, if desirable, or study the behaviour further. Interpreting R^2_{adj} was one way of estimating what proportion of observed variance in intention scores was explained by which behavioural component. There are certain weaknesses with this method and consequently interpretation should be made with caution (Hankins et al., 2000); however, the results presented are in line with what was expressed in the qualitative elicitation interviews, and this can as such be regarded as additional support that they provide a valid and relevant reflection of the true situation.

The attitudes and subjective norms of greatest influence

As described above, direct ATT had the highest influence on the behavioural intention. Underlying this general ATT is the more specific indirect ATT which gives us further insight to drivers and barriers for performing the behaviour. Looking at the Spearman rank correlation coefficient between individual indirect ATT and the behavioural intention score, several significant drivers, positively correlated, were found (Table 3).

Table 3. Spearman rank correlation coefficients (p-value) between the behavioural intention score and the indirect attitudes (ATT) from a Theory of Planned Behaviour study. A positive and significant correlation indicates that the attitude is a driver to perform the behaviour. The studied behaviour was to contact the veterinarian the same day as detecting signs of mild clinical mastitis in a lactating cow. In Finland, the behaviour studied was to take a milk sample and send for bacteriology. The study was performed in Spring 2010 with a questionnaire sent to 400 dairy farmers per country.

INDIRECT ATT	DENMARK	FINLAND	NORWAY	SWEDEN
Will always result in medical treatment	ns	0.26[a] (0.0006)	ns	ns
Results in undesirable veterinary cost	ns	ns[b]	ns	ns
Will agree with veterinarian on treatment	ns	ns[a]	ns	ns
Will prevent reduced milk yield for the rest of the lactation	0.44 (<0.0001)	ns[a]	0.35 (<0.0001)	0.40 (<0.0001)
Leads to concern over extra labour due to treatment	ns	ns	ns	ns
Will lead to quicker recovery of the cow	0.51 (<0.0001)	0.27[c] (0.0003)	0.54 (<0.0001)	0.64 (<0.0001)
Will prevent the cow from becoming three-teated	0.46 (<0.0001)	0.20 (0.0073)	0.42 (<0.0001)	0.40 (<0.0001)
Will benefit from vet's skills and knowledge	0.22 (<0.0001)	ns[a]	ns	ns
Will contribute to a healthy herd	0.34 (<0.0001)	0.26[c] (0.0006)	0.33 (<0.0001)	0.39 (<0.0001)
Will provide useful knowledge about bacterial growth	0.28 (<0.0001)	0.25[c] (0.0009)	0.22 (0.0015)	0.22 (0.0015)
Will lead to concern over less milk in the bulk tank due to treatment	ns	ns[a]	ns	ns

ns = not significant at the 99% significance level.

[a] Questions in Finland where only the behaviour 'contacting the veterinarian' was applicable.

[b] In Finland, the cost for a milk sample.

[c] Questions in Finland where a combination of the behaviours 'contacting the veterinarian' and 'taking a milk sample' were applicable.

The strongest driver in all countries was the belief that contacting the veterinarian upon detecting a cow with MCM would lead to a quicker recovery of the cow. In FI this referred to the situation of contacting the veterinarian or taking a milk sample. The belief that the behaviour would prevent the cow from becoming three-teated was also a strong driver in all four countries. Third on the list in DK, NO and SE was the belief that contacting the veterinarian would prevent reduced milk yield for the rest of the lactation. In FI the third belief was that contacting a veterinarian would always result in medical treatment. This is an expected result for FI where farmers generally first take a milk sample. If the veterinarian is contacted for a visit, this is due

to the desire to treat medically straight away. Looking into these specific behavioural beliefs facilitates the understanding of what factors may be important to emphasise in any written or spoken communication with farmers regarding management of MCM cases.

As presented above, the SN was of less importance than ATT in all four countries (Table 2). Nonetheless, it is interesting to look at the hierarchy of who are the persons with the largest influence as these are likely to be the persons that farmers generally trust and listen to. In DK the non-veterinarian herd management advisor was rated highest, in FI and SE the veterinarian, while in NO family members involved in farming had the highest correlation (Table 4). There could be many factors underlying the reason for a high rating, for example trust and frequency of contact with a certain person. Considering how to make use of important others might help in developing a communication strategy if behaviour modification is desirable.

Table 4. Spearman rank correlation coefficients between behavioural intention score and the indirect subjective norms (SN) from a Theory of Planned Behaviour study. A significant correlation indicates that the SN is an important social referent for whether to perform the behaviour or not. The behaviour of interest was to contact the veterinarian the same day as detecting a lactating cow with signs of mild clinical mastitis. In Finland, the behaviour under study was to take a milk sample and send for bacteriology. The study was performed in Spring 2010 with a questionnaire sent to 400 dairy farmers per country.

INDIRECT SN	DENMARK	FINLAND[a]	NORWAY	SWEDEN
Family members involved in farming	0.39 (<0.0001)	ns	0.44 (<0.0001)	0.25 (0.006)
Employee/relief worker	0.38 (<0.0001)	ns	0.39 (<0.0001)	0.20 (0.0077)
Farming colleagues	0.33 (<0.0001)	ns	0.42 (<0.0001)	0.31 (<0.0001)
Veterinarian	0.36 (<0.0001)	0.39 (<0.0001)	0.43 (<0.0001)	0.40 (<0.0001)
Herd management advisor (non-veterinarian)	0.40 (<0.0001)	0.38 (<0.0001)	0.38 (<0.0001)	0.40 (<0.0001)

ns = not significant at the 99% significance level.

[a] For Finland all the correlations are for the behaviour to take a milk sample.

The TPB approach

In our opinion, using a well established behavioural theory like the TPB was appropriate for the aim of this study as comparable quantitative results were obtained for behaviour, which is otherwise a complex phenomenon to study. It is also attractive to use the methodology as clear guidelines exist. All dairy producers included in this study will have experienced MCM cases and therefore, the answers given are not likely to be based on guesses or assumptions.

A TPB questionnaire tends to be long and complex. The questions need to be worded very precisely and can often be seen as repetitious (Beedell & Rehman, 2000). The questionnaires used in this study were approximately 10 pages long, and as such ran the risk of having a low response rate and creating reply fatigue which would lead to less considered responses. In the

initial data editing, a limited number of responses with many neutral or unanswered questions were removed in an objective way to exclude unsatisfactory responses. When comparing with other TPB studies, a response rate of around 50% in all four countries can be viewed as high. For example, a response rate of around 20-30% has been considered satisfactory (Garforth et al., 2006). Still selection bias cannot be excluded. However, measuring the external validity of our results would be a complex task, taking into consideration not only demographic variables and disease situations on particular farms, but also the intent and behavioural components, i.e. if there was a representative spectrum of existing behaviours and underlying beliefs. The TPB methodology can also be applied to focus group discussions. However, as the main objective of the present study was a between-country comparison of behavioural intention, a quantitative approach was desirable.

CONCLUSION

There are significant differences between the Nordic countries, with regard to the intent to contact a veterinarian on the same day as detecting MCM in a lactating dairy cow, with DK and NO being an exception. In FI, the behaviour under study was to take a milk sample for bacteriological analysis on the same day. This suggests that dairy farmers in these countries have different thresholds for the action that would lead to a disease recording in the respective countries' cattle databases. As such, this may influence the proportion of mastitis cases detected on farm that are captured by the official mastitis incidence figures. This is important to be aware of when comparing official statistics between these countries.

In all four countries, the most important predictor of this intent was the ATT towards contacting the veterinarian, or for FI, to take a milk sample. Underlying this general ATT were the two most important outcome beliefs of the behaviour studied; that it would lead to a quicker recovery of the cow and prevent the cow from becoming three-teated.

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SURVEILLANCE

SYNDROMIC SURVEILLANCE ON BLOOD SAMPLES OF ABORTING COWS FOR EARLY DETECTION OF (EMERGING) INFECTIOUS DISEASES USING DIFFERENT STATISTICAL METHODS

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SUMMARY

The objective of this study was to evaluate whether syndromic surveillance can contribute to the early detection of (emerging) infectious disease outbreaks in the Dutch cattle population using submissions of blood samples of aborting cows. As part of compulsory brucellosis monitoring, blood samples have to be submitted to the Animal Health Service. Submissions from 2007-2010 were available to investigate elevations in the number of samples in time and space by using three different statistical methods: Linear regression, retrospective and prospective space-time cluster analysis.

This study showed that data of blood samples of aborting cows are suitable for syndromic surveillance. All methods used in this study were sensitive for the detection of (emerging) infectious diseases that cause increased number of abortions similar to bluetongue. Prospective space-time analysis on these data can statistically contribute to early detection of local outbreaks of (emerging) infectious diseases that cause abortions.

INTRODUCTION

Traditional detection of disease outbreaks is based on specific disease symptom. Syndromic surveillance focuses on elevations of non-specific disease indicators varying from diarrhoea, abortion, fever and dullness to mortality. Systematic analyses of these non-specific disease indicators allow detection of disease outbreaks before laboratory diagnostics have confirmed the presence of the disease. Syndromic surveillance can accelerate the detection of disease outbreaks and reduce economic costs related to an outbreak as control measures could be implemented at an early stage.

Syndromic surveillance was first introduced in public health since the bioterrorism attacks in 2001 and the SARS epidemic in 2003 (Buehler, 2003). In veterinary health, it is also increasingly used for the early detection of disease outbreaks in animals (Dorea et al., 2011). Different statistical methods can be used to detect elevations of non-specific disease indicators

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in time and/or space. For the detection of elevations in time, different control charts can be used in case there is limited availability of historical data (Lawson and Kleinman, 2005; Stoto et al., 2006). When historical data is available, regression methods can be used. This method allows adjustment of covariates, for example seasonal trends. Usually, an alarm is given when the disease indicator is statistically out of control. However, the choice of the appropriate control limits is sometimes difficult (Williamson and Hudson, 1999). To optimize sensitivity, space-time scan statistics can be used to detect clusters of non-specific disease indicators in time and/or space, i.e. detection of local outbreaks in a certain time interval (Kulldorff, 1997). However, false alarms may lead to wasted resources and a decreased feel of urgency.

In the Netherlands, the Animal Health Service (AHS) collects many syndromic data such as blood samples from aborting cows. Dutch farmers are obliged to submit a sample for brucellosis monitoring from cows that abort between 100 and 260 days of gestation. However, compliance is about 25%. The objective of this study was to evaluate whether syndromic surveillance can contribute to early detection of (emerging) disease dynamics in the Dutch cattle population using the number of submitted blood samples of aborting cows. Therefore, different statistical methods were used to detect elevated numbers of blood samples in time and/or space. A linear regression analysis was chosen to detect elevations in time, as the number of blood samples was normally distributed and data covered a 4-year period. In addition, adjustment for seasonal variation was possible. Since the location of herds was known, it was possible to detect local elevations in the number of submitted blood samples as well. A space-time scan statistic was used to detect elevations in space and time. In this study, the (dis)advantages of these statistical methods are illustrated and discussed.

MATERIALS AND METHODS

Syndrome data collection

Data were available over a period from January 2007 to December 2010 concerning submitted blood samples from aborting cows for brucellosis monitoring (source; AHS). Each record in the database consisted of a unique farm identity number (UFI) related to one specific herd, the identification number of the cow, the date of submission and the result of the test. X-y coordinates of each UFI were used (source; AHS) to determine the location of a herd.

Blood samples originating from herds that participate in voluntary certification programmes for salmonellosis, neosporosis and/or infectious bovine rhinotracheitis (IBR) were tested for these diseases. Blood samples from herds that did not take part in any of these programmes were only tested for *Brucella*. The additional test results were used to explain some elevations in the number of blood samples that were found in our analyses.

Statistical analyses for detection of clusters in time and space

Descriptive statistics were carried out on the number of submitted blood samples. The presence of seasonal differences was tested using a T-test ($P \leq 0.05$). Three methods were used for syndromic surveillance: 1) multivariable linear regression, 2) retrospective space-time cluster analysis and 3) prospective space-time cluster analysis. The characteristics and (dis) advantages of the different statistical methods are illustrated based on the results of the analysis.

Multivariable linear regression

A linear regression analysis was carried out on weekly data from 2007-2010 to detect elevations of blood samples in time. The analysis was repeated on a dataset excluding the 2007 data to set a baseline, which was less influenced by the bluetongue outbreak (from July 25th-December 31st 2007). The number of submitted blood samples (log-transformed) per week was set as dependent variable and seasonal differences in submissions were modelled with a sine-cosine function. The multivariable model that was used for analysing the data was:

$$ln(NBS)_i = \mu_i + \beta_1 sin((2\pi * week_i)/52) + \beta_2 cos((2\pi * week_i)/52) + \varepsilon \quad (1)$$

where,

$\ln(NBS_i)$	= Number of blood samples per week (i) log-transformed
μ_i	= Intercept per week (i)
$\beta_1 sin((2\pi * week_i)/52)$	=Sine function per week (i)
$\beta_1 cos((2\pi * week_i)/52)$	=Cosine function per week i)
ε	= Random error

For each week, the number of expected submissions was determined and compared with the number of observed submissions. Model fit was checked on the residuals (histogram, normal probability plot and skewness-kurtosis test). The threshold for elevated blood samples submission was set at the 5% weeks with the highest difference between observed and expected submissions. The outbreak of bluetongue in 2007 (from July 25th (=first confirmed case in cattle) to December 31st) was used to test the sensitivity of the linear regression analyses. To optimise the specificity of the system, a predetermined threshold for taking follow-up actions was used, namely two or more elevations occurring within four weeks (the chance of occurrence of this event at random was 1% or less) (Williamson and Hudson, 1999).

Retrospective space-time cluster analysis

The spatial scan statistic was used to detect possible elevations (i.e. clusters) of blood samples in time and space. A retrospective space-time cluster analysis was performed for the whole study period from January 2007 to December 2010. The outbreak of bluetongue in 2007 was used to test the sensitivity of the retrospective space-time cluster analysis. To optimize specificity, the threshold for taking follow-up actions was set when a significant result was found at the 1% level ($P \leq 0.01$).

Sensitivity analysis was carried out in which the threshold for taking follow-up actions was set at the 5% level. The number of signals associated to the bluetongue episode that resulted from this threshold was compared to the 1% level threshold. In addition, a moving 4-weekly analysis was carried out and the results were compared to the results of the weekly analysis.

Prospective space-time cluster analyses

In operational syndromic surveillance, prospective space-time cluster analyses at regular time intervals should be carried out to detect new outbreaks at an early stage (Dórea et al., 2011). Compared to retrospective analysis, prospective analyses are timelier. Historical data are

required to build up reliable references (data to which new data are compared). However, no reliable submission data before 2007 was present and therefore it was not possible to simulate prospective analyses for the bluetongue outbreak. Instead, data of 2010 was used to simulate the situation in which the syndromic surveillance would be operational. The spatial scan statistic was run on weekly data from 2008 and 2009, completed with previous weeks in 2010 for each weekly prospective analysis in 2010. The prospective analysis was carried out without data from 2007, because the bluetongue episode considerably influenced the number of submitted blood samples. To optimize specificity, the threshold for taking follow-up actions was set when a significant result was found at the 1% level ($P \leq 0.01$). The main cluster found in the analyses was further investigated to explore the possible causes. Therefore, additional results of herds, submitting samples when participating in voluntary certification programmes for salmonellosis, neosporosis and/or IBR, were used. The proportion of herds with positive result(s) for those diseases was determined in the cluster and compared with the same area in the same period in the previous year. Differences in the proportion of herds with positive result(s) between years were tested with a Proportion test ($P \leq 0.05$).

For both the retrospective and prospective space-time cluster analyses, data were aggregated per week and per 2-digit postal code and a space-time permutation scan statistic was used. For this statistic only case data is needed to estimate the expected number of cases in each space-time window. The herd density and possible time trends in the case data are automatically adjusted for. The spatial scan statistic compares the observed number of cases in circular windows with variable radii in flexible time periods with the expected number of cases based on the geographic distribution of cases in the whole dataset. The maximum spatial and temporal cluster sizes were set at 50% of the population at risk, because then clusters of both small and large sizes were searched for without any pre-selection bias in terms of the cluster size. A circular window shape was chosen. We scanned for 'high rate' clusters of blood samples (i.e. higher number of observed submitted blood samples than would have been expected). For the identification of these clusters, the likelihood function was maximized over all windows. For each window, a likelihood-ratio test statistic was calculated and the window with the maximum value was the most likely cluster. A *P*-value was assigned to the cluster using Monte Carlo hypothesis testing (999 simulations) and considered significant at the 1% level ($P \leq 0.01$). The cluster analyses were carried out using SaTScan v7.0.3 (Kulldorff, 1997).

RESULTS

Descriptive results

Submissions from herds with unknown x-y coordinates were excluded from the analyses (3.2%). From January 2007 to December 2010, 12,386 unique cattle herds submitted in total 47,183 blood samples to the AHS. The weekly number of blood samples ranged from 126 (January 1st -7th 2009) to 362 (November 12th-18th 2007, Table 1). The number of blood samples and unique herds that submitted samples was highest in 2007 and lowest in 2009 (Table 2).

In June and August more samples were submitted (mean 260 samples) than in January-May and September-December (mean 216 samples, T-test, $P \leq 0.01$, Fig. 1).

Table 1. Descriptive statistics of blood samples from aborting cows submitted for brucellosis in the period January 1st 2007 to December 31st 2010 in the Netherlands

	TOTAL NO. SUBMISSIONS	MEAN NO.SUB- MISSIONS PER WEEK	MEDIAN	MIN	IQR	95TH PERCENTILE	MAX
Blood samples	47,183	227	217	126	72	313	362

Table 2. Yearly number of blood samples from aborting cows submitted for brucellosis monitoring and number of Dutch cattle herds that submitted blood samples from January 1st 2007 to December 31st 2010

YEAR	NO. BLOOD SAMPLES	NO. UNIQUE CATTLE HERDS
2007	13,214	6,387
2008	12,421	6,185
2009	10,431	5,543
2010	11,117	5,812

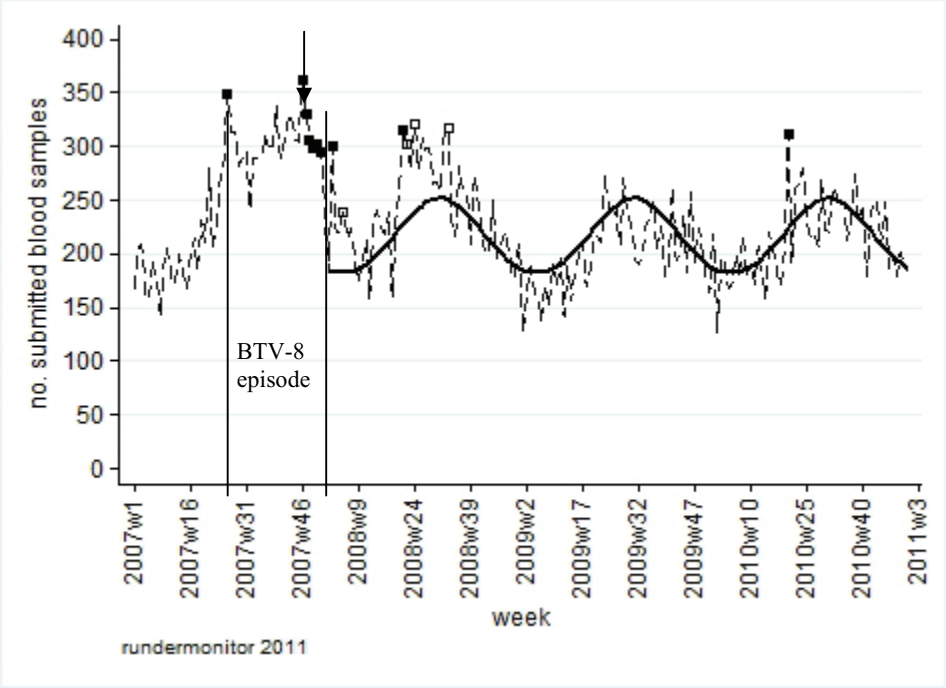

Fig. 1 Observed (dashed line) and expected (straight line) number of submitted blood samples from aborting cows for brucellosis monitoring per week in the period January 1st 2007-December 31st 2010. The solid squares represent the weeks with an elevation of blood samples based on the analysis without correction for the BTV-8 episode and the open squares represent the weeks with an elevation of blood samples with correction for the BTV-8 episode. The solid squares in 2008-2010 represent weeks that were marked as elevations in both regression analyses. The arrow is the first signal that would have required follow-up actions (November 19th-25th, week 47 of 2007).

Elevations of blood samples in time: Linear regression

The histogram and normal probability plot appeared normal and the skewness-kurtosis test was not significant (P=0.21), indicating that the data were normally distributed.

With the linear regression analysis, 10 weeks with elevated numbers of submitted blood samples (solid squares in Fig. 1) were detected. Eight weeks were during the period in which abortions due to bluetongue occurred (June-December 2007). The first elevation was detected in week 26 of 2007 (June 25th –July 1st). Seven signals that would have required follow-up actions (as defined by two or more elevations occurring within four weeks) were found and six signals were in the bluetongue episode. The first signal was found on November 19th 2007 (week 47), the period in which bluetongue quickly spread among Dutch cattle.

Most elevations occurred during the BTV-8 episode or shortly after. In order to determine a baseline submission, it was decided to do the regression analysis without data of 2007. In the period from 2008-2010 no infectious diseases emerged the Netherlands. Based on the second regression analysis, seven weeks with elevated blood samples submission were detected (open squares; Fig. 1). Three signals that would have required follow-up actions were found.

Elevations of submitted blood samples in time and space: retrospective cluster analysis

From January 2007 to December 2010, 11 significant space-time clusters of submitted blood samples were detected that would have required follow-up (Fig. 2). Five clusters (45%) could be related to the bluetongue episode in 2007 (June-December 2007, see bold, underlined numbers in Fig. 2) with the first signal in the weeks from June 25th to July 15th 2007. The cluster belonging to this signal was located in the northern part of the Netherlands, where no BTV-8 infection was yet confirmed.

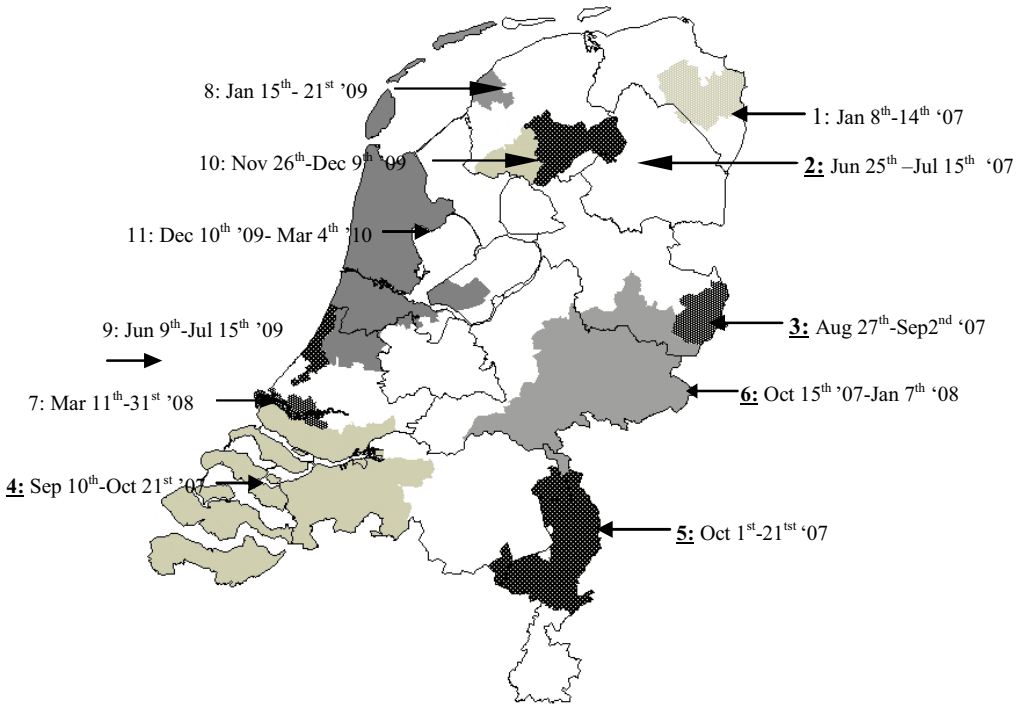

Fig. 2 Significant clusters ($P \leq 0.01$) of submitted blood samples from aborting cows for brucellosis monitoring in the period January 1st 2007-December 31st 2010 per 2-digit postal code in the Netherlands by a weekly retrospective space-time cluster analysis. The bold and underlined clusters were found in the BTV-8 episode (July 25th –December 31st 2007). Clusters are numbered in chronological order.

Sensitivity analysis showed that when the threshold level was set at 5% ($P \leq 0.05$), 17 significant space-time clusters of blood samples were detected that would have required follow-up. Six clusters (35%) could be related to the bluetongue episode in 2007 compared to five clusters when the threshold was set at 1%. This extra cluster was at the end of the bluetongue episode in the week from December 3rd -9th 2007. Raising the threshold produced more signals that were outside the bluetongue episode and produced no earlier signal compared to the 1% level threshold.

When a moving 4-weekly analysis was carried out, 12 significant space-time clusters of submitted blood samples that would have required follow- up were detected. This analysis showed four clusters (33%) that could be related to the bluetongue episode and the first signal was found from June 25th to July 15th 2007 and came from the same area as found with the weekly analysis. This analysis produced more signals that were outside the bluetongue episode and produced the same first signal compared to the weekly analysis.

These sensitivity analyses showed that a threshold of 1% ($P \leq 0.01$) and a weekly analysis fitted our data best and were chosen for prospective space-time cluster analysis.

Elevations of submitted blood samples in time and space: prospective cluster analysis

The 2010 prospective analyses found nine clusters in different areas. Eight out of nine clusters were signalled in one up to four analyses (of in total 52 week-analyses). One 2-digit postal code (no. 13 lying in the province Flevoland, see black dot in Fig. 3) was signalled in 51 of 52 weekly analyses. If syndromic surveillance on submitted blood samples would have been operational in 2010, the first signal from area number 13 should have been given on January 15th 2010 (=first day after the week this cluster was found). This cluster included three 2-digit postal codes, namely 12, 13 and 37 (dark grey areas in Fig. 3). From January 8th to January 14th 2010, 18 blood samples (from eight different herds) were submitted from this area, whereas 2.9 blood samples were expected. Further investigations showed that blood samples of seven herds were tested for neosporosis and two herds (from which 5 and 4 blood samples were submitted resp.) had positive results. In the same period of the previous year (January 8th to January 14th 2009), only one blood sample was submitted from this area, which tested negative for neosporosis (Table 3).

The result of the prospective analysis for the last week in 2010 showed that the cluster found at the start of 2010 was extended with two more 2-digit postal codes, namely 10 and 82 (light grey areas in Fig. 3). The period in which this cluster was found from September 3rd 2009 to December 31st 2010 included 606 blood samples, whereas 504 were expected. There was a tendency for a higher proportion of herds with a positive results for salmonellosis compared to the previous year (Proportion test, $P=0.10$, Table 3).

These results showed that in January 2010, more herds seemed to have neosporosis problems and in December 2010 more herds seemed to have salmonellosis problems than in the previous years (2008-2009). However, a higher proportion of herds with positive results for neosporosis or salmonellosis does not have to be related to an outbreak of those diseases. Some veterinary practices could have stimulated the submission of blood samples from aborting cows in 2010 more than in previous years and/or than in other areas.

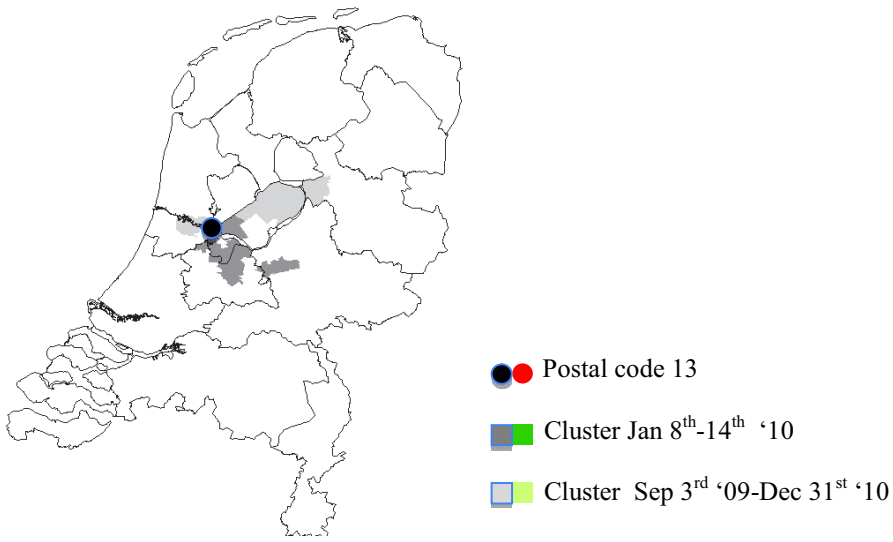

Fig. 3 Repeated significant clustering of submitted blood samples from aborting cows in the period January 1st 2010-December 31st 2010 per 2-digit postal code in the Netherlands by weekly prospective space-time cluster analyses in 2010. The dark grey coloured areas represent the first cluster found in 2010 and the light grey coloured areas represent the extension of the first cluster found in the prospective analysis for the first week of 2011.

Table 3. Description of clusters in number of blood samples from aborting cows submitted for brucellosis monitoring in 2010, compared to the same period and location in 2008/2009 in the Netherlands.

2-DIGIT POSTAL CODE	CLUSTER	PERIOD	NO. BLOOD SAMPLES	NO. HERDS	PROPORTION OF HERDS WITH POSITIVE RESULT(S)		
					salmonellosis	neosporosis	IBR
12,13,37	Yes	8-14 Jan '10	18	8	0% (n=4)	28.5% (n=7)	0% (n=4)
	No	8-14 Jan '09	1	1	0%	0%	-
10, 12,13,37,82	Yes	3 Sep '09 – 31 Dec '10	606	472	8.0% (n=188)	27.5% (n=309)	6.5% (n=247)
	No	3 Sep '08 – 31 Dec '09	456	371	3.2% (n=153)	26.2% (n=260)	5.6% (n=195)

DISCUSSION

In this study, three different statistical methods for surveillance on submitted blood samples of aborting cows were used, to evaluate whether syndromic surveillance on these data can effectively detect outbreaks of (emerging) infectious diseases in time and/or space: 1) multivariable linear regression, 2) retrospective space-time cluster analysis and 3) prospective space-time cluster analyses. We used methods that were suitable for data that cover a couple of years. The bluetongue episode in 2007 was used to test the sensitivity of the linear regression and retrospective space-time cluster analyses.

Our study showed that the linear regression analysis was sensitive to detect elevations of submitted blood samples during the bluetongue episode. Regression is especially suitable for detection of elevations in time, thus when infectious diseases cause a higher submission of blood

samples from different areas at the same time. In addition, seasonal differences can be adjusted. We used a model in which a sine-cosine function was used to adjust for weekly seasonal differences. Perrin et al. (2010) used for example a comparable function to model the mortality rate per week adjusting for seasonal effects. A major assumption to this function is that seasonal trends in submissions will be the same in the future. To increase the accuracy of the model, it is important to update the reference dataset at regular time intervals.

Regression models are not sensitive for the detection of relatively small local outbreaks. This method was not able to detect the start of the bluetongue outbreak in 2007 in cattle. Despite this disadvantage, linear regression is a simple tool for analysing syndromic data and can statistically contribute to monitor timely trends and developments. It can easily be integrated with the traditional surveillance components, needs little computation time and results are quickly produced. In addition, such methods can also be used to estimate the global burden of an epidemic. Perrin et al. (2010) and Santman-Berends et al. (2011) used a Poisson regression model to estimate the additional mortality associated to the bluetongue epidemic in France and the Netherlands respectively.

A spatial scan statistic was used to detect elevations of submissions in time and space. This method has been applied by many others to identify clusters in, for example, a variety of cancers and BSE (Pfeiffer et al., 2008). We used the scan statistic to perform both retrospective and prospective space-time cluster analyses. Space-time cluster analysis is suitable to detect an increase in the number of submitted blood samples from a certain area at a certain time even when the increase is relatively small (Lawson and Kleinman, 2005). The retrospective space-time cluster analysis is valid to investigate if the data are sensitive to detect past outbreaks. The method needs little computation time if data are aggregated on week level and results are quickly produced. The retrospective space-time analysis was able to detect the bluetongue episode more early than the linear regression. However, space-time cluster analysis leads to more false-positive signals (=55% of the signals fell outside the bluetongue episode) than simple linear regression (40% of the signals fell outside the bluetongue episode). A procedure is required to investigate these signals and should be relatively low cost.

The prospective space-time cluster analysis is timelier than the retrospective space-time cluster analysis. However, it requires historical data to build up a reliable reference (data to which new data are compared). No reliable submission data before 2007 were available for this study and therefore it was not possible to simulate a prospective surveillance for the bluetongue outbreak. Weekly prospective space-time cluster analyses for 2010 were carried out instead. An elevation of blood samples was found from the same area in 51 of 52 weekly analyses and the first signal came on January 15th 2010. This elevation was not detected by the traditional surveillance components and in-depth analysis showed more herds with positive results for neosporosis compared to the previous year. However, a higher percentage of herds does not have to be a signal for an outbreak. Some veterinary practices could have stimulated the submission of blood samples from aborting cows in 2010 more than in previous years and/or than in other areas.

For an operational syndromic surveillance on submitted blood samples, prospective analyses should be carried out for early detecting new outbreaks that cause abortion in cows. Prospective methods can run on different time levels. Daily analyses consume a lot of computation time and the results will be available after a couple of days. Prospective methods on submitted blood samples can run very quickly when data are aggregated on week level and are therefore recommended. These methods can be deployed as an additional tool for early detection of recent

emerging and endemic disease outbreaks. In Great Britain, this method was for example successfully used on laboratory submission data to detect potential outbreaks (Hyder et al., 2011). However, this method will produce false alarms. This emphasises the need for a strict protocol defining follow-up actions. In the Netherlands, the integral cattle health surveillance system was set up in 2003 when surveillance information from proactive and reactive components were aggregated and interpreted by cattle health specialists, statisticians and epidemiologists (Van Wuijckhuise et al., 2011; Van Schaik et al., 2011). These representatives meet weekly to discuss the cases that were received. The focus of these meetings is to separate sense and non-sense related to known exotic and new or emerging disease situations. Syndromic surveillance can be integrated within this system quite easily and signals can be put side-by-side along with the other surveillance components and interpreted by specialists. In addition, veterinary practitioners involved, can be contacted to learn about possible reasons for submitting extra samples thereby excluding false-positive signals and reducing follow-up costs.

In the Netherlands, farmers are obliged to submit blood samples of aborting cows between 100-260 days of gestation to the AHS for brucellosis monitoring. All submissions are registered in one database and are available for monitoring and surveillance purposes. However, compliance is about 25%. Nevertheless, syndromic surveillance does not need data representing all cases in the population. Syndromic data has to be sensitive to changes in the level of disease in a population at the start of an outbreak (Yahav and Shmueli, 2007, Dórea, 2011). No correlation was found between bluetongue and abortion in 2007 in the Netherlands (Santman-Berends et al., 2010). However, Elbers et al. (2009) did find an increased abortion rate in the Netherlands due to bluetongue in 2007. Farmers will be triggered to submit blood samples of aborting cows when more consecutive abortions occur in a relatively short time period. Our study showed that elevations of submitted blood samples were detected at the start of the bluetongue outbreak using space-time cluster analysis.

In our study, the 2007 bluetongue episode influenced the results of the linear regression and the prospective space-time cluster analyses (data not shown). The 2007 data were therefore excluded from the analyses. To determine a baseline for submission, it is important to define what the periods that reflect the normal situation are (Lawson and Kleinman, 2005). Including epidemics in the data results in a higher baseline and decrease of sensitivity to detect new epidemics.

It can be concluded that data of blood samples of aborting cows are suitable for syndromic surveillance. All statistical methods used in this study were sensitive for the detection of (emerging) infectious diseases that cause increased number of abortions similar to bluetongue. Linear regression analysis was not suitable for early detection of infectious diseases but can easily be used to monitor timely trends and developments. In addition, regression models can be used to estimate the global burden of an epidemic. Retrospective space-time cluster analysis was more suitable for early detection of local outbreaks of infectious diseases than linear regression. When syndromic surveillance on blood samples will be operational, prospective space-time cluster analyses at regular time intervals are recommended. This method is timelier than the retrospective method and can statistically contribute to the early detection of local outbreaks of emerging infectious diseases or endemic diseases that cause abortions. It can serve as an additional tool for the traditional surveillance components. As this method will produce false alarms a strict protocol defining follow-up actions is needed.

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CONCEPTUAL EVALUATION OF VETERINARY AND PUBLIC HEALTH SURVEILLANCE PROGRAMS: METHOD AND EXAMPLE

F. EL ALLAKI*, M. BIGRAS-POULIN AND A. RAVEL

SUMMARY

We propose a "conceptual" method for the evaluation of veterinary and public health surveillance programmes that is detailed, structured, unbiased, transparent, and based on concepts common to all surveillance systems. This method extracts, based on the programme's documentation, all concepts included in the programme, analyses the logical and chronological relationships between these concepts, and generates the programme's conceptual model. Finally, it compares this conceptual model to a theoretical conceptual model (i.e. theoretical standard). It generates data for assessing both the completeness and coherence of the programme's surveillance concepts.

This paper details the conceptual evaluation method's rationale and process, provides an example of its application, and discusses its advantages and drawbacks in comparison to the other surveillance evaluation methods. This method responds particularly well to the requirements of the Sanitary and Phytosanitary Agreement and the International Health Regulations as it allows for a standardised and less subjective evaluation of a surveillance programme, even prior to its implementation.

INTRODUCTION

Evaluation consists of ascertaining or assessing the worth or merit of something. This involves assigning a value to the thing or object being evaluated, which is done by comparing what should be (e.g., standards, criteria) with what is (Royse, 2010).

As is the case for all surveillance programmes, the evaluation of a population health surveillance programme (PHSP) is carried out in order to make judgments about and/or improve the programme's effectiveness (Klaucke 1992; Chen, 2005). Evaluation aims to determine the value of the PHSP and to make recommendations for improving it. The evaluation consists in comparing the programme's characteristics to requirements, and it may be carried out at different points in the health surveillance process (Health Canada, 2004). Evaluation is an important tool for demonstrating the credibility of a PHSP to stakeholders (e.g., the public, policymakers, the animal industry, trading partners and funding agencies) (Drewe et al., 2011).

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A number of detailed health surveillance evaluation tools for application to public health and veterinary surveillance programmes have been developed by national and international organisations, including the US Centers for Disease and Prevention (CDC) protocol, World Health Organisation (WHO) guidelines, and the Health Canada framework (German et al., 2001; WHO, 1997; Health Canada, 2004). Almost all commonly used health surveillance evaluation tools focus primarily on assessing the performance of the surveillance programme based on a description of the programme, and on a qualitative or quantitative estimation of a varying number (unstandardised) of programme attributes (Stärk, 2003; Drewe et al., 2011). At present, in the field of theory-driven evaluation science, there is considerable interest in assessing the validity of the conceptual framework or theory underlying a programme. This is because recent research shows, for example, that the experiences and perceptions of programme designers (or owners) affect programme design (Brousselle and Champagne, 2011).

All existing public health and veterinary surveillance programmes are based on a set of concepts–ideas about how to carry out the programme, how the programme will work, and what the programme will achieve. Often, these concepts and assumptions are not made explicit. In order to demonstrate that surveillance has a solid conceptual foundation, it is necessary to elicit, formulate and assess these concepts and then compare them to a theoretical standard. Such an analysis constitutes what we refer to as conceptual evaluation.

The objective of this study is to propose a conceptual method for the evaluation of veterinary and public health surveillance programmes that is detailed, structured, unbiased, transparent, and based on universal concepts that should be part of surveillance system of any kind.

MATERIALS AND METHODS

Conceptual evaluation of surveillance programmes

Conceptual evaluation refers to the systematic process of extracting concepts from a text and analysing the relationships between these concepts. The goal is to judge the theoretical framework of the surveillance programme by comparing this framework (i.e. programme's conceptual model) to a theoretical standard (i.e. theoretical conceptual model). In this definition, the programme's "conceptual model" is a schematic conceptual representation of the programme that delineates its concepts and the interconnections between these concepts. By contrast, the "theoretical standard" is a valid schematic representation of concepts and their interconnections, and it represents the conceptual reference for all programmes.

First contact with the evaluated programme's technical team.

The purpose of this first contact with a technical team member is to: (i) explain the objectives of the analysis and its utility, (ii) request access to a document describing in detail the surveillance programme, and (iii) explain the need to re-conduct a second interview session to validate the results and distinguish between missing concepts and concepts that are not documented but present.

Steps in the conceptual evaluation

The proposed conceptual evaluation method consists of four steps:

1 Text analysis: Extracting the Elementary Context Units (ECUs)

2 Creation of the programme's conceptual model

3 Comparison of the programme's conceptual model to the theoretical model

4 Validating interview with a technical team member

1. Text analysis

The main objective of this step is to extract the most significant phrases from the analysed text, or ECUs (Image, 2006). This is done using the software Alceste, which performs a lexical context analysis based on a set of text segments (Image, 2006). The surveillance programme document being analysed has to: (1) be in electronic format, (2) describe all the aspects of the surveillance programme in at least 5 pages, (3) be coherent and homogeneous (focus on surveillance), (4) be written by the programme's designer, and (5) be written in one of the languages that Alceste can analyse.

The extraction of these phrases is conducted using Alceste (Analyse Lexicale par Contexte d'un Ensemble de Segments de Texte) (Image, 2006). Alceste relies on co-occurrence analysis, which is the statistical analysis of frequent word pairs in a text. Alceste can be used to analyse the content of interviews, open-ended survey questions and a diverse range of articles, including those of a scientific nature. It quantifies the text in order to automatically extract its most significant structures (e.g., phrases). Since these significant structures in the text are extracted by Alceste and not by the user or researcher, the introduction of biases in coding and analysis by the user is kept to a minimum. Research has shown that these structures are closely linked to the distribution of words in a text, and that this distribution is rarely random. Alceste segments the analysed text into small context units (CUs) composed of ECUs, and then clusters together context units with similar lexical content into classes (Deaux and Philogène, 2001). Both key words and phrases are ranked in terms of their statistical significance, and both can be traced back to the original text to evaluate their context (Schonhardt-Bailey, 2006). Alceste is very effective at characterising, assimilating, classifying and synthesising texts in an automatic and objective fashion.

Text analysis is carried out in four sub-steps (Image, 2006):

1.1 Preparing the text.

The text describing the surveillance programme is saved as an ASCII file with line breaks. Alceste changes all capital letters at the beginning of the word into lower case, except for words that are completely in upper case (they remain as is and are not analysed). The user removes accents (é), dollar signs ($), hyphens (-) and asterisks (*) from the text. Apostrophes are removed if they are followed by a space. Apostrophes followed by a letter are replaced by underscores (_), and then any underscores followed by an "s" are removed if the "_s" is followed by a space (e.g. "Who's there?" becomes "Who there?") (Image, 2006).

Alceste uses a dictionary to recognise compound words and phrases like "decision-making". If the software does not recognise a compound word, it puts an underscore (_) between the two words (e.g., decision_making) if the user wishes to keep the compound word in the text (Image, 2006).

The text has to be broken down into smaller segments, by the user, in order to detect two different types of context units: (1) Initial Context Units (ICUs), which are pre-existing divisions (e.g., sections or chapters) in the document; and (2) Elementary Context Units (ECUs), which are phrases constructed by the programme and "calibrated" as a function of length and punctuation (Image, 2006).

The ICUs has to be coded using asterisked lines. An ICU represents a pre-existing division of the text (e.g. chapter, section) and has to be specified by the user. The first asterisked line is the first line of the text. All text contained between two asterisked lines constitutes an ICU. An asterisked line is preceded by 4 asterisks (****). It cannot contain more than 240 characters and has to contain as least one asterisked word. Asterisked words contain a maximum of 18 characters, starting with an asterisk (e.g., *****Surveillance_1), and are part of an asterisked line (e.g. **** *Surveillance_1 *Chapter_1). An asterisked word is preceded by at least one space and cannot contain any blanks, punctuation, separators or hyphens (only an underscore was allowed). Finally, note that each ICU is composed of a well-defined number of ECUs (Image, 2006).

1.2 Text segmentation and lemmatisation

Alceste uses the punctuation marks to segment each ICU into phrases or ECUs (Image, 2006). An ECU is a word sequence that integrates a fixed number of "plain-words", as specified by the user in the analysis plan. The programme automatically decides on the appropriate length for each ECU and segments the corpus accordingly. The segmentation is performed based on the punctuation and a priority order for the punctuation signs (. > ? > ! > ; >: > , > space) (Image, 2006).

Words marked with an asterisk are considered to be outside the text and are therefore excluded from the analysis. Alceste recognises all textual forms using its dictionaries: ALC_ MO ("tool-words"), ALC_FO ("full-words"–nouns, adjectives and adverbs), ALC_VR (regular verbs), ALC_VI (irregular verbs) and ALC_FVI (irregular verb endings). It then processes the text and establishes a dictionary of reduced forms of the analysed corpus using the roots of words, whatever their syntactical category. For example, act+ion combines both the adjective active and the nouns action, activity and activities. The main objective of this sub-step is to reduce words to their roots (Image, 2006).

These reduced words are then divided into two subgroups: "analysable" forms (e.g., nouns, verbs and adjectives) and "supplementary" forms (e.g., prepositions, pronouns and conjunctions). The analysable forms are important to the context and are therefore used for classification. Both forms are used for the interpretation of classes (Image, 2006).

1.3 Drawing up the contingency table (reduced forms versus units of context) and descending hierarchical classification

Alceste creates a contingency table, with analysable forms as columns and ECUs as rows. It puts '1' in the contingency cell if the reduced form exists in a given ECU, regardless of the number of times it occurs in that ECU. Thus, we end up with a contingency table of 0s and 1s (Image, 2006).

As ECU length can affect the results, Alceste carries out a double classification by constructing units of context (UCs) of two different lengths. To construct an UC, Alceste concatenates ECUs belonging to each ICU until it has k different analysed forms. In the standard

analysis plan, the parameter k is fixed by the software. Descending hierarchical classification is then applied to the two contingency tables (reduced forms versus units of context) in order to identify classes by finding dichotomies that maximise the chi-square distance. The iterative algorithm continues on each subset of each table until further classes are not significantly different (Image, 2006).

1.4 Describing and interpreting stable classes

Alceste compares both classifications and retains ECUs common to both analyses, which then will constitute the stable classes. It determines the number of stable classes and lists the ECUs that are the most representative of their class. Each ECU is preceded by a number indicating its order in the text and by a chi-square expressing its association to the class. The list of words that is characteristic of each stable class is created. Chi-square analysis is used to determine how strongly a given word is associated with a class, thereby revealing the terms most representative of any given class. Other methods can be used to supplement this analysis of classes, including cross-sorting, factorial correspondence analysis and ascending hierarchical classification (Image, 2006).

2. Creation of the programme's conceptual model

The programme's conceptual model is constructed through the coding of ECUs. The ECU coding plan has to take into account the theory nomenclature in order to facilitate the comparison of models later on. Concretely, the concepts contained in the extracted ECUs are translated into the theory language in order to standardise the concept nomenclature and permit a comparison of the models. The coding plan should encompass all surveillance concepts contained in the theoretical model and also define these concepts. The coding focuses on explicit concepts contained in the ECUs; the researcher should not interpret the hidden content of ECUs. The ECU coding plan also describes the rules for connecting surveillance concepts through logical and chronological relationships.

The conceptual and relational analyses described by Kathleen Carley (1993) can be used to extract the concepts from the ECUs and link them together, thereby constructing the programme's conceptual model.

3. Comparison of the programme's conceptual model to the theoretical model

The programme's conceptual model is compared to the theoretical model in order to determine the completeness of concepts and the relationships among these concepts (coherence of concept relationships) in the surveillance programme. Qualitative data analysis software that allows for the super-positioning of the two models and facilitates their comparison can be used at this stage.

4. Validating interview with a technical team member

The objective of this interview is to determine whether the surveillance programme technical team has taken into consideration the concepts found to be absent from the analysed text. The technical team member is asked to distinguish between missing concepts–concepts that are not present in the model, and non-documented but present concepts–concepts that are, in fact, present in the model even though they are not documented.

Health surveillance theoretical standard

Conceptual evaluation of surveillance programmes aims to compare the surveillance programme's conceptual model to a surveillance theoretical standard. We used the population health surveillance theory (PHST) as a theoretical standard for veterinary and public health surveillance programmes. For the PHST, a surveillance process is composed of the following five steps: (1) Surveillance justification, (2) Problem formulation, (3) Surveillance planning, (4) Surveillance implementation, and (5) Knowledge transformation (information communication and audit) (El Allaki, 2006).

1. Surveillance justification

According to the PHST, the initiation of any surveillance process requires the coexistence of three essential elements: 1) a dissatisfaction; 2) an information need; and 3) a desire to eliminate the dissatisfaction and meet the information need (El Allaki, 2006). Surveillance will continue for as long as these elements persist or new elements are added.

Dissatisfaction is the feeling of psychological discomfort that emerges when a person compares his/her perception of two situations—one representing reality and the other what reality should be. Dissatisfaction, then, is part of the affective dimension of a problem and constitutes the starting point for any surveillance initiative. Information need is ongoing and may be considered as a source of dissatisfaction. The desire to act is a dynamic state that directs action toward a concrete goal. It refers to the ability to initiate action and mobilise resources to develop a surveillance plan and reach its objectives. Four motivational criteria can be used when selecting a problem for surveillance purposes (El Allaki, 2006): (i) need for knowledge about a disease and/or exposure, (ii) epidemiological issues, (iii) economic issues, and (iv) legislative issues. Dissatisfaction, need for information, and a desire to act represent the triggering factors for initiating any surveillance process.

2. Problem formulation:

Formulating the problem is the first step in the knowledge acquisition process. John Dewey (1938) realised the importance of this when he wrote: "It is familiar and significant saying that a problem well put is half-solved" (Dewey, 1938; page 108). Problem formulation is challenging by nature. The degree of difficulty depends on the nature of the problem and the knowledge and experience of those formulating the problem.

Formulating a problem requires describing precisely the dissatisfaction and then describing and defining the real problem. It means precisely identifying the problem and specifying its characteristics. This often requires close cooperation and consensus building among various stakeholders working in different disciplines in order to identify the real problem and reduce divergence in opinion. Eden and Sims (1979) emphasise the "negotiation" among stakeholders that occurs when defining a problem. In this case, consensus decision-making aims for unanimity or substantial agreement; it is a process that maximises the chances of identifying the real problem and then helps in better formulating it. Such consensus building is the responsibility of all participants.

In the context of health surveillance, the following elements have to be included during problem formulation: health problem under surveillance, population affected, area concerned, date when cases first reported, and surveillance objectives. The problem formulation process must be clear, concise and based on consensus (El Allaki, 2006).

3. Surveillance planning

Surveillance planning is a formal methodology for the rational use of material, financial and human resources in producing valid information. Surveillance planning is defined as an ongoing process that provides a framework for making decisions concerning the expected results and the strategies available for solving the surveillance problem. The final step in surveillance planning is drawing up a collective (versus individual) action plan that identifies activities, task division and resource allocation, thereby ensuring that these activities are structured via organised processes of individual and/or collective actions and appreciable results are produced. The success of this step obviously depends on the objectives defined in the problem formulation step. The planning strategy must be consistent with these previously defined objectives. Surveillance is the process of collecting and analysing data in order to generate information and transmit it to those who need it. Based on this definition, it is necessary to consider the following elements during surveillance planning: (i) Data collection and integration plan, (iii) Data analysis plan, and (iii) Communication plan (El Allaki, 2006).

After identifying the main surveillance activities and establishing a plan for each of these activities, it is necessary to link all these activities together via an administrative and organisational plan. Since this plan coordinates all activities related to data collection, integration and analysis, plus information communication, it is finalised after the communication plan has been decided. Health surveillance is a collective activity that requires human, financial and material resources. Resource planning should thus include the following elements: (i) Personnel allocation and training plan, (ii) Financial plan, (iii) Material and work tool needs plan. Elaborating an evaluation plan is the final step in planning (El Allaki, 2006).

4. Surveillance implementation:

Implementing the surveillance programme is the final step in the knowledge acquisition process. The objective of this step is to put the surveillance plan into action (El Allaki, 2006).

5. Knowledge transformation:

Knowledge transformation is the dynamic process of converting knowledge into information. Knowledge in the form of numbers (results of the data analysis) must be expressed in an understandable form, i.e. language. This step also includes information dissemination to the various stakeholders involved and the evaluation of the surveillance programme (El Allaki, 2006).

For the PHST, surveillance is a structured process characterised by interdependence between steps and the chronological execution of steps, i.e. a dynamic and ongoing process. The theory identifies and defines the essential components of surveillance (surveillance concepts) for each step in the process and determines the rules connecting these components. The PHST is formalised in the form of a theoretical conceptual model (i.e. theoretical standard) composed of 185 concepts (El Allaki, 2006).

Example of an application of the conceptual evaluation method

We applied this conceptual evaluation method to a new zoonotic disease surveillance programme that involves both laboratory-based surveillance of enteric diseases in humans and active surveillance of pathogens in food, water, and livestock. This programme is the National Integrated Enteric Pathogen Surveillance Programme, or C-EnterNet, a multi-partner sentinel site surveillance programme facilitated by the Public Health Agency of Canada (PHAC, 2008). C-EnterNet is designed to support activities that lead to a better understanding of the burden of

enteric disease. It consists of comprehensive sentinel site surveillance implemented through local public health units. The specific objectives of C-EnterNet are: 1) detect changes in trends for human enteric disease and in levels of pathogen exposure from food, animal and water sources in a defined population, 2) generate human illness attribution values (proportion of human cases due to exposure via water, food and animals), and 3) improve the analysis, interpretation and reporting of laboratory and epidemiological data for public health, water and agri-food purposes. Additional information on C-EnterNet can be found at: http://www.phac-aspc.gc.ca/c-enternet/index-eng.php

As we carried out our evaluation prior to the programme's implementation, we could only evaluate the first three steps of the PHST. Therefore, the number of surveillance concepts to code for in the analysed text was established a priori at 152.

The first author contacted a C-EnterNet technical team to explain the project and to request a document describing the C-EnterNet programme for use in our conceptual evaluation. We thus obtained the document entitled "A Business Plan for Launching the First C-EnterNet Sentinel Site" (version dated September 24, 2004). We carried out the four steps of the conceptual evaluation method described above. We used Alceste (version 4.7 for Windows, Société Image, Toulouse, France) for the text analysis and ECU extraction. The conceptual and relational analyses, as well as the comparison of programme and theoretical models, were conducted using NVivo (version 2.0, QSR International Pty Ltd, Doncaster, Victoria, Australia).

We followed the eight coding steps developed by Kathleen Carley (1993) for conducting the conceptual analysis. We used a modified approach to Carley's relational analysis. Our step-by-step relational analysis procedure involved the following: 1) establishing the list of concepts to be included in the analysis based on PHST, 2) determining the types of relationships between concepts (both logical and chronological relationships), 3) Coding only the explicit concept relationships, and 4) using the "Model Explorer" feature in NVivo to draw the C-EnterNet surveillance model and facilitate comparisons with the theoretical conceptual model.

We conducted a second interview with the C-EnterNet technical member to present and discuss the results (this interview had the same objectives as those outlined in step 4 of the conceptual evaluation method).

RESULTS

Our conceptual evaluation shows that C-EnterNet contained 61 documented surveillance concepts with a number of valid concept relationships. The interview revealed the existence of an additional 86 concepts that were not documented but present, and confirmed that five surveillance concepts were absent in C-EnterNet's design. These missing concepts were: 1) evaluation planning; 2) external evaluation; 3) self-evaluation; 4) identification of who is to analyse the data at each level in the programme; and 5) clarification of the programme surveillance objectives. We also identified 23 concepts that were not applicable to the design of C-EnterNet. Table 1 summarises our results.

Table 1. Summary of C-EnterNet evaluation results

	SURVEILLANCE JUSTIFICATION (%)	PROBLEM FORMULATION (%)	SURVEILLANCE PLANNING (%)	TOTALNO. OF CONCEPTS (%)
Documented concepts	4 (50)	14 (26)	43 (47)	61 (40)
Concepts not documented but present	4 (50)	38 (72)	44 (48)	86 (57)
Concepts not applicable to C-Enternet	0	6	17	23
Missing concepts	0 (0)	1 (2)	4 (5)	5 (3)
Total number concepts present in the theory	8	59	108	175

The number of concepts included in the first three steps of the surveillance theory was 175 (10 other concepts defined steps 4 and 5 of the surveillance theory; see Table 1). The total number of concepts that should have been represented in C-EnterNet was 152 (175-23 = 152). Consequently, the level of agreement between C-EnterNet and surveillance theory is 97% (147/152 x 100) based on its documented and non-documented concepts.

DISCUSSION

The conceptual evaluation method enables evaluators to extract the conceptual model underlying a health surveillance programme and to determine what concepts and relationships between concepts are missing in the programme. It also identifies non-documented but present concepts, allowing the programme designer to easily identify what needs to be described and clarified in the text, thus improving the quality of the programme's documentation.

The proposed conceptual evaluation method uses a valid and universal theoretical standard in order to carry out a comprehensive conceptual evaluation of a surveillance programme. The use of a theoretical standard can help improving the surveillance design of both veterinary and human public health surveillance programmes. The improvements suggested by the analysis can be incorporated either before or during the implementation phase of the surveillance programme. In short, the conceptual evaluation method allows for both ex-ante and ex-post evaluation.

The conceptual evaluation method minimises the subjectivity of assessments associated with the human reading and interpretation of programme documentation. By relying on the automated extraction of significant phrases or ECUs on which the coding process is based, the conceptual evaluation method minimises human involvement in the analysis.

Many surveillance evaluation tools, such as the US CDC protocol, WHO guidelines, Health Canada framework, OASIS tool and other methods like scoring systems, fault trees and scenario analyses have been proposed for assessing veterinary and public health surveillance programmes

(German et al., 2001; WHO, 1997; Health Canada, 2004; Dufour, 1999; Stärk, 2003; Hendrikx et al., 2011). These evaluation tools are not conceptual evaluation methods as defined in this paper. Almost all existing methods focus on the performance of health surveillance programmes and assess this by measuring a specific set of programme attributes (Stärk, 2003). These evaluation tools place considerable emphasis on the technical aspects of surveillance. Such a focus may penalise surveillance programmes that have a limited budget and low technical infrastructure, yet a valid and coherent theoretical framework.

Our conceptual evaluation method complements other more commonly used evaluation tools. As noted above, many surveillance evaluation tools require a full description of the surveillance programme as one of the first steps in the evaluation process. Another requirement is full implementation of the surveillance programme before the evaluation process. The conceptual evaluation method complements and facilitates these other evaluation tools by improving the quality of programme documentation. As we have demonstrated for the case of C-EnterNet, an ex-ante evaluation using the conceptual evaluation method can lead to better designed programmes, which would have a positive effect on programme performance during the implementation phase.

The conceptual method as proposed above has some limitations. While technically it is possible to apply this method to a number of individual documents describing a given surveillance programme, it would be more difficult for the external evaluator to connect the concepts together (it would be easier for the programme designers). Furthermore, Alceste can only analyse text written in a limited number of languages. This means that the text must be translated into a language supported by the software, and that the users must have familiarity with this language. While we used PHST as the theoretical standard, the proposed method could easily integrate the use of other existing theories or theories that might be developed in the future.

Certainly the ideal would be a comprehensive surveillance evaluation framework for both veterinary and public health surveillance programmes. Such a framework would enable the rational selection of an appropriate strategy for evaluating a surveillance programme based on the surveillance programme's objectives and the evaluation's objectives. In order to develop such a framework, we need to understand: 1) the requirements and strengths/weaknesses of existing evaluation tools, and 2) the degree of complementarity between individual evaluation tools.

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IMPROVING THE EFFICIENCY OF THE SURVEILLANCE SYSTEM FOR HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS SUBTYPE H5N1 IN THAILAND USING SCENARIO TREE MODELLING

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SUMMARY

A surveillance system for highly pathogenic avian influenza virus (HPAIV) H5N1 has been established in Thailand since early 2004 for early infection detection and to enable a prompt response, which comprises passive and active surveillance system components (SSC). Due to scarce resources, this surveillance system needs to be evaluated for its resource efficiency. Scenario tree modelling used in this study aimed to assess the surveillance system and to provide information for decision making for improved resource allocation. A scenario tree model was developed for each SSC and run with 10,000 iterations to estimate the probability of detection of at least one infected farm at different assumed HPAIV H5N1 farm prevalences (0.05%, 0.1% and 0.5%).

The model showed that the active and passive SSCs of the surveillance system for HPAIV H5N1 in Thai poultry have a high and medium level sensitivity, respectively. The sensitivity of passive SSC can improve by increasing level of willingness to report a disease. A reduction in sampling number of active SSCs is a possible option of improved resource allocation.

INTRODUCTION

Highly pathogenic avian influenza virus (HPAIV) subtype H5N1 was first reported in Thailand in early 2004, and a range of targeted measures were taken to control the disease, including stamping out of infected and in-contact flocks and movement restriction (Tiensin et al., 2005). Although there has been no reported avian influenza (AI) case since 2008, the Department of Livestock Development (DLD), the responsible veterinary authority in Thailand, has to be prepared for possible recurrence of AI because there are still reported HPAI H5N1 cases in neighbouring countries (Amonsin et al., 2010; Keawcharoen et al., 2011). Thus, the on-going surveillance system for AI needs to be able to detect the virus at an early stage to allow early implementation of control measures in case of outbreaks and minimise their economic and social impact (Tiensin et al., 2007).

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The surveillance system for HPAIV H5N1 in Thailand consists of six main surveillance system components (SSC), namely 1) outbreak reporting by the public (passive surveillance) supported by outbreak investigation, 2) the 'X-ray' programme (intensive active surveillance; a nationwide census combining clinical and laboratory diagnostic investigation targeted at backyard chickens and free-grazing ducks), 3) pre-movement testing of poultry and fighting cocks, 4) pre-slaughter testing of poultry, 5) 'Health monitoring' (laboratory surveillance every 2 months for breeder and layer farms, and every 3 months for fighting cocks), 6) and compartmentalisation of selected commercial poultry farms (Buranathai et al., 2007; Tiensin et al., 2007). Table 1 summarises the number and frequency of sampling in each active SSC. More than 800,000 pooled cloacal swab samples from the active SSCs are processed for AI diagnosis annually. Due to limited resource availability, the current surveillance system for HPAIV H5N1 needs to be evaluated in terms of its performance and efficiency.

Table 1. The number of samples and sampling frequency for each active SSC: PM (Pre-movement test), PS (Pre-slaughter test), HM ('Health Monitoring Surveillance'), XR ('X-ray surveillance') and CM (Compartmentalisation scheme)

SSC	SPECIES	SAMPLES	FREQUENCY
PM/PS	Chicken, ducks, quails, geese	12 samples (60 birds)/farm	*7-10 days prior to movement*
HM	Breeder farm, egg producing farm, layer hens, ducks and quails	12 samples (60 birds)/farm	*Every 2 months*
	Registered fighting cocks	Individual birds	*Every 3 months*
XR	Backyard chickens	4 samples/village	*At least twice a year*
	Non housing free-grazing ducks	12 samples/ flock	
CM	Broiler/ducks in compartmentalised farms	**(certify the disease-free status)** 15 birds/premises, all premises	*every production cycle*
		(maintain the disease-free status) 20 birds/premise, 5 premises/farm	*every other production cycle*
	Backyard poultry in buffer zones	20 birds/households/farms 5 households or farms in a buffer zone	

The methodology of stochastic scenario tree modelling has been developed to provide evidence for the disease-free status of countries based on random and non-randomly collected surveillance data (Martin et al., 2007a; Martin et al., 2007b). It has been used to evaluate the sensitivity of various animal disease surveillance systems (Frössling et al., 2009; Hadorn et al., 2009; Hadorn and Stärk, 2008; Hadorn et al., 2008; Hernández-Jover et al., 2011; Martin, 2008; Wahlstrom et al., 2011) including AI (Alba et al., 2010; Christensen et al., 2011; Goutard et al., 2011; Knight-Jones et al., 2010). The current study aimed to assess the efficiency and effectiveness of the surveillance system for HPAIV H5N1 in Thailand using stochastic scenario tree modelling. It quantified the detection sensitivity of the surveillance system and identified the strengths and weaknesses of the current system thereby allowing the development of recommendations to improve resource efficiency.

MATERIAL AND METHODS

Reference population

Poultry considered in this study includes backyard chicken, commercial chicken, ducks, geese and quails. Based on the poultry population census in 2009, there were a total of more than 3 million poultry holdings producing more than 300 million birds, mainly as commercial broilers (See Table 2). In this study, broilers, breeders and layer hens were categorised into one single type, commercial chickens because the Thai livestock census does not differentiate between poultry species or farming types.

Table 2. Number of holdings and poultry population in each farm production type in Thailand (the 2009 DLD poultry population census)

POULTRY	NUMBER OF BIRDS (%) (N = 316,488,629)	NUMBER OF HOLDINGS (%) (N = 3,265,355)
Backyard chickens	19.5	84.5
Commercial chickens	69.5	1.7
Ducks	8.7	13.3
Geese	0.04	0.3
Quails	2.2	0
Total	100	100

Scenario tree model

A scenario tree model illustrates the possible events taking part in each SSC from occurrence of infection to detection of the case (Martin et al., 2007b). It can consider random and non-random data and take risk differences in disease infection and proportions of animal populations into account (Martin et al., 2007b). All steps in a SSC are associated with probabilities or proportions used for calculating the sensitivity of the SSC. Figures 1 and 2 outline the steps of events taking part in passive and active SSCs, respectively. The proportions of poultry considered in this study are shown in Table 2.

To perform scenario tree modelling, it is essential to specify the design prevalence of the disease of interest at farm (P*H) and within-farm level (P*U). Table 3 shows the input parameters used in the current scenario tree modelling for HPAIV H5N1.

Given that poultry farms are infected at the assumed design prevalences of HPAIV H5N1, there are differences in risk of HPAIV H5N1 infection in each poultry farm type because of differences in host susceptibility to infection, or type of poultry production system. The relative risk (RR) of HPAIV H5N1 infection for each poultry farm type was represented using beta distributions of input parameters shown in Table 3. Numbers of reported HPAI H5N1 outbreaks in 2004 and number of flocks in each corresponding category were used to estimate risk of HPAIV H5N1 infection for each bird category, which was then divided by the risk of infection in the reference category, the risk of infection in backyard chickens. The RR for different categories was weighted according to the proportion of the poultry population in that category using Eq. (1). These values were adjusted to maintain their relativity and ensure that the average risk for the poultry population stratum is one (Martin et al., 2007b).

Adjusted Risk$_C$ (AR$_C$) = RR$_C$/$\sum$ (RR$_C$ x PrP$_C$) $\text{AdjustedRisk}_c = \frac{RR_C}{\sum_{C=1}^{C}(RR_C \times PrP_C)}$ (1)

Where C represents a particular poultry farm type and PrP is the proportion of the poultry population in poultry type category.

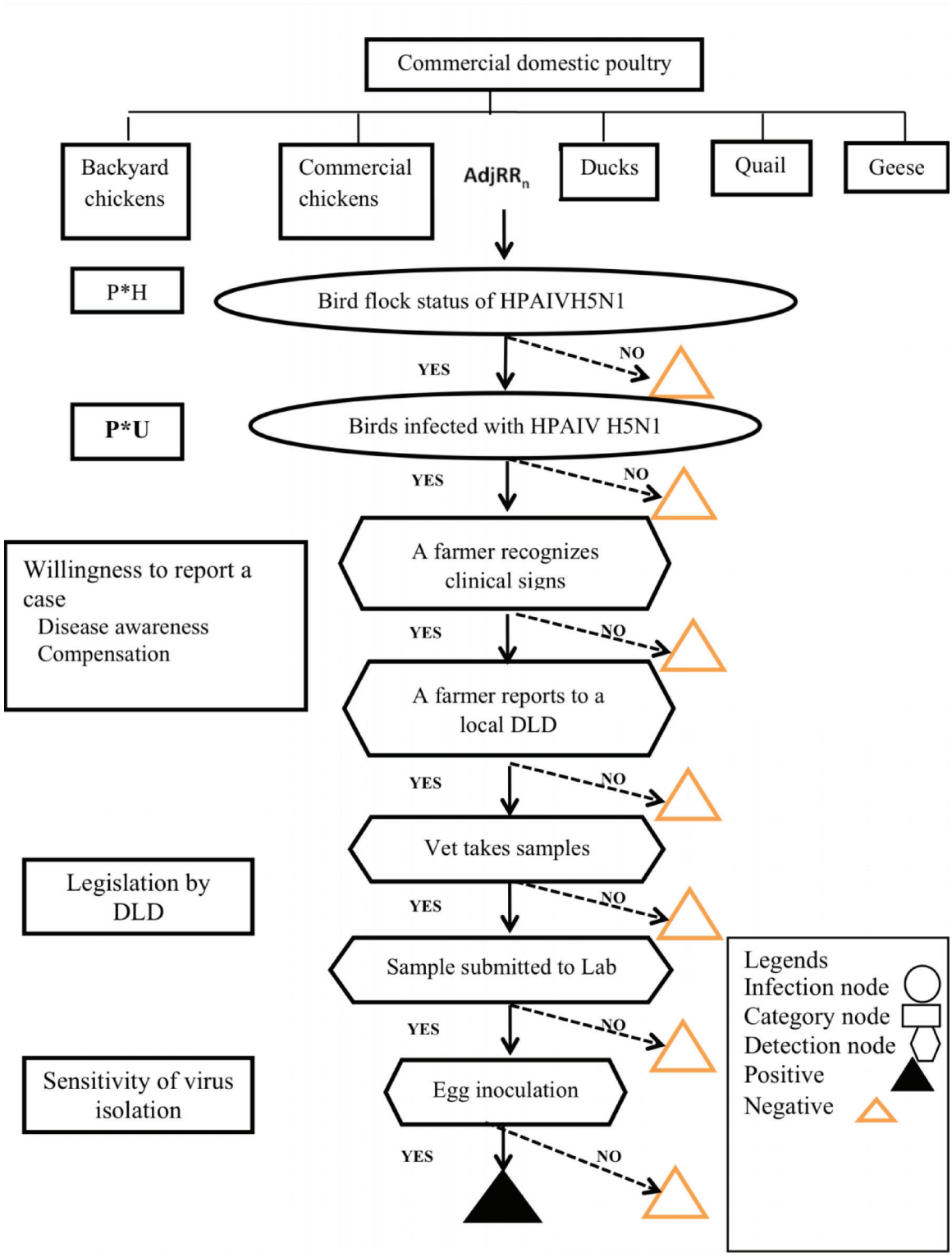

Fig. 4 Scenario tree for passive surveillance of highly pathogenic avian influenza virus (HPAIV) subtype H5N1 in Thai poultry

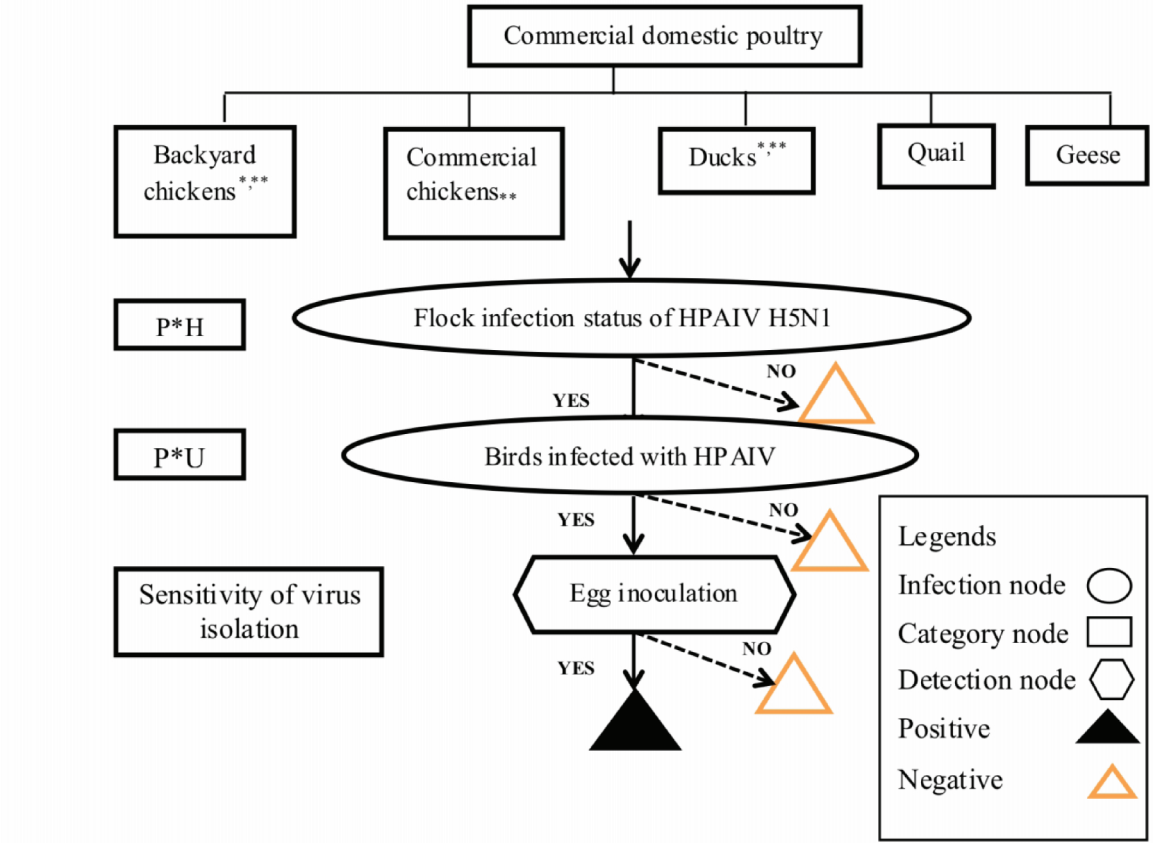

Fig. 5 Example of scenario tree for active surveillance of highly pathogenic avian influenza virus (HPAIV) subtype H5N1 in Thailand, * for X-ray surveillance (backyard chickens and ducks) and ** for the compartmentalisation scheme (backyard chickens, commercial chicken and ducks).

Table 3. The scenario tree model input parameters for the evaluation of the Thai HPAIV H5N1 surveillance system

PARAMETERS	VALUE	SOURCES
Design prevalence		(Alba et al., 2010; Christensen et al., 2011; Knight-Jones et al., 2010)
- Herd level (P*H)	0.5%, 0.1%, 0.05%	
- Within herd (P*U)	30%	
Levels of willingness to report	Pert distribution	Hadorn et al (2009)
	- Low (0.1,0.2,0.3)	Expert opinion
	- Medium (0.4, 0.5, 0.6)	
	- High (0.7,0.8.0.9)	
Sensitivity of egg inoculation	Pert distribution (0.95,0.99,0.995)	Expert opinion
Relative risk	Beta distribution(s+1, n-s+1)	
Relative $Risk_{Backyard}$	$\frac{\text{Beta Backyard (1006, 2,135,646)}}{\text{Beta Backyard (1006, 2,135,646)}}$	Tiensin et al. (2005) DLD records
Relative $Risk_{Commercial\ chickens}$	$\frac{\text{BetaCommercial chicken (200, 82,055)}}{\text{Beta Backyard (1006, 2,135,646)}}$	Tiensin et al. (2005) DLD records
Relative $Risk_{Ducks}$	$\frac{\text{Beta Ducks (479, 683,082,667)}}{\text{Beta Backyard (1006, 2,135,646)}}$	Tiensin et al. (2005) DLD records
Relative $Risk_{Quails}$	$\frac{\text{Beta Quails (40, 2,555)}}{\text{Beta Backyard (1006, 2,135,646)}}$	Tiensin et al. (2005) DLD records
Relative $Risk_{Geese}$	$\frac{\text{BetaGeese (15, 14,672)}}{\text{Beta Backyard (1006, 2,135,646)}}$	Tiensin et al. (2005) DLD records
Relative $Risk_{Low}$	$\frac{\text{Beta Low (798, 1,542,116)}}{\text{Beta Low (798, 1,542,116)}}$	DLD records
Relative $Risk_{Medium}$	$\frac{\text{Beta Medium (605, 105,588)}}{\text{Beta Low (798, 1,542,116)}}$	DLD records
Relative $Risk_{High}$	$\frac{\text{Beta High (330, 38,618)}}{\text{Beta Low (798, 1,542,116)}}$	DLD records

In each poultry farm type, there is a probability of farms/holdings being infected, i.e. the effective probability of HPAIV H5N1 infection (EPI_C) in such a category, where C indicates the poultry farm type. EPI_C was calculated by multiplying P*H with the associated AR_C as shown in Eq. (2)

$$EPI_C = AR_C \times P*H \quad (2)$$

In each branch, proportions of poultry farm types are multiplied by the corresponding EPI_C and probabilities of detection. Probabilities with positive outcome are summed up to gain the system unit sensitivity (SeU), i.e. probability of disease detection from a random sampling process. The sensitivity of the surveillance system (SSe), i.e. the probability of detecting at least one infected farm by a particular SSC, given that the population are infected at the design prevalence, is calculated using (Eq. (3)) (Martin et al., 2007b).

$$SSe = 1 - (1- SeU)^n \quad (3)$$

Where n is the number of units processed in the SSC.

Passive surveillance

All poultry farms were assumed to participate in the passive SSC. The unit of interest in this is poultry keeping households/farms/holdings. Districts of Thailand were classified as either 'high', 'medium' or 'low' geographical HPAIV H5N1 infection risk areas based on their previous history of HPAI H5N1 outbreaks, the density of commercial chickens (broiler, layer hens), free-grazing ducks or migratory birds, and the presence of borders to neighbouring countries. Table 4 summarises the number of districts and their combined poultry population for each geographical risk area.

Table 4. Number of districts and their combined poultry population aggregated by geographical risk area

	LOW	MEDIUM
Districts	520	47
Poultry (birds)	237,218,947	2,783,215
Flocks	1,542,116	105,558

The AR for each geographical HPAI H5N1 infection risk area was estimated using Eq. (1). The number of reported HPAI H5N1 cases and number of poultry holdings in each risk area in 2004 were used to estimate the risk of HPAIV H5N1 infection before dividing it by the risk of HPAIV H5N1 infection in the low geographical risk area (See Table 3).

The probability of detection through passive surveillance, the unit sensitivity (U), was calculated using Eq. (4).

$$U = FR \times WTR \times VS \times LT \quad (4)$$

Where FR is a farmer's ability to recognise clinically infected birds, WTR is a farmer's willingness to report such a recognised case, VS is the probability of a vet taking and submitting samples/carcasses to the local DLD laboratory and LT is the sensitivity of egg inoculation used for laboratory confirmation.

Willingness to report a disease (WTR) is a key parameter in the disease reporting system. WTR in this context means the farmers' knowledge and awareness of disease and their judgment between the perceived personal benefits and costs of reporting considering expected compensation, economic consequences and other factors. After reporting a disease, farmers receive compensation for their culled birds at 75% of the Thai poultry market price. Movement restriction of birds and products imposed within a 10 km radius of the index farm for a 30 day period interrupts poultry trading activities in the affected area resulting in uncompensated economic losses for owners of unaffected farms. Due to the complex interaction between these factors, it is difficult to quantify WTR. For the purpose of this study, DLD staff were interviewed for estimating levels of WTR qualitatively as 'high', 'medium' or 'low', and then converted into the quantitative scales presented in Table 3. WTR for chicken and quail farmers were assumed to be high, medium or low, whereas WTR for duck and geese farmers were

assumed to be medium or low. Chickens and quail are more susceptible to AI infection and likely to show visible clinical signs of the disease, compared with ducks and geese (Brown et al., 2008; Saito, 2009; Songserm et al., 2006). Therefore, chicken and quail farmers are more likely to recognise clinically infected birds than duck and geese farmers. The detection sensitivities of passive surveillance were estimated for each scenario assuming different combinations of levels of WTR; 1) high WTR for chicken and quail farmers and medium WTR for duck and geese farmers, 2) medium WTR for chicken and quail farmers and low WTR for ducks and geese farmers, and 3) low WTR for all farmer types.

Active surveillance

The unit sensitivity in the active SSCs is the sensitivity of egg inoculation used in AI diagnosis. In each active SSC, SeU was estimated as described above. The sensitivity of the j^{th} SSC ($SSCSe_j$), i.e. the probability that at least one bird of the i^{th} farm/holding/household from which a pooled sample was collected produces a positive outcome, if AI infection is present at P*H, was estimated using Eq.(3) and n was the number of pooled samples processed in each SSC. After the sensitivities have been calculated, the combined sensitivity of all active SSCs (SSeAct) can be obtained using (Eq.(5)):

$$\mathrm{SSeAct} = 1 - \prod_{j=1}^{J} (1 - \mathrm{SSCSe_j})\, \mathrm{SSeAct} = 1 - \prod_{j=1}^{J} (1 - \mathrm{SSCSe_j}) \qquad (5)$$

Where j is the number of SSCs contributing to active surveillance and SSCSe*j* is the SSC sensitivity of the j^{th} component.

The combined active SSC model was run using 2 scenarios: 1) the current active SSCs, and 2) the active SSCs using an assumed number of 100,000 samples for each SSC, excluding the compartmentalisation scheme.

A stochastic scenario tree model was developed based on the scenario tree pathways using a spreadsheet (Excel; Microsoft, Redmond, WA) and simulation software (@Risk version 5.7.1; Palisade Corp., Ithaca, NY). The model parameters were entered with their appropriate probability distribution into the model (see Table 3). Data on actual sample numbers tested for HPAIV H5N1 in each active SSC in 2009 were used in the modelling process. Each simulation scenario was run for 10,000 iterations, and subjected to a sensitivity analysis.

RESULTS

The median probabilities of detecting at least one infected farm via passive surveillance were at least 36.7%, 91.3% and 100% in high, medium and low risk areas, respectively, assuming a low willingness to report in all three risk areas and HPAIV H5N1 prevalence of at least 0.5% (See Table 5). With a design prevalence of 0.1% and 0.05% in the high-risk area, the median sensitivity of passive surveillance decreased to 8.7% and 4.5%, respectively. At the same design prevalence level, by improving the levels of WTR the median sensitivity of passive surveillance increased by a factor of 3. Sensitivity analysis showed that the sensitivity of passive surveillance primarily depends on the WTR.

The estimated median sensitivities of all active SSCs were higher than 95% as shown in Table 6. The probability of HPAIV H5N1 detection in poultry through active SSCs remained high when the specified levels of H5N1 farm prevalence changed from 0.05 to 0.5%, mainly as a result of the high test sensitivity of egg inoculation used for the virus isolation and the large number of samples processed in each active SSC. Assuming a number of 100,000 pooled samples processed in each active SSC, excluding the compartmentalisation scheme, the median sensitivities of active SSCs remained high (>95%) at all levels of design H5N1 farm prevalences.

Table 5. The median sensitivity of passive surveillance for detecting at least one infected poultry farm in high, medium and low geographical risk areas of Thailand assuming different levels of HPAIV H5N1 farm prevalence (P*H) and different levels of willing to report (WTR): Sensitivity presented as median (5^{th} percentile; 95^{th} percentile), n = number of poultry flocks in area.

GEOGRAPHICAL AREAS	WTR		DESIGN PREVALENCE BETWEEN FARMS LEVEL (P*H)		
	Chickens & quail	Ducks & geese	P*H (0.5%)	P*H (0.1%)	P*H (0.05%)
High risk (n=38,618)	H	M	0.824 (0.775, 0.867)	0.294 (0.258, 0.332)	0.159 (0.138, 0.182)
	M	L	0.649 (0.583, 0.713)	0.189 (0.160, 0.221)	0.099 (0.084, 0.118)
	L	L	0.367 (0.267, 0.462)	0.087 (0.060, 0.117)	0.045 (0.031, 0.060)
Medium risk (n = 105,558)	H	M	0.999 (0.999, 0.999)	0.842 (0.797, 0.881)	0.602 (0.555, 0.654)
	M	L	0.996 (0.990, 0.998)	0.669 (0.605, 0.732)	0.425 (0.372, 0.482)
	L	L	0.913 (0.810, 0.963)	0.387 (0.283, 0.485)	0.217 (0.153, 0.282)
Low risk (n = 1,542,116)	H	M	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	0.999 (0.999, 0.999)
	M	L	1.00 (1.00, 1.00)	0.999 (0.998, 1.00)	0.999 (0.998, 0.999)
	L	L	1.00 (1.00, 1.00)	0.999 (0.998, 0.999)	0.971 (0.909, 0.992)

DISCUSSION

Stochastic scenario tree modelling provides an effective quantitative methodology to evaluate the disease detection ability of individual surveillance system components as well as of the whole system. The results from the modelling can then be used to make judgements about likelihood of disease/infection presence as such, as well as to consider alternative surveillance system configurations. In the current study, the model was based on a combination of known parameters but due to lack of published information assumptions had to be made on the basis of expert opinion. Despite of this constraint, the structured modelling approach accompanied by transparent presentation of the assumptions still allowed an assessment of the performance of the existing Thai HPAIV H5N1 surveillance system that will be useful for designing a more efficient and potentially more effective system.

The model produced a high probability of active SSCs to detect at least one infected farm, assuming that the infection was present at a very low HPAIV H5N1 prevalence level. The

different active SSCs were developed by the DLD to allow detection of AI infection in different parts of the Thai poultry production system (Buranathai et al., 2007; Tiensin et al., 2007). No case of HPAI H5N1 has been reported since late 2008. Given the high sensitivity of active SSCs, as estimated by the scenario tree models presented here, it suggests that Thailand's poultry production system is free from infection.

Active surveillance usually requires substantial staff and financial resources. In Thailand, more than 800,000 pooled samples have been processed for AI diagnosis annually since 2004, in a situation of apparent absence of HPAI disease. It is therefore unlikely that the current necessary resources will continue to be available, and therefore alternative surveillance system configurations need to be considered.

A possible option is to reduce the number of samples processed in active SSCs. Assuming 100,000 samples processed in each active SSC, the model showed that the overall sensitivity of active SSCs remained high even at the lowest level of the HPAIV H5N1 design prevalence. This suggests that the active surveillance strategy can be modified by taking the risk of AI infection, level of implementation of biosecurity measures, and previous results of AI testing into account. In broiler production, commercial farms with high biosecurity measures and an all-in-all-out system, such as is the case for compartmentalised farms, the number and/or frequency of sampling could be reduced because of the very low risk of AI infection (Alba et al., 2010). For 1-day-old chick and egg producing farms, assuming the farm operates at a high biosecurity standard and has had negative AI test results from continuous 'Health Monitoring Surveillance', sampling for pre-movement or pre-slaughter testing may not be necessary.

Due to its relatively low cost, passive surveillance for HPAIV H5N1 has a key role in the surveillance system as an early warning system for detecting HPAIV H5N1 at a very low prevalence, despite of its low sensitivity. WTR is the most influential parameter, and is determined by several factors, e.g. knowledge and awareness of disease, level of compensation payment, economic or social consequences. The Thai government has attempted to educate people by providing training and education campaigns via television and other media (Chunsuttiwat, 2008). As a result a relatively high level of public understanding of the disease was achieved during the time of major epidemics in 2004, but is likely to have reduced over time due to absence of human and animal cases in Thailand in recent time (Olsen et al., 2005). To maintain passive surveillance sensitivity at an acceptable, tailored incentives needs to be developed for farmers to encourage them to report any suspected outbreaks.

An effective disease reporting system requires a good cooperation between government, poultry farmers and other stakeholders involved in the Thai poultry production system. A low level of compensation for culled birds may discourage poultry farmers to report a disease, as they believe that they would earn more from selling infected birds than from the expected compensation for culled birds. In contrast, a high compensation level may induce farmers to report fraudulent cases or increase their bird flock size before reporting a case to obtain higher compensation payments. It should be noted that the levels of WTR assumed in the current scenario tree models were based expert opinion. This parameter needs to be studied in more detail so that the incentives and disincentives to reporting are better understood and can be considered in the design of compensation policies.

The risks of HPAIV H5N1 infection for different poultry farm types assumed in this study were derived from reported Thai HPAI H5N1 outbreak data from 2004 and the poultry population census data from 2004, which the source data did not allow differentiation between

poultry species and production system types. Since there are known differences in the risk of virus introduction between different production system types, the HPAIV H5N1 infection risk for commercial chickens assumed in the current study is likely to be an overestimate, as this farm type includes commercial vertically integrated farms which operate at strict biosecurity standard and cases of HPAI H5N1 have never been reported from this system (Tiensin et al., 2007).

The scenario tree model developed here can be used to quantify system performance as well as inform the design of more efficient and effective surveillance systems. For example, a reduction in pre-slaughter and pre-movement testing would still achieve the desired overall surveillance system sensitivity. But the design of a system should also consider economic aspects, which were not examined in this study. As the sensitivity of passive surveillance was highly dependent on farmers' willingness to report, understanding the motivation of Thai poultry keepers to report requires further investigation so that tailored information campaigns can be conducted that ensure high levels of farmer cooperation.

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OBSERVATIONAL STUDIES

HOW IMPORTANT IS "NEIGHBOURHOOD" IN THE PERSISTENCE OF BOVINE TUBERCULOSIS IN IRISH CATTLE HERDS?

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SUMMARY

Localised persistence of infection is a key feature of bovine tuberculosis (bTB) among cattle herds in the Republic of Ireland (ROI). A case-control study was conducted on the association between the occurrence of a bTB episode in 2006 in ROI herds, and the occurrence of bTB in previous years among neighbouring herd(s) within 1km, while controlling for own-herd bTB history and other risk factors. Neighbouring herds were grouped into three zones, and bTB incidence measures summarised within each zone and by calendar year (2001-2005). The results highlight an association between bTB and an increased animal incidence within two subsets of neighbouring herds: (i) herds directly contiguous during the previous 2 years, and (ii) herds at a distance of > 25 metres in the previous year. Further studies will be necessary to determine to what extent the association at (i) may be confounded by the existence of a wildlife (badger) source.

INTRODUCTION

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is an economically important disease for the Irish cattle sector (O'Flaherty, 2008). In ROI, a statutory control program is in place whereby all cattle in all herds are tested, at least annually, using the Single Intra-dermal Comparative Cervical Test (SICCT).

A key feature of bTB in both Ireland and the UK is local persistence of infection, leading to the development of recurrent episodes in one or a number of neighbouring herds (Karolemeas et al., 2011; Kelly & More, 2010). Local persistence could be attributed to: residual infection that persists in the index herd, despite testing following initial disclosure (Wolfe et al., 2010), neighbourhood or contiguous spread by cattle-to-cattle transmission over farm boundaries (Denny & Wilesmith 1999), and/or infection of a cluster of herds via a common wildlife source, such as the badger (Griffin et al., 2005).

There have been few published studies to date on the role of "neighbourhood" in local bTB persistence within ROI. On-farm investigations by Veterinary Inspectors into the likely source of bTB infection commonly identified multiple possible sources, with strong evidence supporting a single source only rarely found (O'Keeffe & Driscoll,1997). The following factors have been shown as statistically significant for explaining risk of local persistence:

- bTB status of contiguous herds (Griffin et al., 1996; O'Sullivan & O'Keeffe, 1997; Denny & Wilesmith, 1999)

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- animal incidence within District Electoral Division (i.e. local area of county) (O'Sullivan & O'Keeffe, 1997; Frankena et al., 2007)
- herd incidence within District Electoral Division (Olea-Popelka et al., 2004) and
- badger source (Griffin et al., 1996; Reilly & Courtenay, 2007; Murphy et al., 2011)

An understanding of the relative importance of the factors for local persistence would contribute to improved understanding of bTB epidemiology and improved resource usage in disease control efforts. The aim of this study was to determine the relative importance of "neighbourhood" in the persistence of bTB by investigating herds having a bTB episode in 2006. The occurrence of one or more bTB episodes among neighbouring herds within 1km of a study herd over the period 2001-2005 was used to predict the bTB status of that herd in 2006. The key factors of interest among neighbouring herds were time (in years) since previous episode, and distance (maximum 1000 metres) from a study (own) herd, while controlling for each herd's own bTB history and other risk factors.

MATERIALS AND METHODS

General

Under the national control programme in ROI, all cattle in all herds are tested at least once annually using the SICTT. This involves the injection of purified protein derivative (PPD) from *M. bovis* and *M. avium* into the skin of the animal's neck at two separate sites. At 72 hours after initial injection, the test is interpreted based on the size of the skin reaction. An animal with an increase in skin thickness at the bovine site more than 4 mm greater than the increase at the avian site is classed as a standard reactor. Other animals in the herd with smaller skin (bovine minus avian) increases may be deemed to be (non-standard) reactors. All animals classed as reactors are compulsorily removed, and the herd is 'restricted', *i.e.* no buying or selling of cattle is permitted, except under permit to slaughter. The herd remains restricted until two consecutive clear herd tests have been achieved, the first at 2 months after removal of reactor(s) and the second at 4 months at which point the herd is 'de-restricted' when both tests are clear. A "check-test" is conducted after six months, and in cases of more extensive reactor disclosure, two further check-tests at six-month intervals are carried out after which the herd reverts to annual testing provided that no further reactors occur.

Data assembly and management

Data were sourced from three national databases: the Animal Health Computer System (AHCS) for bTB herd testing data, including skin readings of individual reactors for the period 1999-2006; the Cattle Movement and Monitoring System (CMMS), recording calf births and cattle movements (farm-to-farm or via a cattle mart), and the Land Parcel Information System (LPIS) for herd location in 2005. The data were assembled into a single database at the Centre for Veterinary Epidemiology and Risk Analysis (University College Dublin).

Herd bTB testing data were organised such that all animals removed from a herd as reactors on standard interpretation over the restriction period were attributed to a single record called a "bTB episode". Thus, for the purposes of this study, a "bTB episode" refers to a period of herd restriction during which at least one standard reactor was disclosed. This definition excluded circa 33% of herd restrictions that had no standard reactors (i.e. restricted due to confirmed factory lesion but no other reactors) or animal(s) testing twice inconclusive (of uncertain status).

Study design

The study population was comprised of herds that underwent a herd test in 2006 (active herds) in ROI. A case control study of bTB status in 2006 was conducted, with herds that experienced a new bTB episode in 2006 (denoting a herd having one or more standard reactors) classed as cases, and herds that remained clear of bTB throughout 2006 classed as controls.

Omitted data

Among the 117,298 herds active in 2006, a total of 14,517 (12.4%) herds were excluded: 3,141 where the herd was already restricted on Jan 1st 2006. Among the newly restricted herds in 2006, 1,968 were omitted as having no standard reactor identified. Other herds were excluded due to incomplete data: 8,702 herds had no land parcel data recorded, and 706 herds had no recorded enterprise type. The final study population consisted of 3,909 cases and 98,872 controls.

Exposure classification

The herds surrounding each study herd (called own-herd) were classified into three zones based on their closest associated land fragment:

Zone 1 - within 25 metres (directly contiguous)
Zone 2 - between 26 to 150 meters
Zone 3 - between 151 to 1000 meters

Herds within 25m (zone 1) and between 26 and 150m (zone 2) were identified using ArcView GIS software, based on the closest edge-to-edge distance between parcels. Herds between 151m and 1000m were identified using SQL Server 2005, based on Pythagoras' theorem to calculate the straight-line distance (D) between two points (National Grid co-ordinates of centroid of each parcel), due to GIS computational limitations. To approximate an edge-edge distance, the average distance from the centroid to the edge of the parcel was subtracted from distance (D), based on the radius of a circle of equal area to the parcel.

The main factor of interest in this study was the previous bTB history for neighbouring herds (zones 1-3) over the period 2001-2005 with both herd and animal incidence measures summarised by zone, by year and by zone and year.

Statistical analyses

A multivariable logistic regression model was developed using the bTB status of each herd in 2006 as the outcome measure and herd as the unit of interest (Eq. (1)).

$$Logit\ (P(Y=1 \mid X)) = \mu + \beta_1 X_1 + ... + \beta_n X_n \quad (1)$$

Logit denotes log (P/(1-P)) the log odds ratio, where Y = 1 denotes a case herd and Y = 0 a control herd and where P (Y=1 | X) is the probability that a herd experienced a bTB episode in 2006, while adjusting for a set of risk factors and potential confounders (x). μ is the overall mean.

The following independent variables were considered (Table 1).

Table 1. Overview of own-herd and neighbouring-herd related factors for bTB status of ROI cattle herds in 2006

VARIABLE	DESCRIPTION
Herd Size	The size of the herd at the first herd test in 2006 (all animals; # cows # non-cows).
Enterprise type	Divided into four categories: dairy (dairy cows present), suckler (suckler cows present), beef finisher (no breeding animals), and other (uncertain type) based on the herd classification on AHCS in 2006.
Purchased animals in 2005	Total Animals: The number of animals purchased (all classes) into the herd in 2005. In addition, purchased animals were stratified as follows: i. male or female ii. aged < 12 months or >=12 months when movement occurred iii. moved before 1st of July 05 or moved on/after 1st July 05.
bTB History - Own herd	bTB herd and animal incidence measures were summarised within calendar years (2001, 2002, 2003, 2004 or 2005) For episodes that commenced within each calendar year, the following predictors for bTB history were modelled: A. Presence or absence of a bTB episode B. Count of standard reactors[a] C. Incidence of standard reactors[a]
bTB History - Neighbouring herds	bTB herd and animal incidence measures were summarised over all herds within 3 zones in combination with 5 seperate years, 2001-2005. Within each selected zone, year or zone-year combination, the following predictors for bTB history were modelled: 1. Presence or absence of a bTB episode within zone-year 2. herd incidence[a] expressed as no. of bTB episodes/active herds 3. animal incidence[a] expressed as no. of standard reactors/1000 animal population 4. Maximum episode size[a] (number of standard reactors)
No. of fragments	Number of seperate land fragments[#] belonging to the herd.

[a] Ln transform - natural logarithm (base e)

Univariable logistic regression analyses were performed to investigate the preliminary relationships between predictor variables and bTB episode occurrence in 2006 and to select variables to include in multivariable models. A test for linearity was performed for continuous

variables that were associated with 2006 bTB episode by graphing log-odds against equally sized categories of the continuous variable (Stata command: lintrend). When a visual assessment showed a non-linear trend, the continuous variable was either categorised or transformed before being entered into the model. All variables with a $p < 0.15$ in the univariable analysis were tested for collinearity, including multicollinearity analysis to ensure a mean Variance Inflation Factor (VIF) of <10 before being offered to the multivariable models (Dohoo et al., 2009).

A multivariable logistic regression model, containing all suitable variables identified from the univariable analysis, was then constructed. Model building consisted of a manual backwards elimination. Because the study aimed to include the National herd, all concerns about model power were trivial, so a selection threshold of $p = 0.01$ was used for the elimination of non-significant variables (Wald's test, using likelihood-ratio tests for comparison of nested models). Each time a variable was dropped, the potential for confounding was assessed by noting any changes in the log odds ratios of the remaining variables. Variables that modified the coefficients for the independent variables by 25% or more were classified as confounding and retained in the model (Dohoo et al., 2009). Based on previous research (Wolfe et al., 2010), herd size was also included in all models as a potential confounder. Once the main effects model was obtained, two-way interactions amongst the remaining independent variables were tested on the basis of biological plausibility. Results were expressed as odds ratios (OR) and 95% confidence intervals (CI).

An indicator variable for the county of the herd was forced into the models initially as a fixed effect, and within the final model, as a clustering variable, to account for regional variations in bTB prevalence.

Because of the large number of potential risk factor combinations, subject-related variables were categorised into smaller groups (clusters), and each cluster was analysed separately. The clusters were herd size, purchasing, and previous bTB history (own-herd combined with neighbouring-herds). Per cluster, logistic-regression analysis with backward selection of variables was used, as previously described. To differentiate between non-nested competing models, comparisons of Akaike's information criteria (AIC) and Bayesian Information Criteria (BIC) were used for model assessment, with smaller values preferred (Dohoo et al., 2009).

For the final model, the Hosmer-Lemeshow goodness-of-fit test was used to assess the model fit. Predictive ability was measured by the sensitivity, specificity and the percentage of observations correctly classified by the model; and by ROC curves (area under curve (AUC)). Data manipulation was performed using Microsoft SQL server 2005 (Microsoft, Redmond, CA) and statistical computations were performed in Stata 11.0 (StataCorp, Lakeway Drive, College Station, TX, 2009).

Population attributable fraction

The overall influence of neighbourhood was assessed by calculating the 'population-attributable fraction' (PAF using the weighted 'aflogit' command in Stata (Dohoo et al., 2009). PAF was used to assess the proportion of disease in all herds which could be attributed to the occurrence of bTB episode within previous years among neighbouring herds within 1km.

RESULTS

Descriptive

Of the 3,909 case herds, 1,690 (43.2%) involved only 1 standard reactor, 721 (18.4%) involved 2 standard reactors, and 1,498 (38.2%) had multiple standard reactors over the 2006 episode. Visible-lesions at slaughter among one or more reactor animal(s) were observed in 2,528 (65%) of the case herds. Among the study population, the mean herd size was 58.8 (median 38; range 1 - 2005). The herd size of case herds (n=3,909; mean 97.9; median 73) was significantly larger than control herds (n=98,872; mean 57.3; median 37) as determined by a Wilcoxon rank-sum test for the equality of medians ($p<0.001$).

Measures of bTB occurrence within own herd and within surrounding herds (zones 1-3) for the previous 5 years are shown in Table 2. Over the period 2001-2005, case herds had a higher ($p < 0.001$) mean annual incidence of bTB episodes (8.9) than did control herds (3.9) when all years were combined. Over the same period, neighbouring-herds (zones 1-3) to cases also had a higher ($p < 0.001$) annual incidence of bTB episode (6.7) than did neighbouring-herds (zones 1-3) to control herds (5.0) when all zones and years were combined.

Univariable results

Univariable logistic regression analyses were used to assess both individual and cluster-level variables, resulting in at least one variable being retained ($p<0.01$) for the multivariable analyses from each variable/cluster.

Within the purchasing cluster, all variables were significant ($p<0.001$). Based on competing models, the number of purchased animals aged over 12 months (ln transformed) was retained as the preferred predictor as having the lowest AIC.

Within the cluster herd size, competing models of herd size and cows versus non-cows were assessed, and all factors were significant ($p<0.001$). Overall herd size (ln transformed) was selected for further modelling since it had the lowest AIC.

The variables for enterprise type & herd fragments (ln transformed) were significant ($p<0.001$).

Within the cluster bTB history, predictors of own-herd history, and neighbourhood-herd history were seperately assessed. Each of the sets of predictors A-C of own-herd history (Table 1) over the 5 separate years (2001-2005) were significant ($p<0.001$). Each of the predictors 1-4 (Table 1) for neighbouring-herd bTB history were significant ($p<0.001$) when assessed by zone seperately (years amalgamated) and also by year seperately (zones amalgamated).

Table 2. Frequency of bTB for study herds and zone 1-3 neighbouring herds by year and status of study herd (case or control)

Variable	Year	Study herds		Neighbouring herds of Cases			Neighbouring herds of Controls		
		Case	Control	Zone 1	Zone 2	Zone 3	Zone 1	Zone 2	Zone 3
Active herds	2006	-	-	11	4	31	9	4	29
Animals[a]	2006	98	57	927	312	2,396	662	252	1,888
Presence of bTB episode (% = Yes)	2005	5%	2%	49%	22%	75%	29%	14%	58%
	2004	10%	4%	44%	20%	71%	29%	14%	58%
	2003	10%	4%	46%	23%	72%	32%	16%	61%
	2002	9%	4%	46%	22%	75%	33%	16%	62%
	2001	10%	5%	49%	22%	77%	36%	18%	65%
Herd incidence[b] per 100 herd years	2005	4.6	1.9	7.6	4.6	6.4	4.2	6.3	4.2
	2004	10.1	3.7	6.5	4.6	5.7	4.1	5.6	4.2
	2003	10.4	4.2	7.0	5.2	6.7	4.8	6.1	4.8
	2002	9.3	4.5	7.1	5.6	6.5	5.0	6.7	5.2
	2001	10.4	5.3	7.8	6.1	6.7	5.6	7.1	5.6
Animal incidence[b] per 1000 animal years	2005	1.5	1.1	4.5	3.7	3.5	2.7	2.6	2.6
	2004	3.9	2.0	2.9	2.8	2.7	2.3	2.2	2.3
	2003	4.3	2.4	3.4	3.4	3.1	2.8	2.7	2.8
	2002	4.4	2.7	3.6	3.6	3.7	3.1	3.0	3.1
	2001	4.1	3.1	3.6	3.1	3.5	3.1	3.1	3.0

[a] Average herd size for study herds, or average number of animals within zone for neighbouring herds.
[b] Herd and animal incidence calculated as sum (numerator) / sum (denominator) within zone-year.

Competing models for own herd & neighbouring herd

Clusters of predictor variables for own-herd (A-C) when combined with neighbouring-herd history (1-4) formed a set of 12 competing candidate models (Table 3: A1, A2, A3, A4, B1, B2, B3, B4, C1, C2, C3 & C4) for assessment over a range of zone-years. In an effort to reduce the number of zone-year combinations, the separate contributions of zones 1, 2 & 3 (years amalgamated) and years 2001-2005 (zones amalgamated) were assessed by use of competing models. The assessment was made using the simplest permutation (Table 2: A1), combining dichotomous presence of bTB in own herd (A) with dichotomous presence of bTB in neighbouring herd (1), while controlling for herd size and county (as a fixed effect). Using Model A1, a subset of zone-years consisting of zone 1 (all years), and years 2004-2005 (all zones) resulted in a similar AIC (30,920), and a lower BIC (31,292) than a model containing all zones and all years (AIC = 30,918; BIC = 31,299), and was selected for further modelling.

By use of the reduced subset of zone-years, the 12 competing multivariable models were assessed (Table 3), and Model A3 was selected on the basis of having the lowest AIC (30565).

The relevant bTB predictors for the model (A3) therefore included animal incidence within neighbouring herds over all zones (2004-2005) and zone 1 (all years), combined with presence or absence of any bTB episode within own-herd-year (2001-2005).

Table 3. Evaluation of competing models: neighbouring-herd predictors versus own-herd predictors of previous bTB history in the prediction of bTB episodes in 2006 within ROI Cattle Herds

Model Cluster	Own-Herd bTB 2001-2005	Neighbouring-herd bTB 2001-2005[a]	AIC	BIC	AUC	N
A1	Any episode (yes/no)	Any episode (yes/no)	30729	31140	0.7267	102781
A2	Any episode (yes/no)	Herd Incidence[b]	30656	31066	0.7294	102781
A3	Any episode (yes/no)	Animal Incidence[b]	30565	30975	0.7325	102781
A4	Any episode (yes/no)	Largest episode[b]	30642	31053	0.7295	102781
B1	Count of standard reactors[b]	Any episode (yes/no)	30745	31155	0.7265	102781
B2	Count of standard reactors[b]	Herd Incidence[b]	30672	31082	0.7291	102781
B3	Count of standard reactors[b]	Animal Incidence[b]	30583	30993	0.7322	102781
B4	Count of standard reactors[b]	Largest episode[b]	30660	31071	0.7292	102781
C1	Animal Incidence[b]	Any episode (yes/no)	30833	31243	0.7239	102781
C2	Animal Incidence[b]	Herd Incidence[b]	30757	31167	0.7266	102781
C3	Animal Incidence[b]	Animal Incidence[b]	30668	31078	0.7296	102781
C4	Animal Incidence[b]	Largest episode[b]	30746	31156	0.7266	102781

[a] Year 2005 zones 1- 3 and zone1 years 2001-2004.
[b] ln transformed variable.

Further analysis showed no evidence of multicollinearity amongst any of the variables identified (mean VIF of 1.06). A backwards elimination was conducted with county as a clustering variable to arrive at a final multivariable model. This resulted in the following non-confounding variables being dropped: zone 1 animal incidence in 2002 ($p = 0.70$), zone 1 animal incidence in 2001 ($p = 0.03$), enterprise type ($p = 0.11$), number of farm fragments ($p = 0.02$), zone 1 animal incidence in 2003 ($p = 0.02$). Herd size was a confounder for number of herd fragments, and enterprise type.

The final multivariable model is shown in Table 4. Factors positively associated with a bTB episode in 2006 at $p<0.01$ included an increased animal incidence of bTB within neighbouring herds zones 1-3 in 2005 and zone 1 in 2004, the presence of a bTB episode within own herd within any of the previous 5 years (2001-2005), herd size, and number of animals purchased at age over 12 months in 2005. No interaction was found between the occurrence of bTB in zone 1 herds in 2005 and own herd size. The Hosmer & Lemeshow goodness of fit test indicted that the model fitted the data adequately (Hosmer & Lemeshow, $p = 0.11$).

Table 4. Final multivariable logistic-regression model for the occurrence of a bTB episode in 2006 within ROI cattle herds.

Variable	Exposure scale	Odds Ratio (95% CI)[a]
Neighbourhood bTB animal incidence	Standard reactors per 1000 animal years	
Zone1, 1 year previously	log scale[b] (range 0 - 500)	1.13 (1.01-1.15)
Zone2, 1 year previously	log scale[b] (range 0 - 1,000)	1.05 (1.03-1.07)
Zone3, 1 year previously	log scale[b] (range 0 - 323)	1.13 (1.10-1.17)
Zone1, 2 years previously	log scale[b] (range 0 - 1,000)	1.05 (1.02-1.07)
Previous bTB episode	Yes/No	
None within 5 years	(reference)	1.00
1 year previously		1.47 (1.31-1.66)
2 years previously		1.76 (1.61-1.92)
3 years previously		1.72 (1.53-1.94)
4 years previously		1.37 (1.24-1.50)
5 years previously		1.39 (1.23-1.58)
Herd Size	log scale[b] (range 1 - 2005)	1.59 (1.49-1.69)
Animals purchased	square root transformed (range 0 - 11,709)	1.04 (1.03-1.05)

[a] All Odds ratios are significant ($p<0.001$)
[b] Indicates per unit increase on the log e scale (or equivalent to a 2.7-fold increase), range according to the original scale

Optimal cut-off for model prediction of a 2006 bTB episode was determined from sensitivity-specificity plots. The full logistic model correctly classified 66.2% of the observations when the probability cut-off was set ($p>0.038$) to concurrently maximise both sensitivity (66%) and specificity (66%), but suffered from a low positive predictive value (7.2%).

The contribution that each variable or cluster variable made to the overall predictive ability of the model was assessed by dropping each variable or group of variables, from the model individually and recalculating the AUC. Dropping all neighbouring herd bTB history zones/years reduced the AUC from 0.723 to 0.699, while dropping own herd history for all years reduced AUC to 0.713. Among the model predictors, the greatest reduction of AUC was associated with dropping herd size (AUC reduced to 0.692).

Population attributable fraction (PAF)

The population attributable fraction for having an "increased animal incidence of bTB" among neighbouring herds within 1km was calculated. This required the refitting of the final model with continuous variables recoded as categorical variables. Predictors of neighbouring herd bTB history were divided into 4 quantile groups. For reasons of parsimony, the cutpoints were calculated from the distribution of animal incidence among herds within zone 1 for year 2005. Since for 30% of the observations, the animal incidence was zero, groups were divided at

the 50th, 75th & 90th percentiles (cutpoints 0, 1.3, 6.4, 13.0). Likewise, animals purchased in 2005 aged >12m were divided at the 75th, 90th and 95 percentile (cutpoints 5, 18, 35). Herd size was divided into quartiles (cutpoints 17, 38, 78). The calculated PAF for animal incidence of more than 1.3 standard reactors per 1000 animal years (quartiles 2, 3, 4) within each of the significant zone-years are shown in Table 5. The overall PAF for being within 1 km of an "increased animal incidence" of bTB was 0.35 (95% C.I. 0.25-0.44).

Table 5. Population Attributable Fraction for Zone 1 in Years 2004-2005 and Zones 2-3 in 2005, for an increased animal incidence of > 1.3 standard reactors per 1000 animal years.

bTB zone-year	P.A.F.	Std. Error	95% C. I.	
Zone 1, 2005	0.15	0.04	0.06	0.23
Zone 1, 2004	0.07	0.05	-0.03	0.15
Zone 2, 2005	0.04	0.05	-0.06	0.13
Zone 3, 2005	0.16	0.05	0.05	0.26
Zones 1-3 Years 2004-2005[a]	0.35	0.05	0.25	0.44

[a] Overall PAF < sum of individual zone-years due to confounding.

The respecified model fitted the data adequately for calculation of PAF (Hosmer & Lemeshow test; X^2 =11.3; p=0.08; 10 groups), with a modest decrease in predictive ability from 0.723 to 0.714 as measured by AUC under the ROC curve.

DISCUSSION

In this study logistic regression techniques were applied to quantify the importance of a bTB affected neighbourhood as a risk factor for future bTB episode. From the results, it is concluded that "being within 1km of a zone with increased animal incidence" for either of the two previous years accounted for 35% of the bTB episodes in 2006 within the study population.

Based on previous research, lesion status of reactor(s) was omitted as a predictor variable on the basis that this variable was not predictive of future breakdown (Olea-Popelka et al., 2004; Wolfe et al., 2010). We defend the use of 1 standard reactor as our measure of outcome on the basis of previous research by Good & Duignan (2011), whereby only 6% of herds restricted with a single reactor were considered unconfirmed for bTB on the basis of post-mortem lesion, and subsequent laboratory investigations.

The explanatory variables within this study may provide insight into the sources of local persistence of bTB. However a number of limitations remain. The odds ratios from the logistic model indicate the impact of a specific variable on the risk of a new bTB episode in 2006; whereas the PAF for specific factors takes into account the "effect" indicated by the odds ratio, adjusted for the prevalence of the specific factor. A limitation of the final model is that the metrics of coding history of bTB differed between the own-herd and herds in Zones 1-3; thus the magnitude of the odds ratios are not strictly comparable. The average number of herds within Zones 1, 2 and 3 was 10, 5, and 32 respectively.

Own-herd history

Consistent with previous studies in Ireland (Olea-Popelka et al., 2004; Wolfe et al., 2010), and Great Britain (Karolemeas et al., 2011), the multivariable model confirms that recurrence of bTB within herds is a persistent problem, with odds ratios ranging from 1.39 to 1.76 of having a bTB episode within any of the previous years 2001-2005. The estimated odds ratio of 1.47 for previous year may be a reflection of some of the herds experiencing a new bTB episode in 2005 being ineligible for study participation as they were "already restricted" on January 1st, 2006.

A likely explanation for the finding of a history of one or more bTB episode(s) within the previous 5 years as a risk factor is presence of residually infected animals, or environment within and/or around the herd. In a survival study of previously inconclusive animals, Clegg et al. (2011) found an increased future risk of a positive bTB diagnosis for at least 4 years. Such animals must always be considered as a possible source of within-herd persistence. On farms that restocked following FMD in Great Britain in 2001, Carrique-Mas et al. (2008) found when there was a history of bTB in the preceeding 4 years, bTB risk persisted on those farms following restocking (i.e. a different animal population). This suggests either environmental contamination, the purchase of infected animals for restocking, or the presence of a locally infected badger population.

Contiguous spread by cattle-to cattle transmission

For neighbouring herds within 25m, in 2005 the PAF was 0.15, and 0.07 in 2004 (confidence interval for 2004 includes zero). The available data suggests that transmission directly through over-the-fence contact with zone 1 herds in the previous year cannot be ruled out. Also, over-the-fence contact cannot be ruled out as an explanation for the increased odds within zone 1 in 2004 because of timing of test intervals, and due to imperfect test sensitivity. However the presence of a locally infected badger population cannot be excluded either.

Our analysis was repeated using only zone 1 predictors, years 2004-2005, and model A3 remained as the preferred model. This suggests that modelling for a "large episode next door" does not add useful information about local bTB persistence, so alternative mechanisms such as a common wildlife source must also be considered. These observations are consistent with the work of Griffin et al. (1993) who performed a matched case control study to explain persistent breakdown, and concluded that contact with contiguous herds (direct neighbours) was unlikely to be a risk factor.

In Northern Ireland in a study of dairy herds, Denny & Wilesmith (1999) attributed 40% of breakdowns to the presence of (directly) contiguous neighbour(s) with confirmed bTB. While the PAF from the Northern Ireland study is not directly comparable to the current study due to differing methodologies, it is likely that any PAF for neighbourhood is an overestimate where exposures to a common wildlife source are not taken into account.

Possible infection of herds via a common wildlife source

The calculated PAF for neighbourhood herds at distances greater than 25m, in the previous year were 0.04 for zone 2 (95% confidence interval includes zero), and 0.16 for zone 3. A shared wildlife source is the most plausable explanation for this aspect of disease persistence. The direct role of badgers in transmission of bTB to cattle has been shown in the East Offaly and Four Area studies whereby an area-wide removal of badgers led to a significant reduction in the prevalence of disease in associated cattle herds (Ó'Máirtín et al., 1998; Griffin et al., 2005).

Based on our cutpoint for exposure (1.3 standard reactors per 1,000 animal years), and the actual bTB herd test results for the relevant zone-years, an estimated 63% of the study population were exposed to "an increased animal incidence", and/or the causes of that increased incidence. The population attributable fraction of 0.35 for herds within 1km, suggests that if this neighbourhood risk factor could be removed there would be a 35% reduction in the incidence of 2006 bTB episodes. This reduction assumes that no confounding exists between this "neighbourhood factor" and other factors that impact on future risk.

Other risk factors

Increased herd size was a significant risk factor for bTB episode in 2006, resulting in an increased odds of 1.59 per 2.7-fold increase in herd size. Herd size is widely reported as a risk factor for bTB episodes within previous modelling studies (e.g. Olea-Popelka et al., 2004, Wolfe et al., 2010). Within our model, dropping of herd size resulted in the largest decrease in predictive ability (from 0.723 to 0.692 as measured by change in AUC) of any variable or cluster of variables. The effect of increasing herd size on future bTB episodes may be related either to the own-herd factors varying with herd size (*e.g.* enterprise type, increasing fragmentation, presence of residually infected animal(s)) or to an increased exposure to neighbourhood factors. Given that a larger number of animals are likely to occupy a larger land mass, larger herds are more likely to feature a badger sett, on or adjacent to the farm. Thus, a greater exposure to an infected wildlife source may account for some of the effect of increased herd size within our model.

Other predictors of outcome that were significant included purchasing of animals over 12 months of age (square root transform). The finding of increased bTB risk among older animals is consistent with the work of others in Ireland (Martin et al., 2001) and Great Britain (Reilly & Courtenay, 2007). No benefit was found for modelling male versus female purchased animals, and it is inferred that females may be more likely retained for breeding, whereas males may be more likely destined for beef finishing. Also, no benefit was found for modelling animals moved before versus on/after 1st of July of 2005, and it is inferred that herds that sold animals in the second half of the year may have been more recently tested for bTB.

Now that we have a template, it would be informative to repeat these analyses in areas of the country where badger-to-cattle transmission is likely to have been minimized. This would allow a better estimate of true cattle-to-cattle (herd-to-herd) spread among herds directly contiguous to one-another, and will be pursued as soon as feasible.

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A COHORT STUDY OF BODY WEIGHT AND ENVIRONMENTAL RISK FACTORS FOR CANINE HIP DYSPLASIA AMONG FOUR LARGE BREEDS

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SUMMARY

Canine hip dysplasia (CHD) is a common skeletal disease with a multifactorial background. Prospective studies of dogs from the Newfoundland, Leonberger, Labrador Retriever, and Irish Wolfhound breeds revealed a protective effect of higher body weight (BW) at 3 months of age on the odds of radiological CHD (Krontveit et al., 2010) and that housing and exercise related factors during the first 3 months of life influenced the odds of radiological CHD (Krontveit et al., 2011). The aim of the current analysis was to measure the combined effect of, and potential interactions between, this BW variable and these housing and exercise variables. A protective effect of living at a farm and access to off-leash exercise in park terrain was observed, as well as higher BW at 3 months. Daily use of stairs increased the odds of CHD. Significant interactions were not found. Litter-level clustering was high and significant.

INTRODUCTION

Canine hip dysplasia (CHD) is a common skeletal disease with a multifactorial background. It affects many breeds and causes varying degrees of pain and disability in affected individuals. The cause of CHD is considered complex, and the connection between the genetic predisposition and environmental factors influencing the development is poorly understood. Efforts to reduce the occurrence of CHD have been made by the selection of desired phenotypes based on radiographic screening of dogs at one to two years of age and subsequently imposing breeding recommendations for dogs with radiographic diagnosis of CHD. This can be seen as a pass/fail system for hip joint status given once during the dogs' life. The screening is voluntary, and the results are recorded in national registries. Selection based on radiographic screening has been applied for several decades in a number of different countries.

Prevalence and heritability estimates vary according to the phenotype evaluated, and for the conventional radiological CHD, phenotype heritability estimates are often relatively low and the prevalence commonly considered to be underestimated. Which environmental factors contribute to the development of CHD, and to what extent, are largely unknown. Most studies relating to CHD have been retrospective or experimental and performed in relatively small kennel populations.

A prospective cohort study looking at CHD has been previously carried out in Norway involving large breed dogs, namely the Newfoundland (NF), Leonberger (LEO), Labrador Retriever (LR), and Irish Wolfhound (IW) breeds. Incidence estimates of radiological CHD in this cohort were found to vary considerably between the breeds and NF had the highest occurrence with an 18 months incidence risk of 36% (Krontveit et al., 2010). In LEO, the 18 months incidence risk was 25%, and in LR and IW the 12 months incidence risks were 20%, and

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10%, respectively (Krontveit et al., 2010). A previous analysis of body weight (BW) and growth in this cohort of dogs failed to support the hypothesis that heavy and fast growing individuals were at increased risk of developing CHD. On the contrary, a protective effect of higher BW at 3 months of age was observed (Krontveit et al., 2010). Environmental factors related to housing and exercise conditions during the first 3 months of life were also identified to have impact on the odds of radiological CHD in this cohort of dogs (Krontveit et al., 2011). The aim of the current analysis was to measure the combined effect of and potential interactions between the BW variable at 3 months and the significant housing and exercise variables from the period up until 3 months of age on the odds of being diagnosed with CHD at radiographic screening.

MATERIALS AND METHODS

The study was carried out in agreement with the provisions enforced by the National Animal Research Authority.

Study design

The present study is part of a larger study (the so called *main study*) aimed at investigating the epidemiology of several skeletal diseases: CHD, elbow dysplasia, panosteitis, and osteosarcoma. The *main study* included dogs from the breeds: NF, LR, LEO, and IW. A prospective single cohort study was conducted to investigate factors affecting the radiological occurrence of CHD in growing dogs from the *main study*.

Inclusion of dogs

The sampling procedure and inclusion of dogs in this cohort have been described previously (Krontveit et al., 2010). Puppies born in Norway between November 1998 and June 2001 were eligible for inclusion in the *main study*. All geographic areas of Norway were represented. The breeding stock consisted of dogs born in Norway as well as dogs which had been imported. Inclusion of a litter began when the bitch was mated. All puppies were registered by The Norwegian Kennel Club (NKK). Each breeder, dog owner and veterinarian who participated in the project signed a written agreement of cooperation to comply with the project plan. Not all dogs enrolled in the study continued to completion. Reasons for dropouts included, but were not limited to, death of the dog, relocation of the owners during the study, and export of dogs abroad (Trangerud et al., 2007). In total, 647 dogs from 106 litters from the *main study* were eligible for inclusion in the present study, which is a convenience sample consisting of approximately 25% of the total numbers of litters born from the included breeds in Norway during the sampling period. The inclusion criteria for the present study were that the dogs were radiographically screened for CHD and that they had BW, housing and exercise conditions recorded at least once during the observation period which was from birth to 12 months of age. The dogs were privately owned, and each dog had a housing, exercise, and feeding regimen which was developed by its owner.

Radiographic screening for CHD

The signed written agreement of cooperation and consent encouraged dog owners to have their dogs radiographically screened for CHD. LR and IW breeds were radiographed at 12 months of age, and LEO and NF breeds at 18 months of age, which are the recommended screening ages for these breeds in the NKK. Hip radiographs were taken by a veterinarian and

subsequently sent to NKK for judging irrespective of whether the dogs showed any clinical signs or not. More than 90% of the radiographs were scrutinized by the same experienced veterinary panelist at the NKK. In the present study some of the dogs were living in Sweden and Denmark. The hip radiographs of these dogs were therefore scrutinized by the panelists in The Swedish Kennel Club (SKK) and The Dansk Kennel Club (DKK), respectively. The radiographic panel of The Nordic Kennel Union consists of panelists from the Nordic countries and ensures that the hip radiographs of dogs examined in different Nordic countries are graded according to the same protocol, thus minimizing differences in grading between the Nordic countries. All dogs were sedated before the radiographic examination to achieve complete muscle relaxation. The identity of the dogs (NKK registration number, tattoo or microchip identification number) was photographed onto the film before developing the radiographs. The radiographs were made at 100cm film to focus distance. The dogs were placed in dorsal recumbency with the hind limbs extended and abducted so that the patellae were superimposed over the femora. The entire pelvis and femora including the patellae were included on the radiographs. The Fédération Cynologique Internationale (FCI) five class grading scale was used to classify the hip status of the dogs: A (excellent), B (normal), C (mild dysplasia), D (moderate dysplasia) and E (severe dysplasia). The CHD grades are defined descriptively based on the size of the Norberg angle (NA), degree of subluxation, shape and depth of the acetabulum, and signs of secondary joint disease (Flückiger, 2007). Each hip joint was graded separately, and the final grading was based on the most severely affected hip joint. Dogs graded with mild dysplasia (C), moderate dysplasia (D), and severe dysplasia (E), were considered affected by CHD.

Questionnaires and clinical registrations

Information about each included dog was obtained from three sources: 1) the breeder of the litter, 2) the owner of the puppy, and 3) the veterinarian examining the dog. All three sources completed questionnaires and recorded information in a booklet prepared for each dog. The breeder and owner booklets (in Norwegian) are available at: http://www.nvh.no/Documents/PDF/SportFaMed/breeder_booklet.pdf and http://www.nvh.no/Documents/PDF/SportFaMed/owner_booklet2.pdf, respectively.

The breeders were asked to record the BW in grams for each puppy at birth and on day 3 and 7, and then weekly until 56 days of age. The breeders determined the feeding and housing regimen of the litter, and this information was recorded along with information about any medications and vaccinations. The puppies stayed with the bitch until approximately 8 weeks of age when they were sold to their new owners.

The owners reported information regarding feeding, housing, exercise, and any signs indicative of disease in their dogs. The owner completed the questionnaires and recorded information regarding the dog at specific ages, called “the observational ages”: 3, 4, 6, and 12 months. The owners agreed to have their dogs examined by a veterinarian at visits scheduled specifically for this project at the observational ages. Clinical examination, blood sampling for subsequent analyses, measurement of BW in kg, and routine vaccinations were done at each of the veterinary visits. The breeders, owners, and veterinarians sent in the questionnaires from the booklets to the project leaders at regular intervals in prepaid envelopes.

The factors investigated in the present study were those found to be significant in two previous studies of growth and a study of housing and exercise conditions respectively on the odds of radiological CHD (Krontveit et al., 2010; Krontveit et al., 2011). The variables are described in Table 1. In temperate regions like Norway there are large climatic variations

between the seasons, and the calendar year was divided into four seasons according to the climatic conditions in Norway. March is a winter month in most parts of Norway. Therefore winter is longer than summer and fall, and spring is shorter (Table 1).

Table 1. Definition of the variables investigated in the study of body weight and environmental risk factors for canine hip dysplasia (CHD) in four large breeds.

Variable	Definition	Source of information	Abbreviated name
Breed	Breed of the dog (categorical): Newfoundland, Labrador Retriever, Leonberger, Irish Wolfhound	Breeder	Breed
Litter size	Number of puppies in each litter (continuous)	Breeder	Litter size
Season of birth[a]	Season of birth of the litter (categorical): winter (December-March), spring (April-May), summer (June-August), fall (September-November)	Breeder	Season
Type of house[a]	Type of building in which the dogs live with the breeder (categorical): single family house or farm/small farm	Breeder	Breeder house
Use of stairs[a]	Daily use of stairs in the period until 3 months of age, owner data (dichotomous)	Owner	Stairs
Off-leash park[a]	Daily off-leash exercise in park terrain until 3 months of age, owner data (dichotomous)	Owner	Free park
Body weight[b]	Body weight in kg at 3 months of age, veterinarian data (continuous)	Veterinarian	BW 3 mo

[a] Variables used in the study of housing and exercise conditions (Krontveit et al., 2011)
[b] Variables used in the study of growth (Krontveit et al., 2010)

Statistical analysis

The software Stata 11 was used for all analyses. For descriptive purposes the number of observations within each categorical and dichotomous variable was calculated as well as the mean and range for the continuous variables. The distribution by breed of CHD free and affected dogs was estimated. For the purpose of the multivariable analyses the dependent variable (CHD

status) was reclassified into 2 categories: free (grades A and B) and affected (grades C, D, and E). Therefore, a logistic regression model of the relationship between predictors and CHD was considered appropriate. The dogs in the study were clustered into litters. The assumption of independence between observations was therefore violated and a random intercept for litter was included in a generalized linear mixed model. A random intercept for breeder was also considered, but because the breeder: litter ratio was close to one, the breeder level was omitted.

Collinearity was evaluated by pairwise correlations for continuous variables, Goodman and Kruskal`s gamma for ordinal and dichotomous variables and the *phi* coefficient for nominal variables. When collinearity was detected between two predictors, the predictor with least missing data was selected for further analyses. Lowess curves were used to assess the linearity of the relationship between the continuous variables and the logit of the outcome. The Stata command *xtmelogit* using adaptive quadrature was used for the multilevel modeling and the model was constructed using manual backward elimination. Predictor variables were retained in the model when the P-value was < 0.05. Potential confounding and intervening variables were assessed after evaluation of a tentative causal diagram. Changes of more than 20% in the coefficients in the model with the potential confounder present were also used as indications of confounding. Interactions between significant predictors were tested by adding an interaction term to the model, and the interaction term was retained if $P < 0.01$, as judged by the likelihood ratio test (LRT) comparing models with and without the interaction term. Following manual backward elimination, the model was built again by forward selection by offering the excluded variables one at a time. A variable was considered to be intervening if adding it removed the entire effect of another variable and if the intervening variable lay on the causal path between the factor and the outcome. Intervening variables were excluded. The LRT was used to evaluate the significance of the random litter effect in models with and without random litter effect, but containing the same fixed effects. The LRT was considered significant at $P=0.05$ and was a one-sided test. The LRT was used to evaluate differences between categories of categorical predictors and the Stata command *lincom* was used to conduct contrasts among the categories. The between litter variance (σ^2_{litter}) was estimated from the final model. The intraclass correlation coefficient (ICC) was calculated (Eq. 1) using the latent variable approach, assuming that dog level variance (σ^2) is constant at $\pi^2/3$ (Dohoo et al., 2009).

$$ICC= \sigma^2_{litter}/ \sigma^2 + \sigma^2_{litter} \quad (1)$$

To evaluate and assess the fit of the final multi-level model, residuals were estimated at the individual dog level, as well as at the litter level. Litter level residuals were plotted against both predicted and fitted values to assess the assumptions of homoscedasticity and normality (Dohoo et al., 2009).

RESULTS

A total of 501 dogs (260 females, 241 males) fulfilled the inclusion criteria. Collinearity was not detected between any of the variables. All of the tested variables, except season, were significantly associated with CHD status ($P<0.05$) and thus retained in the model. Distribution of dogs free and affected by CHD for each breed is presented in Table 2 and results from the

multivariable model with descriptive statistics for the variables are presented in Table 3. The LRT for the variable breed was significant (P<0.001). A protective effect of being a LR, LEO, and IW dog compared to a NF dog was found, although not significant for LEO (Table 3). The difference between the LR and LEO breeds was significant (OR 0.21, P=0.005), while the differences between the LR and IW breeds (OR 0.66, P=0.62) and LEO and IW breeds (OR 3.10, P=0.09) were not significant. Breeders situated on farms/small farms and dogs exercising daily in park terrain until three months of age were also found to be protective against radiological CHD (Table 3). Daily use of stairs until three months of age increased the odds of a radiological CHD diagnosis. Litter size was forced into the model as a potential confounder. The ICC was 0.21 and significant (P=0.002) (Table 3). None of the tested interactions were significant at P<0.01 level, but the interaction term between stairs and BW at 3 months was approaching significance (P=0.03) and had an OR of 1.19 indicating that the negative effect of the daily use of stairs was more pronounced for the heavier dogs. The assumptions of homoscedasticity and normality were met as evaluated by the litter level residuals. No extreme values were found in the dog level residuals.

Table 2. Number and percentage of dogs classified as free or affected by canine hip dysplasia (CHD) in a study of body weight and environmental risk factors for CHD in four large breeds.

Breed	Radiological CHD status	
	Free, N (%)	Affected, N (%)
Newfoundland	80 (64.0)	45 (36.0)
Labrador Retriever	106 (79.7)	27 (20.3)
Leonberger	135 (75.0)	45 (25.0)
Irish Wolfhound	57 (90.5)	6 (9.5)

Table 3. Descriptive statistics and results from the multivariable random effects logistic regression model of body weight and environmental risk factors for canine hip dysplasia (CHD) in four large breeds.

Variable	Categories	N (%)	Mean (range)	Estimate	OR	95% CI	P-value
Breed	Newfoundland	125 (25.0)		-	1.00	-	-
	Labrador Retriever	133 (26.5)		-2.04	0.13	0.04, 0.40	<0.001
	Leonberger	180 (35.9)		-0.51	0.60	0.25, 1.45	0.26
	Irish Wolfhound	63 (12.6)		-1.66	0.19	0.05, 0.74	0.02
Litter size			9 (1, 15)	-0.13	0.88	0.76, 1.02	0.09
Breeder house[a]	House	364 (76.3)		-	1.0	-	-
	Farm/small farm	113 (23.7)		-1.17	0.31	0.13, 0.76	0.01
Stairs[a]	No	294 (61.6)		-	1.0	-	-
	Yes	183 (38.4)		0.83	2.29	1.29, 4.06	0.004
Free park[a]	No	360 (75.5)		-	1.0	-	-
	Yes	117 (24.5)		-1.17	0.31	0.15, 0.66	0.002
BW 3 mos			16.1 (6.8, 27)	-0.14	0.87	0.77, 0.97	0.02
Rho				0.21	-	0.09, 0.44	0.002

[a] Data were missing for 24 observations

DISCUSSION

In a previous study involving this cohort of large breed dogs it was found that higher BW at 3 months of age was protective against radiological CHD at screening (Krontveit et al., 2010). Furthermore, it was found that being born during spring or summer, living on a farm/small holding in the breeder period, and getting free exercise in park terrain until 3 months of age protected against radiological CHD while the daily use of stairs in until 3 months increased the odds (Krontveit et al., 2011). In the present study, the effect of BW at 3 months and the housing and exercise related variables as well as breed and season of birth were combined to evaluate their effect and possible interactions between the BW variable and the housing and exercise variables.

Compared to NF, LEO dogs had around half the odds of radiological CHD (OR 0.60), but the difference was not statistically significant. LR and IW breeds had significantly reduced odds compared to NF with ORs of 0.13 and 0.19, respectively. Based upon the model in the current study, it appears that when taking all study variables into account, the LR, IW, and LEO breeds had almost equal odds of radiological CHD, although large differences in incidence (20%, 10%, and 25% respectively) were found in a previous study (Krontveit et al., 2010). It might be that given the housing and exercise variables and BW at 3 months these breeds have almost the same odds of radiological CHD.

One of the aims in the current study was to evaluate the combined effect of the BW variable and the housing and exercise variables. When combining these variables the effects were almost as similar as in the previous studies except that the effect of season of birth was not significant. If the breeder house type was farm/small holding this reduced the odds of radiological CHD (OR 0.31) as did daily exercise in park terrain until 3 months of age (OR 0.31). Daily use of stairs during the same time-period doubled the odds (OR 2.29), and higher BW at 3 months of age seemed protective (OR 0.87). A possible explanation for the effect of breeder house-type is related to outdoor environment and exercise conditions. The puppies' opportunity to get off-leash exercise outdoors on a regular basis might be greater when they are reared in a rural area on a farm or small holding compared to in a single family house in more urban areas. The beneficial effect of off-leash exercise in the current study might be due to increased muscle development and strength. Dogs with high pelvic muscle mass have been found to be more likely to have normal hip joints (Cardinet 3rd et al., 1997; Lust et al., 1972; Riser & Shirer, 1967). Stairs are designed for human adults and the steps can be too high and too slippery for a small puppy. Climbing as well as descent of stairs in combination with the immature neuromuscular function and coordination of young puppies (Lopez et al., 2006) can be hypothesized to create too much load on immature joints and thus promote abnormal development and an explanation for the observed negative effects of the use of stairs up to the age of 3 months in the present study. The protective effect of increased BW at 3 months can be hypothesized to be as a result of increased muscle mass and/or increased bone mass in dogs.

The other aim of the current study was to evaluate if there were any interactions between BW at 3 months and the housing and exercise variables. A more restrictive p-value was applied when evaluating the significance of interaction terms because a large number of comparisons were made (17). The interaction between BW at 3 months and stairs was approaching significance (OR 1.19, P = 0.03) indicating that the negative effect of stairs was more pronounced with higher BW at 3 months. If the hypothesized negative effect of the daily use of stairs results in too much load on the joints thus promoting development of CHD, then it is plausible that this negative effect is more pronounced with increasing BW.

The high level of clustering at the litter level (20%) is most probably a result of common genetic background and common environment within a litter both before and after birth. Breeders commonly make management recommendations to owners who buy puppies and this might make litter effects important even after weaning. The environmental variance, i.e. all variation of non-genetic origin, is greater in our study population than in dogs living in kennels under standardized conditions. Genotype-environment interactions have been described for several traits in many species (Lillehammer, 2008). Genotype-environment interactions are considered important when individuals of a population are reared under different conditions (Falconer & Mackay, 1996) such as the dogs in our cohort, and might also contribute to the observed high litter variance.

All Norwegian breeders of the 4 breeds in this study were offered to participate in the *main study* during the inclusion period, and there were no restrictions regarding household or environment. In Norway the NKK registered population is believed to include more than 90% of the purebred dogs. Of the total registered population of dogs in NKK the *main study* included 36% of NF litters, 7.6% of LR litters, 41.3% of LEO litters, and 66.7% of IW litters born in Norway during the sampling period. The current study sample consists of approximately 80% of the dogs included in the *main study*. Thus, the study is a large scale prospective cohort study of dogs living in private homes. Cohort studies are generally considered to have high relevance to real-world situations and a high external validity (Dohoo et al., 2009). However, the study included only purebred dogs of large size, and the results derived from the study might not be relevant for smaller breeds. The inclusion criterion of radiological CHD screening might be a source of selection bias. If dog owners who screen their dog have different attitudes than dog owners who do not, and if the probability of being screened also varied across the levels of other factors of interest such as breed, then a bias could occur. However, the most common cause for not screening was death of the dog at a young age. High socio-economic status can increase willingness to participate in observational studies (Dohoo et al., 2009) and if the socio-economic status of the dog owners is associated with both the dogs' exposures and outcome then a selection bias could be present. Other sources of selection bias are non-response and loss to follow-up. Strategies aimed at reducing these sources of bias were getting the owners to sign contracts, conducting regular follow-ups during the study, and contacting non-responders by telephone. The most common cause of non-response was death of the dog.

Misclassification of the outcome might be a source of bias in this study. The standard hip-extended radiographic projection was the basis for the outcome variable. The degree of hip joint laxity cannot always be evaluated reliably using this method and false negative diagnoses can occur although breed differences are present (Farese et al., 1998; Flückiger et al., 1999; Kapatkin et al., 2004; Powers et al., 2010; Smith et al., 1990; Smith et al., 1993). The low sensitivity can be a source of non-differential misclassification, and if the number of false negative diagnoses also varies across predictors of interest, e.g. breed, then differential misclassification can occur. False negative CHD diagnoses have been found in this cohort of dogs (Krontveit et al., 2012) and these findings are in line with several other studies (Farese et al., 1998; Flückiger et al., 1999; Kapatkin et al., 2004; Powers et al., 2010; Smith et al., 1990; Smith et al., 1993). The vast majority of the hip radiographs in the present study were, however, graded by a single panelist and this reduces the inter-observer variation and thus some of the misclassification bias (Verhoeven et al., 2009). The age of the dog at screening can influence the screening result. Older dogs tend to get more severe grades than younger dogs because secondary changes in the hip joint are more common with increasing age (Hou et al., 2010; Leppanen et al., 2000; Maki et al., 2000; Ohlerth et al., 1998; Swenson et al., 1997; Wood &

Lakhani, 2003). The NF and LEO breeds were 6 months older when they were screened, and this might be another source of differential misclassification.

In conclusion, it appears that access to varied exercise (off-leash in parks and living in rural housing) has a protective effect against CHD development among these large breed dogs in Norway. The protective effect of higher BW at 3 months of age can be speculated to be a result of increased muscle mass which is beneficial for hip joint development. We propose that the findings might be generalized to similar breeds in other regions, and could be used for primary prevention of CHD in the future.

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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, The Netherlands	van Klink
2002	Cambridge	Wood & Newton
2003	Warwick	Green
2004	Martigny, Switzerland	Stärk
2005	Nairn	Gunn
2006	Exeter	Peeler
2007	Dipoli, Finland	Virtala & Alban
2008	Liverpool	Pinchbeck & Robinson
2009	London	Verheyen & Pfeiffer
2010	Nantes, France	Fourichon & Hoch
2011	Leipzig, Germany	Thulke & Lange
2012	Glasgow	Parkin & Others

PAST PRESIDENTS

1982-'83	G. Davies
1983-'84	P.R. Ellis
1984-'85	G. Gettinby
1985-'86	R.W.J. Plenderleith
1986-'87	M.V. Thrusfield
1987-'88	K.S. Howe
1988-'89	A.M. Russell
1989-'90	S.G. McIlroy
1990-'91	J.E.T. Jones
1991-'92	J.M. Booth
1992-'93	E.A. Goodall
1993-'94	R.G. Eddy
1994-'95	J.T. Done
1995-'96	G.J. Gunn
1996-'97	M.S. Richards
1997-'98	J.D. Collins
1998-'99	F.D. Menzies
1999-'00	K.L. Morgan
2000-'01	S.W.J. Reid
2001-'02	A.D. Paterson
2002-'03	L.E. Green
2003-'04	J.L.N. Wood
2004-'05	E.G.M. van Klink
2005-'06	D.J. Mellor
2006-'07	E. J. Peeler
2007-'08	J. R Newton
2008-'09	L. Alban
2009-'10	D.U. Pfeiffer
2010-'11	L.A. Kelly

EXECUTIVE COMMITTEE 2011-2012

C. Fourichon (President), L. A. Kelly (Senior Vice- President), T.D.H. Parkin (Junior Vice-President), K. Mintiens (Honorary Secretary), K. Verheyen (Honorary Treasurer), A. Lindberg, L. Rosenbaum Nielsen, H-H Thulke, M. Brennan, T. Martinez (co-opted), and Beatriz Martinez-Lopez (co-opted)

Honorary Auditors: Dominic Mellor & Fraser Menzies

LIFE MEMBERS

J.M. Booth, M.J. Clarkson, J.D Collins, G. Davies, J.T. Done, R.G. Eddy, P.R. Ellis, E.A. Goodall, G. Gettinby, K.S. Howe, M.E. Hugh-Jones, W. Martin, F. Menzies, A.M. Russell, M.V. Thrusfield, J. Wilesmith

PLENARY TALKS

Year	Gareth Davies Lecture	Conference Opening Plenary
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 s From science to policy - the case of classical swine fever (CSF) control	Dominic Mellor The trouble with epidemiology: the tyranny of numbers
2010	David Waltner-Toews Beyond one world, one health and ecohealth…what's out there?	James Wood From pathogen adaption to host ecology: epidemiological and experimental contributions to the understanding of emerging infectious diseases
2009	Jørgen Westergaard The interaction between veterinary science, legistlation and management in animal disease control in the European Union	Katharina Stärk Food safety challenges in a global market – are we ready?
2008	Paul Fine Infectious disease eradication – meanings and implications	Kenton Morgan For the benefit of Mr Kite
2007	Yrjö Gröhn Food supply veterinary medicine: Modelling of production, health and food safety	Laura Green Improving Animal Health
2006	David Galligan From partial budgets to real options - concepts in animal health economics	Nigel French Understanding human exposure to zoonoses from food and the environment: The application of molecular tools and modeling
2005	Bill Reilly: From TB to VTEC: The changing epidemiology of foodborne zoonoses	Simon More: Towards eradication of bovine tuberculosis in Ireland: A 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 web site, http://www.svepm.org.uk/, or from the Secretary or Treasurer.

Please send this form to the Society's Treasurer:

Dr Kristien Verheyen
The Royal Veterinary College
Hawkshead Lane
North Mymms
Hatfield
Herts, AL9 7TA
UK

TEL +44 (0) 1707 666 625
FAX +44 (0) 1707 666 574
Email: kverheyen@rvc.ac.uk

Please turn over

INTEREST GROUPS

Please tick appropriate boxes to indicate your interests:

- ☐ Analytical Epidemiology (Observational Studies)
- ☐ Quantitative Epidemiology & Statistical Techniques (Incl. Statistical/Mathematical Modelling)
- ☐ Herd/Flock Level Disease Control Strategies
- ☐ National/International Disease Control Policy
- ☐ Sero-Epidemiology
- ☐ Herd Health and Productivity Systems
- ☐ Disease Nomenclature and Epidemiological Terminology
- ☐ Economic Effects of Disease on Animal Production
- ☐ Veterinary Public Health and Food Hygiene
- ☐ Computing, including data logging
- ☐ Computer Programming *per se*
- ☐ Population and Animal Disease Databases
- ☐ Information System Design
- ☐ Geographical Information Systems (GIS)
- ☐ Risk Analysis

CONSTITUTION AND RULES

NAME

1. The society will be named the Society for Veterinary Epidemiology and Preventive Medicine.

OBJECTS

2. The objects of the Society will be to promote veterinary epidemiology and preventive medicine.

MEMBERSHIP

3. Membership will be open to persons either actively engaged or interested in veterinary epidemiology and preventive medicine.
4. Membership is conditional on the return to the Secretary of a completed application form and subscription equivalent to the rate for one calendar year. Subsequent subscriptions fall due on the first day of May each year.
5. Non-payment of subscription for six months will be interpreted as resignation from the Society.

OFFICERS OF THE SOCIETY

6. The Officers of the Society will be President, Senior Vice-President, Junior Vice-President, Honorary Secretary and Honorary Treasurer. Officers will be elected annually at the Annual General Meeting, with the exception of the President and Senior Vice-President who will assume office. No officer can continue in the same office for longer than six years.

COMMITTEE

7. The Executive Committee of the Society normally will comprise the officers of the Society and not more than four ordinary elected members. However, the Committee will have powers of co-option.

ELECTION

8. The election of office bearers and ordinary committee members will take place at the Annual General Meeting. Ordinary members of the Executive Committee will be elected for a period of three years. Retiring members of the Executive Committee will be eligible for re-election. Members will receive nomination forms with notification of the Annual General Meeting. Completed nomination forms, including the signatures of a proposer, seconder, and the nominee, will be returned to the Secretary at least 21 days before the date of the Annual General Meeting. Unless a nomination is unopposed, election will be by secret ballot at the Annual General Meeting. Only in the event of there being no nomination for any vacant post will the Chairman take nominations at the Annual General Meeting. Tellers will be appointed by unanimous agreement of the Annual General Meeting.

FINANCE

9. An annual subscription will be paid by each member in advance on the first day of May each year. The amount will be decided at the annual general meeting and will be decided by a simple majority vote of members present at the Annual General Meeting.

10. The Honorary Treasurer will receive, for the use of the Society, all monies payable to it and from such monies will pay all sums payable by the Society. He will keep account of all such receipts and payments in a manner directed by the Executive Committee. All monies received by the Society will be paid into such a bank as may be decided by the Executive Committee of the Society and in the name of the Society. All cheques will be signed by either the Honorary Treasurer or the Honorary Secretary.
11. Two auditors will be appointed annually by members at the Annual General Meeting. The audited accounts and balance sheet will be circulated to members with the notice concerning the Annual General Meeting and will be presented to the meeting.

MEETINGS

12. Ordinary general meetings of the Society will be held at such a time as the Executive Committee may decide on the recommendations of members. The Annual General Meeting will be held in conjunction with an ordinary general meeting.

GUESTS

13. Members may invite non-members to ordinary general meetings.

PUBLICATION

14. The proceedings of the meetings of the Society will not be reported either in part or in whole without the written permission of the Executive Committee.
15. The Society may produce publications at the discretion of the Executive Committee.

GENERAL

16. All meetings will be convened by notice at least 21 days before the meeting.
17. The President will preside at all general and executive meetings or, in his absence, the Senior Vice-President or, in his absence, the Junior Vice-President or, in his absence, the Honorary Secretary or, in his absence, the Honorary Treasurer. Failing any of these, the members present will elect one of their number to preside as Chairman.
18. The conduct of all business transacted will be under the control of the Chairman, to whom all remarks must be addressed and whose ruling on a point of order, or on the admissibility of an explanation, will be final and will not be open to discussion at the meeting at which it is delivered. However, this rule will not preclude any member from raising any question upon the ruling of the chair by notice of motion.
19. In case of an equal division of votes, the Chairman of the meeting will have a second and casting vote.
20. All members on election will be supplied with a copy of this constitution.
21. No alteration will be made to these rules except by a two-thirds majority of those members voting at an annual general meeting of the Society, and then only if notice of intention to alter the constitution concerned will have appeared in the notice convening the meeting. A quorum will constitute twenty per cent of members.
22. Any matter not provided for in this constitution will be dealt with at the discretion of the Executive Committee.

April, 1982
Revised March, 1985; April, 1988; November 1994
Corrected January 1997; April 2002