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ECONOMICS 2

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ECONOMICS 1

EPIDEMIOLOGICAL AND ECONOMIC ASSESSMENT OF DIFFERENT CONTROL STRATEGIES AGAINST NEOSPORA CANINUM IN DAIRY CATTLE IN SWITZERLAND

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SUMMARY

Mathematical and economic models were used to assess the epidemiological and economic impact of the control strategies 'testing and culling of seropositive animals', 'discontinued breeding from offspring of seropositive cows', 'chemotherapy of calves' and 'vaccination of susceptible and infected animals' on the *Neospora caninum* prevalence in Swiss dairy cattle. Each strategy considered different sub-scenarios with regard to the frequency of diagnostic testing and the animal group under consideration. A dynamic deterministic 'Susceptible-Infected' model was developed to simulate the impact of the different control strategies on the prevalence in the population. The results of this model were then used as inputs in @Risk spreadsheet models to estimate the direct disease costs, the implementation costs and the cost-effectiveness of the strategies mentioned. The median current annual losses due to *N. caninum* in the Swiss dairy cattle population were estimated to be 13 million Swiss Francs (CHF). Several policies reduced prevalence effectively and rapidly. However, economic analyses revealed that only two control strategies were beneficial: 'chemotherapy of all female calves' and 'discontinued breeding from offspring of seropositive cows', which at present would be the preferential control strategy.

INTRODUCTION

Neospora caninum, a protozoan parasite, is one of the most important infectious causes of abortion in cattle worldwide. The major route of transmission involves reactivation of a persistent infection and subsequent transplacental invasion of the embryo (=vertical or endogenous infection mode) (Pare et al., 1996; Schares et al., 1998). The transplacental infection may provoke abortion, although in most cases a calf without clinical symptoms is born and harbours the parasite for its whole life (Pare et al., 1996; Thurmond and Hietala, 1997). Cattle can also become infected postnatally by horizontal transmission (=exogenous transmission mode) (McAllister et al., 1996; Hietala and Thurmond, 1999).

Since 2001 *N. caninum* has been registered as a notifiable disease in Switzerland, but so far, no national control program for *N. caninum* has been set up. As resources for national control programs are limited, it is vital to understand the population dynamics and economic consequences of control options fully to support the decision making process of policy makers. *N. caninum* infection in a cattle population can be influenced by different control measures that are applicable now or potentially in the future: culling of infected animals, selective breeding in

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infected herds, chemotherapy and vaccination. Little information is available on the metaphylactic use of drugs to address the problem, but previous studies delivered promising results (Kritzner et al., 2002; Gottstein et al., 2005). A commercial, killed protozoan vaccine for use in cattle (NeoGuard™ and Bovilis Neoguard®, www.intervet.com) is available in various countries, but its efficacy has been questioned (Andrianarivo et al., 2000; Heuer et al., 2004; Romero et al., 2004). In Europe and Switzerland, neither vaccination nor chemotherapy have been assessed reliably and validated for field application. Throughout the world, *N. caninum* causes substantial economic losses in cattle attributable directly to abortion (Anderson et al., 1991), reduced milk yield (Hernandez et al., 2001), premature culling (Thurmond & Hietala, 1996) and reduced postweaning weight gain in beef calves (Barling et al., 2000).

The present study aimed to assess the epidemiological and economic impact of different control strategies in the female Swiss dairy cattle population. Firstly, the impact of the following control strategies on the prevalence of *N. caninum* was evaluated:

i. Testing and culling of seropositive cattle

ii. Discontinued breeding from offspring of seropositive cows

iii. Chemotherapy of calves from seropositive cows

iv. Vaccination of susceptible and infected cattle.

Secondly, the results were used as inputs in a spreadsheet model, which aimed (a) to calculate the current losses related to *N. caninum* in dairy cattle in Switzerland and (b) to assess the costs and benefits of selected control strategies against *N. caninum* in Switzerland to permit comparison.

MATERIALS AND METHODS

The epidemiological and economic simulation models were based on available demographic and seroprevalence data from Switzerland, supplemented by data from published literature. The study population included all female Swiss dairy cattle. Figures on the total number of dairy cattle in Switzerland, pregnancy, birth and culling rates were derived from livestock demographic data obtained from the Swiss Animal Movement Database (TVD). Various input parameters such as milk yield, milk price, slaughter weights, market values of cattle, veterinary service costs, etc. were gathered from different sources ('Swiss Milk Statistics', Swiss Federal Statistical Office, Swiss Farmers' Union, Society of Swiss Veterinarians, Institute of Parasitology, University of Bern).

All models were run in time steps of one year and with a constant population size over a time period of 25 years using the modeling software Vensim© Professional32 Version 5.4a (Ventana Systems, Inc., Harvard, USA) and the @Risk software for Excel version 4.5 (Palisade Corporation, Newfield, NY, USA).

Part 1: the epidemiological model

The baseline scenario (model 1; status quo without control measures): Twelve age classes of one year each were set up (0 to <1; 1 to <2; 2 to <3; …10 to <11; ≥11years), each defined by its own set of pregnancy and culling rates. There is no evidence for the existence of animals which have recovered from infection (either remaining seropositive or reverting to seronegative), or for naturally immune seronegative animals. Such classes have been omitted, therefore, and the model was restricted to susceptible (S) and infected animals (I).

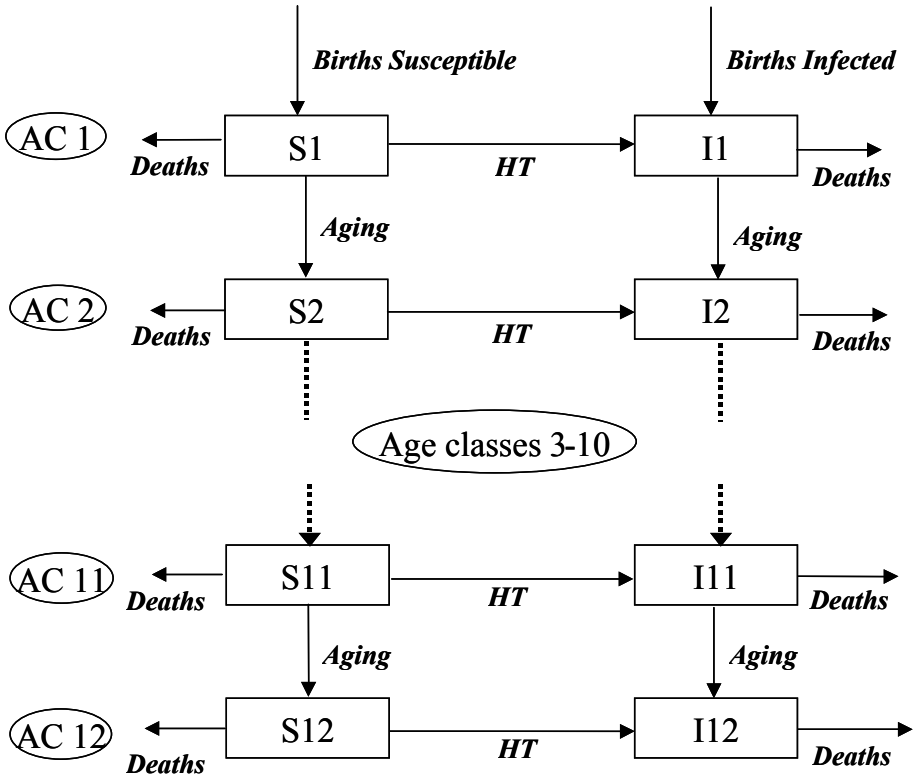

Fig. 1 Reference model for the simulation of *N. caninum* infection dynamics in the Swiss dairy cattle population. AC=age class, HT=Horizontal transmission, I=Infected, S=Susceptible

Figure 1 shows the structure of the baseline model. The boxes S1 to S12 represent the compartments of the susceptible, uninfected, seronegative animals by age class. The boxes I1 to I12 represent the compartments of the infected, seropositive animals by age class. The initial values for each age class were derived from the TVD data, assuming an endemic seroprevalence of 12% (Gottstein et al., 1998; Sager et al., 2001).

The number of births per age class and time step of susceptible and infected animals was calculated by subtracting the abortion rate of susceptible and infected animals from the pregnancy rate and multiplying the result by the number of susceptible or infected animals of the respective age class. All births from each age class flowed either into the first compartment of susceptible animals, *S1*, or into the first compartment of infected animals, *I1*. Vertical transmission from an infected cow to its calf was accommodated by multiplying the total number of births among infected animals, *BI*, by a vertical transmission factor *vtf* (baseline value=0.9). Flows into the S1 compartment were the calves of all susceptible cows, *BS*, plus the uninfected calves of infected mothers, *BI*(1-vtf)*. Flows into the I1 compartment were the infected calves of infected cows, *BI*vtf*, plus the horizontally infected animals from S1.

The age class-specific culling rates, cr_X, were the rates per time step at which animals were removed from the compartments. The culling rates of the infected compartments and the last susceptible compartment were set to constant. Assuming a stable population size, the culling rates of the susceptible compartments were allowed to vary depending on the constant inflows and outflows of the model, i.e. the total number of births and the deaths of all infected compartments and the last susceptible compartment.

The horizontal transmission process, *HT*, from susceptible to infected cattle was modeled using two independent parameters that determined the rate at which susceptible individuals became infected. The first was a prevalence dependent factor, *pf*, which was multiplied by the prevalence in the adult population. The second, *uf*, accounted for the per capita force of infection from unknown transmission factors such as putative *N. caninum* cycles in wildlife. Iterative sensitivity testing was performed to determine the values of *uf* and *pf* that allowed a steady state seroprevalence of 12% (*pf*=0.028; *uf* =0.001181) to be reached.

To account for the aging process in the population, in each time step susceptible or infected animals that were not horizontally infected or culled moved over to the next age class.

Simulation of control strategies: The steady state of the baseline model was chosen as the starting point for all intervention strategies. Various variables in the model were modified to assess the impact of the control strategy on the endemic seroprevalence of 12%.

In the test-and-cull model (model 2), four sub-scenarios were considered. Moreover, with regard to the economic assessment of the control strategies, certain sub-scenarios were modeled with a more and less extensive serological testing procedure: (2a) culling of all infected, seropositive animals with (2ai) and without (2aii) yearly serological testing; (2b) culling of all infected, seropositive fertile animals with (2bi) and without (2bii) yearly serological testing; (2c) culling of all infected, seropositive animals that aborted for any reason; (2d) culling of all infected, seropositive animals that experienced an *N. caninum*-induced abortion. For sub-scenarios 2a and 2b, the culling rates of the animals that tested seropositive were set to 1. In sub-scenario 2c, only the seropositive cows that aborted were removed from the population. In sub-scenario 2d, only the cows that experienced an *N. caninum*-induced abortion were eliminated.

In the discontinued breeding from offspring model (model 3), three different sub-scenarios were considered: (3a) no breeding from offspring of all infected, seropositive animals with (3ai) and without (3aii) yearly serological testing; (3b) no breeding from offspring of infected, seropositive animals that aborted for any reason; (3c) no breeding from offspring of infected, seropositive animals that experienced a *N. caninum*-induced abortion. These sub-scenarios were simulated by excluding from breeding the animals that were tested seropositive (birth rate=0).

In the chemotherapy model (model 4), two possible options were considered: (4a) chemotherapy of newborn calves from infected, seropositive cows with (4ai) and without (4aii) yearly serological testing; (4b) chemotherapy of all newborn calves in the population, irrespective of the serological status of their dams (i.e. without any serological testing). For sub-scenario 4ai and 4aii, all calves from seropositive cows were chemotherapeutically treated and the flows of calves into the compartments S1 and I1 were modified to account for the efficacy of medication (baseline value=0.6; hypothetical value). In sub-scenario 4b, all calves were treated yearly without previously testing the cows.

In the vaccination model (model 5), the effect of a *N. caninum*-vaccination strategy in Switzerland was assessed on the assumption that a potential vaccine is able to prevent abortions caused by *N. caninum* in cattle and to reduce vertical and horizontal transmission in vaccinated animals to some degree. The protocol implied annual vaccination of all susceptible and infected cattle in the population without previous serological testing. Assumptions were made on the efficacy of the vaccine in preventing *N. caninum* abortion (baseline value=0.6), on the efficacy of the vaccine in preventing horizontal transmission (baseline value=0.7) and on the probability of vertical transmission in vaccinated infected adult cattle (baseline value=0.4).

Part 2: the economic model

The prevalence data and the number of infected and susceptible animals of the strategies that showed a decrease in prevalence of at least 2% over the whole simulation period, i.e. 2ai, 2aii, 2bi, 2bii, 2c, 3ai, 3aii, 4ai, 4aii, 4b and 5, were transferred to the economic spreadsheet models.

Simulation model to calculate the overall losses caused by *N. caninum*: The reproductive losses included the losses due to abortion and the related veterinary treatment costs. The total abortion loss in the population per year and scenario was calculated by multiplying the abortion rate by the number of affected animals and the loss per abortion. For each abortion, the cost of the veterinary service and the therapy cost were taken into account. The veterinary treatment cost was the product of abortion rate, number of affected animals and veterinary service plus therapy and insemination costs.

The losses due to reduced milk yield (ML) were calculated relying on previous studies which examined the effect of *N. caninum* seropositivity on the milk production in dairy cows. To account for the uncertainty of the parameter 'rate of reduced milk yield' (rmy) in infected cattle, a Pert probability distribution was defined (min=0, most likely=0.02, max=0.04). The ML were then $N*P*my*p_{milk}*rmy$, where N is the number of cows, P the prevalence, my the milk yield and p_{milk} the milk price.

The losses due to premature culling (PCL) were also determined according to available literature. To account for the uncertainty of the parameter 'rate of premature culling' (rpc), a Pert probability distribution was defined (min=0, most likely=0.008, max=0.016). The PCL were then calculated by multiplying the number of animals that were prematurely culled by the replacement cost. The replacement cost was the market value minus the slaughter value of the animal.

Simulation model to calculate the implementation costs of putative control strategies: The costs of each control strategy were calculated by estimating the sampling, laboratory, replacement, medication and vaccination costs using the input parameters listed in Table 1.

The test-and-cull strategy required the testing of all animals of a specified age group. In scenario 2a and 2b, where all animals (cows + heifers + female offspring=C+H+O) or all adult animals (C+H), respectively, were tested, all dairy farms had to be visited to take the blood samples. The SC was the number of dairy farms multiplied by the price of a veterinary visit (vt_{mass}) plus the product of the animals in the population (C+H+O) and the price of the blood sampling (bs_{mass}). The LC was calculated by multiplying the price of the serological test (lt) by the number of animals sampled. The cattle that had to be culled were all seropositive animals. The RC resulted from the difference between the slaughter value and the market value of the

culled animals. In scenario 2c, only the seropositive, aborting cows were culled. Thus, only the farms with abortions were visited, and the rates for individual sampling, rather than for mass sampling, were applied.

Table 1. Input parameters used to calculate the implementation costs per control strategy

Cost	Input parameter	[CHF]
Sampling cost SC	Veterinary visit in case of mass sampling (vt_{mass})	28
	Veterinary visit in case of individual sampling (vt_{ind})	32
	Blood sampling in case of mass sampling (bs_{mass})	8.5
	Blood sampling in case of individual sampling (bs_{ind})	16
Laboratory cost LC	Serological examination of one sample (lt)	28
Replacement cost RC	Market value-slaughter value	
Medication cost MC	Price/dose (oral administration), p_{drug}	6
Vaccination cost VC	Price/vaccine (plus injection), p_{vacc}	8

The SC and LC in scenario 3a of the discontinued breeding from offspring model were calculated as in the test-and-cull strategy. The calculation of the RC was more complex. In the first year of the control program, the replacement cost of a calf was defined as the difference between the market value of a dairy offspring (o_{dairy}) and the slaughter value of a dairy offspring (so_{dairy}). Assuming that in the following years a seropositive cow would be inseminated with a beef rather than a dairy breed, the replacement cost for years 2 to 4 would be the difference between the market value of a dairy calf and the slaughter value of a crossbred offspring (so_{cross}). After four years of the control programme, the farm management would have adapted to the new situation and the RC would decrease to zero.

The LC and SC for scenario 4a of the chemotherapy model were calculated as above. In addition, the MC for the treatment of female calves from seropositive mothers was calculated. In scenario 4a, the number of female offspring from seropositive cows was multiplied by the price of the drug (p_{drug}) and the number of administered doses (n_{drug}), which was defined as a Pert probability distribution (min=1, most likely=2, max=3). In scenario 4b, the cows were not tested but all the female offspring were treated (only MC considered).

In the vaccination model, all female dairy cattle were vaccinated without previous testing. As the vaccine was administered by a veterinarian, not only p_{vacc}, but also the veterinary visit (vt_{ind}) was accounted for. All dairy farms were visited once per vaccination round. The number of vaccinated animals was obtained by multiplying the number of cattle by the vaccination coverage ($cge=0.9$), i.e. the proportion of cattle covered by a national vaccination program. The number of vaccinated animals was multiplied by p_{vacc}. The result was multiplied by the number of administered doses per year (n_{vacc}=2).

Cost benefit analysis of control programs: In a final step, the benefits (=prevented losses of a control scenario in comparison to the baseline scenario) and costs per control strategy were compared by calculating the benefit-cost ratio (BCR) and the net present value (NPV) over a time period of 25 years and with a discount rate of 3%.

RESULTS

Part 1: Simulation of control measures

There was no relevant difference between the more and less extensive testing options i and ii per scenario. A policy of culling all seropositive animals (sub-scenarios 2ai and 2aii) or seropositive fertile animals in the population (2bi and 2bii) was very effective as it rapidly reduced the prevalence from 12% to <4% in the first year of simulation, after which prevalence declined steadily until it came to endemic levels of <1%. Culling all seropositive, aborting animals (2c) was clearly less effective and prevalence declined to only 5.8% at the end of the simulation period. With an end prevalence of 10.4 % the policy of culling seropositive animals that had experienced a *N. caninum*-abortion (2d) was the least effective culling strategy.

A policy of discontinued breeding from offspring of infected, seropositive cows (3ai and 3aii) had a slower impact on the prevalence when compared with the most effective culling scenarios, but it also reduced the prevalence of infection in the population to a comparable level (<2% at the end of the simulation period). The effect of the strategies of not breeding from offspring of seropositive aborting (3b) and seropositive *Neospora* aborting (3c) animals did not lead to a marked reduction in the prevalence.

A policy of chemotherapy of calves from seropositive dams (4ai and 4aii) or of all calves without previously testing the dams (4b) reduced the seroprevalence of *N. caninum* more slowly than culling and discontinued breeding from offspring, but still showed a useful decline in prevalence over time (<4% after 15 years of simulation).

A policy of vaccinating all animals in the population was very effective. Prevalence decreased rapidly from 12% to 2% in the first three years and then declined steadily to 0.2% by year 25 of the simulation.

Part 2: Economic model

Losses due to *N. caninum* without control strategy: Table 2 presents the losses due to *N. caninum* in the Swiss dairy cow population for the situation of endemic equilibrium (seroprevalence = 12%). The total losses were about 13 million Swiss Francs (CHF) per year. The major part of the total loss was caused by reduced milk yield and abortion, whereas the veterinary and premature culling cost accounted for less.

Table 2: Losses due to *N. caninum* in the Swiss dairy cattle population (seroprevalence 12%).

LOSS TYPE	MEDIAN LOSSES [1000CHF]	95% CI
Abortion	2960	(1616; 4308)
Veterinary service	406	(356; 457)
Reduced milk yield	9197	(2649; 17216)
Premature culling	796	(189; 1772)
Total	13403	(6634; 21572)

Economic outcomes of the different control strategies: Table 3 shows the discounted costs, discounted benefits, BCRs and NPVs per control strategy. The control strategies testing and culling of seropositive (2a) and seropositive fertile (2b) cattle and vaccination (5) had the

highest benefits. The control strategy of discontinued breeding from offspring of seropositive cows (3a) gave moderate benefits, whereas the control strategies of testing and culling seropositive aborted cows (2c) and chemotherapy of calves (4ai, 4aii, 4b) produced the lowest benefits. The scenarios with the more extensive testing (2ai, 2bi, 3ai and 4ai) had the highest total discounted costs of all control strategies, whereas the less extensive testing (2aii, 2bii, 3aii, 4aii) produced only moderate costs. The scenarios with the lowest costs were scenarios 4b and 2c. With a discount rate of 3%, only the control strategies 3aii and 4b had a BCR greater than 1 and a positive NPV.

Table 3: Summary results of different control strategies against *N. caninum* in the Swiss dairy cattle population: The median (M) total discounted benefits and costs, the net present value (NPV) and the benefit cost ratio (BCR) with their 95% confidence limits.

	DISCOUNTED BENEFITS [MILLION CHF]		DISCOUNTED COSTS [MILLION CHF]		NPV [MILLION CHF]		BCR	
CS	M	95% CI	M	95% CI	M	95% CI	M	95% CI
2ai[a]	227	(198; 258)	1193	(896; 1691)	-965	(-1463; -665)	0.19	(0.13; 0.26)
2aii[a]	216	(186; 247)	303	(234; 376)	-87	(-165; -11)	0.71	(0.55; 0.95)
2bi[b]	211	(182; 242)	980	(670; 1489)	-770	(-1276; -456)	0.21	(0.14; 0.33)
2bii[b]	214	(184; 244)	322	(244; 422)	-109	(-212; -24)	0.66	(0.49; 0.90)
2c[c]	66	(30; 102)	96	(53; 139)	-29	(-85; 26)	0.69	(0.29; 1.44)
3ai[d]	159	(126; 193)	468	(451; 481)	-308	(-344; -270)	0.34	(0.27; 0.41)
3aii[d]	152	(120; 186)	133	(121; 145)	20	(-16; 55)	1.15	(0.89; 1.43)
4ai[e]	121	(86; 157)	468	(461; 475)	-347	(-383; - 311)	0.26	(0.18; 0.33)
4aii[e]	115	(80; 150)	145	(144; 146)	-30	(-65; 6)	0.79	(0.55; 1.04)
4b[e]	125	(91; 160)	48	(31; 65)	77	(39; 116)	2.62	(1.68; 4.32)
5[f]	210	(181; 241)	363	(347; 379)	-153	(-188; -117)	0.58	(0.49; 0.67)

[a] Testing and culling of all seropositive cattle with and without yearly serological testing
[b] Testing and culling of seropositive, fertile cattle with and without yearly serological testing
[c] Culling of seropositive aborted cows
[d] Discontinued breeding from offspring of seropositive cows with and without yearly serological testing
[e] Chemotherapy of offspring from seropositive cows with and without yearly serological testing
[f] Vaccination of female susceptible and infected animals

DISCUSSION

Model limitations

An important issue to consider when studying *Neospora* control strategies is the quality of the input parameters used. One major limitation of the epidemiological model was the lack of reliable data concerning the horizontal transmission process. A more specific model for long-term simulations would include the life cycle of *N. caninum* in a definitive host population. In a previous study conducted in Switzerland, 3,289 fecal samples from 249 dogs were examined for the presence of *N. caninum* oocysts. *Hammondia*/*Neospora*-like-organisms were detected in 25 samples of 24 dogs, but the presence of *N. caninum* DNA could not be confirmed by PCR (Sager et al., 2005). These results indicate that the importance of horizontal infection through dogs in Switzerland is low. Another uncertainty of the present study was the lack of reliable data

concerning the control strategies of medication and vaccination. A range of assumptions was made regarding the efficacy, the protocol and the price of vaccination and medication. To estimate better the epidemiological and economic effects of such control strategies on *N. caninum*, improved knowledge about the mechanisms of such substances is needed.

Current losses due to *N. caninum* in the Swiss dairy cattle population

The losses due to *N. caninum* in Switzerland have been shown to be about CHF 13 million per year, assuming an endemic seroprevalence of 12%. This is consistent with the losses estimated for New Zealand (CHF 14 million), with 900,000 cows at risk (Pfeiffer et al., 1997) and with those for the Netherlands (CHF 30 million), with 1.5 million dairy cows (Hogeveen et al., 2003). The results of the simulations strongly support the widespread view that *N. caninum* abortions are economically detrimental to the Swiss dairy industry.

Control strategies

Different control strategies reduced the prevalence quickly and effectively. However, only two strategies were shown to be economically interesting. Comparison of the cost and benefits of each control strategy revealed that yearly testing of all animals in the population was not a viable approach. For each control strategy, it yielded benefit-cost ratios (BCR) smaller than 1 and negative net present values (NPV), whereas less extensive options all resulted in clearly better BCRs and NPVs.

The strategies of culling all seropositive and seropositive fertile cattle were very effective in reducing the prevalence in the population quickly. As the horizontal transmission factor was defined not only by a prevalence-dependent factor, but also by an unknown factor, which was a constant, age-class independent per capita force of infection, the prevalence did not decline to zero, which is consistent with the findings of French (French et al., 1999). To eradicate the disease in the population completely, it would be necessary to know the complete life cycle of *N. caninum* and to control the horizontal transmission process as well. The results of the economic analysis demonstrated that none of the test-and-cull strategies was beneficial.

The policy of keeping infected cattle in a herd but ceasing to breed from their offspring was shown to be effective in reducing the prevalence of infection. However, such a policy would not be as effective as culling because it would block only vertical transmission and infected, fertile animals could still form part of the horizontal transmission cycle. The economic assessment revealed that a policy of discontinued breeding from offspring of infected cattle was a viable strategy. The major advantage of this control strategy would be that the cows remained in the population and so no clinically healthy animal was culled. Moreover, the genome of seropositive animals of high genetic merit could be preserved by using embryo transfer as described in several studies (Baillargeon et al., 2001; Landmann et al., 2002; Campero et al., 2003).

The chemotherapy of calves of infected cows with a putative new drug was shown to reduce prevalence efficiently over time. Only the strategy ‘chemotherapy of all calves in the population’ (without previously testing the dams) was shown to be economically beneficial. Overall, it was the control strategy with the highest BCR and NPV and the lowest costs. The costs were minimal because it was assumed that all offspring were treated and drugs were administered by the farmer. However, chemotherapy of calves would be successful only if the drug were efficacious. As in the discontinued offspring model, only the vertical transmission can

be blocked, and cows could still be part of the horizontal transmission cycle. One objection against this method may be that healthy offspring would be treated also.

A useful vaccine against *N. caninum* would preferably reduce abortion and both horizontal and vertical transmission. With the hypothetical efficacies assumed for this strategy, prevalence was reduced rapidly. There were considerable vaccination costs comprising the vaccine and the veterinary costs. These costs exceeded the benefits substantially, i.e. economically, vaccination was not a recommendable strategy. The situation would, however, be different if vaccination could be applied by farmers themselves.

Conclusions

For the Swiss dairy producers, an estimated loss of CHF 13 millions per year attributable to *N. caninum* is considered to be economically important. Various strategies were shown to reduce prevalence quickly and efficiently. However, none of the modeled intervention strategies was able to bring the prevalence to zero as long as the horizontal transmission was not controlled. As long as the horizontal transmission process is not fully understood, it is advisable to take hygienic measures to minimize the risk of infection by dogs. In terms of viability, the following two control strategies revealed benefit cost ratios above 1 and positive net present values: (1) discontinued breeding from offspring of seropositive cows, and (2) chemotherapy of all offspring. As there is no suitable medication currently on the market, the control strategy to be followed at present would be 'discontinued breeding strategy'. However, because medication produces the best BCR, it might be worth delaying a national *Neospora* control campaign until more specific data about the medication (efficacy, market release) is available.

The availability of more reliable data concerning the epidemiology of the disease, as well as the efficacy of medication and vaccination would increase the accuracy of the predictions of the model. Future research should focus on the efficacy of medication and vaccination and the disease dynamics in an end host population.

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STOCHASTIC SIMULATION OF A MILK QUALITY ASSURANCE PROGRAMME FOR PARATUBERCULOSIS: WITHIN-HERD INFECTION DYNAMICS AND ECONOMICS

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SUMMARY

A milk quality assurance programme for *Mycobacterium avium* subsp. *paratuberculosis* (Map) in dairy herds was simulated with a stochastic simulation model. Herds were certified as 'low-Map bulk milk' if, with a certain probability, the concentration of Map in bulk milk did not exceed a maximum acceptable concentration (MAC; based on pasteurisation studies). The programme started with an initial assessment; test-negative herds entered a surveillance procedure and test-positive herds a control procedure. The aim of this study was to evaluate the epidemiological and economic effects of various test schemes and preventive management measures in a simulated population of closed dairy herds.

The simulations showed that herd examinations by ELISA effectively ensure the quality of 'low-Map bulk milk': >96% of certified herds were below the MAC. Preventive management measures considerably increased the number of 'low-Map bulk milk' herds. Culling based on biennial faecal culture was more effective than culling based on annual ELISA. Average total discounted costs for 20-year participation in a programme consisting of initial assessment by ELISA, surveillance by biennial ELISA and control by biennial faecal culture were €6,000 per herd. On average, additional preventive measures increased these costs to €40,000 per herd.

This study showed that a bulk milk quality assurance programme for closed dairy herds is feasible, and provided information on the cost-effectiveness of different programmes.

INTRODUCTION

Mycobacterium avium subsp. *paratuberculosis* (Map) infections in cattle are of concern to the dairy industry due to the as-yet-unresolved issue of its potential role in Crohn's disease in humans (Anon. 2000, Chacon et al. 2004, Herrewegh et al 2004). If Map is implicated, then milk is a possible vehicle of transmission of the organism to humans, because Map has been detected in raw milk and may not be effectively inactivated by pasteurisation (Sweeney et al., 1992b, Streeter et al. 1995, Grant et al. 1996, Millar et al.1996, Sung and Collins 1998, Grant et al. 1999, Giese and Ahrens 2000, Corti and Stephan 2002, Gao et al. 2002, Grant et al. 2002a & b, McDonald et al. 2005, Pillai & Jayarao 2002, Sevilla et al. 2002). A milk quality assurance programme for paratuberculosis in dairy herds may reduce the potential risk of transmission of Map to humans through consumption of milk and milk products.

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Certification-and-surveillance programmes for Map-free herds have been developed in several countries (Kennedy et al. 2001). These programmes generally aim at a low-risk trade in cattle. In the Netherlands, a certification-and-surveillance programme has been developed in which herds can obtain 'Map-free' status following five negative annual herd examinations (the first herd examination by ELISA and faecal culture of ELISA-positive animals, the 2nd to 5th examination by pooled faecal culture; Benedictus et al., 1999). Control programmes for Map infected herds generally aim at elimination of Map in these herds. Because of their aims, these certification, surveillance and control programmes are inherently expensive, and participation is often restricted to a minority of herds. By July 1st 2005, only 473 of approximately 23,000 Dutch dairy herds had obtained 'Map-free' status. However, the goal of a milk quality assurance programme is to reduce the concentration of Map in bulk milk rather than eradication of Map. Herds in a milk quality assurance programme can be certified as 'low-Map bulk milk' if, with a certain probability, the concentration of Map organisms in bulk milk (cMapobm) does not exceed a pre-set maximum acceptable concentration. This does not necessarily mean that the herd is free of Map infection. Such a milk quality assurance programme might possibly be run at considerably lower cost than the current Dutch certification, surveillance and control programme. The aim of this study was to simulate different milk quality assurance programmes to evaluate their epidemiological effects and economic consequences for a population of Dutch dairy herds.

A milk quality assurance programme starts with an initial assessment; test-negative herds enter a surveillance procedure and test-positive herds enter a control procedure. The control procedure aims to suppress the infection in the herds, such that the milk quality can be guaranteed and the herd can move to the surveillance procedure. Different milk quality assurance programmes were simulated with a stochastic model JohneSSim (Groenendaal et al. 2002). Various alternative test schemes based on herd examinations by serology (ELISA) or individual faecal culture (IFC) were simulated. All programmes were simulated with and without preventive management measures taken by all participating herds.

MATERIALS AND METHODS

The JohneSSim model

The JohneSSim model is a stochastic and dynamic simulation model that simulates (a) the herd dynamics, (b) the disease dynamics within the herd, (c) the control of Johne's disease and (d) the economic consequences in a typical Dutch dairy herd over a 20-year period. The model and its use to study certification-and-surveillance programmes have been described in detail (Groenendaal et al. 2002, Weber et al. 2004). Repeated runs of the model provide insight into the variation in outcome at the farm level. Results at a higher aggregation level (e.g. national level) are obtained by simulating different types of dairy herd and aggregating the results according to their relative abundance. Both infected and uninfected herds are simulated.

Assumptions in JohneSSim model for present study

All herds were assumed to be closed (i.e. no purchase of animals and no new introductions of Map). Herd-size was assumed to be initially 65 adults (≥ 2 yr.), and to increase by 5% per annum. Eighty to 100% of heifer calves were raised in the herd, while a surplus of heifers was sold shortly before 1st calving. Mean annual milk production was 8000 kg. Initially, 30% of herds were assumed to contain Map infected animals, based on a recent study in the Netherlands

(van Weering, personal communication, 2004). The assumed distribution of the initial within-herd true prevalence in infected herds is shown in Fig. 1. Economic assumptions on losses caused by infection with Map, costs of participation in the quality assurance programme, and costs of preventive management measures are shown in Tables 1-3. All costs were discounted at a real interest rate (approximated by interest rate minus inflation rate) of 5% per year. Assumptions on test characteristics are shown in Table 4. Preventive management in the simulated herds reflects the current distribution of management practices in the Dutch dairy industry ('background' management; Groenendaal et al. 2002). Assumptions on effectiveness of additional preventive management measures, superimposed on the 'background' management, have been described in detail previously (Groenendaal et al. 2002). By default, effective separation of young stock from adult cattle was assumed to reduce infections through faecal contamination of the environment by 90%.

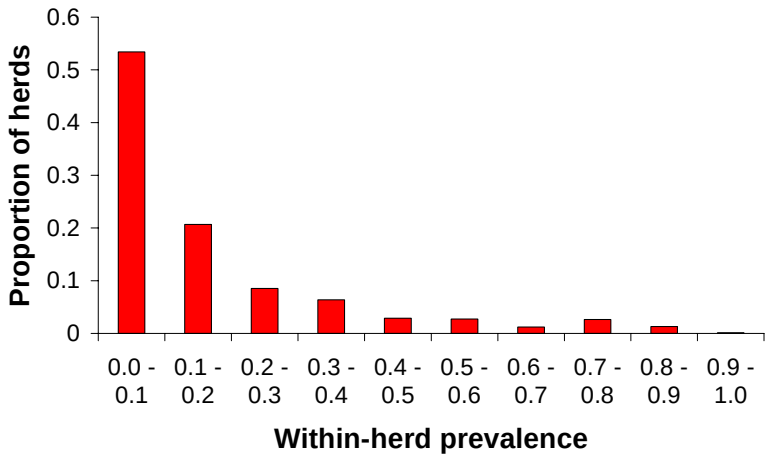

Fig. 1 Assumed distribution of within-herd true prevalence in infected herds at the start of simulations.

Table 1. Assumptions on losses caused by infection with Map. Losses did not include effects of a potential reduction in milk consumption due to consumer concerns.

	CATEGORY	COSTS (EURO)
Milk production	Reduction depends on infection state: 5% (mildly infected subclinical) to 20% (highly infected clinical)	0.08 / kg
Treatment	Treatment clinical case	30
Reduced slaughter value	Standard slaughter value (per cow):	448.75
	Reduction depends on infection state (slightly infected 5%, highly infected subclinical 10%, highly infected clinical 100%)	
Missed future income	Retention Pay Off, depending on parity, month in lactation and production level assuming no alternative use of production factors	- 111.63 to 1431.23

Table 2. Variable costs (Euro) of participation in the bulk milk quality assurance programme. Subscription costs were 90 Euro per year. Costs do not include Value Added Tax (VAT for subscription and laboratory tests = 6%; VAT on other costs 19%).

TEST / ACTION	VETERINARIAN COSTS	TRANSPORT COSTS	LABORATORY COSTS	
			PER SUBMISSION	PER TEST
Veterinarian's visit	22			
IFC	2.75 per animal	10	7.80	30.00 per animal
ELISA	2.75 per animal	10	7.80	6.15 per animal

Table 3. Assumed costs (Euro) of preventive management measures (including labour at 18.21 Euro per hour). Fifty percent of the costs of additional preventive management measures superimposed on the 'background' management (Groenendaal et al. 2002) were attributed to the control of paratuberculosis.

CATEGORY		LOSS OR COSTS
Calving	Costs of cleaning per year	€ 100 per year
	Extra labour (hygiene, milking own dam) per calving	Giving colostrum of own dam € 9.11
Milk replacer	280 litres of artificial milk, 8 litre of milk replacer per kg milkpowder, costs of milkpowder € 1.30 per kg, value of bulkmilk €0.20 per litre.	42 litre instead of rest milk = € 6.83 238 litre instead of bulkmilk = - € 9.11 Total = - € 2.28
Hygiene barrier	Between adult stock and young stock	€ 726.71 per year (including labour)
Roughage	Better quality roughage, straw etc. during housing in summer season only.	€ 39.03 for calves 0 – 6 months
Housing	Separate housing of animals 0 – 70 days (initially 5 animals)	€ 487.5 per year; 5% increment per year
	Separate housing of animals 70 – 180 days (initially 7 animals)	€ 682.5 per year; 5% increment per year
Calving	Separate housing of animals 180 – 360 days (initially 9 animals)	€ 877.5 per year; 5% increment per year

Table 4. Assumptions on sensitivity (Se) and specificity (Sp) of individual faecal culture (IFC) and ELISA.

	STAGE OF INFECTION	IFC	ELISA
Se	Latently infected	0	0.01
	Mildly infectious	0.40	0.10
	Highly infectious	0.95	0.60
Sp	Not infected	1	0.997 [(a)]

(a) van Maanen et al. (2002).

Shedding of Map in milk

The assumptions made on shedding of Map in milk depending on the stage of infection are shown in Table 5. These assumptions were based on the available quantitative data on direct shedding of Map in milk, faecal contamination of milk and shedding of Map in faeces (Chiodini et al 1984, Stadhouders and Jørgensen 1990, van der Giessen et al. 1992, Sweeney et al. 1992a

& b, Streeter et al. 1995, Millar et al. 1996, Rossiter and Burhans 1996, Nauta and van der Giessen 1998, Giese and Ahrens 2000, Pearce et al. 2001, Corti and Stephan 2002, McDonald et al. 2005, Grant et al 2002a,b, Pillai & Jayarao 2002, Sevilla et al 2002, Rademaker, personal communication 2004, Stehman, personal communication, 2004).

Table 5. Assumed concentration of Map in milk for each stage of the infection-and-disease process in adult cattle. (Total Map in milk = direct shedding + faecal contamination ∗ Map in faeces. Faecal contamination was assumed to be 0.04 gram/litre milk.)

STAGE	PROPORTION OF ANIMALS	DIRECT SHEDDING OF MAP IN MILK (ORGANISMS PER LITRE)	MAP IN FAECES (ORGANISMS PER GRAM)	TOTAL MAP IN MILK (ORGANISMS PER LITRE)
Latently infected		0	0	0
Mildly infected	0.8	0	0	0
	0.2	0	10^2	4
Highly infected	0.6	10^2	10^2	10^2
	0.24	10^2	10^4	$5 \cdot 10^2$
	0.16	10^2	10^7	$4 \cdot 10^5$
Clinical disease		10^4	10^9	$4 \cdot 10^7$

Acceptable concentration of Map organisms in milk

The concentration of Map organisms in on-farm bulk milk that can be considered acceptable has not been defined. No quantitative data are available on the probability of human disease after exposure to Map (either alive or dead). Therefore, in the present study, it was assumed that no viable Map organisms would be present after commercial pasteurization. Sung and Collins (1998) concluded that Map may survive HTST pasteurisation when the initial organism concentration is greater than 10^4 cells per litre. The authors believe that no studies indicated that Map may survive HTST pasteurisation when the initial concentration of Map organisms in milk is less than 10^4 cells per litre. Therefore, in this study, a concentration of Map organisms in milk less than 10^3 per litre was considered acceptable and allowing some safety margin.

Bulk milk quality assurance programmes

At each testing point, the model estimated the Map content of bulk milk, using the proportions of the herd infected to different degrees and therefore the likely concentration of Map in milk (Table 5) and the characteristics of the test (Table 4). The test result was negative only if the cMapobm was $<10^3$/l with a certain probability. Herds were initially assessed two years after the start of the simulations, and assigned a status based on the results of this assessment: test-negative herds were classified as 'green' and test-positive herds as 'red'. Thereafter, 'green' herds were regularly monitored in a surveillance scheme, whereas a control scheme was applied to 'red' herds, in which test-positive cattle and their last-born offspring were culled. Thus, 'green' herds were herds with a high probability that cMapobm was $<10^3$/l.

Various alternative test schemes for the initial assessment (i), surveillance (s) and control (c) were simulated (Table 6). The number of negative herd examinations required for a 'red' herd to become 'green' was determined by the probability that cMapobm was $<10^3$/l. A test-negative 'red' herd became 'green' if this probability was equal to, or higher than, the probability for a 'green' herd immediately after the intake procedure to have a cMapobm $<10^3$/l.

All programmes were simulated with and without additional preventive management measures superimposed by all participating herds on their 'background' management. The following additional preventive measures were applied: improved hygiene around birth, colostrum from own dam only, feeding of artificial milk replacer only, and effective separation of young stock from adult cows from birth to the end of the first year.

Table 6. Simulated test schemes for initial assessment (i), surveillance (s) and control (c). In the initial assessment and the surveillance procedure, a positive ELISA result was confirmed by individual faecal culture (IFC); IFC positive cattle and their lastborn calf were culled. In the control procedure, all ELISA or IFC positive cattle were culled.

SCHEME	INITIAL ASSESSMENT		SURVEILLANCE			CONTROL			
	TEST (ONCE)	ANIMALS	TEST	INTERVAL	ANIMALS	TEST	INTERVAL	ANIMALS	TEST
i1-s1-c1	ELISA	All, ≥3 yr	ELISA	1 yr	All, ≥3 yr	ELISA	1 yr	All, ≥3 yr	ELISA
i1-s1-c7	ELISA	All, ≥3 yr	ELISA	1 yr	All, ≥3 yr	IFC	2 yr	All, ≥2 yr	ELISA
i1-s2-c1	ELISA	All, ≥3 yr	ELISA	2 yr	All, ≥3 yr	ELISA	1 yr	All, ≥3 yr	ELISA
i1-s2-c7	ELISA	All, ≥3 yr	ELISA	2 yr	All, ≥3 yr	IFC	2 yr	All, ≥2 yr	ELISA

Model output

In the present study, relevant herd-specific outcomes over time were the within-herd true prevalence, test-result prevalence, cMapobm, and costs spent on the quality assurance programme. Relevant outcomes over time on the aggregate level included the proportion of 'green' herds, the average concentration of Map in bulk milk from 'green' herds, the proportion of 'green' herds with a cMapobm of $<10^3$/l, and costs spent on the bulk milk quality assurance programme (including herd examinations, subscription costs, preventive measures and cull of infected animals).

Sensitivity analyses

The influence of various input parameters on the study results was analysed, by changing one parameter at the time. These sensitivity analyses were performed with test scheme i1-s1-c1, with or without additional preventive management measures taken in the herds. These sensitivity analyses were: (1) By default, the numbers of Map organisms in milk (Table 5, last column). Alternatively, these were multiplied by 10^6 to study the effect of this insecure parameter. (2) By default, preventive management measures reduced the probability of infection through the environment by 90%. Alternatively, this reduction was only 50%. (3) By default, the initial within-herd true prevalence was 0.30. Alternatively, a prevalence of 0.56 was simulated. (4) By default, a number of negative herd-examinations were required for a 'red' herd to become 'green' (based on the probability for such herd to have a cMapobm of $<10^3$/l). Alternatively, only one negative herd-examination was required to become 'green'.

RESULTS

Simulated bulk milk quality assurance programmes

At the initial assessment (scheme i1: ELISA, all cattle ≥3 yr), 90% of all herds were test-negative and classified as 'green'. The remaining 10% of herds were test-positive (i.e. ~35% of the infected herds at that time, and none of the non-infected herds) and therefore classified as

'red'. The within-herd prevalence of infection in adult cattle in 'green' and 'red' herds at the initial assessment is shown in Figure 2A. The concomitant distribution of the cMapobm is shown in Figure 2B. Immediately after the initial assessment (with scheme i1), 98% of 'green' herds had a cMapobm of $<10^3$/l. During control in 'red' herds, two consecutive negative herd-examinations by IFC or six consecutive negative herd-examinations by ELISA were required to reach the same probability of having a cMapobm of $<10^3$/l. Therefore, by default, 'red' herds were re-classified as 'green' only after two consecutive negative herd-examinations by IFC, or six consecutive negative herd-examinations by ELISA.

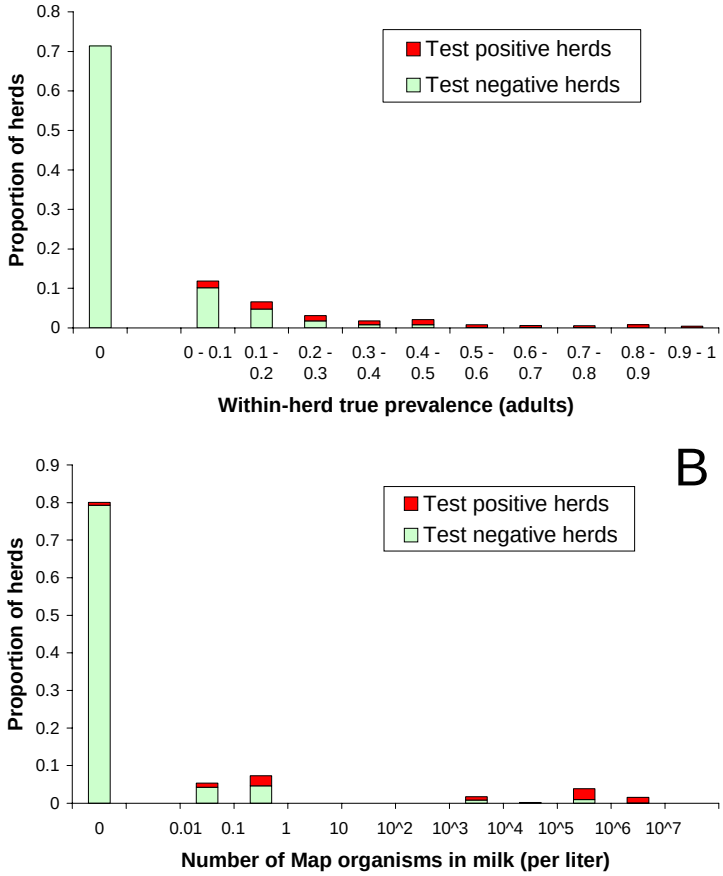

Fig. 2 Estimated within-herd prevalence of infection in adult cattle (A) and estimated cMapobm (B) immediately after the initial assessment in simulated herds that were test-positive ('red') and test-negative ('green') at the initial assessment, using scheme i1 (ELISA, all cattle ≥3 yr).

The proportion of herds classified as 'green' decreased over time if no additional preventive management measures were taken (Fig 3A). However, if additional preventive measures were taken, this proportion first decreased, but, thereafter, increased towards 86% - 99%, depending on the test scheme used (Fig 3B). Additional preventive measures were pivotal for 'red' herds to become 'green'. Furthermore, these measures reduced the proportion of 'green' herds that lost their status. If additional preventive measures were taken, culling based on biennial IFC was more effective than culling based on annual ELISA.

The estimated average cMapobm in 'green' herds did not decrease below 10^3/l before year 8 to 15, depending on the scheme used and whether or not additional preventive measures were

taken. The proportion of 'green' herds with a cMapobm of $<10^3$/l increased towards 100% in year 20 (Fig. 4).

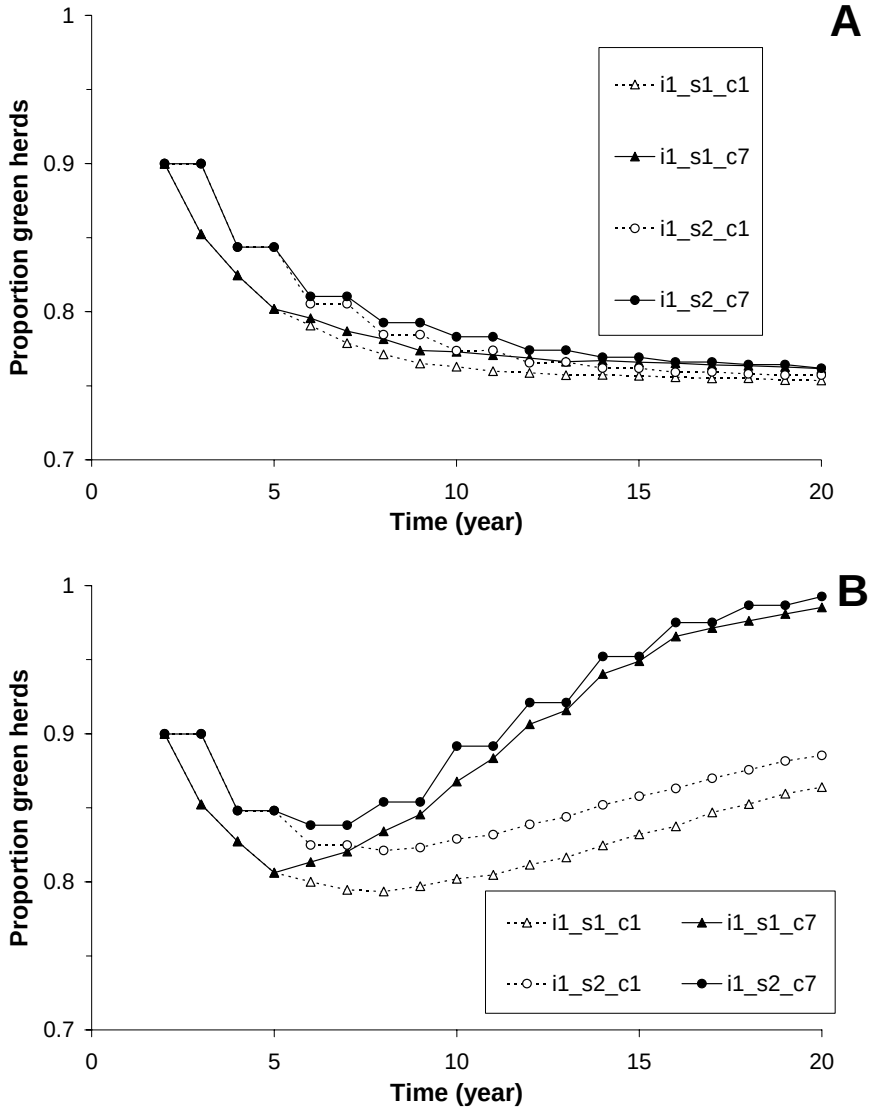

Fig. 3 Proportion of herds that are classified as 'green' over time. (A) Without, and (B) With additional preventive measures. Test schemes are defined in Table 6.

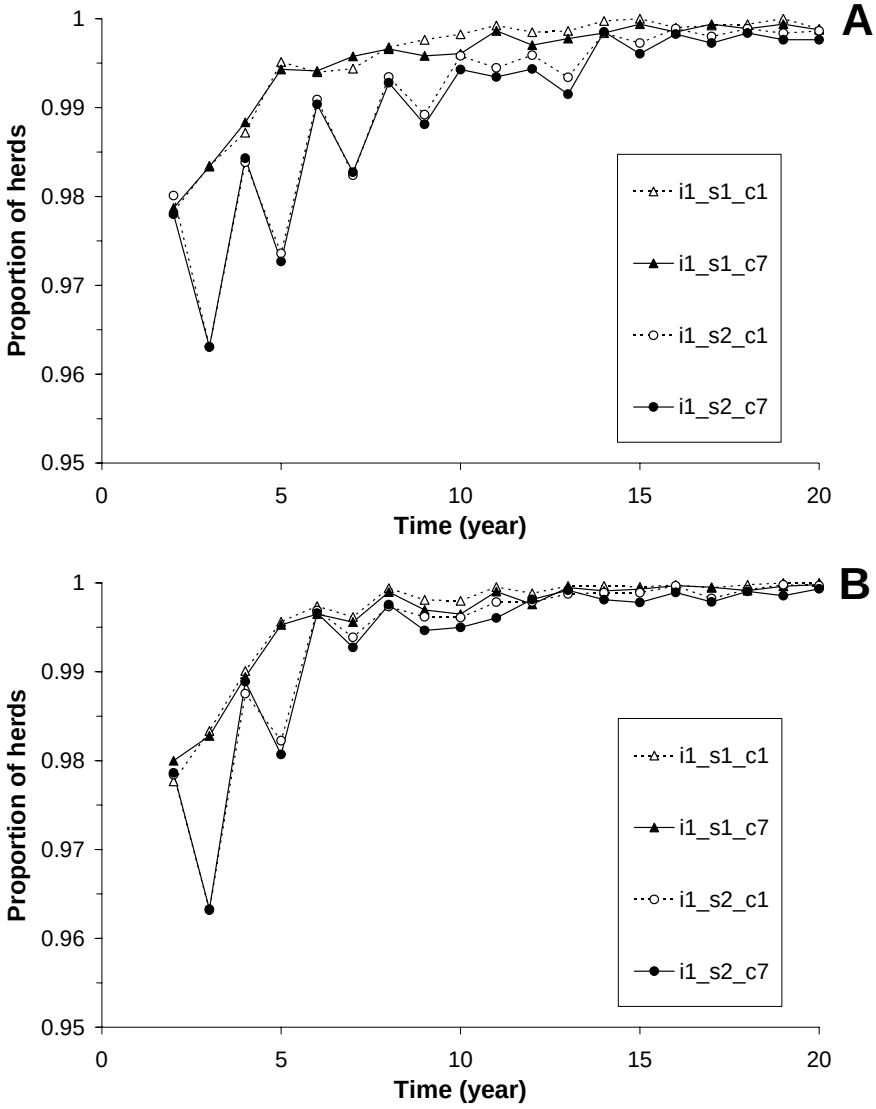

Fig. 4 Proportion of 'green' herds with a cMapobm of $<10^3$/l. (A) Without, and (B) With additional preventive measures. Test schemes are defined in Table 6.

The median cumulative discounted costs during the 20 simulated years for schemes without additional preventive measures ranged from 6,000 to 10,000 Euro (Fig. 5). For schemes with additional preventive measures these costs were higher, ranging from 40,000 to 44,000 Euro. However, the 90% range of costs was much broader without additional preventive measures. Therefore, for some schemes, the 95% percentile of costs were higher without than with additional preventive measures.

Sensitivity analyses

If additional preventive measures were taken but their effect was assumed to be 50% effective in reducing transmission through the environment instead of the default value of 90%, the proportion of herds certified as 'green' after 20 years was 81% instead of 86%. However, not taking any additional preventive measures resulted in only 75% of herds being certified as 'green'. Moreover, decreasing the reduction in transmission through the environment had no effect on the proportion of 'green' herds with a cMapobm of $<10^3$/l.

If the default level of contamination of milk with Map was multiplied by 10^6, the proportion of 'green' herds with a cMapobm of $<10^3$/l was reduced by up to 10% during the first years after intake. However, beyond approximately year 10 (i.e. 8 years after the initial assessment), the effect was very small (Fig. 6).

If a higher initial within-herd prevalence of 0.56 instead of 0.30 was assumed, the proportion of 'green' herds in year 20 was reduced by 21% (54% instead of 75% with additional preventive management measures; 75% instead of 86% without additional preventive measures). The proportion of 'green' herds with a cMapobm of $<10^3$/l during the first years of the simulations was decreased by up to 2%, but this decrease was small beyond year 10. Effects on the cumulative discounted costs up to year 20 were negligible.

By default, six negative herd examinations by ELISA were required for a 'red' herd to be re-classified as 'green'. Alternatively, if only one negative herd examination by ELISA was required, then over 99% of herds were classified as 'green' in year 20 (instead of 86%), if additional preventive management measures were taken. The reason is, of course, that 'red' herds move to the pool of 'green' herds sooner. If no additional preventive measures were taken, there was only a minor effect on the proportion of 'green' herds. However, the bulk milk 'quality' of 'green' herds was lower: the proportion of 'green' herds with a cMapobm of $<10^3$/l was reduced by up to 2% if only one negative herd examination by ELISA was required instead of six.

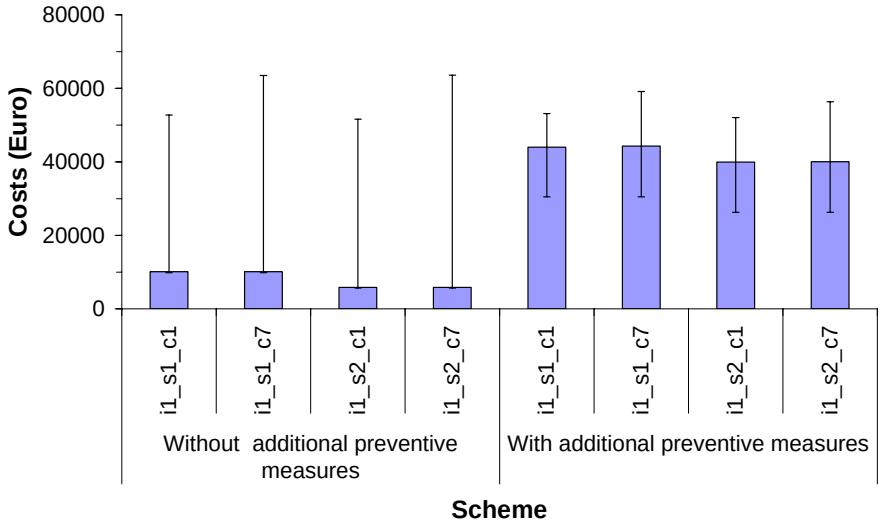

Fig. 5 Median cumulative discounted costs per herd up to year 20 (averaged over all 'green' and 'red' herds). Error bars indicate the 5% to 95% range. Test schemes are defined in Table 6.

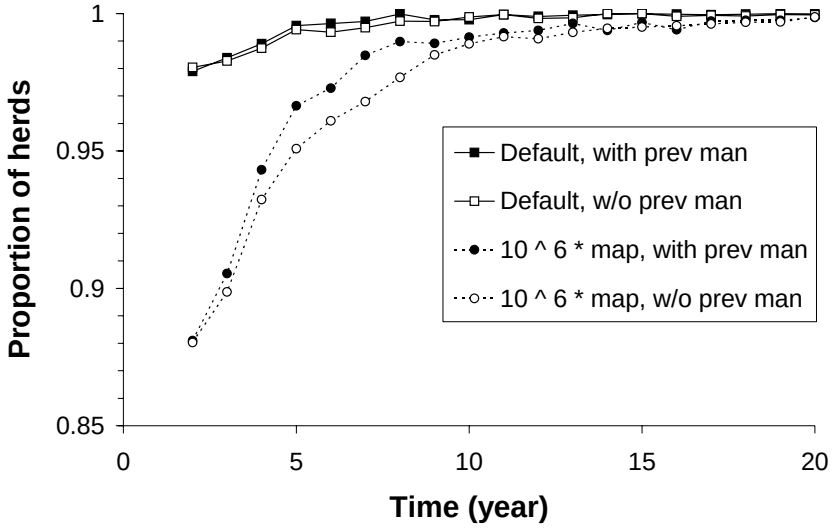

Fig. 6 Proportion of 'green' herds with a cMapobm of $<10^3$/l in sensitivity analysis for the effect of contamination of milk with Map, using scheme i1-s1-c1, with or without (w/o) preventive management measures (prev man). Default concentrations of Map organisms in milk are given in Table 5. Alternatively, these concentrations were multiplied by 10^6.

DISCUSSION

The authors believe that this is the first modelling study into a bulk milk quality assurance programme for paratuberculosis in dairy herds. By aiming at suppression of Map to guarantee milk quality, such a milk quality assurance programme can be run at considerably lower cost than certification, surveillance and control programmes aiming at a low-risk trade in cattle and elimination of Map from herds.

Key elements in a successful bulk milk quality assurance programme are preventive measures to reduce the risk of introduction of Map in participating herds (including trade restrictions), preventive management measures to reduce the risk of within-herd spread of Map, and the intake, surveillance, and control procedures. The present study was restricted to closed herds. Effects of animal trade have been analysed separately using a mathematical model (van Roermund et al. 2005). In the present study, additional preventive management measures to reduce within-herd spread of Map were found to have a major effect on the proportion of herds that can be certified as 'low-Map bulk milk' (i.e. 'green' in this study). These management measures were pivotal for test-positive ('red') herds to become certified as 'low-Map bulk milk' ('green'). However, these measures had only a minor effect on the bulk milk quality of 'low-Map bulk milk' herds ('green'). The intake, surveillance and control procedures should preferably be based on quantification of the concentration of Map organisms in bulk milk. However, the authors believe that techniques routinely to quantify Map in large numbers of bulk milk samples are not yet available. Therefore, procedures for initial assessment surveillance and control were simulated based on tests at the animal-level (ELISA, faecal culture). The results showed that herd examinations by ELISA for intake and surveillance effectively ensure the quality of 'low-Map bulk milk': >96% of simulated certified herds (increasing to >99% after 10 years) were below a cMapobm of 10^3/l. However, culling of test-positive animals and their last-born offspring based on biennial faecal culture was more effective than culling based on annual ELISA.

Important assumptions were made in the present study because of uncertainty and lack of information. However, assumptions considered to be most critical were studied in the sensitivity analyses. Due to deficiencies in the current methodology, it has so far been impossible accurately to quantify Map organisms in milk from a dairy herd infected with paratuberculosis (Dundee et al, 2001; Grant et al, 2002a). For instance, colony forming units cannot simply be translated to concentrations of Map organisms, because of clumping of Map in specimens and insensitivity of culture. The sensitivity analyses showed that a 10^6 fold increase in the assumed concentration of Map in milk from infected animals would initially decrease the number of certified 'low-Map bulk milk' ('green') herds with indeed a cMapobm of $<10^3$/l by 10%. However, such high concentrations of Map organisms in milk are probably not biologically plausible (for example, a clinical animal would then shed $4 \cdot 10^{12}$ Map/litre of milk). Even so, the effects of such an increase in Map organisms in milk from infected animals on the bulk milk quality of 'green' herds were very small beyond year 10 (i.e. 8 years after the intake procedure).

It is concluded that a bulk milk quality assurance programme for paratuberculosis in closed dairy herds is feasible. Preventive management measures should be recommended to participants, because they considerably increase the probability of obtaining and keeping a 'low-Map bulk milk' status in the long term. Serology is sufficient for initial assessment and surveillance in the programme. However, for control in test-positive herds, culling based on faecal culture results is more effective than culling based on ELISA results. The present study provided decision-makers with information on the cost-effectiveness of different programmes.

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EMERGING DISEASES

A SPATIOTEMPORAL STUDY OF A WEST NILE VIRUS OUTBREAK IN HORSES IN CAMARGUE, FRANCE

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SUMMARY

West Nile fever is a flaviviral infection transmitted by mosquitoes. Natural foci of West Nile Virus (WNV) infections are mainly situated in wetland ecosystems and are characterized by the bird-mosquito transmission cycle. Since the outbreak in 2000, the Camargue area is considered as an endemic zone for WNV. In August 2004, another WNV epizootic was declared. In this paper, a spatio-temporal study of this outbreak with the aim of identifying environmental conditions that favour the re-emergence of the disease in horses is presented.

Thirty two cases of WNV infection were laboratory confirmed between August 5th and October 14th 2004. Fifty six other stables were selected from the centre's database and surveyed to ensure that no neurological signs had been observed in horses during the epizootic period. A SPOT-4 satellite image (13 July 2003) was classified into 14 landscape categories to identify environmental risk factors. The landscapes were quantified in a circle of 1 kilometre around the stables and cases and controls were compared using Mann-Whitney U tests.

The epizootic lasted for 4 weeks between weeks 39 and 42 inclusively. The spatio-temporal study was conducted with SaTScan software. Two clusters were identified (risk ratio [RR] = 3.4). The landscapes within the clusters comprised higher proportions of wet meadows (25% prevalence; RR = 1.3) and dry lawns (7%; RR = 2.1), but lower proportions of open water (6%; RR = 0.3) and woodlands (0.7%; RR = 0.2) compared to the area of study. Within one kilometre around the stables, higher proportions of open water and dry lawns were observed for the cases (6.7 ± 5.1% and 6.5 ± 4.9% respectively) than for the controls (3.1 ± 6.7 % and 3.6 ± 2.2 respectively; P = 0.032).

INTRODUCTION

West Nile fever is a flaviviral infection transmitted by mosquitoes. Transmission cycles of West Nile virus (WNV) involve wild birds as principal reservoir hosts and mosquitoes as vectors (Komar, 2000). The virus has been isolated from 43 mosquito species, predominantly of the genus *Culex*. Birds usually do not show any signs when infected. High, long term viraemia, sufficient to infect vector mosquitoes has been observed. Migratory birds are instrumental in the introduction of the virus to temperate areas during spring migrations (Rappole & Hubalek, 2003).

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Mammals, including humans and horses are incidental and dead-end hosts. Infected horses can exhibit signs of biphasic fever, followed by encephalomyelitis with staggering gait, hind limb weakness and paralysis, often leading to recumbency and death (Ostlund et al., 2000). The frequency of inapparent infection in association with cases of clinical disease is difficult to establish.

Natural foci of WNV infections are mainly situated in wetland ecosystems (river deltas or flood plains) and are characterized by the bird-mosquito transmission cycle. In Europe, West Nile virus circulation is confined to two basic types of cycles and ecosystems (Komar, 2000):

- rural (sylvatic) cycle: wild, usually wetland birds and ornithophilic ('bird-liking') mosquitoes,

- urban cycle: synanthropic or domestic birds and mosquitoes feeding on both birds and humans.

The first European isolations of the virus were recorded in 1963, from patients and mosquitoes in the Rhone delta, and from patients and ticks (*Hyalomma marginatum*) in the Volga delta (Hannoun et al., 1964; Platonov et al., 2001). WNV was subsequently isolated in Portugal, Slovakia, Moldavia, Ukraine, Hungary, Romania, Czechland and Italy (Hubalek & Halouzka, 1999). In France, two outbreaks were reported in the Camargue region, Southern France, during the summer of 1962 (Joubert et al., 1970) and during the late summer and autumn 2000 (Murgue et al., 2001). No abnormal bird mortality was reported.

Since the outbreak in 2000, the Camargue area is considered an endemic zone for WNV. The Camargue is a region situated in the south of France, along the Rhone river delta. From an ecological point of view, the Camargue presents diversified environments, consisting of dry areas irrigated by canals, ditches and wetlands (Hoffmann et al., 1968; Blondel & Isenmann, 1981). These biotopes are favourable to both mosquitoes, which are potential vectors for the WNV and populations of sedentary and migratory birds, which are potential reservoirs. More than 300 migratory and resident bird species are observed in this area, which is also home to thousands of horses.

In 1964, WNV was isolated from *Culex modestus* (Hannoun et al., 1964) determining this species as a vector of WNV in France. Since then, no entomological studies and sequential samples collected have shown any other infected species (Murgue et al., 2001). Recent studies have investigated the potential of a variety of mosquito species to serve as the epidemic vectors of WN virus in Camargue (Porphyre et al., 2003). These potential vectors include *Culex modestus Culex pipiens*, *Ochlerotatus caspius* and *Aedes vexans*. Both *Aedes* and *Culex* species females are aggressive towards mammals as well as birds. Environmental conditions control the formation and multiplication of vector breeding habitats, and the dynamics of vectors controls the virus transmission and circulation within vertebrate populations (Porphyre et al., 2005). Controlling populations of these potential vectors is of great interest to health officials, mosquito control workers and the public at large.

In August 2004, another WNV epizootic was declared. The vector in the present outbreak is still unknown. In this paper, a spatio-temporal study of this outbreak with the aim of identifying environmental conditions that favour the re-emergence of the disease in horses is presented. Geographic information system technology allows the examination of remotely sensed landscape elements that relate to vector abundance and therefore transmission risk. So,

identification of landscape elements that predispose humans and horses to risk of WNV transmission is important for understanding and controlling the disease.

MATERIALS AND METHODS

Study area

The target population included all horses living in Camargue. Due to the existence of semi-wild horses in this area, the target population actually meant all horses seen by the veterinary surgeons in the study area. The last census of the livestock, conducted by the Ministry of Agriculture in 2000, estimated the horse population of the Camargue to be approximately 7,000 animals, located in an area of around 3600 square kilometres.

Clinical data

Data on the neurological cases were collected through the passive surveillance system implemented by the veterinary practitioners and health authorities. In order to improve the passive surveillance, the authors developed, in 2004, an integrated electronic system based on the department's existing infrastructure for secure web-based electronic health information interchange with sentinel veterinary practitioners. The first neurological case was declared on the network on 23rd August, 2004 with a further two cases registered on 27th August by the same practitioner. A total of 57 horses with neurological signs were reported by 12 practitioners, between August 23rd, 2004 and October 30th, 2005.

Neurological cases were classified as confirmed WNV when the response to the ELISA IgM test was positive by the 'Agence Française de Sécurité Sanitaire des Aliments' (AFSSA). They were classified as probable WNV cases when the ELISA tests were positive for IgG and negative for IgM. Among the 57 neurological cases, 32 were confirmed as WNV infection by laboratory confirmation (56%) and 5 were classified as probable cases (9%).

Fifty six other stables were selected from the centre's database and surveyed to ensure that no neurological signs had been observed in horses during the epizootic period. These latter stables constituted the control sample for the statistical analysis. Cases and controls stables were located using a Global Positioning System (GPS).

Environmental data

A SPOT-4 satellite image (acquired 13th July 2003) was used to record the environmental data. The SPOT-4 sensor measures the spectral reflectance in the visible, near infra-red and mid-infrared wavelengths and covers an area of 3600 square km in size with 20 metres resolution. The mid-infrared channel is very useful in vegetation studies. The pixels of the image were classified into 14 landscape categories. The selected categories were then associated with mosquito, birds and horses densities. Landscape elements identified included: open water, rice fields, reeds, cereals, vineyards, woodlands, wet meadows, salt ponds, sparse and small vegetation in brackish swamps (low and salted "sansouire": *arthrocnemum glaucum)*, dense and higher vegetation in less brackish swamps (high and wet 'sansouire': *arthrocnemum fructicosum*), dry lawns, dry bushes, buildings and bare ground.

A validated method was used for the classification. A set of training data with known ground conditions was generated. Forty training zones were visited and half of the samples were

used as a training set for the parameter estimation phase. The other half was used as a validation set for calculation of a confusion matrix in order to assess the accuracy of the classification for each landscape category. The image was then imported into a Geographic Information System and the landscapes were quantified in a circle of one kilometer radius around the stables.

Statistical analyses

Cluster identification: The spatio-temporal study was conducted with the SaTScan software (http://www.satscan.org/). The scan statistic has been widely used in the veterinary literature during recent years (Kulldorf, 2004). The scan statistic is a cluster-detection test, able to both locate and test the significance of clusters. The procedure was based on a Bernouilli model. Briefly, the test sequentially centred circles in each location where information is available and compares the ratio of cases to controls for the area inside the circle with the ratio outside the circle. The maximum radius of the circle is variable, and may be based on some knowledge about the epidemiology of the disease or on the purpose of the study. One drawback associated with this technique is that it assumes that clusters are circular.

Risk factor identification: To avoid the bias due to the possible co-existence of two different mechanisms of transmission in the wet or in the dry zone of Camargue, the study of the environmental factors was restricted to the wet areas. The ratios of percentages, ε, were calculated in order to compare the proportions of landscapes in the wet area with the proportions of the landscapes around the stables or within the clusters. For the area i and the landscape category j (LC_j), $\varepsilon = (\%\ LC_{ij}\ )/\ (\%L_j$ in the Camargue wet area).

Cases and controls were selected so that their corresponding buffer zones were disjoined. A bivariate Mann Whitney-U analysis was conducted Followed by a multivariate analysis involving generalized linear modelling (GLM) with Stata® software fitted using an automatic ascendant alogorithm.

RESULTS

Spatio-temporal study of the outbreak

The epizootic lasted for 4 weeks between week 39 and week 42 of 2004. The spatio-temporal analysis of the outbreak gave the best results for a spatial window of nine kilometres and a temporal window of 18 days. Two clusters were identified (risk ratio [RR] = 3.35). Their spatio-temporal characteristics and locations are presented in Table 1 and Fig. 1. The results obtained with the probable cases were quite similar.

Environmental risk factor analysis

Accurracy of the classification: A confusion matrix was generated to compare the results of SPOT image's classification with field sample sites. The classification procedure gave an overall estimation of 87.2% accuracy. Open water, rice fields, wet 'sansouire', reeds and woodlands had an index of accuracy between 80 and 100%. Low 'sansouire' was confused with wet 'sansouire' and its index was 62%. The indices for dry lawns, wet meadows and vineyards were respectively 53%, 47% and 6%. Dry lawns were confused with cereals and wet meadows with wet 'sansouire'.

Table 1. Description of the two clusters identified by the SatScan procedure for the 2004 WNV outbreak in horses in Camargue (coordinates are given in the projected system UTM WGS84 31N)

	Radius (km)	Center coordinate Y	Center coordinate X	Time frame		Number of cases	P-value
Confirmed cases							
Cluster 1	4.03	43.484348	4.38706	August 25	September 11	8	0.009
Cluster 2	8.93	43.573162	4.35913	September 13	September 30	10	0.029
Probable cases							
Cluster 1	5.01	43.478798	4.39908	August 25	September 11	8	0.042
Cluster 2	8.93	43.573162	4.35913	September 13	September 30	11	0.004

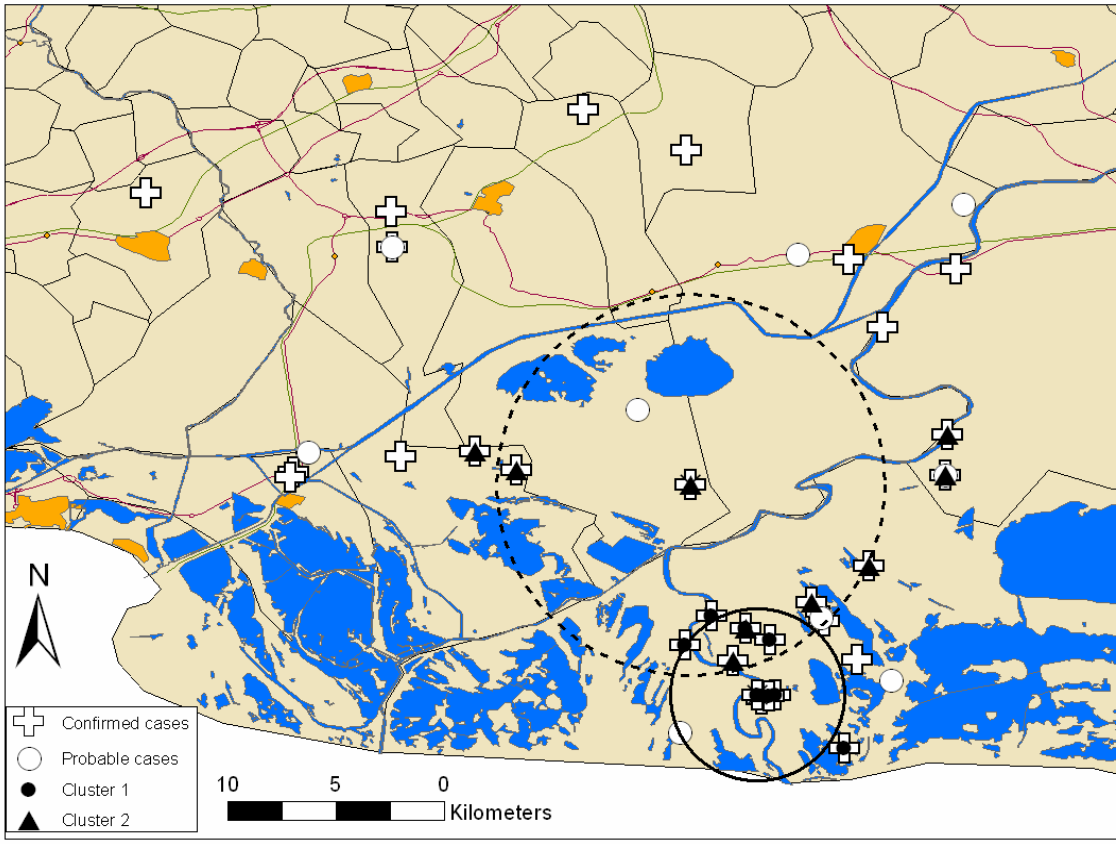

Fig. 1 2004 WNV outbreak in horses: location of clusters of WNV disease in horses identified by the SatScan procedure; maximum spatial window was set to 9 kilometers and maximum temporal window was < 50% of the study period

Descriptive analysis: More than 60% of biotopes in the area of study were representative of wetlands: Camargue area comprises 20% open water, 11% rice fields, 13% reeds, 13% of low and salted 'sansouire' and 8% of high and wet 'sansouire'. The percentages of wet meadows, cereals, dry lawns and woodlands were respectively 14%, 5%, 3% and 3%. Within 1 kilometre around the stables, the lanscapes were characterized by higher proportions of wet meadows (22% prevalence; RR = 1.55), reeds (19%; RR = 1.43), cereals (7%; RR = 1.54), dry lawns (5%; RR = 1.38) and woodlands (4%; RR = 1.44). A lower proportion of open water was observed around the stables (4%; RR = 0.21) compared to the area of study.

The landscapes within the clusters comprised higher proportions of wet meadows (25%; RR = 1.33) and dry lawns (7%; RR = 2.07), but lower proportions of open water (6%; RR = 0.31) and woodlands (0.7%; RR = 0.23) compared to the area of study.

Case control analyses: For the statistical analyses, the environmental data from the cases belonging to each cluster were averaged and these were then considered as single points. Finally, 7 cases and 15 controls were included in the analysis (Fig. 2). The results of the bivariate analysis between cases and controls suggested that a lower proportion of open water was associated with the cases (P = 0.09, non significant)

Woodlands and wet 'sansouire' were highly correlated with low and salted 'sansouire' (respectively -53% and 68%), and rice fields were highly correlated with wet meadows (-72%). Thus, further analyses were conducted with the following variables: open water, reeds, rice fields, wet 'sansouire', woodlands and dry lawns. Finally, dry lawns and open water were positively associated with the cases (P = 0.032 and P = 0.064 respectively).

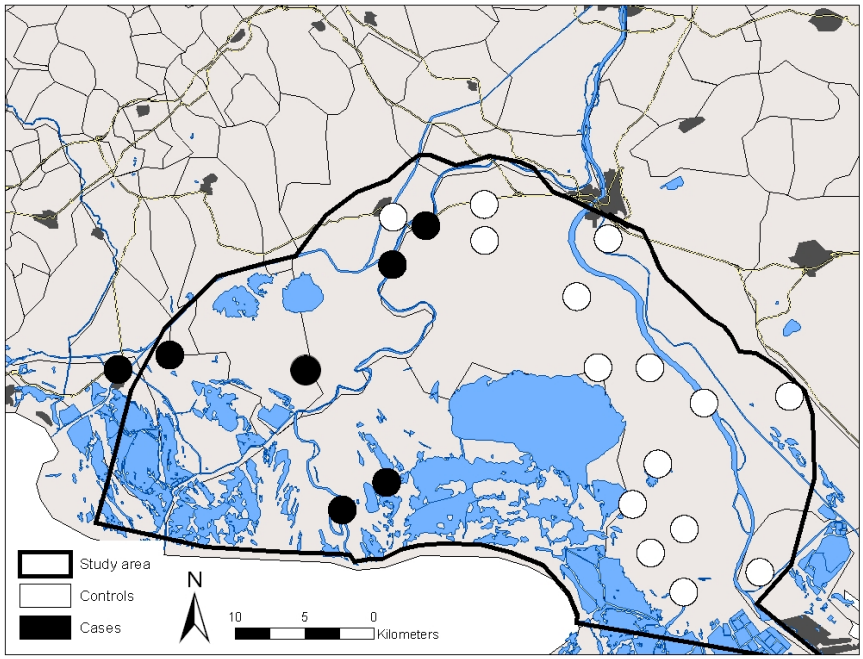

Fig. 2. Delimitation of the humid zone of Camargue, where the analysis of environmental risk factors for WNV disease was implemented and location of the cases and controls selected for the statistical analyses

DISCUSSION

The aim of this study was firstly to investigate possible clustering of disease occurrence and using remotely sensed landscape information to determine environmental elements that predispose horses to WNV transmission. This kind of ecological approach to the study of infectious agents has been used recently to answer epidemiologically relevant questions in a variety of arthropod-borne disease systems (Thomson & Connor, 2000).

The scan statistic has frequently been used for spatio-temporal analysis of disease patterns in veterinary epidemiology (Ward & Carpenter, 2000). Problems identified by these authors included difficulty defining the scanning "window" to be used in analyses. Ideally, the scanning window should be defined on the basis of the disease biology. In the Camargue area, potential vectors of WNV are mosquito species of the genera *Aedes* and/or *Culex*. *Aedes* adults migrate long distances from their breeding places, 10 to 20 kilometers being rather common, till 45 kilometers in autumn. *Culex* mosquitoes migrate short distances, maximum distance flight is thought to be less than five kilometers (Pratt & Moore, 1993). So, the spatial windows to be tested were defined sequentially from 1 to 20 kilometers radius. The temporal window was set to reach a maximum size of 50% of the study period. Finally, two clusters were identified, both in the wet area of Camargue, near the town 'Les Saintes Maries de la Mer'. The first one was 4 kilometers radius, the second nine kilometers. These distances are compatible with a vector having a rather short distance flight.

The potential vector mosquito species produce eggs in specific habitats (Pratt & Moore, 1993). *Aedes* larval habitats are constituted by brackish temporary ponds and low 'sansouire' in Camargue (till 38 g/L of salt, mean 10 g/L). Preferential larval habitats for *Culex modestus* species are quiet permanent wet areas, for example rice fields, reeds, or ponds containing low level salt, like the wet 'sansouire' (< 2g/L). Woodlands, dry bushes and open water are biotopes attractive for resting and nesting birds. In Camargue, the practice of waterfowl hunting has recently conducted in the intensifying management of hunting marshes (Tamisier & Grillas, 1994). Finally, dry lawns and wet meadows constitute a source of forage for the livestock. The use of a SPOT-4 image, of 20 meters resolution, and the definition of the landscape categories aimed at identifying the preferential habitats for the main actors of the WNV transmission cycle, vectors, reservoir hosts and incidental hosts. Unfortunately, at the date of the study, images acquired in 2004 were not available and environmental data were collected from an image taken in 2003. For this preliminary approach, it was assumed that the landscape cover wasnot too different from one year to another, except for the categories related to the presence of water. The date of the 13th July 2003 was chosen because it should give a good representation of the landscape cover just before the period of WNV transmission.

The confusion matrix was used to provide a site-specific assessment of the correspondence between image classification and ground conditions (Foody, 2002). The global accuracy index of the classification was satisfactory. The most important errors were encountered with wet meadows, which were confused with wet 'sansouire'. These errors were understandable because of the comparable structures and chlorophyll activity of these landscapes categories. Moreover, the quantity of water in these biotopes can vary each day. These variations could lead to difficulties for the identification of the interface between wet and dryer zones, especially if ground surveys are not updated at the date of acquisition of the satellite image.

Generalized linear models are used to model data with non-normally distributed errors (Thomson and Connor, 2000). The Bernouilli distribution is well adapted for a dichotomous

dependant variable. Underlying this statistical technique are the two basic assumptions that all observations are independent and identically distributed. However, this is rarely true for spatial data, where observations that are close in space have a higher probability of being similar than observations far apart. In order to approach this assumption of independence, the cases and controls were selected according the following criteria: the buffer zone of each observation was distinct; the landscapes surrounding the cases belonging to one of the clusters were averaged and each cluster was resumed to one case observation.

Finally, higher proportions of dry lawns and open water in the vicinity of horses were identified as risk factors associated with WNV cases. Open water is favourable for the presence of both birds and mosquitoes. But the low number of cases available for statistical analysis renders it difficult to draw further conclusions on the possible influence of environmental risk factors for West Nile virus disease in horses. Further studies should be conducted to investigate hypotheses related to different potential vectors in the wet area of Camargue.

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ANALYSIS OF A SINGLE FARM OUTBREAK OF POSTWEANING MULTISYSTEMIC WASTING SYNDROME (PMWS) USING ROUTINE PRODUCTION DATA TO IDENTIFY INDIVIDUAL AND GROUP-LEVEL RISK FACTORS FOR DISEASE

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SUMMARY

Routinely collected farm-level production data from a single farm outbreak of postweaning multisystemic wasting syndrome (PMWS) were used to investigate risk factors for clinical presentation of disease at the individual- and group-level among postweaning pigs. A generalised linear mixed model with litter incorporated as a random effect identified a reduced risk of PMWS amongst Landrace breed, females, pigs from heavier litters and pigs that were weaned later in the outbreak. Risk factors for an individual animal developing PMWS included larger weaning groups, sow parity and a strong association between two batches of imported boar semen and PMWS, with descendents of these boars at significantly higher risk. This work has demonstrated how routinely collected farm data can provide a cost effective source of epidemiological data for investigating animal disease.

INTRODUCTION

Postweaning multisystemic wasting syndrome (PMWS) was first described in Canada in 1996 (Harding & Clark, 1997; Harding et al, 1998). Since then it has been reported from most of the major pig-producing countries of the world including North America (Harding & Clark, 1997; Harding et al, 1998), Spain (Segales et al., 1997), France (Le Cann et al., 1997), UK (Potter, 2000; Gresham et al., 2000), Sweden (Hasslung et al., 2005) New Zealand (Loth, 2005), parts of Asia (Choi & Chae, 1999) and Eastern Europe (Kiss et al., 2000). The disease was first identified on the UK mainland in 1999 and since then has slowly been spreading north from its original foci in East Anglia. It has since spread to the north of England and into Scotland despite attempts to control it (Muirhead, 2002). PMWS is now endemic in the UK and is one of the major causes of wasting disease in pigs in the country. Pig movements and mixing have been implicated in the spread of PMWS, largely through corporate pig farms but with circumstantial evidence for horizontal spread to nearby farms via animal vectors (e.g. birds) or semen, equipment and clothing (Mackinnon, 2000). The economic impact of the disease due to mortality or the production of non-marketable pigs is considerable and mortality on farms can be as high as 20% from weaning to fattening (Madec et al., 2000; Gresham et al., 2003).

PMWS is characterised by progressive weight loss and sometimes other signs such as respiratory distress, scouring and jaundice, with highest mortality in the 6 to 12-week-old age

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group. Although infection with porcine cirvovirus type 2 (PCV-2) appears to be necessary for the development of clinical PMWS, PCV-2 is also found in herds which do not have recognised signs of PMWS and additional factors are likely to be involved in the progression to clinical disease. Furthermore PCV2 is virtually endemic in the pig population and has been so for many years before the appearance of clinical PMWS (Magar et al., 2000).

Epidemiological studies carried out in France showed deviations in 'best-management' in severely affected farms and the implementation of Madec et al (2000) 20-point plan significantly reduced the mortality in several severely affected herds (Rose et al., 2003). Further studies to explore risk factors for PMWS in farrow-to-finish farms have also highlighted the role of management and the environment in determining the risk of PMWS (Rose et al., 2003). Changes to improve hygiene and reduce stress have been proposed as a means of reducing the impact of PMWS including measures to reduce mixing pigs, adequate pig flow (all in-all out) and pig density (Segales & Domingo 2002).

During a study of PMWS conducted by the Veterinary Laboratories Agency in 2003, one of the participating farms had an outbreak of the disease. This provided a unique opportunity to examine on-farm risk factors using retrospectively collected data and because the outbreak had already occurred, routinely collected herd production data was used to identify risk factors. This not only provided a large amount of data for analysis but also enabled an exploration of how such data can contribute to epidemiological studies with minor cost or additional data collection.

MATERIALS AND METHODS

Study population

The study population comprised all post-weaning pigs that were present on a single breeding unit in the UK at the time of the outbreak. The farm had previously been PMWS-free and maintained a high standard of hygiene and biosecurity, including operating a closed herd using only home bred boars and gilts. The AI studs from which semen were sometimes used were free of both PMWS and porcine reproductive and respiratory syndrome virus (PRRSV) and the only notable importation of semen from an external source was a year prior to the outbreak when the farm received three batches of imported semen from abroad. PMWS was diagnosed by the farm's private veterinary surgeon and confirmed by submission of affected pigs to VLA for *post mortem* examination. An aggressive culling policy was instituted in an attempt to control the disease and the eartag numbers and details of all euthanased pigs were recorded weekly, enabling the tracing of cases. The dataset did not include clinical or pathological findings, but it was assumed that all these pigs were PMWS cases. According to farm records, less than 10 pigs died before culling started. Pathological results from pigs submitted to VLA for study purposes confirmed that no PMWS was present in March, 4 months prior to the outbreak but in July 2003 when the first cases were detected the farm was confirmed as PMWS and PCV2 positive.

Data collection

A large amount of data were collected daily as part of the farms own recording system to monitor production parameters, and the farm generously allowed the VLA access to their computerised records as well as paper records kept on the farm. Each pig on the farm is identified by a unique identifier with individuals linked to their litter (and thus litter-level variables) by the unique litter ID. All data from paper records were entered by trained data entry staff at the VLA with checks made before data analysis commenced. Information was extracted

from the on-farm databases by the farms own staff and sent to the VLA electronically. Data were stored as MicroSoft Access databases and transferred to STATA 8.0 (Stata Corporation) for data manipulation and statistical analysis.

Statistical analysis

Due to the large number of possible risk factors and lack of knoweldge of *a priori* risk factors the data were analysed in a case control type study with logistic regression methods used to determine adjusted odds ratios for each variable. Cases were those pigs that were identified by the farm as having PMWS and subsequently euthanased and control individuals were all non-PMWS pigs from the same weaning cohorts. This yielded a total of 1232 PMWS cases and 12466 unaffected controls weaned between March 2003 and October 2004.

All variables significant at $p \leq 0.25$ in univariate analyses were incorporated into a multilevel (random or mixed effects) logistic regression model which was then subjected to a backward elimination modelling process. Non-significant (Wald's test $p > 0.05$) variables were removed sequentially to determine the final model. It was expected that non-independence would occur in the data due to similarities between pigs in the same litter and so, litter was included as a random effect in the model. Failure to take into account such clustering in data tends to lead to underestimation of the standard errors of parameter values and hence overestimation of the variable effects (Dohoo et al., 2003). Several authors recommend using more than one method of model fitting when attempting to fit multilevel models and if good concordance is achieved then one can have confidence in the results (Vigre et al., 2004). Two methods were used in this study to estimate parameter values. Penalized-quasi-likelihood (PQL) estimates were calculated in R using the *glmmPQL* command, and maximum likelihood (ML) estimates based on a numerical integration procedure were computed in STATA using the *gllamm* macro (www.gllamm.org). Model coefficients and standard errors estimated using the two methods were compared.

This two-level model (pigs within litters) was then extended to a three-level random effects model to allow for non-independence between litters (second level) from the same sow or boar (third level). To test the significance of the random effects terms, the log-likelihood of the full (three-level) was compared to the log-likelihood of refitted models without the effect of interest using the likelihood ratio test (Dohoo et al., 2003).

Random effect variance estimates were used to calculate intraclass correlation coefficients (ICC) for each level of clustering. The ICC is the correlation between two observations within a cluster (e.g. pigs within litters), and was estimated using a simple approximation method based on latent variables. This also gives an estimate of the proportion of variance at each level and can be used to examine the variation between clusters in the disease probabilities (Dohoo et al., 2003, Snijders & Bosker, 1999).

RESULTS

Prior to the identification of PMWS on the unit, all production parameters on the farm were good and within normal range. Farm records indicate that postweaning mortality was stable at approximately 5% until the start of the outbreak at which point there was a sharp increase in postweaning mortality to 33% within the first month. Figure 1 plots the number of PMWS cases by the week in which the pigs were weaned. This figure provides a good approximation to the

epidemic curve which was unavailable due to reporting delays at the start of the outbreak, although as shown below, the age of cases was not constant over the outbreak period.

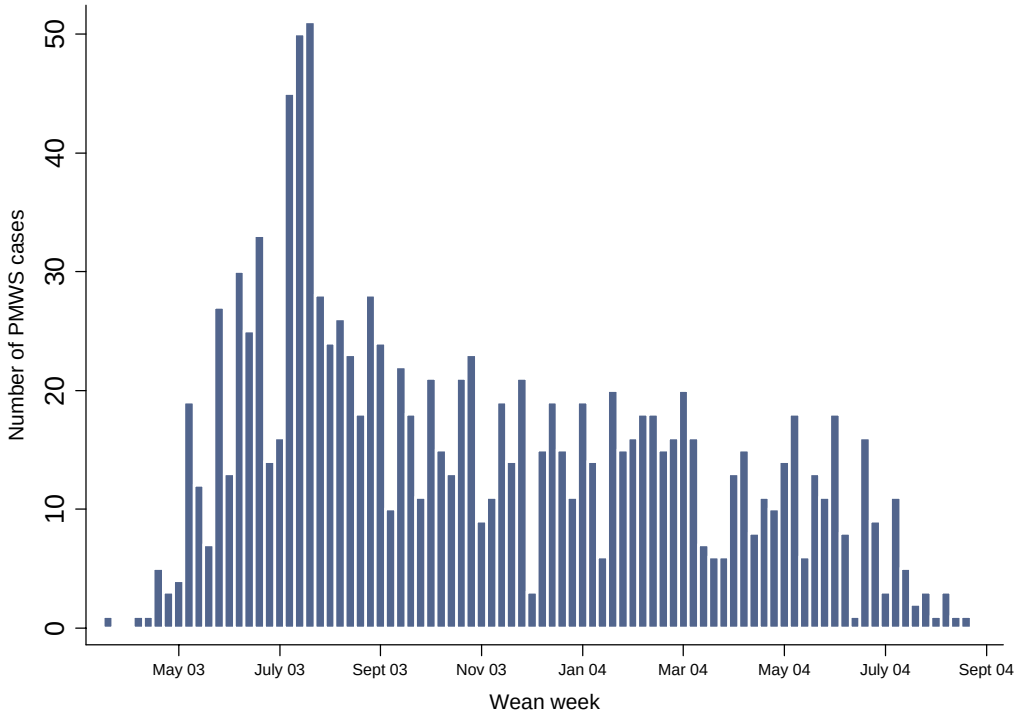

Fig. 1 The number of pigs that went on to develop PMWS by week of weaning. If pigs are assumed to develop the condition at roughly the same age, this should be an accurate representation of the epidemic curve.

Age of PMWS cases

Figure 2 shows the age of PMWS cases each month for the duration of the outbreak with the fitted regression line and 95% confidence intervals also presented. The first month (July) was excluded as pigs were significantly older at death due to recording delays. There is a clear and significant increase in the age of cases from approx 60 days in the first months of the outbreak to approx 75 days in the last month (November 2004). This is a difference of 2 weeks in the mean age of cases with a shift in the peak age at infection from 8-9 weeks to 10-11 weeks. Although the difference is not a large one, it may have significant economic implications if pigs are dying later.

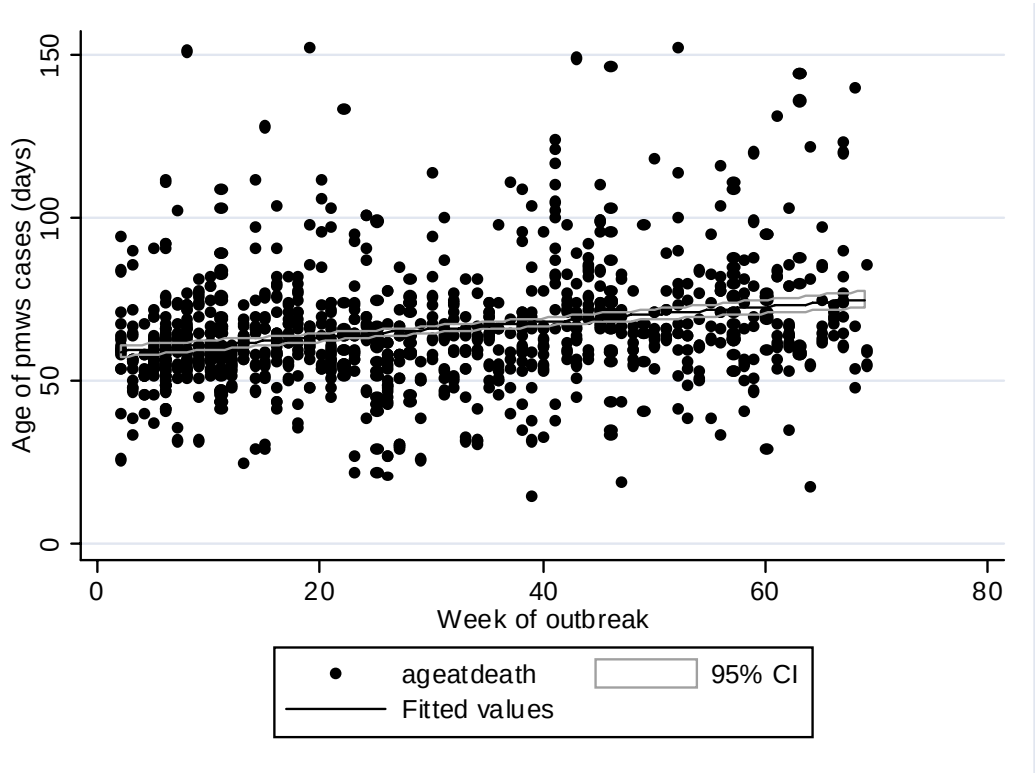

Fig. 2 Mean age of death of PMWS cases in days (α=58.51, β=0.24 R^2=0.06, p=0.000) by week of outbreak.

Risk factor analysis

Univariate analyses were carried out to examine each potential risk factor individually and its association with PMWS. With the exception of whether artificial insemination (AI) was used and whether any piglets in the litter were mummified, all variables were significantly associated with PMWS at the univariate level. Live litter size and litter weight were highly correlated so mean pig weight was calculated (live litter weight/number born alive in the litter) and used to remove the problem of correlated variables.

Table 1 shows the final results for the multilevel models with litter as a random effect using maximum likelihood for parameter estimation. The estimates of the coefficients were similar using PQL estimation, suggesting that reasonable confidence can be placed in the parameter estimates (results not shown).

There was a significant association between sex and the risk of PMWS, with females at reduced risk compared to males (OR=0.83). Breed was also significant in the final model with Large Whites at approximately twice the risk compared to Landrace (OR=0.56). This breed effect was supported by the fact that overall, the PMWS mortality rate was approximately 7.18% in Landrace and 13.6% in Large Whites. One possible explanation for this effect may be explained by the results for the imported boar semen as a risk factor. Imported Boar 1 was a Landrace boar and relatedness to this boar was not significantly associated with the risk of PMWS. For the other two Large White boars (boars 2 and 3) there was a highly significant relationship, with progeny of either of these two at a much higher risk of PMWS (OR=1.91 & 2.44 respectively). Two randomly selected boars (one Landrace, one Large White) were also

included but relatedness to either of these was not a significant risk factor. Week of weaning was included in the final model to control for the decreased risk of PMWS over time. This variable was categorised as four categories of 20 weeks.

A higher mean piglet birth weight (total litter weight/number born alive in litter) was only protective in the highest category, when the mean weight of pigs in a litter was >1.85kg, but there was a trend for a decreasing risk of PMWS with increasing mean piglet weight.

The number of pigs weaned from a litter (as opposed to per batch as described below) gave an indication of the size of the litter prior to weaning as it would take into account any changes in litter size after farrowing due to fostering or pre-weaning mortality. Although significant at the univariate level, this variable was not significant in the multilevel model. Likewise, the number born alive, dead, and the number of returns to service were not significant in the multivariable model. However, it is not possible to use these data to differentiate between failure to conceive or early abortion and resorption, which may represent different risks.

Table 1. Multivariable logistic regression results using a multilevel model with clustering on litter

VARIABLE	LEVEL	%PMWS	COEF	SE	OR	95% CI	P
Breed	Large White (LW)	13.60					
	Landrace	7.18	-0.58	0.12	0.56	0.444-0.712	0.000
Sex	Male	9.59					
	Female	8.38	-0.18	0.07	0.83	0.734-0.951	0.007
Week weaned	≤20	9.17					
	21-40	12.72	0.43	0.15	1.54	1.161-2.051	0.003
	41-60	9.44	0.07	0.16	1.07	0.788-1.462	0.654
	61-86	5.40	-0.51	0.16	0.60	0.439-0.814	0.001
Mean piglet weight (kg)	≤1.44	7.98					
	1.45-1.64	8.64	-0.08	0.13	0.92	0.715-1.197	0.553
	1.65-1.84	9.40	-0.24	0.14	0.79	0.602-1.031	0.082
	≥1.85	13.08	-0.50	0.14	0.60	0.457-0.800	0.000
Parity	≤2	9.54					
	≥3	8.42	0.25	0.10	1.28	1.045-1.566	0.017
Imported Boar 2 (LW)	No	8.49					
	Yes	19.46	0.65	0.21	1.91	1.262-2.891	0.002
Imported Boar 3 (LW)	No	8.54					
	Yes	22.10	0.89	0.24	2.43	1.527-3.893	0.000
Weekly Cohort size	≤147	7.75					
	148-167	7.78	0.08	0.14	1.08	0.823-1.418	0.526
	168-188	11.05	0.47	0.13	1.59	1.228-2.070	0.000
	≥189	9.11	0.31	0.16	1.37	0.999-1.872	0.050

The total number of pigs weaned in a batch was used as a proxy for crowding in the post weaning accommodation. Where a large number were weaned in a particular week, the housing was likely to be more crowded which may consequently have caused stress and affected pig health. Only when more than 167 pigs were weaned was there a significant increase in the risk of PMWS.

The variance of the random effects for the two level model was 1.208 (0.124), giving an estimated ICC of 0.236. This is the proportion of the overall variance that occurs at the litter level, or the correlation between individuals in the same litter. If the random effects variance, and hence ICC is low and close to zero then there is no additional variation between litters any variation that does exist litters can be explained by other effects included in the model, whereas if it is large and positive then there is a high degree of clustering with classes being very different (Dohoo et al., 2003). In this case approx 24% of the total variance could be explained at the litter level.

To investigate whether there was also clustering on sow or boar, two further models were fitted. One included both sow and litter as random effects, the other boar and litter. Coefficient estimates were very similar using both the two- (litter only) and three-level models (boar or sow in addition to litter). Both random effects (litter and boar or sow) coefficients were highly significant with $p<0.001$ in both models suggesting that in there may be additional clustering of PMWS in the offspring of certain sows and boars. Model comparisons with and without the third level also support this.

DISCUSSION

Epidemiological studies designed to examine new and emerging infections of livestock are often costly due to the lack of prior information regarding disease aetiology and risk factors. The work presented here has shown how routinely collected data, gathered with minimal cost, may provided a cost effective first step in answering some of the questions and highlights the wealth of data that can be available from pig producers. This analysis has focused on describing the major features of a single farm PMWS outbreak and has highlighted several factors that may be associated with the risk of the disease.

General hygiene parameters could not be investigated in this study but changes made on the farm after the discovery of PMWS are likely to have had a beneficial effect in reducing the transmission intensity and lowering mortality. However, as noted in other studies (Madec et al., 2000; Gresham et al 2003) the postweaning mortality is still significantly greater than prior to the outbreak. The presumed reduction in transmission may also be reflected in the increased age of cases as the outbreak progressed. This increase in the mean age of cases may be due to a number of factors but it is unlikely to be due to slower identification of cases as farm staff maintained the euthanasia policy for PMWS cases and if anything, identification may have improved as staff became more experienced in identifying the early stages of the disease. It is also plausible that if PMWS is caused by an infectious agent, then this pattern may reflect the reduction in transmission intensity described above. An increase in the mean age at which the burden of disease occurs is typical of a reduction in the transmission intensity (Anderson & May, 1999). After the initial introduction of the disease into the susceptible population the transmission rates are high and the pigs encounter high exposure at young ages. As some of the population acquire a degree of immunity the rate of transmission is slowed and it takes longer for an individual to reach the same level of exposure and hence the burden of disease is shifted

to an older age group (Anderson & May, 1999). This is typical of infectious diseases and hints at an infectious agent as a primary cause of PMWS. If acquired immunity does not play a significant role in this disease, the interventions applied by the farm after the initial diagnosis may have been effective in reducing transmission rates but not eliminating the disease from the population.

Although the cause of the outbreak has not been determined in the present analysis, a number of significant risk factors have been identified. The increased risk to males noticed here has been reported elsewhere (Rodriguez-Arrioja et al., 2002). In the study of Rodriguez-Arrioja et al. (2002) there was an observed difference in the mortality rates of males and females, and because male pigs were castrated, this procedure was implicated as a risk factor for PMWS. In the present study the farm did not castrate males suggesting that males may be at increased risk regardless of whether they were castrated or not.

This study showed no association between PMWS and reproductive disorders. There was no increase in the number born dead or mummified in the litter and an individuals risk, and likewise the sows were not more likely to return to service prior to having a PMWS affected litter. This supports the findings of other authors (Rose et al., 2003).

Several previous studies have noted that there appears to be a litter effect in PMWS infection with some litters heavily affected and others apparently unaffected (Madec et al., 2000; Rodriguez-Arrioja et al., 2002). The litter effect was also observed in the present study with some litters heavily affected and others having no clinical PMWS.

The inclusion of whether pigs were direct descendants of the imported semen as a risk factor also highlighted the fact that the progeny of certain boars may be at greater risk. The offspring from the Landrace boar were not at increased risk but those offspring of the two Large White boars were and it is possible that this may also account for the breed difference. However, all the progeny of these boars present at the time of the outbreak were second generation and none of the first generation descendants developed PMWS. Furthermore, some pigs not related to these boars developed PMWS, suggesting additional factors were necessary for clinical disease. This would not be what was expected if PMWS was caused directly by some agent present in the semen and it may be that there is a genetic effect related to susceptibility that plays a role in the presentation of clinical PMWS. The presence of a 'factor X' either through co-factors or genetic susceptibility might explain the lack of disease in the first generation if some factor contributing to a sufficient cause of PMWS was not yet present on the farm. Madec et al. (2000) suggested that it was the sow that played a key role as the sow is supposed to the reservoir for circovirus and other pathogens found on commercial rearing units. The significant effect of parity on the risk of PMWS also seems to point to other external factors acting on litters to influence the risk of PMWS. PCV2 has been found in the semen of infected boars (Kim et al., 2001; Hamel et al., 2000) and has been implicated in the spread of PMWS in the UK (Mackinnon, 2000).

A number of assumptions have been made in the analyses presented here, the major one being that no fostering (i.e. moving piglets between litters) occurred and so all weaning data and much of the litter-level data have been linked to individuals through the litter ID. Farm records suggested that limited fostering did occur but it proved to be impossible to confidently identify either the individual pigs that had been moved or those litters that had received fostered pigs. However, the data showed that a number of litters weaned more than were born, indicating some movement of pigs between litters or recording errors. Previous studies have shown a high level of cross-fostering to be a risk factor for PMWS (Rose et al., 2003), but the limited data shown

here did not demonstrate any significant effect. Other misclassification may have occurred because all pigs that were not euthanased or did not die of PMWS were presumed to be negative for the disease in this analysis.

In conclusion, this study has identified a number of significant risk factors for PMWS using routinely collected farm production data. The dynamics of the disease on the farm has been explored and concurs with the findings from many other studies that PMWS emerges relatively quickly once introduced to a unit and is epidemic in nature with fast spread through the population. Once the initial outbreak is over the disease settles down but postweaning mortality remains relatively high compared to pre-outbreak levels. The disease may stay on a unit for years, sometimes at a severe level (Madec et al., 2000). There is some suggestion of vertical transmission through the sow or boar or probably both, but the rapid spread of the disease suggests some horizontal transmission may also occur. This study has also demonstrated how routinely collected data, in addition to simple recording systems to record affected individuals, can be used to identify risk factors for new and emerging diseases. Where studies are carried out retrospectively, this type of analysis may provide a reliable and relatively cheap source of data for epidemiological studies.

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PUBLIC HEALTH

COLONY FORMING UNITS OR PREVALENCE: HOW TO USE EXPERIMENTAL DATA IN PREVALENCE SIMULATION MODELLING

B. VOSOUGH AHMADI*, A.G.J. VELTHUIS, H. HOGEVEEN AND R.B.M. HUIRNE

SUMMARY

The effectiveness of antimicrobial decontamination methods in slaughterhouses can be expressed as reduction in number of colony forming units (CFU) counts and as a reduction in prevalence of contaminated end products. In many risk assessments the contamination status of the food products are modelled, with prevalence as output parameter. To use experimental microbiological data in these models, indicating the CFU reduction after an applied intervention, these data should be translated into a probabilistic input parameter. A methodology is presented to calculate such a probability from experimental data. Using this methodology it is demonstrated that the effectiveness of decontamination methods varies with the initial number of bacteria present on the carcase. And in case of a high initial concentration of bacteria (>log 5), the elimination probability will be zero even if a very powerful decontamination method is applied.

INTRODUCTION

Carcase antimicrobial-decontamination methods are considered as slaughterhouse interventions against enteric pathogens such as *E.coli* O157:H7 (VTEC) (Koohmaraie et al., 2005). The effectiveness of decontamination methods is an element that should be considered in a cost-effectiveness analysis. Two measures of effectiveness of decontamination methods at the slaughterhouse can be distinguished: (i) reducing the fraction (i.e., prevalence) of contaminated carcasses and (ii) reducing the number of bacterial colony forming units (CFU) on a carcase. When focusing on food safety problems related to the enteric pathogens that may contaminate meat, models that predict the number of CFU counts (see for example Ebel et al., 2004; Nauta, 2001) are suggested. However, such models require a large number of input variables and thus many assumptions. Prevalence simulation models are often used to estimate the effectiveness of intervention strategies to reduce the fraction of carcasses contaminated by enteric pathogens (see for example Alban & Stärk, 2005; van der Gaag et al., 2004b). The advantage of prevalence simulation models is that there are less input variables and thus fewer assumptions are needed.

Results of experimental studies are often expressed in terms of log reduction of CFU counts on the surface of the meat (Juneja & Sofos, 2002; Phebus et al., 1997; Retzlaff et al., 2004). If it is desirable to use these data in a prevalence simulation model, an approach needs to be developed to convert the reported log reduction to an elimination probability, which is the

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probability of eliminating all bacteria from surface of the meat using decontamination methods. Although, there have been some efforts to translate a pathogen reduction into an elimination probability (SCVPH, 1998, 2003) they were not satisfying. Their focus was mainly on converting percentage reduction of CFU counts by decontamination methods into the proportion of positive carcasses and not on translating the experimental data to an elimination probability. More studies are therefore needed to introduce and examine other approaches. In this paper, the authors demonstrate a modelling approach that can be used to translate an experimentally measured log reduction (of decontamination methods) to an elimination probability. Such elimination probability can be used in a prevalence simulation model to evaluate the effectiveness of decontamination methods. In the following sections first the modelling approach is presented and then, using some published data for the initial number of bacteria and the antimicrobial effects of the decontamination methods, this modelling approach is illustrated in a prevalence model.

MATERIALS AND METHODS

Modelling approach

The aim of this modelling approach is to estimate the elimination probability for antimicrobial decontamination methods, given different values of the initial number of pathogens on the surface of the carcasses. The reduced number of CFU resulting from implementing a decontamination method is translated into the probability of having zero bacteria (i.e. the elimination probability) using the first element of a poisson distribution. The expected number of CFU per carcase after intervention equals the initial number of CFU on each carcase minus the reported CFU reduction due to that intervention. Let *EP* denote the estimated elimination probability, μ the initial number of CFU on the whole carcase and λ the reduction in number of CFU on the whole carcase. The elimination probability can be calculated using following equation:

$$EP = e^{-(\mu-\lambda)} \tag{1}$$

Using Eq. (1) the relation between *EP*, μ and λ has been illustrated (Fig 2). For this illustration, seven different decontamination methods with antimicrobial effectiveness varying from one to seven log reduction of CFU (log 1 to log 7) were assumed. The results of this methodology are given in the result and discussion section.

Application

The modelling approach described above, was developed to investigate the effectiveness of interventions (in terms of reduction in prevalence) against *E.coli* VTEC in Dutch dairy-beef industrial slaughterhouses (Vosough Ahmadi et al., YEAR?). Five carcase-antimicrobial decontamination methods, hot-water wash, lactic-acid rinse, steam vacuum, steam pasteurization and gamma irradiation including their combinations were examined. With a Monte Carlo simulation the elimination probabilities for the decontamination methods were calculated using published data for antimicrobial effectiveness of the decontamination methods and the initial number of bacteria on the surface of the beef carcase (Fig 1). The area separated by the dashed line in Fig. 1 is the model to estimate the elimination probability presented in this paper. The output of this model serves as input in the prevalence simulation model, which uses binomial processes (Vosough Ahmadi et al., YEAR?). The initial number of bacteria (CFU) on each

carcase was simulated by multiplying two distributions: the amount of transferred manure in grams to the carcase (beta distribution) and the concentration of VTEC in one gram of manure (cumulative distribution). The used data and distributions were based on a VTEC risk assessment (Table 1, Nauta, 2001). A beta distribution was chosen to describe the carcase contamination with manure after fitting the results of expert estimates to a series of probability distributions (Nauta, 2001). The parameters α and β were used to express the level of carcase contamination with manure and its variability per carcase. A cumulative distribution was used to include the uncertainty related to the concentration of VTEC in a gram of manure, based on data reported by Zhao et al. (1995). In the mentioned study, VTEC concentrations in the faeces of 31 positive calves were measured from a survey of dairy herds in the U.S.

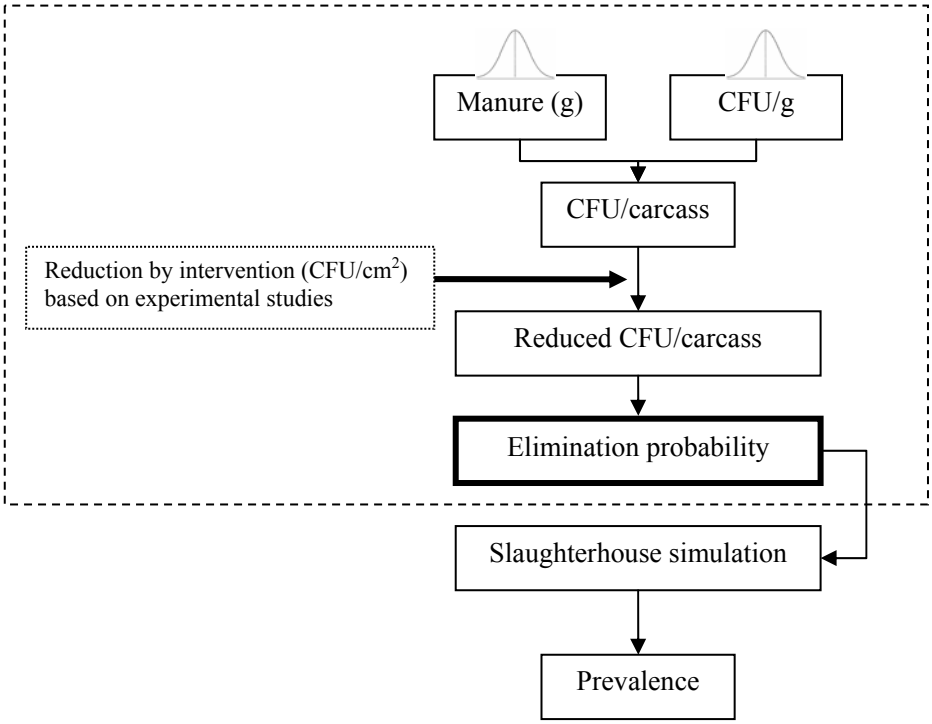

Fig. 1 Schematic view of VTEC simulation model for beef-carcasses at slaughter

For the simulations it was assumed that each carcase has a total surface of 32,000 cm^2 and that each quarter receives equally one fourth of the total faeces. The expected number of CFU per quarter when interventions are applied equals the initial number of CFU (μ) on each quarter minus the reported reduction (λ) due to a specific intervention (Phebus et al., 1997). The reduced number of bacterial counts resulting from a reduction due to intervention is calculated by Eq. (1). The mean elimination probabilities were determined using 10,000 iterations and were used as inputs in the VTEC prevalence simulation. The model was built in Microsoft Excel spreadsheet using @Risk add-in software.

Table 1. Description of variables and distributions used in a VTEC simulation model for beef-carcasses at slaughter

VARIABLE	DISTRIBUTION	VALUES
Concentration of bacteria (log CFU) in gram of manure	Cumulative [a]	$\{X_i$: 0, 2, 3, 4, 5, 6$\}$ $\{P_i$: 0.00, 0.46, 0.53, 0.87, 0.96, 1.00$\}$
Gram of manure on each carcase	Beta [b]	Max: 10.1 α: 0.395, β: 2.47

[a] @Risk function: RiskCumul(0,6, {2,3,4,5},{0.469, 0.531, 0.875, 0.969})
[b] @Risk function: Max * RiskBeta(α, β)

RESULTS AND DISCUSSION

Figure 2 shows the elimination probabilities for the seven assumed categories of decontamination methods (log 1 to log 7) with different values for the initial number of bacteria present on the carcase. The elimination probability will be zero when applying a weak decontamination method (log 1 reduction in CFU) on a carcase that is initially contaminated with more that 68 CFU (log 1.8). At that level of initial contamination, more powerful decontamination methods give a high elimination probability of infection. However, with a higher level of initial contamination, also more powerful decontamination methods may give zero elimination probability. The elimination probability for decontamination methods with antimicrobial effects of log 6 and log 7 will be zero only if the initial CFU count is higher than one million. These results imply that in the case of having very high initial concentration of bacteria on the carcasses (>log 5), the elimination probability can be zero even if a powerful decontamination method is applied. This means that interventions will have no effect on the reduction of the prevalence of contaminated carcasses. However, these decontamination methods still give an important improvement of the beef safety by reducing the CFU counts.

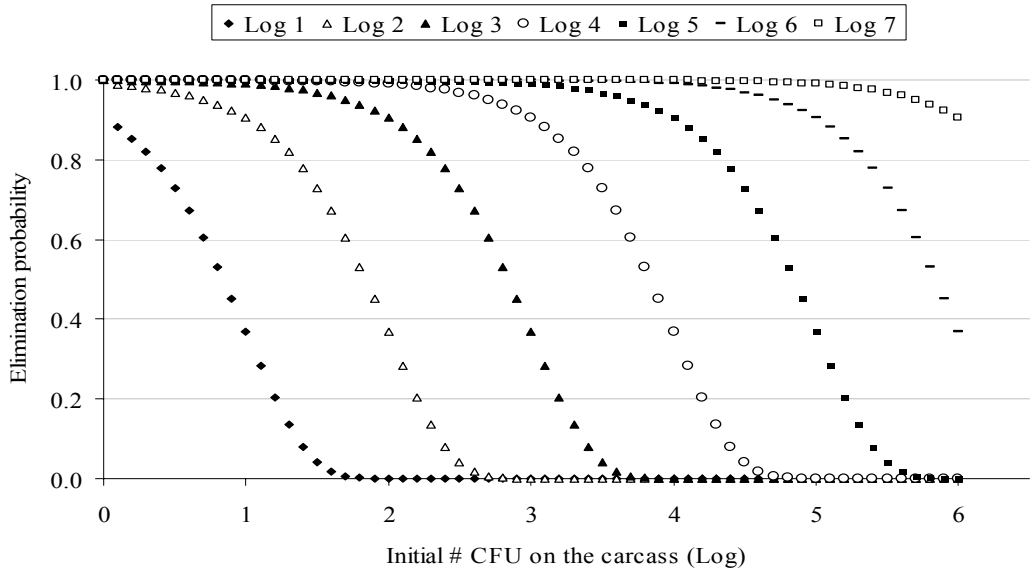

Fig. 2 Elimination probabilities for seven decontamination categories graphed against different levels of initial number of CFU on a beef-carcase at slaughter in log scale

Figure 2 also shows that the elimination probability will be higher than zero when the initial number of bacteria is low (<log 1.8), and therefore a prevalence reduction can be expected using these methods. The majority of the decontamination methods have an elimination probability greater than 90% in the case of having up to 10 CFU (log 1) as initial number of bacteria. These values decline by increasing the initial bacterial load. This result implies that control of the initial contamination of the carcase is effective in two ways. It lowers the prevalence of contaminated carcasses directly and it increases the elimination probability of existing infections.

In the application part of the modelling approach explained in this paper, the data on initial number of bacteria on the carcase and experimental data on antimicrobial effects of decontamination methods were used to estimate the mean elimination probability for each decontamination method. The eventual goal was to use the estimated elimination probabilities in a prevalence simulation model to estimate the effectiveness of decontamination methods in reducing the prevalence of contaminated beef-carcase quarters. To get stable output, the elimination probabilities for the five decontamination methods were calculated with 10,000 iterations. The antimicrobial effects (input) and mean values of elimination probabilities (output) of the five decontamination methods are presented in Table 2. The practical meaning of these values is that, for example, when hot-water wash is used as intervention, a contaminated beef-carcase quarter will have a 34% probability of changing from positive to negative. In this way, these values can be used in prevalence simulation models that are developed based on binomial processes. Because of the low initial number of bacteria coming from the two mentioned distributions, in most cases the most-likely values for the elimination probabilities were close to one. Thus, choosing the mean of the distribution assures us to consider the tail of the distribution.

Table 2. Mean elimination probability for five decontamination methods applied to reduce the amount of VTEC on beef-carcasses obtained through a simulation……

DECONTAMINATION	MEAN REDUCTION [a] (log CFU/cm^2)	MEAN ELIMINATION PROBABILITY (%)
Hot-water wash (W)	0.75 $\pm$ 0.49	34.69
Lactic-acid rinse (L)	2.70 $\pm$ 0.49	68.75
Steam vacuum (V)	3.11 $\pm$ 0.49	77.00
Steam pasteurization (S)	3.53 $\pm$ 0.49	83.17
Irradiation (Ir)	6.00 $\pm$ 0.49 [b]	99.48

[a] Mean reduction in VTEC population (log CFU/cm^2) $\pm$ standard error of mean (Phebus et al., 1997).
[b] Molins et. al.(Molins et al., 2001), the same standard error as the other methods is assumed.

In general, the reduction in prevalence depends highly on the initial number of bacteria on the surface as well as the antimicrobial power of decontamination method used. The antimicrobial power of decontamination methods depends on different factors such as the technical strength of decontamination methods to destroy the bacterial germ, time and place of intervention (in the slaughter line) as well the type of the bacteria and its adherence characteristics to the meat surface. Therefore, both prevalence reduction and CFU reduction effects should be considered when the "effectiveness" of decontamination methods is concerned. In the majority of the cost-effectiveness analyses on the interventions against enteric bacteria, the main focus is only on one of the mentioned effects. For example Jensen et al., (1998) consider only CFU reduction and van der Gaag et al., (2004a) consider only prevalence reduction as the basis of their economic analysis. This may lead to an underestimation of

effectiveness (in case of focusing only on experimentally measured CFU reduction) or overestimation (in case of focusing only on prevalence reduction). Thus efforts should be done to consider these factors together in such studies.

In the relatively simple simulation model described in this paper, the initial number of CFU was determined based on distributions for the amount of manure and the concentration of CFU in the manure. These distributions were based on the Dutch expert's opinion and literature (Nauta, 2001; Zhao et al., 1995). Because the type of the distributions was based data fitting, as explained by Nauta (2001), these distributions may vary in other countries and conditions. Therefore the elimination probability calculated for each decontamination method might be different for different countries and conditions. This is mainly due to the hygienic measures in the slaughterhouses that allows or prevents the transmission of manure to the carcasses. Also this depends on the concentration of CFU bacteria shed into the manure. Farming practice and the situation of different countries are important factors for concentration of bacteria shed in the manure.

CONCLUSIONS: PREVALENCE VERSUS CFU MODELLING

Considering the prevalence versus CFU modelling issue, on one hand it can be observed that industry, regulatory agencies and consumers focus on the fraction (prevalence) of contaminated end products. Also many scientific studies focus only on prevalence. As it was mentioned before, in the case of a low initial contamination (i.e. lower than 1.8 log CFU count), focusing on prevalence can be a good approach without modelling or considering the CFU counts. This seems a valid assumption for the common slaughter practice in most of the developed countries. However risky events such as gut rupture during the evisceration, which can lead to the release of a large number of bacteria on the carcase, can make this assumption invalid even in the best manufacturing practices at slaughterhouses.

On the other hand public health authorities and farm-to-fork risk assessors are very much concerned about the exact number of CFU present on the surface of the meat. As the infectious dose for some of the enteric pathogens such as *E.coli* VTEC is very low, even one bacterium has a great importance. Therefore, from this point of view studies that consider prevalence as their main criterion do not sufficiently address the problem. In this case the result of the effectiveness analysis may become biased because of the overestimation of the effectiveness.

Thus, it can be concluded that in the effectiveness analysis of decontamination methods the expected number of CFU on the carcasses along with the consideration of the expected prevalence of contaminated carcasses should come together. The best way to this is to develop a CFU model that estimates the number of transmitted bacteria to the end product and thus implicitly estimates the prevalence of contaminated product as well. An alternative way that presented in this paper is modelling the elimination probabilities based on initial CFU contamination and feed them as input to a prevalence simulation model to calculate the prevalence reductions due to specific decontamination methods.

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AN ANALYSIS OF SINGLE INTRADERMAL COMPARATIVE CERVICAL TEST (SICCT) COVERAGE IN THE GB CATTLE POPULATION

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SUMMARY

Cattle herds in Great Britain (GB) are routinely tested for bovine tuberculosis (bTB) using the single intradermal comparative cervical test (SICCT). The interval between testing varies according to the bTB history of the parish in which the herd resides and may be one, two, three or four years, unless a reactor is detected in which case a cascade of tests ensues.

An analysis of the likely coverage of bovine tuberculosis (bTB) testing in cattle in GB was conducted to explore a number of variables such as: age, testing regime of parish of residence, breed and herd type that are likely to affect whether and how often animals are tested for bTB in their lifetimes. In addition, the constitution of the untested population was explored to ascertain whether there is a potential reservoir of undetected bTB infection.

The results indicate that the majority of animals are not tested for bTB in their life. The frequency of testing was highly skewed and a small number of animals were tested on more than 15 occasions. Although many untested animals die before 30 months of age, the number of untested animals exceeds the number tested at all ages. There is not a simple relationship between movement and testing (i.e. animals are not moving 'away' from tests) or between herd type and testing.

INTRODUCTION

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is of increasing concern in Great Britain due to the reported increase in prevalence and incidence since the 1980s. The principal control and surveillance tool currently used in GB is the single intradermal comparative cervical test (SICCT), one of the variants of the skin test.

Testing has two purposes: surveillance and control. 'Surveillance' testing is conducted in order to disclose infection, and provide data on the incidence and prevalence of infection. On-farm surveillance (routine) testing is supplemented by routine meat inspection of cattle at slaughter. Control testing is conducted on herds found to be infected, in order to eliminate the infection from the herd. The interaction between the transmission dynamics of infection and such a testing programme has been discussed previously (Medley, 2003). Essentially, more frequent testing provides earlier disclosure of infection and hence better control and surveillance

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(Richards & Wilesmith, 1989) whilst decreasing testing frequency results in an increase in intra-herd prevalence and a decrease in the disclosed incidence.

For practical reasons, the current testing programme is organised and operated at the herd level. Cattle movement is an important factor in determining the frequency of testing for individual animals since movement between herds may result in animals either avoiding tests, or being tested more frequently. Survival of animals is also important in that animals may die before being tested or before they are able to transmit the infection to other animals. In both cases, it is possible for a significant proportion of cattle to avoid testing during their lifetime, and consequently if derived from infected herds, for these animals to be a reservoir of infection that allows *M. bovis* to persist in the GB cattle population, although slaughterhouse inspection suggests that this is not a widespread problem. (See below: Slaughterhouse monitoring)

In this paper, the aim is to understand the outcome of a herd level programme at the individual animal level by examining the outcome of the interactions between survival, movement and herd testing.

First, the individual elements of the programme in GB are described. Cohort data on survival, movement and testing are then extracted by combining two national databases, and analyse the data with respect to the testing experienced by individual animals during their observed lives.

The Elements of the Current Programme

SICCT and its interpretation: The test discloses an immunological reaction to *M. bovis*, in cattle. It is administered by bovine tuberculin being injected into the neck of an animal and any resultant reaction in the form of an increasing skin thickness being measured 72 hours later. Because of the problems of non-specific reactions, avian tuberculin is injected simultaneously at a separate site and it is the comparative difference between the reactions, to the two antigens, that determines the classification of the bovine animal into reactor, inconclusive or clear. An increase in skin thickness in the bovine animal site of more than 4mm greater than in the avian site results in the animal being classified as a reactor at standard interpretation.

The system used to apply the SICCT and the interpretation of results is complicated (Green & Cornell, 2005). Briefly, any reactors in a herd are slaughtered and then subject to both *post mortem* (*pm*) examination and the cultural analysis of a set of pre-determined samples. If either the *pm* examination results in tuberculosis lesions being identified or the culture work results in the growth of *M.bovis*, then the animal is classified as having confirmed infection. The herd is then subject to the rules for a confirmed breakdown, meaning that movement restrictions will not be lifted until 2 clear herd tests, 60 days apart have been achieved. At least the first test subsequent to the disclosing test, for a confirmed breakdown will be performed at severe interpretation, meaning that a relative increase at the bovine tuberculin site of more than 2mm results in the animal being classified as a reactor, thus increasing the sensitivity of the skin test. If no confirmed animals are identified at the disclosing test and any subsequent test, then the breakdown is classified as unconfirmed and restrictions will be lifted after one clear 60-day herd test at standard interpretation.

Slaughterhouse monitoring: All slaughtered animals are subject to examination for bTB lesions at slaughter. In 2004, out of 1,702 confirmed new breakdowns, 194 (11.4%) were first disclosed by discovery of lesions at slaughterhouse (DEFRA, 2004).

Herd Level Testing: Essentially herd testing falls into two categories: surveillance testing (routine tests) and those tests associated with disease control. Disease control tests are not only performed on the incident herd, but may include tests on neighbouring herds (contiguous tests) and the testing of herds to which or from which the incident herd has purchased or sold cattle (tracing tests). From an epidemiological viewpoint, contiguous herd and tracing tests can be regarded as either control or targeted surveillance.

The minimum frequency at which surveillance testing takes place is determined by the parish testing frequency of the parish in which the herd resides. The number of herds within a parish is variable (mean number 8, range 1-248). The parish testing frequency (the frequency at which surveillance testing takes place) varies between 1 test every year (annual) and 1 test every 4 years (quadrennial).

The testing regime is determined by EU directive 64/432/EEC, which sets the minimum frequency according to the proportion of confirmed breakdowns that have occurred in a parish in the previous 6 years. The increase in the incidence of bTB over recent years has resulted in an increase in yearly tested parishes over time.

Table 1. Distribution of parishes, herds and animals by test frequency, November 2005

Inter-test Interval (years)		**1**	**2**	**3**	**4**	**Totals**
Parishes	**No.**	1,909	1,286	101	9743	13,039
	%	14.64	9.86	0.77	74.72	100
Herds	**No.**	22,650	12,677	681	55,990	91,998
	%	24.62	13.78	0.74	60.86	100
Animals	**No.**	2,252,384	1,091,022	61,557	4,867,555	8,272,518
	%	27.23	13.19	0.74	58.84	100

Within yearly tested parishes all herds are tested; however outside these areas certain herds may be exempt from testing. For example fattening herds, where no breeding takes place and where all animals go direct to slaughter are exempt from testing. For yearly tested herds, all animals over six weeks of age should be tested, whilst in other herds, surveillance testing is normally restricted to the adult portion of the herd. Conversely, individual herds outside annual testing parishes may be subject to annual testing if they are deemed to pose an increased public health risk (e.g. producer retailers of unpasteurised milk) or animal health risk (e.g. cattle dealers or bull hirer herds).

Databases

Data relating to bovine TB (bTB) is recorded on DEFRA's disease recording database VetNet. A drawback of this database, from an epidemiological perspective, is that at the individual animal level it only records details of Reactors (R), Inconclusive Reactors (IRs) – animals whose reaction is indeterminate and Direct Contacts (DCs) – i.e. animals that are slaughtered because they pose a disease risk due to their close proximity to infected animals. Consequently, two sets of denominator are not recorded: those cattle not tested and details of those cattle that are tested, but are not R, IR or DC (although the total tested can be calculated).

The Cattle Tracing System (CTS) is a DEFRA database set up in response to Bovine Spongiform Encephalopathy (BSE) to record the births, deaths and movements of GB cattle. It has been in operation since 1998, but the compulsory recording of all cattle has only been a legal obligation since January 2001. Data before this date are of variable completeness and accuracy.

Thus, it is theoretically possible to obtain information on both the denominator populations described above. CTS provides details of an animal's life history (i.e. which herds an animal resided in at which times), and VetNet provides details of the testing of herds. This provides the novel opportunity to evaluate the TB testing programme's coverage at the level of the national cattle population. In particular, this approach permits access to animals that are not tested.

MATERIALS AND METHODS

Data extraction

From previous work, the VLA has established a method of obtaining the movement life histories of animals from the CTS data (Mitchell et al., 2005). Animals whose movement histories are 'illogical' or clearly incorrect are excluded. It was this data set downloaded on 6th July 2005 that was used in the analysis. Animals for which no death was recorded were assumed to be still alive at this date.

As the CTS data is currently of high completeness and accuracy for only 4.5 years (January 2001 – July 2005), and this period does not extend over the longest life expectancy of cattle, three different random samples of animals were taken from CTS:

1. **A**: A random sample of 96,862 animals born in 2000. These animals may or may not have been dead on 06/07/05.
2. **B**: A random sample of 97,429 animals that died in 2004.
3. **C**: A random sample of 87,217 animals with approximately equal numbers born in the five cohorts 1998 – 2002.

It should be noted that each of the three cohorts will include animals that lived through the period of the Foot and Mouth Disease (FMD) epidemic (February – November 2001), and that bTB testing was suspended for much of this time.

The random selection process did not exclude the possibility of the same animal being in the three different cohorts. 616 animals were in both cohorts A and C, 385 animals were in both cohorts B and C, and 83 animals were in both cohorts A and B. No animals were in all three cohorts.

The life history of each animal in each of the three subsets was followed on CTS, and by linking to the bTB testing data it was possible to ascertain for each holding that an animal resided on whether that holding was tested for bTB during that time period. If the holding was tested and the test type and the age of the animal during that time period were such that the animal should have been tested, then it was assumed that the animal had been tested. All tests where the majority of the herd was tested (herd tests) were considered e.g. routine herd tests (VE-RHT); whole herd tests (VE-WHT); contiguous tests (VE-CON) and short interval tests (VE-SI). The analysis could not take into account tests where only individual animals within the herd were tested (animal tests) e.g. inconclusive reactor tests (VE-IR).

Simulation to estimate the expected number of tests per bovine animal

A simulation of testing was conducted for cohort A. For each herd a date for its first test after 1/1/2000 was allocated by selecting at random from a uniform distribution on the interval: (0,365) for one-year testing, (0,730) for two-year testing, (0,1096) for three year testing and (0,1461) for four yearly testing. Subsequent testing was scheduled exactly according to the appropriate test interval until 06/07/05. Each animal in the herd was then considered tested if the test date lay between its birth and death dates (or 06/07/05 if it was still alive) and the animal's age was greater than 182 days, except it was assumed that tests scheduled between February and November 2001 inclusive were not performed due to the FMD epidemic. This simulation provides the expected number of tests per animal assuming that there is no movement and that testing is conducted exactly as prescribed.

RESULTS

Data validity

It is important that the characteristics of the sample population mirror that of the cattle population as a whole. Probably the most important attributes are age, the parish-testing regime within which the animal's natal herd falls and the month of birth distribution of the sample population. Age is considered because the age of an animal at death will clearly affect its probability of having been tested during its lifetime. Month of birth is considered because there is a seasonality associated with testing, with an autumn peak in testing associated with cattle being brought in from grazing.

A comparison of the age at death distribution for the three cohorts is shown in Fig. 1. All three cohorts show appreciable similarity, although the 2004 death cohort has a higher proportion of older animals, by virtue of the fact that all animals by definition have lived their full life expectancy. These ages at death distributions are very similar to those found previously for the cattle population of GB (Mitchell et al., 2005). Similarly, the distributions of month of birth were consistent with that of the cattle population of GB, showing a winter minimum and a spring maximum of births, (Fig. 2).

Figure 3 shows a comparison of the parish testing frequency distribution into which the samples were born and the distribution for the national herd in 2000. Again the cohorts show similar distributions both with each other and with the population data.

From the comparisons, it was concluded that the three samples were each representative of the GB cattle population, as recorded on CTS.

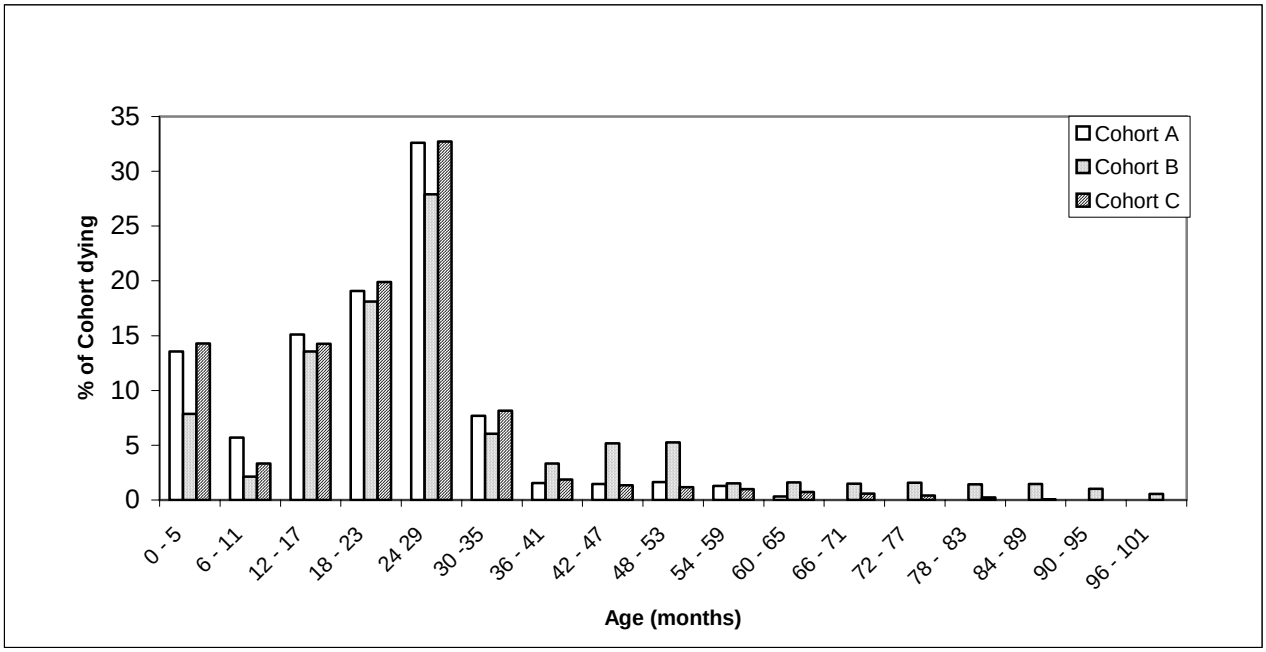

Fig. 1 Age at death

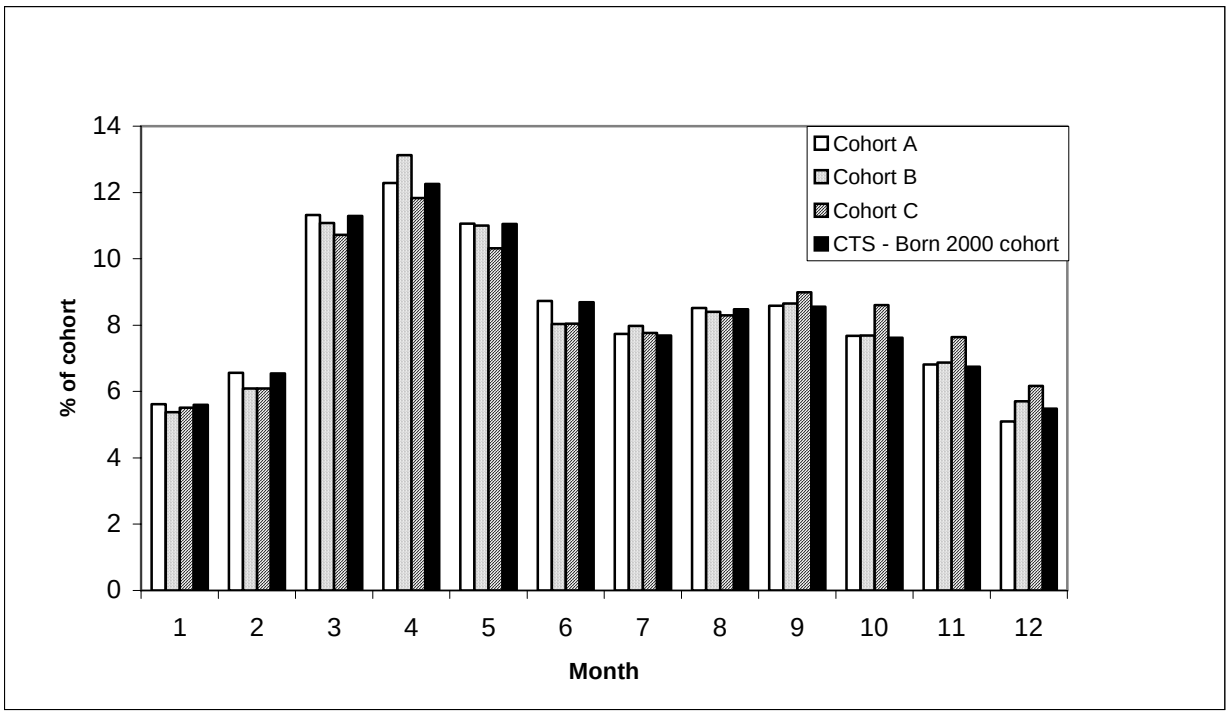

Fig. 2 Month of birth distribution

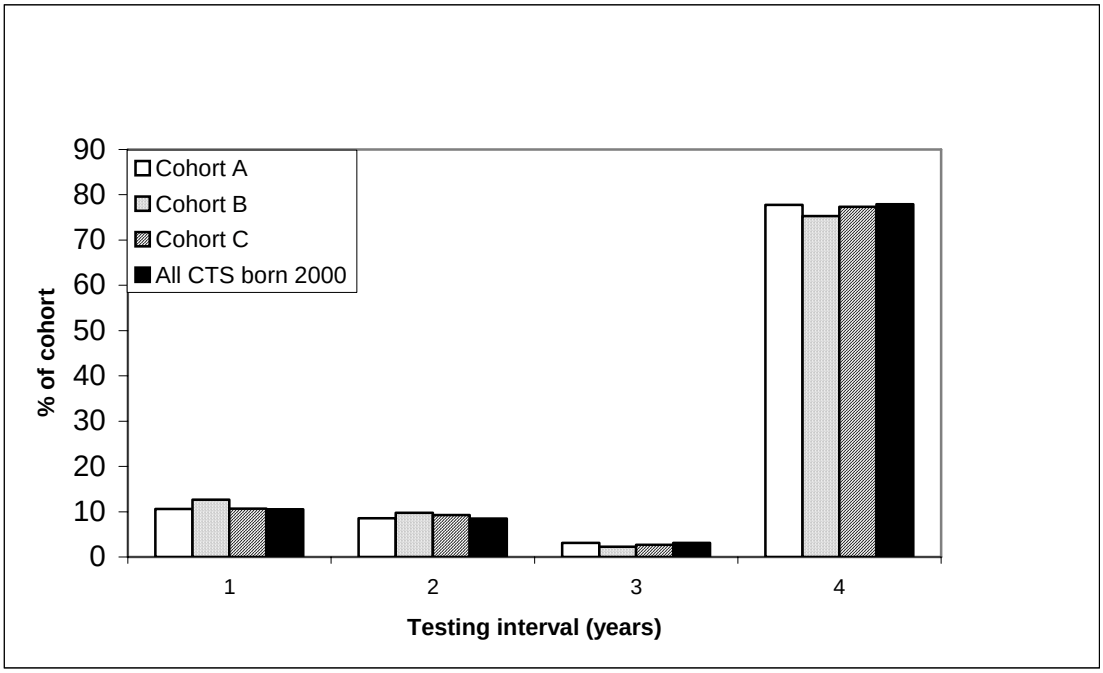

Fig. 3 The distribution of parish testing intervals into which animals are born

Distribution of tests per animal

Table 2 and Fig. 4 summarise the frequency distribution of numbers of tests per animal derived from the three cohorts. Only a minority of animals are ever tested during their lifetime: 14.64%, 28.34% and 17.20% for the birth, death and mixed cohorts respectively. The 2004 (death) cohort appears to have been subject to more testing than the other two cohorts. Figure 5 shows a more detailed distribution for the cohort born in 2000; in addition this graph also shows for each number of times tested, the average number of these tests that were associated with a breakdown.

The processes of birth, death and testing in terms of time and age dependency are described. From here on, only the first test for an animal is considered, i.e. multiple tests are ignored.

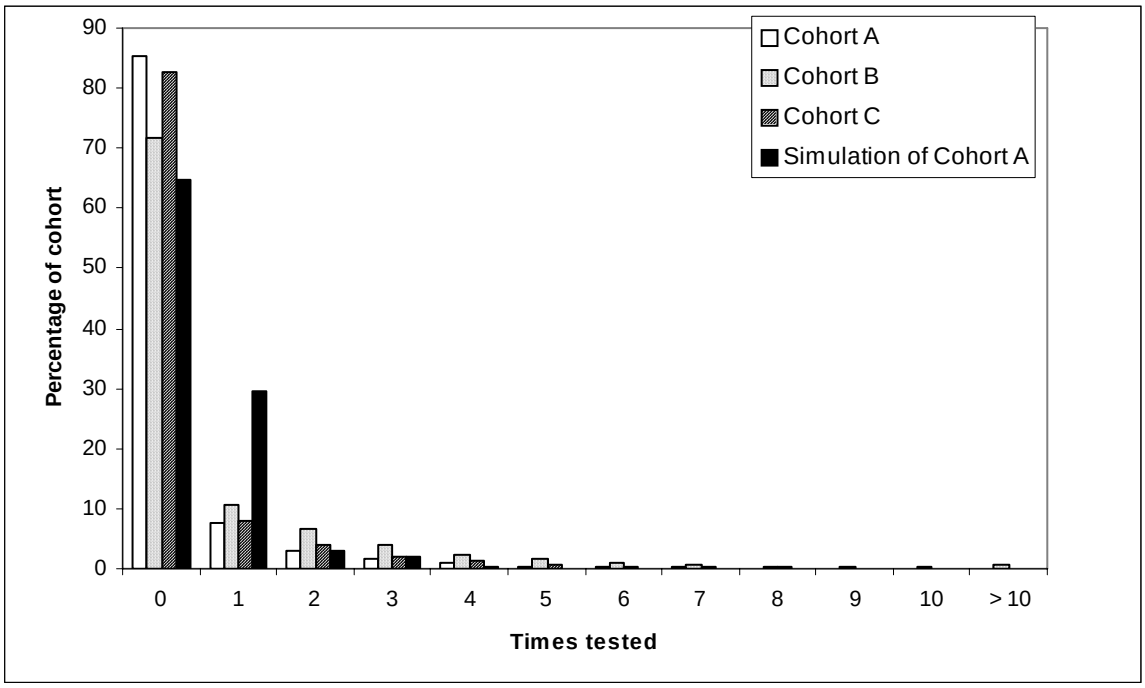

Fig. 4 The frequency distribution of tests per animal

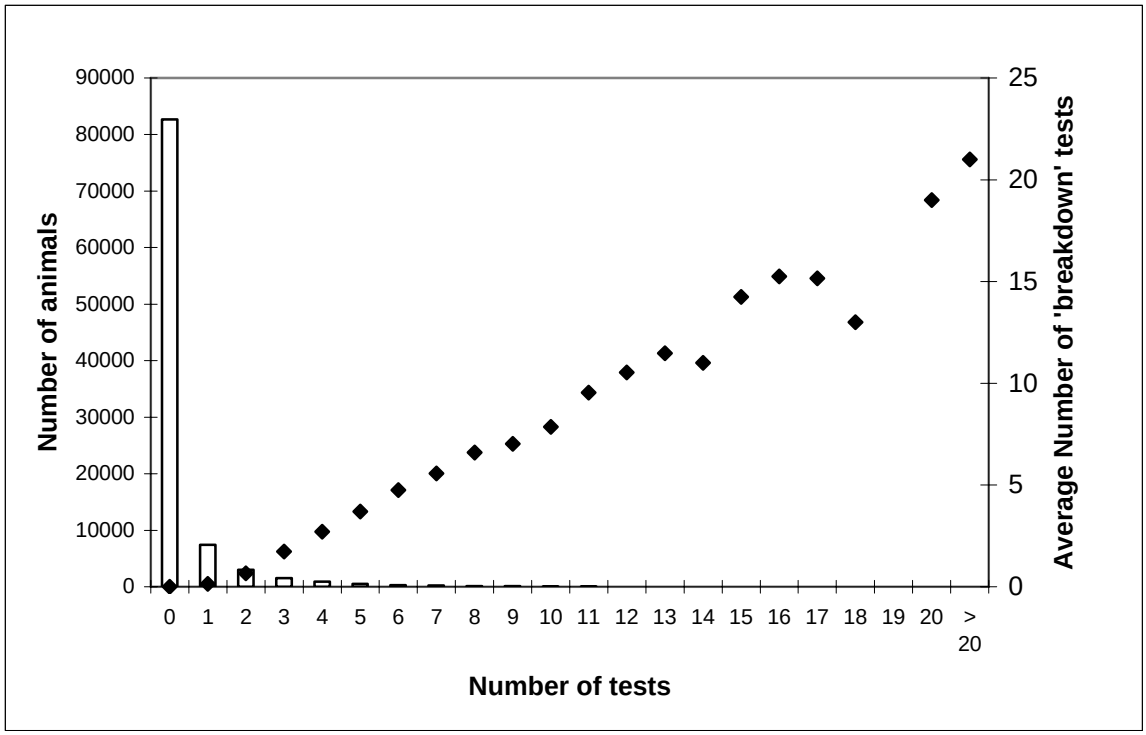

Fig. 5 Cohort A (born in 2000) – The distribution of the number of tests per animal (bars) and the average number of 'breakdown tests' (points)

Table 2. Frequency distributions of total number of tests per animal per premise

Cohort	No. Tests	Number of locations on which cattle were tested[1]					Number of Animals	% of Animals
		0	1	2	3	4	Total	
A (Born 2000)	0	82682					82682	85.4
	1		7424	0	0	0	7424	7.7
	2		2477	529	0	0	3006	3.1
	3		1239	263	19	0	1521	1.6
	4		733	168	9	0	910	0.9
	5+		1048	227	33	11	1319	1.4
	Total		95603	1187	61	11	96862	100
B (Died 2004)	0	63424					63424	72.2
	1		9456	0	0	0	9456	10.8
	2		4798	1098	0	0	5896	6.7
	3		2412	735	59	0	3206	3.6
	4		1516	496	63	0	2075	2.4
	5+		2750	931	101	33	3815	4.3
	Total		84356	3260	223	33	87872	100
C (Born 1998-2003)	0	72218					72218	82.8
	1		6910	0	0	0	6910	7.9
	2		2883	585	0	0	3468	4.0
	3		1404	361	34	0	1799	2.1
	4		869	250	20	1	1140	1.3
	5+		1239	384	42	17	1682	1.9
	Total		85523	1580	96	18	87217	100

[1] Cattle locations are defined as County-Parish-Herd-Holding (CPHH) numbers

Time-dependent patterns

Figure 6 shows the time series of birth, death and first testing derived from the three cohorts. The seasonality of births in GB is evident. The seasonality of testing is really only visible for the

mixed cohort, but in every cohort the severe curtailing of testing during the foot and mouth epidemic (Feb 2001 to Sept 2001) can be seen. Note that 6393 animals have been excluded from cohort B (died in 2004) for which the birth date is unsure.

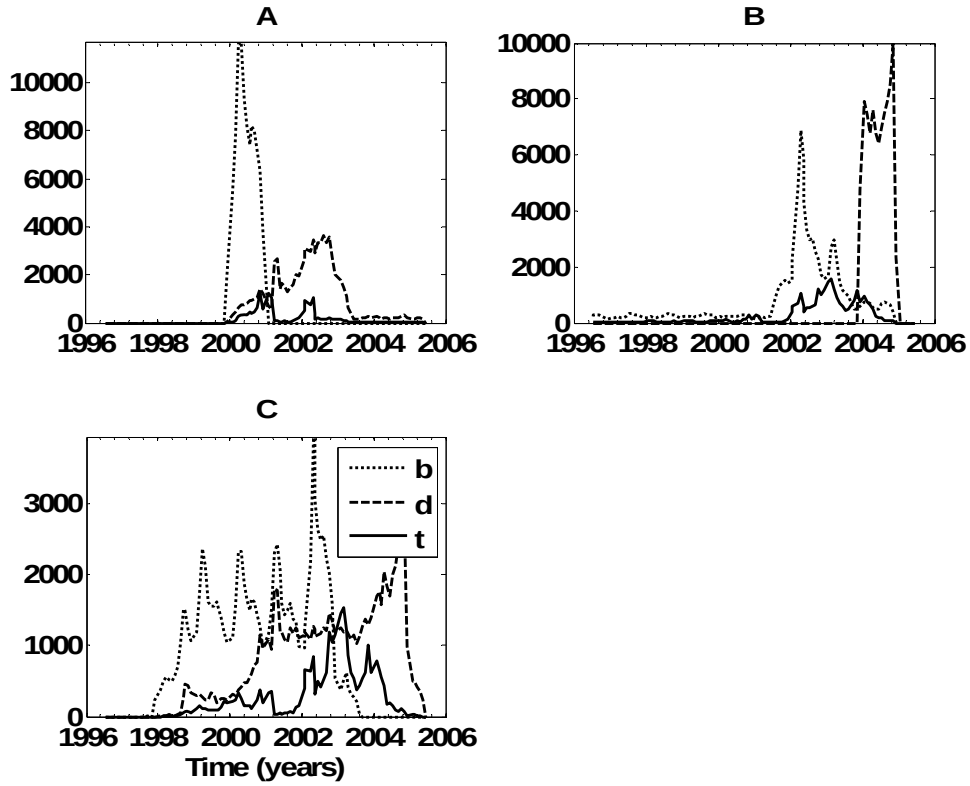

Fig. 6 Time series for three cohorts of British cattle: A (born 2000), B (died 2004) and C (born 1998-2002). The lines show the monthly numbers of births (b) (dotted lines), deaths (d) (dashed) and first testing (t) (solid).

Age-dependent demography

Figure 7 shows the age-dependent patterns of survival and death derived from the three cohorts. The oldest animal occurs in cohort B, dying at 3,086 days (~8.5yrs), with the other two cohorts showing approximately 20% survival at the observation time (July 2005). The death rates are presented simply as the number dying in each month of age as a proportion of those surviving to the start of each month. Cohort B shows increasing hazard as all individuals eventually die, whereas the other cohorts show constant or decreasing hazards, although the sample sizes diminish rapidly as these cohorts are censored. Animals in cohort A are censored between 1,648 – 2,013 days of age.

The death rate shows a marked peak at about 2.4yrs old, presumably brought about by the Over Thirty Month Rule (OTM), which prevents individuals older than this age entering the food chain (as a preventive measure against BSE). This is clearly seen in Fig. 8 which differentiates between sexes; the high death rate is largely due to the death of males at this age, note the different scales. There is also a relatively high peak during the first few months of life. Over the age of 30 months there is an almost constant death rate of 1% – 3% per month, with a slightly increasing trend.

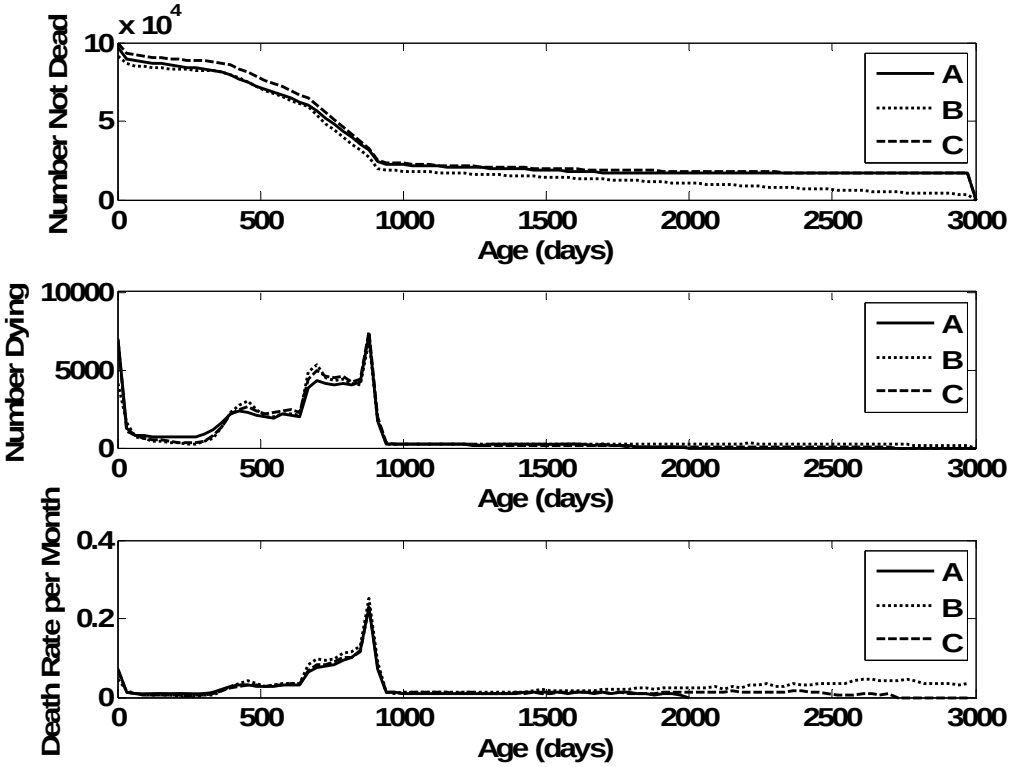

Fig. 7 Age-dependent survival and death rates for the three cohorts of British cattle selected for a study on bovine tuberculosis.

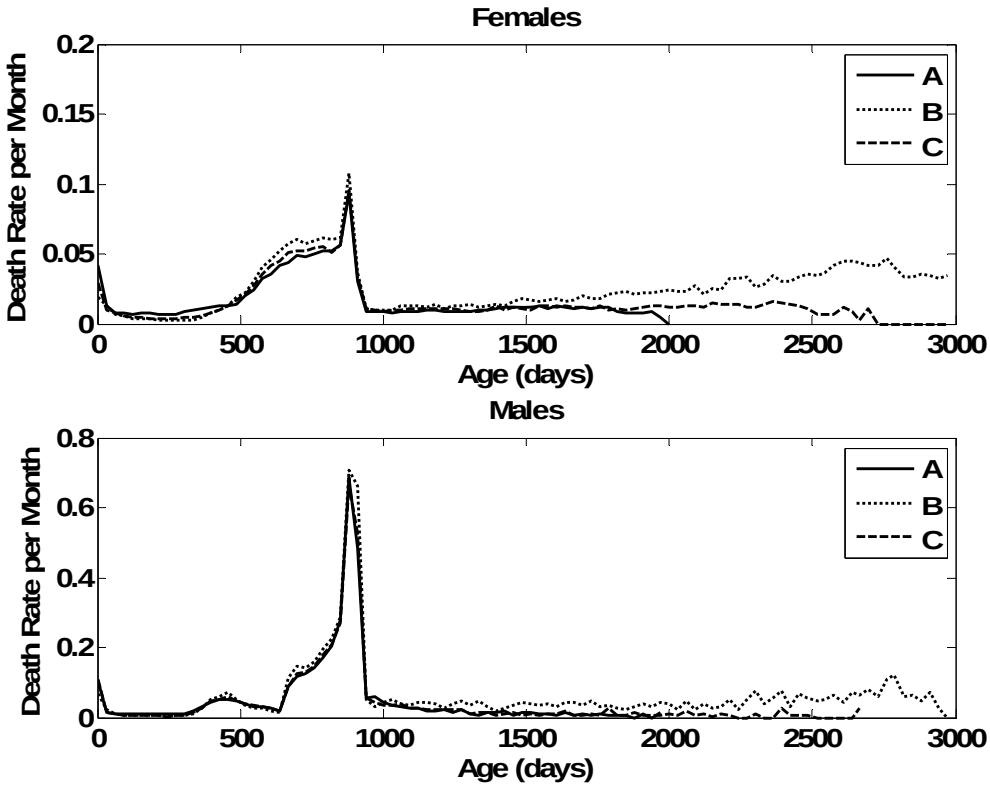

Fig. 8 Age-dependent death rates for the three cohorts by sex

Age-dependent testing

Figure 9 shows the calculated proportions of the surviving members of each cohort that experienced their first test in each month. Testing rates are highest for the first 1.5yrs of life, peak at about 2%, and then drop markedly to a roughly constant rate of 0.5% per month. Note that because of right censoring, the estimates of testing in later life will be most accurate for cohort B. The dramatic drop in testing rates in cohort A between 2 – 18mths is presumably due to the FMD epidemic during which testing for bTB was reduced. There is no difference between sexes in terms of the gross patterns, although males appear to be more likely to have a first bTB test than females at all ages (data not shown). When short interval control tests are excluded, this pattern is unchanged because control tests follow surveillance tests, i.e. surveillance tests are the first test.

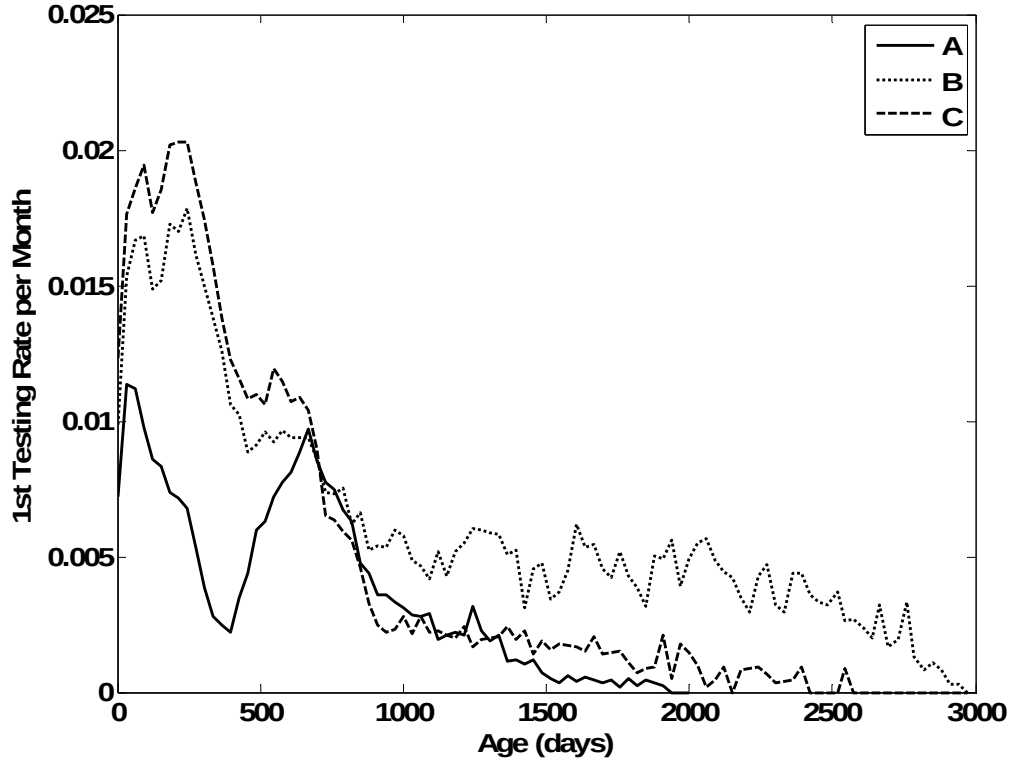

Fig. 9 The age-dependent rate at which animals are subject to their first test for the three cohorts.

Testing and Survival

The low level of observed testing might be explained if those untested in their lifetimes died earlier. Figure 10 shows the death rates estimated from the populations of ever tested (at least once) and never tested during their lifetimes. There is little systematic difference between these two sub-populations in the first three years of life. Although the never tested suffer a slightly increased death during earlier years in cohort A, this would be expected on the basis of the selection between the sub-populations, i.e. there is no evidence that animals are untested because of their short life expectancy.

By contrast, the never tested in cohort B have a much-reduced survival (higher death) after 30mths. This suggests that once into adulthood, the risk of first test is random, and that dying prior to testing is the principal reason for failing to be tested. For the other two cohorts, the ever

tested appear to have, if anything, a greater risk of death after 30mths. The higher death rates of ever tested individuals during the peak of OTM related deaths are related to the fact that males are more likely to be tested than females.

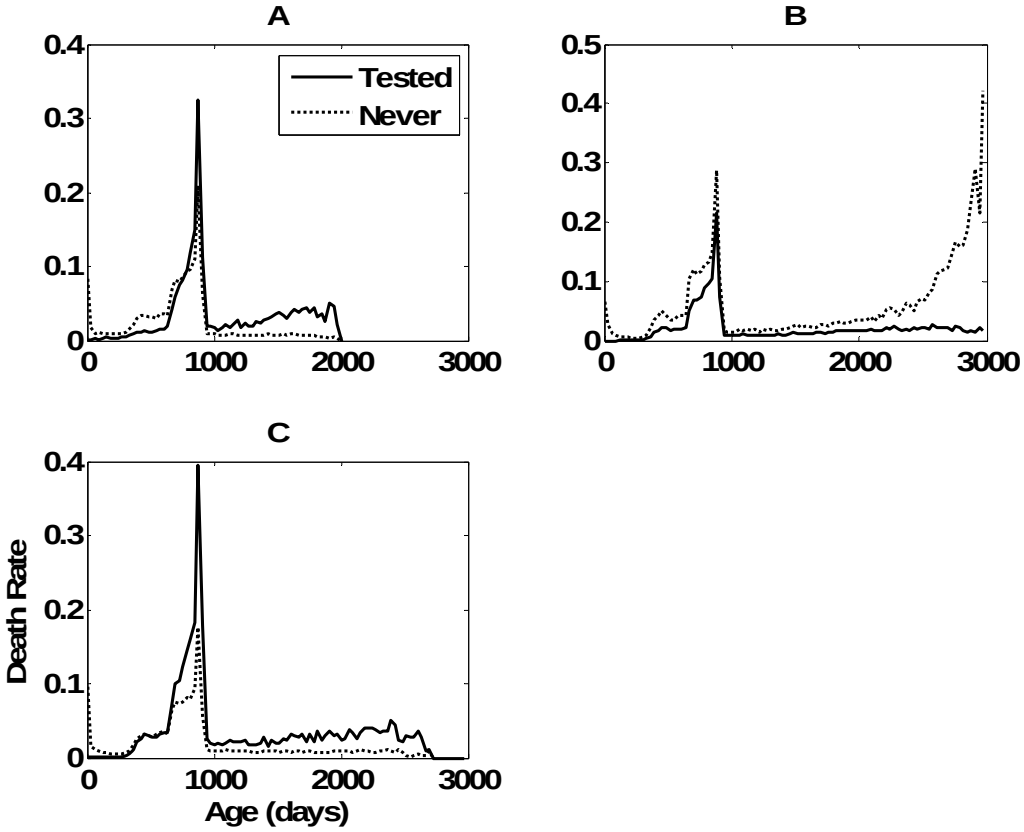

Fig. 10 The estimated death rates by ever (solid line) and never tested (dotted) for the three cohorts.

Testing Effort

Table 3 summarises the same information given in Table 2 and Fig. 4 in terms of the distribution of testing effort. Cohorts A and C were subject to 30,355 and 34,836 tests respectively, compared to 75,439 in cohort B. This apparent doubling of testing effort results in twice as many animals receiving at least one test, but also results in increasing the numbers receiving many tests (e.g. 0.5% of the 2000 cohort were tested 5 times, whereas it was 1.59% of the 2004 cohort).

Characteristically of an over dispersed distribution, the majority of tests are conducted on a minority of animals. Approximately 50% of all surveillance tests are conducted on animals receiving three or more tests in their lives.

Table 3. The distribution of testing effort

Cohort	No. of Tests / Animals	No. of Tests[1]	%Tests[2]	% Animals[3]	Cumulative % Tests	Cumulative % Animals
2000	*1*	7424	24.5	7.7	24.5	7.7
	2	6012	19.8	3.1	44.3	10.8
	3	4563	15.0	1.6	59.3	12.3
	4	3640	12.0	0.9	71.3	13.3
	5	2410	7.9	0.5	79.2	13.8
	6	1722	5.7	0.3	84.9	14.1
	7	1456	4.8	0.2	89.7	14.3
	8	784	2.6	0.1	92.3	14.4
	9	864	2.9	0.1	95.1	14.5
	10+	1480	4.9	0.1	100.0	14.6
	Total	30355	100.0	14.6		
2004	*1*	10279	13.6	10.6	13.6	10.6
	2	13156	17.4	6.8	31.1	17.3
	3	11280	15.0	3.9	46.0	21.2
	4	9576	12.7	2.5	58.7	23.6
	5	7745	10.3	1.6	69.0	25.2
	6	6030	8.0	1.0	77.0	26.2
	7	4620	6.1	0.7	83.1	26.9
	8	3352	4.4	0.4	87.5	27.4
	9	2511	3.3	0.3	90.9	27.6
	10+	6890	9.1	0.7	100.0	28.3
	Total	75439	100.0	28.3		
Mixed	*1*	6910	19.8	7.9	19.8	7.9
	2	6936	19.9	4.0	39.8	11.9
	3	5397	15.5	2.1	55.2	14.0
	4	4560	13.1	1.3	68.3	15.3
	5	3150	9.0	0.7	77.4	16.0
	6	2280	6.5	0.4	83.9	16.4
	7	1722	4.9	0.3	88.9	16.7
	8	1160	3.3	0.2	92.2	16.9
	9	801	2.3	0.1	94.5	17.0
	10+	1920	5.5	0.2	100.0	17.2
	Total	34836	100.0	17.2		

1. The number of disclosing tests performed on animals that had the specified number of tests.
2. The percentage of all disclosing tests that were performed on animals that had the specified number of tests.
3. The percentage of animals in the cohort that had the specified number of tests.

DISCUSSION

The main observations that can be drawn from these analyses are as follows. First, the majority of animals are never tested. Second, the distribution of tests per animal is highly skewed with a relatively small proportion of animals being tested many times. Thirdly, a simulation model predicts that many more animals should be tested than is actually the case.

The highly skewed distribution of the number of times tested (figures 4 and 5) can be explained by the correlation between the number of times tested and the average number of breakdown tests. Figure 5 shows that a relatively small number of animals are caught up in multi-breakdown herds and consequently tested many times.

The difference between the cohort results and the simulation results may be explained by a number of factors. First, the simulation model assumed the theoretical (tested every 1, 2, 3, or 4 years) testing regimes, whereas Fig. 12 shows that these testing targets have not in the past been met. Second, the simulation model assumed that all herds should be tested. In fact, exemptions exist in situations which DEFRA believe the herd does not pose any human or veterinary health risk. For example, herds outside yearly tested regions where all stock go straight to slaughter may be excluded from testing. The effect of the 2001 FMD outbreak on TB testing (Fig. 13) was taken into account in the model.

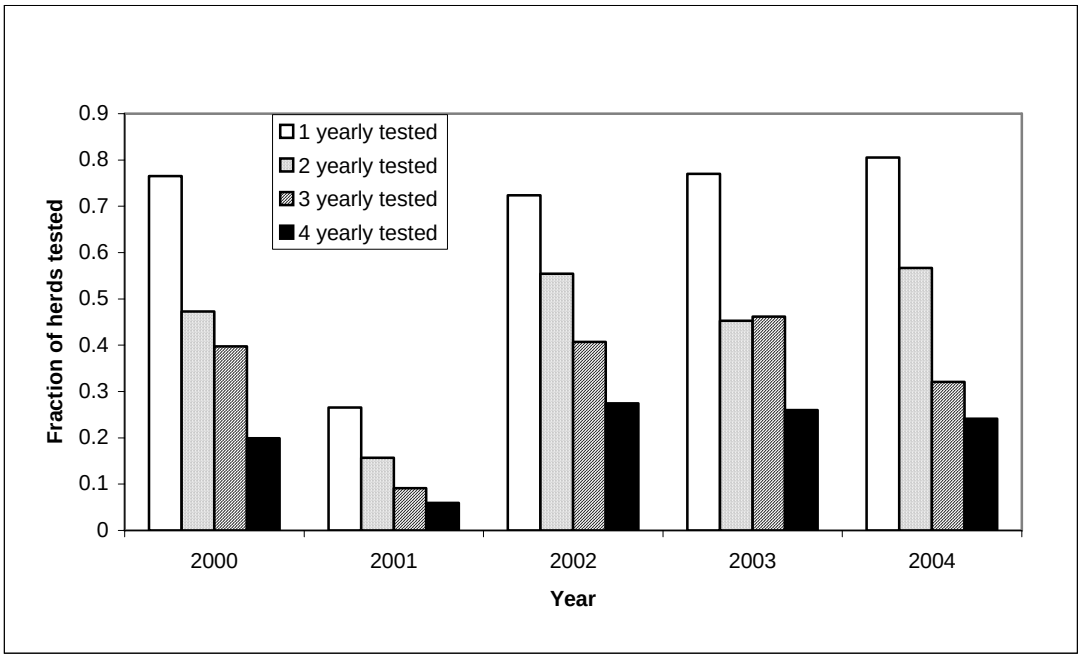

Fig. 12 Fraction of herds tested under each testing regime

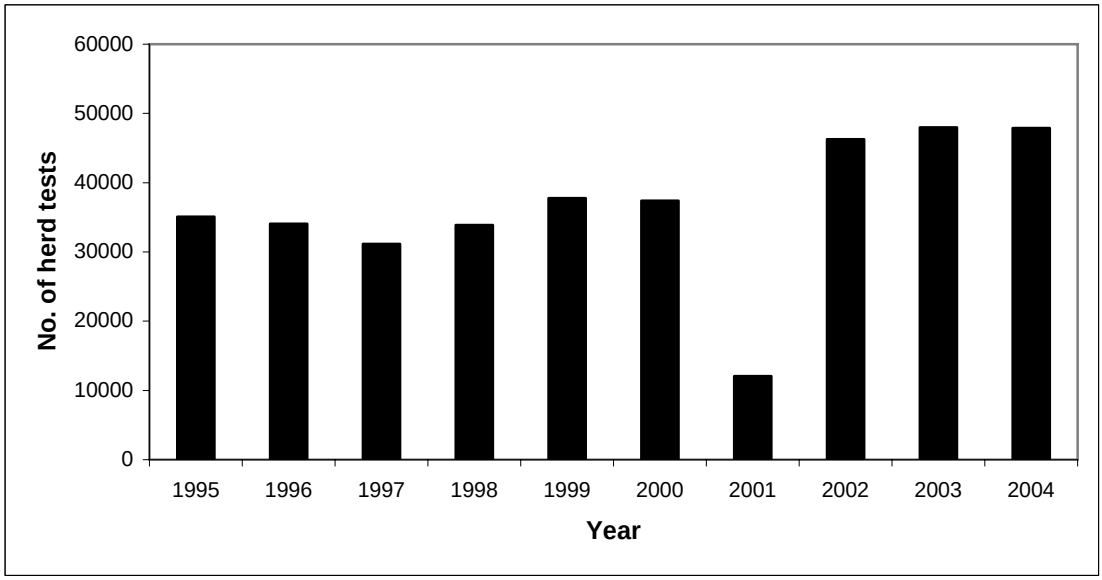

Fig. 13. Number of herd tests carried out per year

It should be noted that two recent policy changes by DEFRA should have a positive effect on the lifetime chances of an animal being tested. It would be expected that the ending of the OTM rule, will lead to a longer life-expectancy for GB cattle and the introduction of pre-movement testing will increase the total number of bTB tests performed.

The three cohorts produce very similar results, and each has strengths. The 2004 (death) cohort provides the best information on later life, which appears to be important in terms of the lifetime chances of being tested. However, it is also subject to the greatest error in terms of recording date of birth. Individuals have been excluded for which the date of birth is not recorded, but there is a greater potential for error in those recorded prior to 2000.

Given the large number of individuals that appear to die untested for bTB, there would appear to be a potential for undisclosed cattle reservoirs of bTB, although to date slaughterhouse monitoring does not suggest that such a reservoir exists. Between 300 and 500 cattle carcases are reported every year in GB by meat inspectors as having suspect TB lesions, out of more than 2.5 million cattle slaughtered. Of those, only 50% are subsequently confirmed as *M.bovis* infections by bacterial culture. A transmission dynamic model is required to determine the likelihood of such bTB reservoirs occurring. Such an approach could also be used to investigate the optimum distribution of tests per animal, and the circumstances under which such a highly targeted programme would be warranted.

ACKNOWLEDGEMENTS

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A RISK ASSESSMENT MODEL FOR ESCHERICHIA COLI IN CATTLE AND SHEEP

K.F. STACEY*, D.J. PARSONS, K.H. CHRISTIANSEN AND C.H. BURTON

SUMMARY

Escherichia coli O157:H7 (VTEC O157) persists in being a threat to food safety. The mechanisms behind the spread of VTEC O157 are complex and poorly understood. The objective of this study was to construct a model to simulate the propagation of VTEC O157 in cattle and sheep on the farm and to use the model to both test the effect of different interventions and to develop understanding of the underlying processes including the identification of areas that could benefit from further research. Using the shorthand that "risk" means the probability of carrying VTEC O157 to the abattoir, key conclusions included: decreasing the group size decreases the risk independently of stocking density; mixing sheep and cattle increases the risk in both groups; merging groups of animals of the same species into larger groups increases the risk substantially and increasing stocking density increases the risk independently of group size.

INTRODUCTION

Food poisoning is a major cause of public concern over food safety. VTEC O157 was first identified as a human pathogen in 1982 in two outbreaks of gastrointestinal illness associated with the consumption of hamburgers from a fast food chain in the United States. The organism is now recognised as an important cause of food-borne disease in several countries including the UK. VTEC O157 may cause severe disease and death in humans. Notifications in England and Wales have risen from just over 14,000 in 1982 to over 83,000 in 1996. Anxiety has been further heightened by two recent outbreaks of VTEC O157 in South Wales in September 2005 (117 cases) and in the Brecon area in November 2005 (12 cases). The Pennington report (Pennington Group, 1997) stated '…it is generally accepted that the main reservoir of VTEC O157 exists in cattle, and possibly sheep farms…' and recommended further investigation and research, and an education/awareness programme for farm workers.

The objective of this study was to construct a model to simulate the propagation of VTEC O157 in cattle and sheep on the farm and to use the model to both test the effect of different interventions on the farm and to develop understanding of the underlying processes including the identification of areas that could benefit from further research. A stochastic simulation model was developed within which risks can be calculated and the consequences of actions can be explored. An extensive literature review gathered from farm visits were used to construct the model. A consultation workshop with experts from outside the project was held before construction of the model to review the proposed structure and concepts (Parsons, 2001).

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The simulation model contains two aspects:

- a general, deterministic simulation of the interactions between animals, feed, enclosures and waste on cattle and sheep farms to study the impact of different factors;

- a stochastic simulation of the epidemiology of VTEC O157 on such farms to study the risk that animals for the abattoir are carrying VTEC O157.

Each run of the simulation results in one possible outcome of the system with values for prevalence and distributions of shedding of VTEC O157 throughout the year for each group of animals. By performing batches of runs, distributions of outcomes are obtained, which represent the range of possibilities arising from the assumptions in the model. The impact of changes to the management of the farm or to the underlying assumptions can be examined and the most significant ones identified. The model is designed to be flexible, for example it can be changed from cattle to sheep through a few settings in the input file. It is also easy to extend or modify if required in the future.

MATERIALS AND METHODS

Farm model

The first step in the modelling was to create a farm model to provide a general, flexible structure to describe the movements of animals, their interactions, feeding, waste handling and other factors likely to influence the transmission of VTEC O157. A single model that is flexible enough to represent almost any conventional type of cattle or sheep farm has been developed. This is a simulation model in which a farm is made up of two types of entity: groups and resources. A farm is then a collection of interacting groups and resources. The specification of an individual farm is defined by input files. These input files specify the number of fields, yards, barns and the number of animals in each age group on the farm. They also specify the characteristics of each enclosure, each group of animals, storage and movement of waste and movement of animals within and on and off the farm. Meteorological data and the parameters of the model, such as the decay rate of VTEC O157 in different environments are also given in an input file.

A group of animals is a set of animals of similar age that are normally kept together and treated alike. Groups may sometimes be merged, for example if purchased stock are integrated with farm-bred animals, or split, for example if some young are retained when others are culled. Two or more groups may share the same enclosure at some times, and be separated at other times. Each group has a set of attributes as shown in Fig 1.

Some of these attributes are fixed (e.g. name and type), some change with time (e.g. age, weight, feed intake), and others change at particular events (e.g. enclosure). Simple models are used for the time-dependent variables, with coefficients that depend on the type of animals. Weight is linearly dependent on age, up to a maximum for the type. Feed intake is a power law function of weight. Water intake is proportional to feed intake. Faeces production is linearly dependent on feed intake

GROUP
Name Type (sheep, cattle) Age Enclosure Number of animals Weight of 1 animal Feed intake per animal Water intake per animal Rate of production of faeces per animal

Fig. 1 Group attributes EXTEND

A resource is anything else on the farm, such as a field, a building, a slurry store or a feed trough; the resources of most interest are those that can act as pools or vectors for VTEC O157. Resources will often be shared by several groups of animal, so they may be a route for cross infection. Four types of resources are distinguished in the model: enclosures (further divided into fields, yards and barns), waste stores, feed troughs and water troughs. Again each type of entity has a set of attributes as shown in Fig. 2.

ENCLOSURE	WATER TROUGH	FEED TROUGH	WASTE STORE
Name Type Number of groups List of groups Area Area grazed Area of silage Frequency of cleaning Proportion of each feed type to be fed (concentrate, silage, hay, grass)	Volume Cleaning interval	Type of feed (concentrate, hay, silage)	Mass of contents

Fig. 2 Resource attributes EXTEND

A single run of the model simulates a period of one or more years using a fixed time step of 1 week. At the start of a run the set of entities is created with the number of each type and their attributes set to values set by data files. To take a simple example, starting on 1st January, a fattening farm may have one group of cattle bought the previous spring and currently housed in a single barn with water and feed troughs. The waste is collected in a single slurry store, and there are four fields, of which two will be closed for silage in the spring.

At each time step, the time-dependent attributes are updated. Other variables are updated through the linkage between groups and resources, for example the volume of slurry in a waste store depends on the rate of production of all the animal groups in the enclosures feeding it. Transitions also take place that affect the groups and resources. These transitions are also defined by data files. In the simple example above, the slurry store is emptied by spreading on the silage fields some weeks before they are due to be cut, the cattle are turned out onto one of the fields in the spring, and new young stock are bought in and grazed on the other field. Later in

the year, after two cuts of silage, the grazing animals are rotated onto the aftermaths. In the autumn the older group is sent to the abattoir while the younger one is housed for the winter.

Animals leaving the farm for sale as livestock, culling or the abattoir are handled by moving them into special groups which are emptied at the end of the time step. This allows relevant information on the animals leaving the farm to be collated. After 12 months the model is back to the initial state, with the new group of animals replacing the old one. For the purposes of the epidemiology/risk model, the farm model is always set up to simulate a steady state, that is, with identical numbers and ages of animals in each group at the same time each year.

Epidemiology/risk model

The epidemiology model simulates the carriage and transmission of VTEC O157 within and between groups of animals and resources. It was constructed by extending the entities described in the farm model to have additional attributes related to VTEC O157. Clearly, the most important is the presence of the organism in the animals in a group. The state variable used is the shedding rate of VTEC O157 in the faeces, which is the variable that is observed in field studies and is the source of contamination for other animals, slurry/manure and the immediate environment. Stewart *et al*. (2002) found very similar counts in the faeces and colon contents so the shedding rate is a good proxy for the level of infection in the colon.

In contrast to the farm model, which is entirely deterministic, the epidemiological model has to deal with variability, so it is a stochastic simulation: many of the transitions in the model are described by probabilities. Some of the processes in the model are temperature dependent. A set of 30 years weather data from Birmingham was gathered and reduced to 30 years of weekly mean temperatures. For each week in a run of the simulation, one of the 30 weekly mean temperatures for that week was selected at random. To generate distributions of outcomes, at least 100 runs of the model were carried out for each scenario. Each run simulated 10 years with the farm model set up in steady-state, to allow the stochastic epidemiological model to stabilise. The results were sampled from the last year only.

Terminology: Although it may not be strictly correct, the term infection will be used to describe the colonisation of the gut of animals by VTEC O157. Contamination will be used to refer to the presence of VTEC O157 in reservoirs. Prevalence is the proportion of animals in the group or on the farm shedding at a point in time. Where necessary, it will be qualified by farm or group. Specific shedding rate is the amount of VTEC O157 shed in a unit of faeces (CFU/g, where CFU = colony forming units). It may be averaged over a group or the farm, in which case it will be qualified by farm mean or group mean. Shedding rate is the rate at which an animal is shedding, that is the product of specific shedding rate and excretion rate; units CFU/day. It may be aggregated or averaged over a farm or group, in which case it will be qualified by farm or group, total or mean.

Attributes: The main attributes added to the entities in the model to describe the epidemiology were those quantifying the infection of groups or contamination of reservoirs. Both the prevalence and the level of shedding are important when considering the risk at the abattoir, so each group is described by the number of animals with a specific shedding rate in each of a series of logarithmic ranges: 0, 0-10, ... 10^7-10^8 CFU/g, from which the prevalence and shedding rate can easily be calculated. For the reservoirs, the important variable is the load of VTEC O157 (CFU/g or CFU/m^2 as appropriate). For each entity, it is necessary to consider the growth or decay of VTEC O157 and transfers to and from other entities. In the case of animal

groups, cross infection within each group is also important. Each of the major elements will now be considered. The model works with groups of animals with respect to farm management and on an individual basis with respect to transmission of VTEC, but where the individuals within the group are distinguished from each other only by their shedding status (CFU/g).

Growth/decay in resources: Cattle, and possibly sheep, are currently recognised as the principal reservoirs responsible for the proliferation of VTEC O157 on farms (Wallace, 1999). There is some evidence that the VTEC O157 population may increase in the short term in contaminated feed (Fenlon et al., 2000), water (Reinders et al., 1999), cattle faeces (Wang et al., 1996; Kudva et al., 1998) and soil (Gagliardi & Karns, 2000). However, as this is within one time period of the model, the effect is considered negligible. Thus, within resources, it is only necessary to consider the decay of the population. There are some differences between enclosures, feed troughs and water troughs that are reflected in the coefficients in the model.

The survival of VTEC O157 has been found to be shorter at high temperatures in cattle faeces (Wang et al., 1996; Kudva et al., 1998), sheep faeces (Kudva et al., 1998) and water (Rice & Johnson, 2000). This is modelled as temperature dependent exponential decay. Expressed in log units, the decrease in load is linearly dependent on temperature. Because feed troughs are emptied frequently, it is assumed that there is no survival of VTEC O157 on the time scale of the model.

Growth/decay in animal groups: Infection of an animal is characterised by a rapid rise in the shedding rate, over a period of a few days, followed by a decline over a period of several weeks (Sanderson et al., 1999; Buchko et al., 2000). The rise time is shorter than the 1 week time step used in the model, so it can be neglected: in the model, infected animals rise immediately to their maximum shedding rate. From the few detailed studies for which data in individual animals are reported (Sanderson et al., 1999), it is clear that the peak specific shedding rate is variable. This is simulated in the model by transition to a varying peak shedding level. The decrease in shedding is simulated as a stochastic exponential decay. For cattle this leads to a distribution of infection durations where the mode is 5 weeks and survival beyond 8 weeks is very rare. For sheep the peak shedding is lower and the initial decline is slightly slower.

Contamination of resources: For each enclosure the rate of contamination by animals (CFU m^{-2} day^{-1}) is proportional to the total shedding rate of the groups occupying the enclosure. Contamination by slurry spreading is proportional to the application rate and the load in the slurry. When a yard or barn is cleaned, the load is set to 0. The contamination of a slurry store is calculated by mixing the contents of the store with the incoming slurry. When emptied, the load is set to 0.

For each water trough the rate of contamination by animals (CFU m^{-2} day^{-1}) is proportional to the total of the products of mean specific shedding rate and water consumption rate of each of the groups using it. When cleaned, the load is set to 0. As noted above, it is assumed that in the feed trough there is no persistence of contamination on the time scale of the model. For each feed trough, the level of contamination is proportional to the sum of the products of mean specific shedding rate and concentrate consumption for the groups using it in that time step. Although silage and hay may be contaminated by VTEC O157 through slurry spreading, the available information shows that the way that the silage or hay is made has a greater effect on the level of contamination at the time it is fed to the animals (Reinders *et al*., 1999; Fenlon *et al*., 2000). The model therefore does not simulate a link between slurry spreading and contamination, but allows the level of contamination to be set as for concentrates.

Transmission from resources to animals: For yards and barns, each animal receives a dose that is proportional to the product of the total feed intake and the level of contamination in the enclosure (CFU/m^2). In fields, because the animals are grazing, there is an additional, larger, dose proportional to the product of the grass consumption and the contamination level. The dose is lower for sheep than cattle because sheep graze more selectively. In addition, each enclosure has a fixed background level, which may be zero, to represent exposure to other sources, such as wild birds and animals. Each animal receives an additional dose proportional to the product of feed intake and the background level. The dose received by an animal from a water or feed trough is proportional to the product of the water or feed intake and the load in the trough.

Transmission between animals: General animal to animal transmission between animals sharing an enclosure is based on the number of contacts made. Each animal is in contact with a number of other randomly selected animals, where the contact rate (number of contacts it makes each day) is fixed. From each animal with which it has contact it receives a dose proportional to that animal's specific shedding rate.

Infection of animals and the dose-response relationship: Experimental and field studies indicate that cattle do not develop any significant immunity to colonisation by VTEC O157 (Shere *et al*., 1998; Sanderson *et al*., 1999). Also initial tests of this hypothesis using the model found that it made sustained infection of farms much less likely and reproduced the results of field studies less well. In the results reported no immunity was assumed. Thus the same transition matrix was used for naïve and previously infected animals. Reinfection during shedding was very rare. Therefore within the model it is assumed that animals cannot be reinfected while shedding.

For animals that are not shedding, the total dose of VTEC O157 received by all the routes discussed above is calculated. The probability of infection is then related to this dose by the dose-response relationship. There is no available data for natural infections from which to derive this relationship, and only limited data from artificial infections created by dosing animals with laboratory cultures. Working with small groups of animals, these typically show an increase in the probability of infection with dose, and a decrease with age of the host (Cray and Moon, 1995; Cornick *et al*., 2000, Kudva *et al*., 1995). All these studies used very small groups of animals, so the estimates of the proportions are subject to large errors. Given the sparsity of the data, the simplest approach that gave reasonable agreement was to standardise the dose by dividing by the host mass. This means that there is about 1 order of magnitude decrease in the standardised dose in lambs or calves from weaning to slaughter, and two orders of magnitude from weaned lambs to mature cattle. In the model, it is assumed that animals are immune until weaning, without making any assumptions about the mechanism.

The form of the dose response relationship is a Beta-Poisson distribution, which has been used in risk assessment models for organisms causing food-poisoning (e.g. Brown, 1998). The parameters were adjusted to give infection rates similar to those quoted above. Figure 3 shows the dose-response curves for host masses of 5, 50 and 500 kg.

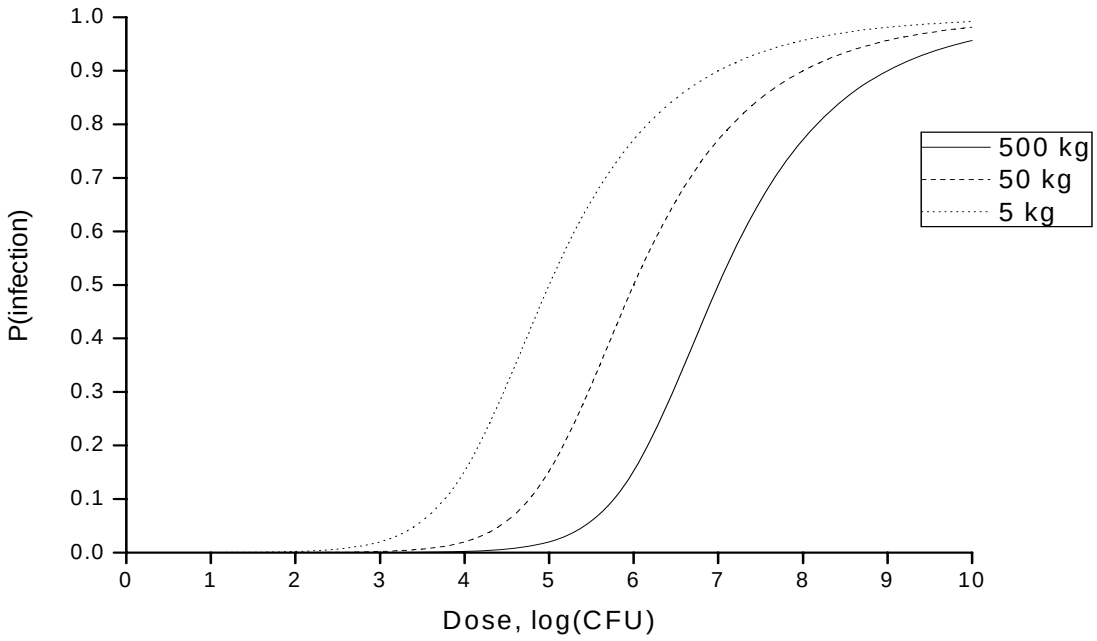

Fig. 3 Dose-response curves for 3 host masses EXTEND

Parameterisation

There were two main stages in setting the parameter values within the model. Wherever possible, data from experiments at the level of individual processes was used. For many of the relationships in the farm systems model, such as intake, slurry production and weight, this was straightforward and it was possible to use well-known approximations. There were also useful data on features such as the survival of VTEC O157 in slurry, soil, etc., from which reasonable extrapolations could be made to other conditions. There were limited data from which rough estimates could be made for other parameters, such as the transition parameters (for infection) and dose-response, including the effect of age.

The remaining parameters were set by considering the overall behaviour of the model in comparison with farm observations. For example, new infections on farms that have been clear for some months are uncommon, but do occur, so the background level of infectivity was set to give a few infections per year. Where some routes of transmission from resources had better data than others, they were adjusted collectively to give reasonable relative effects. Similarly, the transmission rates from the general environment and from grazing were set so that the prevalences during grazing and housing on the grass-based farms were similar.

RESULTS AND DISCUSSION

Farm system specifications

Beef breeder-finisher farm:

Herd: A breeding herd of 10 heifers and 40 cows kept for a total of 5 parturitions, calving in week 40 (1 October); replacement breeders bought in week 40 at 86 weeks. Fattening 50 calves

per year on an 18 month system (so 2 overlapping groups of calves), slaughtering older group in week 22.

Enclosures: 75 ha of grass and 3 barns; the breeders and the two groups of calves grazed and housed separately; in spring 45 ha reserved for silage, reduced to 30 ha on week 24 (1st cut) and all grazed from week 31 (2nd cut). Each enclosure supplied by 1 water trough of 0.5 m^2. Grazing from weeks 18-38 (30 April-17 September). Slurry cleaned from barns every 2 weeks; water troughs cleaned every 5 weeks.

Slurry management: 60% of total spread on all grassland on week 14; remainder spread on weeks 22 (on grassland previously used by abattoir group) and 25 (silage aftermath).

As an example of the model output, Fig. 4 shows the variation in prevalence with time across the complete herd. Week 1 corresponds to the first week of January. The two lines show the mean of 100 runs and the results of a single run. The single run shows an outbreak during the spring and summer. The average is fairly smooth and includes the results of many runs where there was no outbreak. It shows a gradual decline throughout the summer followed by a rise when the animals are rehoused. The apparent peak in the mean in the early summer is mainly the result of the transfer of the older animals to the abattoir: in this scenario they have a lower prevalence than the other groups, so their removal increases the mean value.

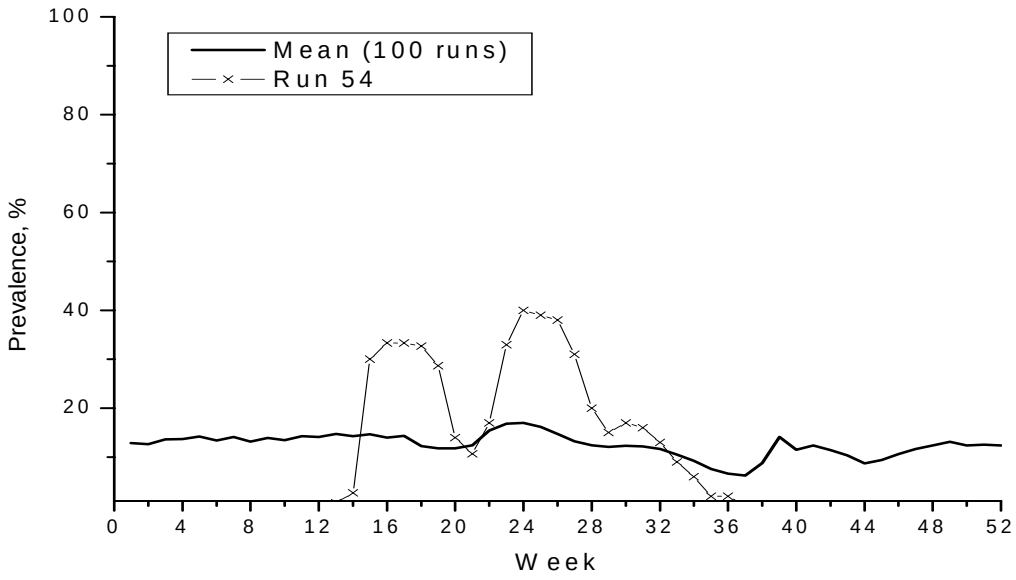

Fig. 4 Simulated variation of VTEC O157 prevalence with time on a beef breeder-finisher farm

Beef fattener farm:

Herd: Group of 50 calves bought aged 9 weeks in week 48 (26 November) and sold at age 79 weeks (18 months) in week 14, maximum stock of 100 calves for 6 months of the year.

Enclosures: 25 ha of grass and 2 barns (young and year-old calved houses separately); in spring 15 ha reserved for silage, reduced to 10 ha on week 24 (1st cut) and all grazed from week 31 (2nd cut). Each enclosure supplied by 1 water trough of 0.5 m^2. Grazing from weeks 18-38

(30 April-17 September). Slurry cleaned from barns every 2 weeks; water troughs cleaned every 5 weeks.

Slurry management: 60% of total spread on all grassland on week 14; remainder spread in week 25 on silage aftermath.

Sheep farm:

Flock: One group of 100 ewes, producing an average of 1.5 lambs per year for 5 years. Lambing in week 13; old ewes culled in week 21; in-lamb ewes bought in week 1; lambs going to abattoir in week 40.

Enclosures: Single field of 10 ha; ewes held in 1 barn from week 12 to week 14.

Manure: spread on a separate field.

This farm was designed to give results representative of lowland sheep production. Lowland farms produce the majority of the sheep meat. The ranking of the prevalences obtained for this farm is likely to be indicative of that expected on upland and hill farms and so are likely to be similarly influenced by the interventions considered.

Mixed beef and sheep farm:

Herd: As for beef fattener farm (groups of 50 calves)

Flock: As for sheep farm.

Enclosures: Single field of 30 ha; separate barns

Alternative: Field of 25 ha for calves; field of 10 ha for sheep; separate barns

Manure: spread on a separate field.

This farm considered the effects on the prevalence of VTEC O157 in both species in the case where all the grazing was shared (and inter-specific contact was assumed to be as likely as intra-specific) compared with the same stock kept in separate fields. Other management variables were the same as for the single species farms, except that manure was not spread on grazing fields to avoid confounding with the effect of mixing.

Calibration and validation

Validation of models of this type and complexity is always difficult, because suitable experiments can rarely be conducted, so it is necessary to test against survey data collected for different purposes with many uncontrolled variables. Much of the results from the literature review consisted of details of the ecology of VTEC O157 on farms, reviewed by several authors (Hancock et al., 1998; Wallace, 1999; Synge, 2000; Hancock et al., 2001; Rasmussen & Casey, 2001). The results of the model were compared with the results of the literature review, as reported below.

VTEC O157 is present on virtually all cattle and sheep farms in Great Britain, although intermittently. VTEC O157 was present at some time in almost all the simulated farms in the later years (i.e. not as a result of initial conditions). In a recent survey of dairy and beef farms in England and Wales, Paiba et al. (2003) found that the overall animal prevalence was 4.7%

across all farm types, or 10.5% in fattener herds, which is consistent with several other surveys reported in the literature review. The mean prevalence shown in Fig. 4 is close to the value for fattener herds; in the abattoir group it was 9.6%. The other results for mean animal prevalence are reasonably consistent with the observations. However, Paiba et al. also reported that the herd prevalence (proportion of herds with 1 or more animals shedding) was 44% and the within herd prevalence was 10.2%. The corresponding values for the abattoir group in the model are 21% and 44%. Therefore, the model is underestimating herd prevalence and overestimating within herd prevalence, which together result in a reasonable estimate for animal prevalence. Comparable recent data for sheep are fewer, but suggest that the prevalence is lower in sheep (Small et al., 2002).

In agreement with the results from the literature review, in the model cattle can have more than one episode of VTEC O157 colonisation and shedding; and shedding and duration are highly variable amongst individuals. These features are borne out in the results of the simulations.

Shedding of VTEC O157 by cattle tends to be seasonal with animal prevalence being higher between late spring and early autumn than in winter. There is substantial variability in the seasonal pattern between surveys. Figure 3 shows one instance in which shedding was high in late spring and summer, and low in winter, though the mean for this scenario (also shown) was fairly uniform, with small peaks after spring turnout and autumn housing, with a decline in late summer. Seasonal patterns are confounded by changes between grazing and housing, and by the timing of purchases, births and slaughter.

VTEC O157 may survive in faeces, manure, soil and water for many weeks. In the simulated farms, population decay rates for VTEC O157 in faeces, manure, soil and water were of the order of 1 log unit per week, producing survival times of several weeks.

Scenario analysis

Many of the parameters in the model had to be estimated from inadequate data, so a sensitivity analysis was conducted to investigate which were likely to have a significant effect on the outcomes, and therefore would be the most useful to investigate further experimentally.

The scenario analysis was carried out using the beef breeder-finisher farm described previously, because it uses all aspects of the model. For the baseline, all the parameters were set to their default values. Each of the parameters selected for analysis was then increased and decreased individually. Parameters related to transmission rates or concentrations were normally multiplied and divided by 10. The magnitude of the changes in the decay rate exponents was 0.1. The animal to animal contact rate was normally 5 per day and values of 1 and 20 were tested.

In each case the changes were assessed by considering the magnitude of the change in the mean prevalence (averaged over 200 runs) from the standard mean prevalence (averaged over 300 runs) at 4 points in the year: weeks 9, 22, 35 and 45. The first and last are when the animals are housed, the other two are during grazing. The standard mean prevalences at the 4 dates were 14.8%, 16.5%, 13.57% and 11.3%. Splitting each trial into 2 batches of 100 allowed the variability resulting from the stochasticity of the model to be assessed: the mean absolute differences between sets were 1.93%, 2.03%, 1.52% and 1.55% respectively for the four dates. Table 1 shows the results of the sensitivity analysis for the parameters where the magnitude of

the difference is greater than twice the mean for one or more of the tests. Any difference whose magnitude is greater than twice the mean is highlighted.

It is clear that, for the sizes of changes made, most of the parameters relating to transmission of VTEC O157 to the animals have larger effects than the decay rates. The one related to grazing is particularly sensitive. Of the decay rates, the one for grassland is the most significant. This is an area where data are very difficult to obtain, and therefore where further research might be particularly helpful.

Table 1. Results of scenario analysis, showing the change in the mean prevalence produced by increasing or decreasing each parameter

	Week 9		Week 22		Week 32		Week 45	
	Increase	Decrease	Increase	Decrease	Increase	Decrease	Increase	Decrease
Barn decay rate	+2.11	0	+0.26	- 0.48	+1.99	**-3.24**	+1.86	+2.31
Grassland decay rate	-1.04	**+7.00**	-2.46	**+9.98**	-2.99	**+7.55**	-0.70	**+4.82**
Slurry store decay rate	+0.23	+2.23	-0.30	+3.35	+0.05	**+4.03**	+2.33	**+3.55**
Transmission from enclosure	**+6.45**	**-12.9**	**+6.61**	**-13.9**	**+6.96**	**-10.7**	**+8.38**	**-8.89**
Transmission from grass	**+40.3**	**-9.20**	**+69.7**	**-14.9**	**+58.0**	**-13.3**	**+42.3**	**-9.92**
Animal-animal contact rate	**+5.4**	-1.31	**+9.28**	-2.79	**+8.30**	-2.41	**+5.55**	-1.45
Faeces dose per contact	**+8.4**	-2.90	**+16.7**	**-5.40**	**+14.3**	**-4.65**	**+8.89**	-2.05

Effect of interventions on simulated farms

Method of analysis: Because the key variables are the prevalence and shedding rate in the abattoir group, the results will concentrate on these variables. A program was written in Genstat (Payne et al. 1987) to process the output file, extract the required information and present it graphically. Figure 5 shows the frequency distribution of prevalence obtained from one batch of 100 runs for the base case of the beef breeder-finisher farm. Although the presentation of frequency distributions as histograms is familiar, it is difficult to compare distributions in this form. Therefore first order stochastic dominance was used to test the effect of interventions on the risk of different levels of prevalence and shedding levels in cattle or sheep being sent to the abattoir, from each type of farm.

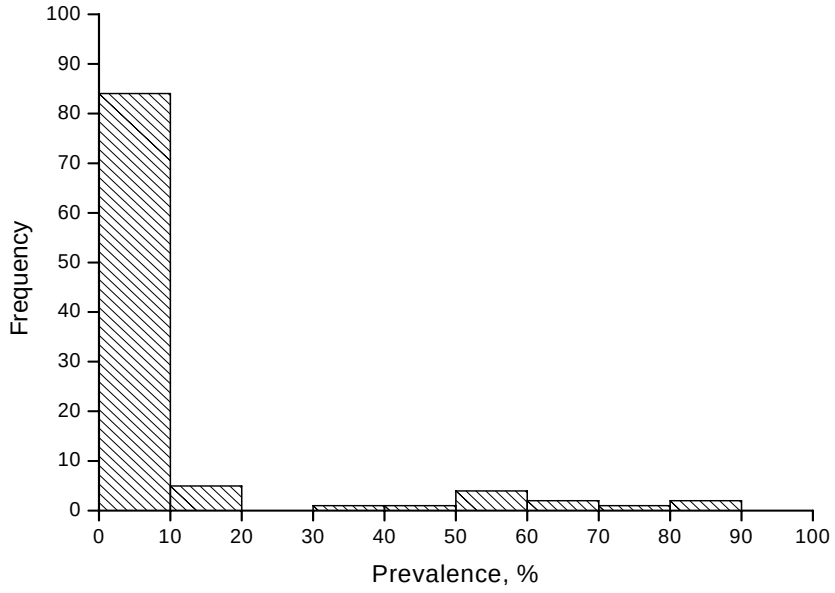

Fig. 5 Distribution of VTEC O157 prevalence in SPECIES? an abattoir group based upon simulation (100 runs)

To assess first order stochastic dominance (hereafter dominance), the data are plotted as cumulative frequency distributions (CFDs). Figure 6 illustrates this for the beef breeder-finisher farm: the solid line is the distribution shown in Fig. 5. For a given prevalence or mean specific shedding level, the value of the CFD is the proportion of simulated farms for which the concentration was at or below that value. For example in Fig. 6, about 90% of farms have a prevalence below 20%. Conversely it is straight forward to read percentage points, such as the median.

Dominance is then determined through the comparison of two CFDs for the same variable before and after an intervention, as shown in Fig. 6. If one curve lies wholly to the left or above the other, it shows that the frequency with which any given value is exceeded is lower than for the other curve. It is said to dominate it, because it is clearly a preferable outcome. In the example shown, where the base case assumes that all purchased animals are free of VTEC O157, whereas the alternative assumes that 5% of the incoming stock are shedding, the base case is clearly (and predictably) preferable.

For each farm several possible interventions were tested to identify those having a substantial effect. Batches of 100 runs were performed for each intervention. The stochastic dominance approach was then used to rank the interventions. The results of interventions tested on the beef fattener farm, the sheep farm and the mixed farm are reported here.

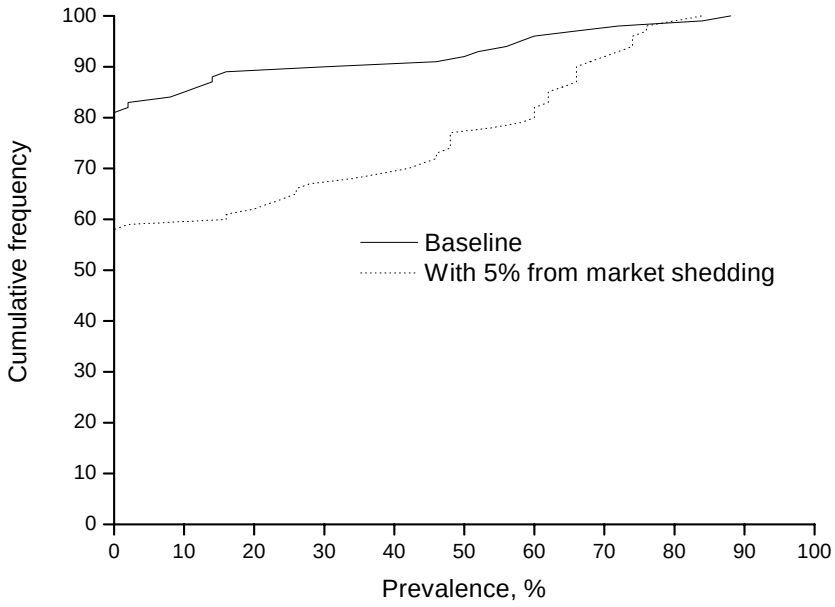

Fig. 6 Simulated cumulative frequency distributions for baseline scenario and with 5% of cattle brought from market shedding VTEC O157

Beef fattener farm: Table 2 shows the results of 9 different interventions on the beef fattener farm, ranked according to their impact on prevalence in the abattoir group. An item in bold dominates the one below it; an item in italics does not achieve first-order dominance, but is dominant over most of the range. Only those interventions for which either partial or complete dominance is achieved are reported here. The mean animal prevalence values are also shown. Where there is first order dominance, the mean of the dominant item must be smaller. Occasionally, where dominance is not achieved and a judgement has to be made, the ordering of the means may be different, because they can be biased by a few uncommon outcomes. The table also shows the rank obtained by considering stochastic dominance of the shedding rate and the value of the mean specific shedding rate. The same approach was taken for the remaining farms.

When the farm size is changed, the number of groups is kept constant and the number of animals and the enclosure areas are changed in proportion, so this was considering the effect of group size independent of stocking density.

Farm size has a strong impact, probably because an outbreak is less likely to be sustained within a small population of animals. The effect of buying uninfected animals is high up in the ranking. This is likely to be especially important in farms such as this where all the animals are purchased. Reducing the contact rate (which could be a consequence of lower stocking density or different handling practices) is beneficial. The effect of very frequent barn cleaning is clearly beneficial, though this assumes that all VTEC O157 are removed, which is highly unlikely in practice. Conversely less frequent cleaning had a highly detrimental effect.

Mixing the groups in the winter, whilst housed, had a severely detrimental impact. Increasing the background level of VTEC O157, which could reflect general hygiene or carriage by vermin and other animals has a fairly strong effect. Synge (2002) found that the presence of

wild geese on the farm was a risk factor; this would be a background source in the model. The timing of slaughter has some effect. The effects on shedding rate were broadly similar.

Table 2. Ranking of management factors on beef fattener farm by effect on prevalence in the abattoir group add AGENT according to the simulation..

Intervention	Mean animal prevalence, %	Rank by shedding rate	Mean specific shedding, CFU/g
Barn clean interval 1 week	1.9	1	408
Farm size -50%	4.9	2	5920
Prevalence in purchased stock 0%	7.8	2	6327
Contact rate = 1/d	14	4	11881
Slaughter 3 weeks earlier	12	5	5514
Base (5% purchased stock prevalence)	14	7	11165
Background level +50%	24	15	21121
Barn cleaning interval 11 weeks	32	17	26866
Farm size +50%	46	18	41251
All animals mixed in winter	51	19	51846

An item in bold dominates the one below it; an item in italics does not achieve first-order dominance, but is dominant over most of the range.

Sheep farm: The results for the simulated sheep farm are shown in Table 3. Stocking density is increased by reducing the area of grazing and keeping the number of animals constant. As with the beef fattener herd farm size is increased independently of stocking density. Because the rates of shedding amongst sheep are very low, the effects of measures expected to reduce them are masked by the variability of the results. However, it can be seen that increasing farm size, stocking density and contact rate produced marked increases in the prevalence and the converse measures tended to reduce it. The effect of contact rate is greater than on the cattle farm, possibly because of the greater number of animals.

Table 3. Ranking of management factors on sheep farm by effect on prevalence in the abattoir group

Intervention	Mean animal prevalence, %	Rank by shedding rate	Mean specific shedding, CFU/g
Stocking density -25%	0.25	1	25
Farm size -50%	0.43	1	196
Base (2% purchased stock prevalence)	0.88	3	367
Farm size +50%	1.3	3	361
Stocking density +25%	2.8	7	1038
Contact rate 20/d	5.8	7	1158

An item in bold dominates the one below it.

Mixed beef and sheep farm: Table 4 shows the effects on mean prevalence and shedding in both the lambs and calves. Mixing with cattle caused a substantial increase in prevalence and shedding amongst the lambs. The CFDs showed that separate grazing was dominant in both

variables for lambs. Mixing also caused a substantial increase in prevalence amongst the calves, though the effect on mean shedding was fairly small. Examination of the CFDs for calves showed that separate grazing was dominant when measured by prevalence, but a few farms with high mean shedding rates meant that the curves for shedding rate crossed, which also explained the small effect on the mean. The effect is greater than would be expected from an increase in stocking rate alone which was relatively small in this comparison.

Table 4. Effect on lambs and calves of mixed grazing

Group	Mixing	Mean animal prevalence, %	Mean specific shedding, CFU/g
Lambs	Fully mixed	5	576
Lambs	Separate grazing	0.9	138
Calves	Fully mixed	22	13439
Calves	Separate grazing	10	12796

CONCLUSIONS

In a simulation study of this type, where the system being simulated is complex and good data are sparse, a certain amount of care is needed when interpreting the results, which necessarily reflect the assumptions built into the model. These assumptions were tested and discussed with other scientists working in the subject during the model development to try to ensure that they were well founded, and they are outlined previously. There are very few data sets in which the dynamics of infection are studied in sufficient detail, including quantification of organisms, to be useful in constructing models. In general, it is found that epidemiological models predict the relative magnitudes of the effects of interventions more accurately than the absolute values of variables such as prevalence. Such models are still helpful, because the need is usually to identify the variables that have the most effect on the outcome, as it was in this study.

The main conclusions from the study are related using the shorthand that “risk” means the probability of carrying VTEC O157 to the abattoir and are as follows. Sheep have a lower risk than cattle. Mixing cattle and sheep increases the risk in both groups. Merging groups of animals of the same species into larger groups increases the risk substantially. Bringing in cattle with a prevalence of 5% increases the risk substantially. Increasing farm/group size increases the risk, independently of stocking density. Increasing stocking density increases the risk, independently of group size. A very high level of barn hygiene reduces the risk. Background sources of VTEC O157 in the model have some effect on the prevalence and shedding; these could be influenced in practice by the presence of wild animals, etc., carrying the organism.

The parameters to which the model is most sensitive (and hence those that it is most important to quantify) are the ones related to: transmission from grass and enclosures to animals; contact between animals; and pathogen survival on grass, in slurry and in barns. Another important area is the course of the infection within the animal, represented in the model by transition probabilities for the shedding rate.

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EPIDEMIOLOGICAL TOOLS 1

PARAMETER ESTIMATION OF A MATHEMATICAL MODEL OF CASEOUS LYMPHADENITIS TRANSMISSION IN SHEEP

K. M. O'REILLY*, L. E. GREEN, F. E. MALONE AND G. F. MEDLEY

SUMMARY

Caseous lymphadenitis (CLA) is an infectious disease of sheep, prevalent in most sheep producing countries which was introduced to the UK sheep population in 1991. Here a mathematical model of CLA transmission is presented with parameters estimated using maximum likelihood estimation. In the model, disease has been categorized into overt and respiratory abscesses. Abscesses in either location may result in clinical symptoms and infectiousness. Assumptions of the model were that overt abscesses resolve and respiratory abscesses remain for life. The model simulation was compared to data from four flocks in Northern Ireland which were culled because of CLA infection.

The model estimates that most (99%) sheep infected develop abscesses. Approximately 73% of sheep with overt abscesses recovered from infection. The average rate to respiratory infectiousness was estimated to be 1/69 days. As the first mathematical model of CLA infection, the parameter estimates point towards aspects of infection that could be utilised in control.

INTRODUCTION

Caseous lymphadenitis (CLA) is a chronic disease of sheep and goats caused by the bacterium *Corynebacterium pseudotuberculosis*. It is characterised by abscesses in lymph nodes (LN), subcutaneous tissues and other organs (Pepin et al., 1994). Disease may be observed as large swellings in overtly located LNs that may discharge infectious material containing *C. pseudotuberculosis* through broken skin. A proportion of infected sheep have abscesses in the lungs and associated LNs, which has been shown to contaminate the respiratory tract with *C. pseudotuberculosis* (Robertson, 1980). CLA is hypothesised to be transmitted through one of three routes (Seddon, 1929): overt infectiousness may cause new overt abscesses in susceptible sheep and respiratory infectiousness may cause respiratory or overt abscesses. The mode of the three routes of transmission has been suggested as: direct contact between sheep for overt to overt transmission; indirect contact (via infected feed or by rubbing) for overt to overt transmission; aerosol spread for respiratory to respiratory transmission; and ingestion for respiratory to overt transmission (where contaminated material from the lungs is coughed onto feedstuff). Secondary abscesses may develop from these primary abscesses; *C. pseudotuberculosis* can survive intracellularly (Hard, 1970) which may aid in development of abscesses away from draining LNs of the site of entry or lungs.

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In countries where CLA infection in sheep is endemic, large economic losses have been attributed to the disease. For example CLA infection reduces wool production in the first year of infection; this was estimated to cost the Australian wool industry AUS $10 million annually (Paton, 1988). In the United States CLA has been described as the most prevalent and economically important disease of sheep (Stoops et al., 1984). It was therefore a concern when CLA infection was first reported in UK sheep in 1991 (Robins, 1991). Results from a postal questionnaire indicated that confirmed cases of CLA (reported by veterinarians) had increased in incidence from 1990-1999 (Binns et al., 2002).

Control measures include preventive vaccination and culling sheep that were either clinically diseased or serology positive (Paton et al., 2003; Schreuder et al., 1994). So far farm-specific control of infection in the UK and other countries has varied in effectiveness (Baird, 2002; Paton et al., 2003) . Control methods can be optimised before being implemented on a host population. Here a mathematical model has been developed and parameters estimated to test optimal control strategies.

To estimate the parameters of the model, data from four flocks from Northern Ireland were used. These flocks were naturally infected with *C. pseudotuberculosis* during 1999-2000 (Malone et al., in press). Either the flock owners notified the Department of Agriculture and Rural Affairs for Northern Ireland (DARDNI) inspectors, or the DARDNI inspectors notified the flock owners as a result of backward tracing from other infected flocks. Maximum likelihood estimation (MLE) was used to compare the model to the data and identify parameter values that fitted the data. Hypotheses of the route of transmission were tested. The null model assumed transmission was identical in each flock. For each test a route of transmission (contact, aerosol or ingestion, all described later) specific to each flock was estimated and the log-likelihood compared to the null model. Should flock-specific transmission be accepted factors specific to each farm may have influenced transmission, this may include management practices such as stocking density.

As maximum likelihood estimates were computed for many combinations of parameter values, a likelihood profile was used to locate the boundaries of individual parameters. This method was suggested by Cox and Medley (1989), where the parameter values were randomly generated and resultant log-likelihood values used to compare the model to the data and provide confidence intervals.

MATERIALS AND METHODS

CLA transmission model

Sheep with CLA infection are reported to remain infected for a considerable time without becoming infectious (Pepin et al., 1994). Therefore a standard susceptible-exposed-infectious-recovered model (Anderson & May, 1991; Gomes et al., 2004) was adapted to incorporate further necessary assumptions of CLA transmission and infection. Abscess location was divided into overt and respiratory abscesses. Overt abscess include the parotid LN, submandibular LN, prefemoral LN, popliteal LN and mammary LN; all of which may rupture externally and be detectable upon clinical examination for disease, and after rupture the model assumes sheep may no longer be infected. Respiratory abscesses are those located in the lungs, mediastinal LN and bronchial LN; should these abscesses erode into the respiratory tract the airways may become contaminated with *C. pseudotuberculosis* (Robertson, 1980). Sheep were assigned to one of

seven mutually exclusive compartments where each compartment represents a proportion of the host population (Fig. 1);

Susceptible (*S*): Sheep are naïve to *C. pseudotuberculosis* infection. When infected at a rate λ, a proportion (s) of infected sheep enter a non-infectious abscess class (F or C), and the remaining infected sheep (1-s) move to the immune class (I). Pepin *et al.* (1991) report that 93% of experimentally infected lambs with observed lesions developed abscesses. Of those that develop abscesses, a proportion (r) develop primary respiratory abscesses, and the remainder (1-r) develop primary overt abscesses; the site of development of abscesses depends upon the mode of transmission. When abscesses are present in both sites, secondary spread from the primary site of abscessation is assumed.

Overt non-infectious abscesses (*C*): An infected sheep has one or more abscesses in overt sites. Sheep may later develop infectiousness in these overt sites and move to the overt infectious class (O) at a rate τ_O. It was assumed that the average time to clinical abscesses (estimated as 49 days in Pepin et al. (1988)) was an approximation for the rate to overt infectiousness.

Respiratory non-infectious abscesses (*F*): An infected sheep has one or more abscesses in respiratory sites. Infectiousness may develop (at a rate τ_R) and the individual moves to the respiratory infectious class (R). τ_R has not been investigated experimentally.

Overt (*O*): Overt abscesses have ruptured the skin and the sheep is infectious. These abscesses will eventually resolve at a rate φ (days) resulting in the sheep no longer being infectious. Field observations suggest the time to healing of abscesses was approximately 21 days (Binns, personal communication); this was accepted as an estimation of φ. A proportion (p) will move to the immune class (I) as no other abscesses are present. In experimental infection necropsied sheep generally have more than one abscess (Pepin et al., 1988), therefore not all sheep that have a resolving abscess will move to the immune class. If there is more than one abscess in an infected sheep (due to multiple primary abscesses or secondary spread of *C. pseudotuberculosis*), a proportion (1-p)q will move to the overt non-infectious abscess class (C) and the remainder (1-p)(1-q) will move to the respiratory non-infectious abscess class (F).

Respiratory (*R*): Respiratory abscesses are infectious and infectiousness is assumed to be life-long. Secondary overt abscesses may develop infectiousness and the individual moves into the overt and respiratory class (B) at a rate τ_R.

Overt and Respiratory (*B*): Overt and respiratory abscesses are both infectious. Overt abscesses resolve after φ days, and the individual moves back to the respiratory abscessed class (R).

Immune (I): An individual has recovered from infection (either non-visible lesions or overt abscesses). Reinfection studies and vaccination experiments provide evidence of strong immunity to reinfection (Hodgson et al., 1994; Robertson, 1980), and immunity to reinfection is assumed to be life-long.

The rate of infection (λ) is the per capita rate of acquisition of infection (Anderson & May, 1991). As transmission may occur through three routes the rate of infection will depend upon three transmission coefficients and proportions infectious. Abscesses caused by contact with sheep that were overtly infectious occur through transmission described as contact transmission (β), resulting in overt abscesses. Overt abscesses may also arise from respiratory abscesses,

which are assumed to occur from ingestion of *C. pseudotuberculosis* (described as ingestion transmission, κ). Abscesses located in respiratory sites may have been caused by aerosol spread of *C. pseudotuberculosis* from sheep with respiratory abscesses, referred as aerosol transmission (π). Combining the three routes of transmission and the compartments defining infectious sheep for these routes, the rate of infection is defined as;

$$\lambda = \beta(O + B) + (\pi + \kappa)(R + B) \tag{1}$$

The proportion of sheep infected that develop either respiratory or overt abscesses depends upon the route of transmission. Therefore the proportion (r) that develop respiratory abscesses and the proportion (1-r) that develop overt abscesses are given by;

$$r = \frac{\pi(B + R)}{\lambda} \tag{2}$$

$$1 - r = \frac{\beta(O + B) + \kappa(R + B)}{\lambda} \tag{3}$$

The above assumptions and flock demography produce a set of deterministic non-linear ordinary differential equations with respect to time;

$$\begin{aligned}
\frac{dS}{dt} &= -S(\lambda + \mu) + \mu \\
\frac{dI}{dt} &= S\lambda(1 - s) + Op\,\varphi - I\mu \\
\frac{dF}{dt} &= S\lambda sr + O\varphi(1 - p)(1 - q) - F(\tau_R + \mu) \\
\frac{dC}{dt} &= S\lambda s(1 - r) + O\varphi(1 - p)q - C(\tau_O + \mu) \\
\frac{dR}{dt} &= F\tau_R + B\varphi - R(\tau_B + \mu) \\
\frac{dO}{dt} &= C\tau_O - O(\varphi + \mu) \\
\frac{dB}{dt} &= R\tau_B - B(\varphi + \mu)
\end{aligned}$$

The values of the transmission coefficients (β, π and κ) are unknown, and are estimated from data. Sensitivity analysis has identified s, q and p as parameters that have a large effect on the model output. In addition, the parameters τ_R and τ_B have not been investigated experimentally, and therefore are estimated here. The parameters τ_O and φ did not affect model simulations greatly; therefore the estimates reported (τ_O=1/49 days and φ=1/21 days) are used in the simulations.

Data of CLA infected sheep

The data used here are from four flocks where sheep were removed because of CLA infection (Malone et al., in press). For each sheep culled the ear-tag number, sex, year of birth, serology (not analysed here), bacteriology and post-mortem examination results were recorded.

Only the breeding ewe group and associated rams of each flock are analysed. The post-mortem examination results were categorised according to the location of abscesses as in the transmission model. Four categories were used; no abscesses, overt abscesses, respiratory abscesses, and sheep with abscesses in both locations.

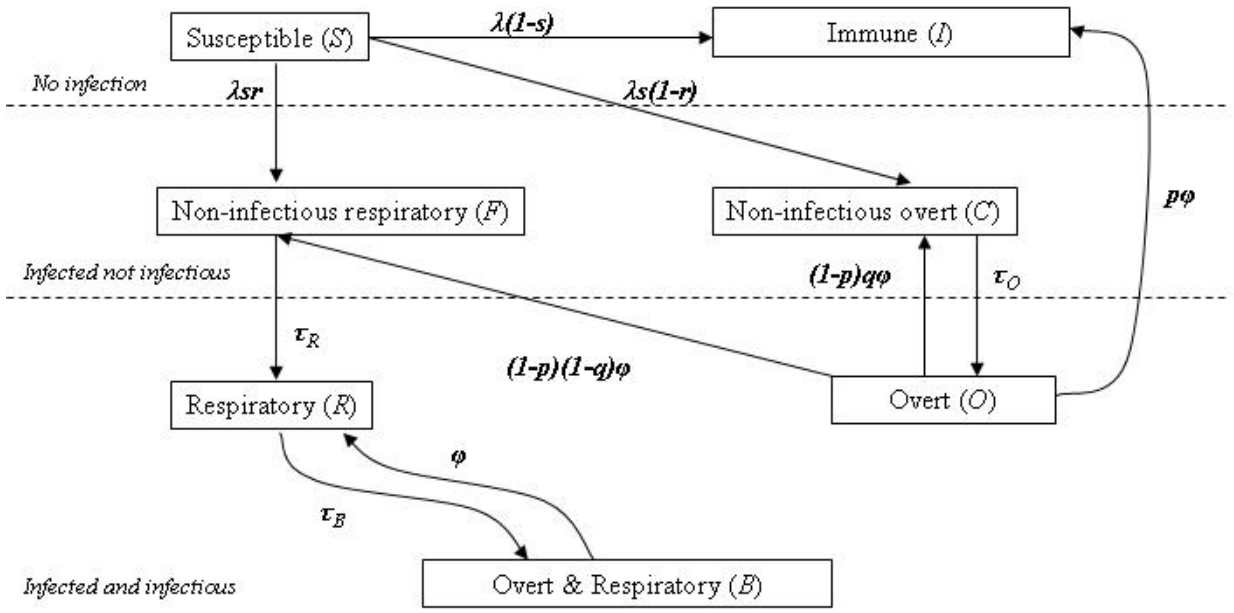

Fig. 1 Caseous lymphadenitis transmission model. The parameters of the model are identified in Table 1

Sheep were initially removed from each flock because of clinical signs of CLA disease; it was later decided that all in-contact ewes and rams would be removed. Therefore the data were divided into sheep culled as a result of clinical examinations and sheep culled as in-contacts. Sheep removed because of current clinical signs were generally culled weeks (rather than days) later, whereas sheep removed as in-contacts were culled on the day they were removed. When examined *post mortem*, some sheep specified as clinically diseased had no abscesses (35 of 100 sheep); the time between removal because of clinical disease and examination *post mortem* was long enough (20-62 days) for overt abscesses to resolve. Therefore the post-mortem examination data were extrapolated back to the time when sheep were removed from flock; assuming that those with no abscesses at post-mortem examination had previous overt abscesses (Table 1).

When sheep were removed from the flock without clinical inspection, fewer sheep were abscessed than when removed because of clinical disease (Table 2). Of the four flocks, 36% of sheep in Flock A, 51% of Flock B, 39% of Flock C, and 14% of Flock D were abscessed. The proportion of sheep with each type of abscess (overt or respiratory) did not differ greatly between flocks when examined *post mortem*. These samples of sheep were assumed to represent the prevalence of infection in the remaining flock.

Parameter Estimation

The flock data was pooled for parameter estimation resulting in 6 longitudinal data points for Flock A, 8 for Flock B, 4 for Flock C and 4 for Flock D, totalling 22 data points of 240 breeding ewes and rams.

The data was categorised according to abscess location, with no information about infectiousness. Therefore to compare the data to the model, assumptions were made regarding the infectiousness of individuals with abscesses. Sheep with overt abscesses were compared

Table 1. Estimated infection status of ewes and rams in each flock when the flock was inspected for CLA disease

Flock	Time PI (days)[a]	Number in flock	Clinical disease[b]	Respiratory abscesses	Overt abscesses	Abscesses in both locations
A	323	62	4 (6)	1	0	3
	337	58	12 (21)	2	7	3
	364	46	6 (13)	1	4	1
B	244	84	22 (26)	5	13	4
	269	62	27 (44)	4	18	5
C	666	55	16 (29)	5	8	3
	702	39	8 (21)	1	4	3
D	247	40	5 (13)	0	3	2

[a] Post introduction (PI) of CLA that ewes and rams were inspected for disease
[b] That were subsequently removed from the flock (%)

with the *C* and *O* classes of the transmission model, sheep with respiratory abscesses were compared with the *F* and *R* classes of the model and sheep with both type of abscesses were compared with the *B* class in the CLA model. Preliminary analysis indicated that the best-fitting simulations were produced when 2 sheep from Flock A, 1 sheep from Flock B, 1 sheep from Flock C and 1 sheep from Flock D were assumed as infectious. These initial conditions were used for the following simulations.

The data and model with associated assumptions were compared using a multinomial distribution (Cox & Hinkley, 1979; Hilborn & Mangel, 1997). Each individual is placed in a category k_i (for $i = 1,\ldots,4$) according to abscess location. The proportion predicted by the model parameters in each category is denoted by p_i (where $\Sigma\, p_i = 1$). For each set of parameter values this allows calculation of the likelihood of the observations. The parameter estimation identifies the set of parameter values that maximize the likelihood of the model given the data. The model and parameter estimation was carried out in MATLAB (version 7.0.1.).

Hypotheses of flock-specific variability of each route of transmission were tested. The starting parameter values for each hypothesis were the best-fitting parameter values without flock variation (the null model). For each hypothesis the transmission coefficients were allowed to vary for each flock in the simulations. The best-fitting parameters were estimated for each hypothesis and corresponding log-likelihood values compared with the null model (allowing for the increase in degrees of freedom). When the full model had a significantly ($P<0.05$) improved fit to the data, the hypothesis was accepted.

Table 2. Data of (in-contact) ewes and rams of each flock culled without clinical inspection

Flock	Time PI[a] (days)	Number removed	No abscess	Respiratory abscesses	Overt abscesses	Abscesses in both locations	Proportion infected
A	440	14	11	1	1	1	0.21
	456	14	6	4	3	1	0.57
	462	11	8	0	3	0	0.27
B	317	10	6	2	0	2	0.40
	324	9	4	0	5	0	0.56
	328	2	2	0	0	0	1.00
	330	5	3	0	2	0	0.40
	331	4	0	2	1	1	1.00
	336	5	2	3	0	0	0.60
C	764	15	9	0	5	1	0.40
	770	16	10	1	5	0	0.38
D	339	14	10	1	1	2	0.29
	345	11	10	0	1	0	0.09
	365	10	10	0	0	0	0.00
Total		*140*	*91 (0.65)*[b]	*14 (0.10)*	*27 (0.19)*	*8 (0.06)*	*0.35*

[a] Post introduction (PI) of CLA that sheep were removed from the flock
[b] Proportion of sheep

Several thousand parameter combinations distributed around the best-fitting parameter values were fitted to the data to obtain a likelihood profile of the parameters. Where the resultant log-likelihood was not significantly (P<0.05) different from the null log-likelihood the minimum and maximum values of each parameter were used as the 95% confidence interval.

RESULTS

In the null model, contact transmission was higher in value than respiratory or ingestion transmission (Table 3). The coefficient for contact transmission (β) was estimated at 0.0792 (per susceptible sheep per infected sheep per day), whilst respiratory (π) and ingestion (κ) transmission coefficients were 0.0004 and 0.0052 respectively.

Flock-specific contact transmission was accepted as a hypothesis; aerosol and ingestion transmission were not accepted (Table 3). The contact transmission coefficient of Flocks A, C, and D were similar whilst Flock B had a much higher transmission coefficient. The ingestion transmission coefficient was estimated to be higher in value than in the null model at 0.0113 and the respiratory transmission coefficient was estimated to be 0.0005. A higher proportion of those infected developed abscesses (s=0.9959). The rate of respiratory infectiousness was estimated to be 1/69 days and additional overt infectiousness was 1/10 days. An example of the model simulation and data of Flock A is illustrated in Figure 2. The data of Flock A shows an initial high proportion with no abscesses when the flock was inspected (circles), followed by an increase in the proportion infected at the time when sheep were removed irrespective of clinical signs (squares). Sudden changes in each proportion infected in the simulations denote removal of sheep.

Table 3. Maximum likelihood estimates of parameter values for the null model and each hypothesis tested. The null model has one value for each of the transmission coefficients whilst each of the hypotheses tested have four coefficients specific to each flock.

Parameters	Null model	Flock-specific transmission		
Transmission coefficients [a]		*contact*	*aerosol*	*ingestion*
β	0.0792	n/a	0.0863	0.0837
π	0.0004	0.0005	n/a	0.0007
κ	0.005	0.0113	0.0001	n/a
Flock-specific [a]		***β***	***π***	***κ***
A	n/a	0.0194	0.0006	0.0837
B	n/a	0.0922	0.0006	0.0194
C	n/a	0.0248	0.0003	0.0103
D	n/a	0.0239	0.0006	0.0003
Proportions				
s	0.8247	0.9959	0.7826	0.7609
p	0.7434	0.7286	0.7472	0.7387
q	0.0351	0.0101	0.0301	0.0374
Rate (days)				
τ_R	1/45	1/69	1/58	1/47
τ_B	1/23	1/10	1/18	1/23
Log-likelihood	*-165.51*	*-112.50*	*-164.96*	*-163.14*

[a] units for transmission coefficients are; per susceptible sheep per infected sheep per day

Table 4. Summary of parameter confidence intervals of the flock-specific contact transmission model

Parameters	Best-fitting value	95 % Confidence interval	
Coefficients [a]		Lower	Upper
β_A	0.0194	0.0154	0.0260
β_B	0.0922	0.0798	0.1108
β_C	0.0248	0.0090	0.0379
β_D	0.0239	0.0081	0.0407
π	0.0005	0.0007	0.0308
κ	0.0118	2×10^{-7}	0.0060
Proportions			
s	0.9959	0.9406	1.0000
p	0.7286	0.6078	0.8166
q	0.0101	0.0003	0.0310
Rate			
τ_R *(days)*	1/69	1/37	1/135
τ_B *(days)*	1/10	1/7	1/17

[a] units for transmission coefficients are; per susceptible sheep per infected sheep per day

Parameters values were randomly generated 58,174 times; producing 1039 combinations of parameter values that were not significantly (P<0.05) different to the best fitting combination. The 95% confidence interval for β_A was 0.0154-0.0260, for β_B was 0.0798-0.1108, for β_C was

0.0090-0.0379 and for β_D was 0.0080-0.0407 without considering any associations with other parameters (Table 4). The contact transmission coefficients β_A, β_C and β_D were significantly (P<0.05) different to β_B.

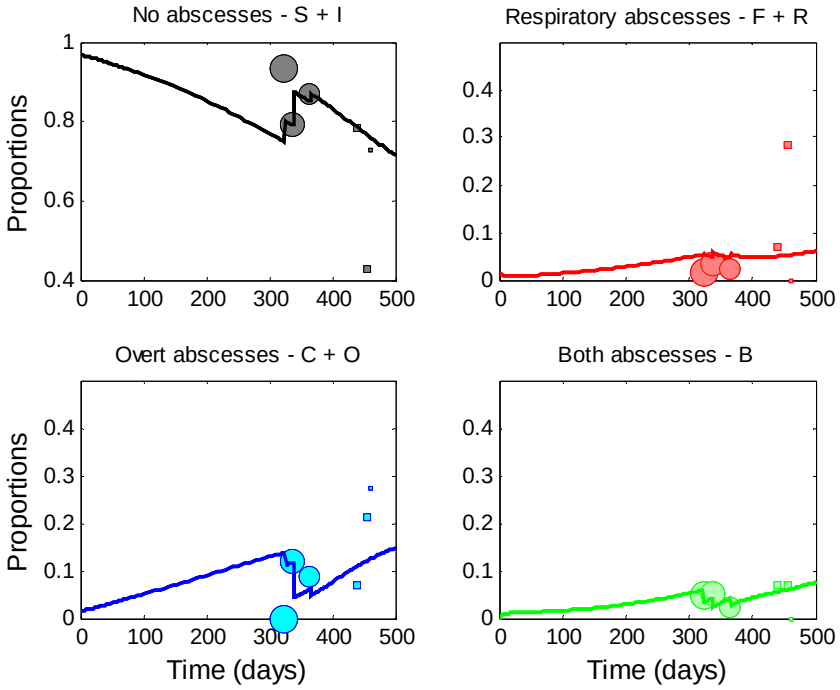

Fig. 2 Plot of data from Flock A and model with best fitting parameters (Circles; sheep removed due to clinical disease, squares; sheep removed irrespective of clinical signs). Size of circles and squares illustrate sample size.

DISCUSSION

The CLA transmission model incorporates two main features; an initial epidemic of overt abscesses followed by a gradual increase in respiratory abscesses. Contact transmission was the highest transmission coefficient. This implies that overt abscesses may cause most infection, but this needs to be confirmed using simulations. Respiratory transmission, although estimated to be lower in value than contact transmission, had an important effect as the respiratory abscesses caused were assumed not to resolve and to remain infectious.

The proportion of infected sheep that develop abscesses (s = 0.9959) was similar to the estimated 0.93 reported by Pepin *et al.* (1991) in experimentally infected lambs. A high value of s illustrates that most infection resulted in the formation of abscesses. Immunity is most likely occur through resolved overt abscesses (as p=0.73), rather than infection that did not lead to abscessation. The remainder of sheep (0.27) remain abscessed; most (0.99) of these sheep were abscessed in respiratory sites and less than 0.01 had only overt abscesses. The model parameters illustrate that sheep with repeated overt abscesses were likely to have additional respiratory infection.

Given the presence of non-infectious respiratory abscesses, the estimated rate of respiratory infectiousness was 1/63 days. If respiratory abscesses were infectious, additional overt infectiousness develops at a rate 1/10 days (when the sheep moves to the both class). Assuming

that overt infection resolves at a rate of 1/21 days, respiratory infectious sheep have an approximate 31 day cycle of developing overt infectiousness.

The hypothesis testing indicates that further study of transmission is necessary. Flock-specific contact transmission indicated a lower transmission coefficient in Flocks A, C, and D when compared to Flock B. The higher prevalence of abscesses (51%) in Flock B when compared with the other flocks (36%, 39%, 14% abscessed from Flocks A, C and D respectively) indicate a higher rate of transmission, despite Flocks A and C being infected for a longer period of time. The high transmission coefficient of Flock B is due to a higher proportion of sheep observed with clinical disease when first inspected (0.26 and 0.44 of breeding ewes and rams were clinically diseased at 244 and 269 days post-introduction of infection respectively). However none of the other flocks were examined for clinical disease at this time. It may be that as other flocks were not inspected this early on in the infectious process, a higher proportion infected was never recorded and therefore cannot be modelled. There remains a level of uncertainty of the hypotheses tested.

The data was suitable for parameter estimation of the CLA model as the proportions with each type of abscess were recorded. However the data was variable, some variability will be due to the routes of transmission and exposure to infection as described by the model, but there was unaccounted variability. The assumption of independence between samples of a multinomial distribution was not met; this may affect the log-likelihood values generated.

The aim of developing the CLA model was to quantitatively investigate control of infection. The parameters estimated need to be explored further to investigate how the parameter values will affect the model output. Once this has been fully investigated, the model can be used to investigate control methods for CLA infection.

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EFFECT OF VITAMIN E SUPPLEMENTATION ON UDDER HEALTH: A META-ANALSYIS

M. NIELEN*, N. MOYO, C.L.J.J. KRUITWAGEN AND A.C. BEYNEN

SUMMARY

This meta-analysis quantifies the effect of vitamin E supplementation on udder health. In total 14 randomised clinical trials were available, yielding 12 records for clinical mastitis (CM), 5 for somatic cell count (SCC) and 9 for intra mammary infection (IMI). The relative risk (RR) was calculated for CM and IMI incidence and the mean difference (MD) of the natural logarithm of the SCC (lnSCC) for SCC.

All udder health parameters improved with vitamin E supplementation; RR for CM was 0.70 (.59 - .83), MD lnSCC was –0.35 (-.68 – 0) and RR for IMI was .86 (.73 – 1.02). Publication bias resulted in fewer 'negative' studies than expected. However, control vitamin E levels were relatively high and records were analysed at cow level, all leading to an underestimation of the effect. The findings thus seemed robust. With better knowledge of vitamin E metabolism, field interventions seem indicated to improve udder health.

INTRODUCTION

Vitamin E is a fat-soluble, sub-cellular and cellular membrane antioxidant, which provides stability and prevents undesirable per oxidation of membrane lipids. Vitamin E's physiological status has been associated with susceptibility and response to infectious diseases like mastitis (Smith et al., 1984, Weiss et al., 1997).

Several studies have reported the relationship between vitamin E status and udder health indicators such as the incidence of clinical mastitis (CM), and sub clinical mastitis as shown by somatic cell counts (SCC) and/or intra-mammary infection (IMI). The association between vitamin E status and udder health indicators from these studies ranged from protective (Smith et al., 1984, Weiss et al., 1997), through no-relation (Batra et al., 1992, LeBlanc at al., 2002) to an unfavourable effect (Erskine et al., 1987, Batra et al., 1992). The goal of the current study was to carry out a meta-analysis on the available literature to quantify the size and significance of the effect of vitamin E supplementation on udder health.

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MATERIALS AND METHODS

Inclusion criteria, search strategy, record selection and data manipulation

Studies had to meet the following inclusion criteria; 1) one or more groups of Vitamin E levels were being compared of which one could be classified as the control group; 2) Indicator of udder health status like SCC, IMI, CM; 3) Plasma or serum vitamin E cow/herd status, or ratios of these to cholesterol or total lipids; 4) Vitamin E per cow or herd dosage, route of dosage and period of administration; 5) Duration of the study; 6) ruminant studies.

Only randomised clinical trials and field studies were considered, thereby excluding small experimental studies. Papers with at least an abstract in English and in German, Dutch, English, Chinese, Japanese, Turkish, Serbo-Croatian published between 1984 and 2003 were located through a literature search that was based on 1) examination of computerized scientific directories and 2) cross-referencing of citations in retrieved papers. Databases used included Agricola, Beast CD, Vet CD, Medline and Web of Science. Potentially eligible articles were selected after examination of the titles and abstracts generated from the searches, for those meeting the eligibility criteria full article texts were sought.

Data from studies that were originally recorded at quarter level were converted to animal level according to the assumption that each infected cow would on average harbour 1.5 infected quarters (National Mastitis Council (1996). When several vitamin level groups were compared, the comparisons were taken as independent trials (records) with the lowest level serving as the control for all the other vitamin levels of the same study.

Statistical analysis

For cohort or randomised clinical trials (RTC) carried out longitudinally, the relative risk (RR) was considered to be the best measure of effect. The mean difference (MD) was used to evaluate the effect of Vitamin E on the SCC, however after normalizing SCC by natural logarithm (lnSCC). Two by two tables were created per record on vitamin E supplementation versus non-supplementation for both CM and IMI. The MD lnSCC between vitamin E supplemented and control groups was also calculated.

The resulting data were entered in a chronological manner into Comprehensive Meta-analysis® software, to draw out any time (year of publication) trend. To obtain the combined measure of effect inverse variance or Maentel-Haenszel (MH) method (Dohoo et al., 2003) and Hedge's adjusted g (Rosnow et al., 2000) were used for RR and MD respectively. The MH method was used whenever any empty cells were observed in any of the two by two cross tabulations. Heterogeneity between studies was tested using a χ^2 procedure. If heterogeneity was present, a random effect model was applied to summarize the data, else a fixed effect model. Funnel plots, which are essentially a scatter plot of the effect measure (RR or MD) against the standard error, were used to visually check for publication bias. The extent of publication bias was quantified using the correlation coefficient.

A weighted univariable meta-regression with the effect measures (RR, MD) as dependent and various explanatory variables of heterogeneity was carried out using SPSS®. The inverse of the variance was used as the weighting factor for each record, RR was assumed to be a continuous dependent variable. Explanatory variables that were studied included; vitamin E plasma level in

the control group or in the treatment group or the difference between the two groups; concurrent Selenium supplementation (yes/no); background level of CM or IMI in the control group; duration of the vitamin E supplementation in days.

RESULTS

The search initially resulted in a total of 34 papers on the relationship between vitamin E and udder health indicators. Of those 34 papers 7 were excluded because no group comparisons were available, and 13 were excluded because of unclear or unsatisfactory study design or because of missing data. Only 14 papers were eligible for use in various sub-analyses, with a total of 18 records. The cross-sectional studies were left out from the final meta-analysis, which was thus based on 14 records from 10 RCT studies (Table 1).

In 12 records the incidence of CM was available, which averaged 39 cases (range 9 to 100) per 100 exposed animals in the control groups while vitamin E supplementation reduced the incidence of CM to an average of 23 (range 1 to 70) cases of CM per 100 exposed animals. For 6 records the vitamin E plasma levels were 3.79 μg/ml (2.35 to 4.95) in the supplemented and 2.69 μg/ml (2 to 3.77) in the control group.

Studies on the relationship between vitamin E supplementation and SCC are the scarcest, with 5 records total. SCCs averaged 300 x 1000 cells/ml (range, 43 to 600) and 100 x 1000 cells/ml (range, 25 to 300) in control and supplemented groups, respectively. For 4 records the supplemented groups had vitamin E plasma levels of 4.40 μg/ml (2.96 to 4.95), where the control groups had 3.07μg/ml (2.14 to 3.77).

In total 9 records included the incidence of IMI, which averaged 51 cases (range 12 to 100) per 100 exposed cows in the control groups. Vitamin E did on average reduce the risk of IMI to 36 (range zero to 85) cases of IMI per 100 exposed cows although the records were heterogeneous. For 3 records vitamin E plasma levels were calculated (similar the CM).

Forest plots are shown in Figs 1, 2, 3, for the three different udder health indicators CM, SCC, and IMI. Vitamin E supplementation led to a significant 30% reduction in the risk of CM (RR=. 70 (.59-.83)) as shown in Fig. 1. The pooled estimate showed a significant .35 units reduction in lnSCC (MD= -.35 (-.69- -.01)) with vitamin E supplementation (Fig. 2). Finally, vitamin E supplementation was on average followed by a non significant 14% reduction in the risk of IMI (RR= .86 (.73-1.02) (Fig. 3).

Table 1. Characteristics of 18 records from 14 papers included in the meta-analysis on the relation between udder health indicators (CM = clinical mastitis, SCC = somatic cell count, IMI = intra mammary infection) and vitamin E supplementation (IU = international units)

1st author year	#cows treat/control	Udder health indicator	IU Vitamin E Treat/control	Status µg/ml[b] Treat/control	Vit E route	Other species / quarter level	Observation period pp
Le Blanc 2002	574/568	CM, SCC	3000 / placebo	2.96 / 2.14	Injection		30
Baldi 2000	14/14	SCC	2000 / 1000	4.85 / 3.25	Diet		14
Valle 2000	26/26	CM, IMI	1000 / none	-	Diet		30
Valle 2000	26/26	CM, IMI	1000,3000 / none	-	Diet		30
Morgante 1999	25/25	CM, SCC, IMI	250 / placebo	-	Injection	Ewes	150
Weiss 1997	21/22	CM, IMI	1000&500 / 100	2.35 / 2	Diet	Quarter	7
Weiss 1997	19/22	CM, IMI	100,4000,2000 /100	3.84 / 2	Diet	Quarter	7
Erskine 1997	204/216	CM	3000 / placebo	3.94 / 2.98	Injection		30
Liu Yu 1995	48/39	CM	500 / none	-	Diet		300
Nizamlioglu 1993[a]	14/20	SCC, IMI	-	0.71 / 0.68	-		-
Batra 1992	108/95	CM, IMI	1000&500 / none	3.84 / 3.11	Diet		305
Batra 1992	58/60	SCC	1000&500 / none	3.84 / 3.11	Diet		280
Nizamlioglu 1992[a]	15/25	SCC, IMI	-	0.70 / 0.30	-	Ewes	-
Erskine 1987[a]	679/690	SCC, IMI	-	4.85 / 4.21	-		-
Atroshi 1986[a]	20/21	SCC	-	6 / 4.84	-		-
Smith 1985	27/28	CM, SCC, IMI	2&88 mg/kg/d / none	4.95 / 3.77	Diet	Quarter	305
Smith 1984	21/20	CM, IMI	.74 g/cow/d / .32	-	Diet	Quarter	305
Smith 1984	20/20	CM, IMI	.74 g/cow/d / .32	-	Diet	Quarter	305

[a]Cross-sectional
[b]plasma/serum vitamin E levels

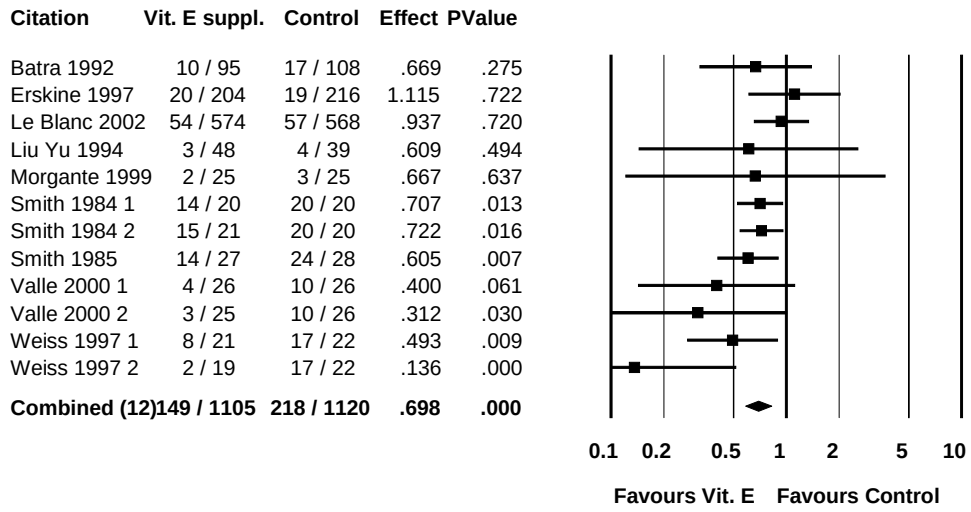

Fig. 1 Forest plot of records with the udder health parameter clinical mastitis (CM) showing recalculated relative risks (RR) and 95% confidence intervals per record, as well as the combined RR

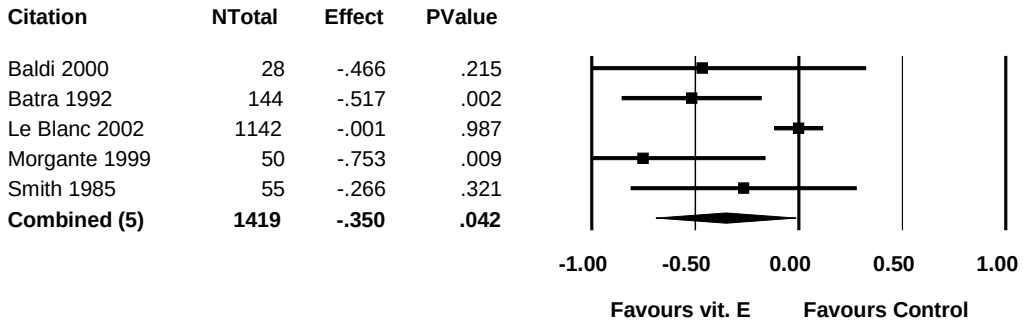

Fig. 2 Forest plot of records with the udder health parameter somatic cell count (SCC) recalculated as mean difference (MD) in the natural logarithmic scale (lnSCC) and 95% confidence intervals per record, as well as the combined MD

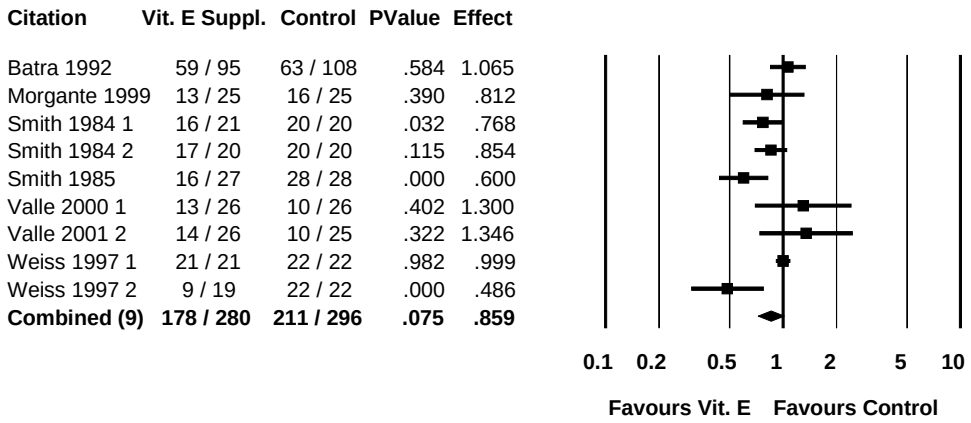

Fig. 3 Forest plot of records with the udder health parameter intra mammary infection (IMI) showing recalculated relative risks (RR) and 95% confidence intervals per record, as well as the combined RR

Significant heterogeneity was observed with SCC and IMI (results not shown), and therefore a random effect model approach was used to calculate the combined effect measures over the records. No year trend was observed across records in all udder health indicators. Funnel plots (results not shown) revealed publication bias as indicated by an absence of smaller studies (high standard error) with a "negative" effect of vitamin E supplementation for all udder health indicators.

Meta-regression

The univariable meta-regression indicated trends for causes of heterogeneity between studies as shown in Table 2. The number of records was too low to contemplate multivariable models.

Table 2. Results of univariable meta-regression models to evaluate potential causes of heterogeneity (P value < .25).

Dependent variable	Predictor variable	# records	coefficient	P value	R^2
RR of CM	Background CM	12	-0.227	0.093	0.26
	Duration suppl days	12	-0.001	0.218	0.15
MD of lnSCC	Vit E status control	4	-0.339	0.128	0.76
	Vit E status treat	4	-0.233	0.039	0.92
	Diff vit E treat-control	4	-0.577	0.002	0.99
RR of IMI	Se suppl yes/no	7	-0.255	0.09	0.47

Table 2 shows that 26% and 15% of variation in RR of CM was related to background (control) CM incidence and duration of supplementation, respectively. Based on 4 records only, an amazingly high percentages of the variation in MD lnSCC was related to absolute vitamin E status levels in the control and treatment group per record (76% to 99%). For IMI, concurrent selenium supplementation was related to 49% of the variation in the RR.

DISCUSSION

Search results

Not many studies, abstracted in the English language, addressed the relationship between Vitamin E and mammary gland health indicators with at least two levels of vitamin E administration to be compared. Many literature reviews have been written that do not reflect the paucity in properly carried out studies or the possibility of publication bias. No previous attempt to quantify the observed varied effects of Vitamin E supplementation across studies was carried out.

Two kinds of study designs were identified, longitudinal and cross-sectional. The meta-analysis showed that studies with a cross-sectional design tended to have higher estimates of effect measures than RCT's did (results not shown). In cross-sectional studies the effect of vitamin E is probably highly confounded by other factors. In a herd high producers are more likely to have altered udder health indicators and more likely to be fed more concentrates, but also more likely to have lower or higher body vitamin E status than other herd mates depending on the diet composition.

Funnel plots showed an even distribution of records with their standard errors (SE), with records based on large data sets showing small SE, and vice versa. However, publication bias was evident in all sub- analysis as smaller studies that showed no protective effect ("negative" results) were missing. There were also high and negative correlations between effect measures and their SE, which further expose the bias towards a mainly protective effect in smaller studies. All this points to the fact that among small studies (high SE) only those with highly significant and 'positive' results were published and may suggest that the effect measures in this study could have resulted in over-estimates of the true summary effect compared to if all studies had been included. However, 60, 56 and 48 small null effect studies would have been needed evaluating CM, SCC and IMI in that order, for the observed summarised effects to be reduced to null effect. The null effect studies used for this bias evaluation were assumed to be of equal sample size to the smallest record used in each of the udder health indicator meta-analyses.

Udder health indicators

Surprisingly large effects of vitamin E levels on udder health indicators were observed in this meta-analysis, particularly in the light of the control vitamin E levels. The reported basic (control) dietary levels of vitamin E in all studies were way above the 1988 NRC recommended levels (15 IU/kg DIM), which are the minimum requirement to prevent overt signs of deficiency and guarantee reasonable animal performance. For proper immune function intake levels were suggested higher at 1000 IU/cow (Weiss, 1998, Hogan et al., 1993). Within the SCC analysis, control groups with low SCC already had high vitamin E status (Batra et al., 1992, Baldi et al., 2000) while supplemental groups with high SCC had a relatively low vitamin E status, as indicated in the meta-regression as well.

The unit of measurement (cow, quarter) in the original study influenced results. Because vitamin E acts systemically it seemed proper to carry out the analysis at animal rather than quarter level, thereby assuming that the probability of a primary infection of any of the quarters is the same. Conversion to cow level always led to a tendency towards the null effect when compared to analysing those studies in their original quarter level measurements (results not shown). This apparent reduced protective effect suggested differential misclassification as the number of infected quarters per cow may be lower in vitamin E supplemented than in control cows.

Vitamin E supplementation

In most records the total vitamin E intake could not be ascertained due to incomplete information of the basic diet and feeding regimes. Vitamin E kinetics is largely unknown, more-so for different routes of administration. The same status of vitamin E as measured in plasma/serum or these as ratios to total lipids may have different implications following parenteral versus oral administration. Following oral administration all plasma/serum vitamin E has passed through the liver, while for parenteral these may include pre-hepatic and post-hepatic forms of the vitamin. The different efficacies of these pools of vitamin E need to be characterized as these may result in heterogeneity of results even for the same vitamin E "status".

The scarcity of information on the kinetics of vitamin E has led to discrepancies between follow up periods in many studies. Most studies started recording udder health indicators at supplementation during the dry period, when it is not certain if the observed parameters can already be related to the supplemented Vitamin E. Udder health was recorded for various lengths of time post partum, which can lead to underestimation of effect measures if the observation period was longer than the expected effect of vitamin E supplementation.

Research is still going on to validate Vitamin E assays, although it is generally agreed that plasma/serum or the ratio of these to total lipids as represented by cholesterol suffices. Plasma/serum levels of vitamin E are prone to plunge during the periparturient period (Goff & Stabel, 1990, Weiss et al., 1994) and could confound anti-oxidant measurements around this period. This drop seems to be relatively steeper the higher the pre-periparturient levels of Vitamin E (Bass et al., 2001). Plasma/serum vitamin E levels are subject to more fluctuations with the stage of lactation (Herdt et al., 1996) when compared to the more robust plasma/cholesterol ratio. The relative physiological importance of the various indicators of vitamin E status need to be determined as it is still vague whether immune function is depended on the absolute blood levels of vitamin E, its enrichment in the fatty fraction of blood or on some other levels in neutrophils and red blood cells.

CONCLUSION

The results of the meta-analysis support the hypothesis that vitamin E supplementation during the dry and early lactation periods is associated with lower CM, SCC and IMI. However optimal dosages, routes of administration, when to start or stop and costs versus benefits will need to be determined in the field again, because the studies described a wide variety in all factors.

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A STOCHASTIC SIMULATION MODEL TO DETERMINE THE SAMPLE SIZE OF CONSECUTIVE NATIONAL SURVEYS TO DEMONSTRATE FREEDOM FROM BOVINE HERPES VIRUS 1 (BoHV1) INFECTION

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SUMMARY

The paper describes the basic structure and application of a generic stochastic model for risk-based sample size calculation of national surveys to document freedom from contagious diseases. The model accounts for residual domestic infection and characteristics of different import pathways. Disease spread potential between two consecutive surveys was considered, either from undetected infections within the domestic population or from herds that bought imported infected animals. The sensitivity analysis revealed that the number of residual infected domestic herds and the multiplicative between-survey-spread factor were strongly correlated with the pre-survey probability of freedom from infection. Using the example of infectious bovine rhinotracheitis (IBR) it was shown, how to accurately estimate the pre-survey probability of freedom from infection. With this model, a generic tool becomes available which can be adapted to changing conditions related to either importing or exporting countries.

INTRODUCTION

Establishing that populations are free from pathogens is vital in the context of contagious animal disease control. According to the SPS agreement (Anonymous, 1998) countries are no longer allowed to restrict the import of animals and animal products for reasons other than science-based evidence that this import could cause negative effects on the indigenous human, animal or plant population. In order to be able to obtain special health guarantees from trading partners, countries therefore need to document their infection-free status. Most countries rely on import risk assessment (RA) and surveillance systems including repeated serological surveys to substantiate claims of freedom from disease or infection. The aim of such surveys is to document with a certain level of confidence that the infection prevalence is below the defined threshold level.

In 2002, Hadorn and co-workers showed how to integrate knowledge obtained from surveys conducted in the previous year and how to combine this information to the risk of disease introduction (Hadorn et al., 2002). The concept consisted of a time-related decrease of the confidence in the results of the last survey. In order to quantify the amount of confidence left after one year, quantitative and qualitative risk assessment methods were used. A limitation of this deterministic model was that the potential risk of undetected infected herds spreading the

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infectious agent in-between two surveys in the population under study was not considered. This limited the approach to surveys conducted on non-highly contagious diseases.

The aim of this study was therefore to incorporate the spread of infection to determine the pre-survey probability of freedom (pre-SPF), and in consequence the minimal required sample size, for national surveys to demonstrate freedom from rapidly spreading infections. This new model is of a generic type and can be easily adapted to a wide range of infectious agents. The use and parameterisation of the model were illustrated on the example of bovine herpes virus 1 (BoHV1), the causative agent of infectious bovine rhinotracheitis (IBR).

MATERIALS AND METHODS

The stochastic model presented here is a combination of quantitative RA components and disease transmission components to simulate the spread of disease within a country, as well as the probability of introduction of the infectious agent. Data used as input for BoHV1 originated from the surveillance programme, import and trading statistics, literature as well as expert opinion on infection dynamics (Appendix 1). International guidelines of import risk analysis were followed (Murray, 2002). Figure 1 describes the stochastic model's basic structure. It consists of four modules: 1) disease spread from undetected infected domestic herds 2) introduction of infection due to import from a country with an equivalent livestock health status, 3) introduction of infection due to import from a country with a lower livestock health status, and 4) the summarizing module representing the total burden of infection for the domestic population. The output of the model is a probability distribution of the updated herd prevalence prior to the following survey (pre-survey prevalence). This prevalence was used to derive the pre-SPF from infection in the national livestock population and, subsequently, to calculate the sample size for the consecutive survey. Uncertainty and variability were incorporated using probability distributions of parameters and Monte Carlo simulation (@Risk, Palisade Tools, Version 4.5.2.).

Data on bovine herpes virus 1

The eradication of clinical cases related to BoHV1 was accomplished in 1992. Currently, Switzerland is officially free from BoHV1. Since 1994, Switzerland has conducted annual serological surveys to substantiate claims of freedom from BoHV1. From 2002 onwards, quantitative and qualitative risk assessments, together with the information from the last year's survey, provided the basis for risk-based sample size calculations for consecutive surveys. Detailed data on Swiss BoHV1surveys are shown in Table 2.

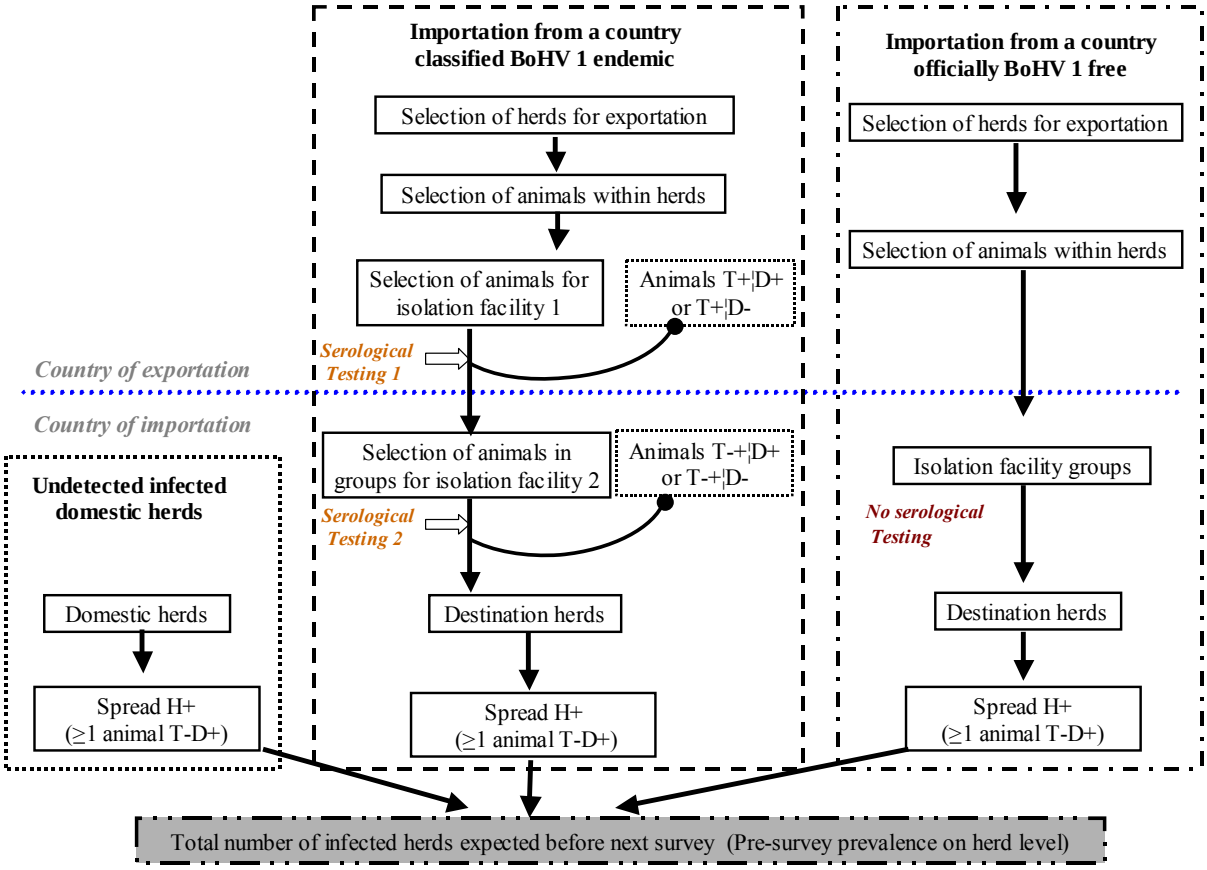

Fig. 1 Basic structure of the stochastic simulation model to estimate the pre-survey probability of freedom from infection as applied to BoHV1. Three main pathways of introduction of the infectious agent into the susceptible domestic population. The import components of the model reflect the regulations for breeding-cattle import according to 2004/558/EC♣.

Undetected domestic infection

An undetected residual infection and potential spread in the domestic population was assumed. The range for the residual herd prevalence (*HP*) was sampled from a beta distribution. The distribution was defined by the previous survey size (*N*) and detected positive herds in previous survey (*s*), beta (*s*+1; *N*–*s*+1).

The potential between-survey spread was interpreted as a multiplicative transmission factor which would approximately predict annual changes in *HP* due to disease spread between herds. This between-survey spread factor (BSSF) was calculated for annual periods between two consecutive surveys from the means (μ) of *HP* beta distributions:

$$\text{BSSF}_{\text{year n}} = \mu \text{ beta } (HP_{\text{year n+1}}) / \mu \text{ beta } (HP_{\text{year n}}) \quad (1)$$

All annual BSSF values were calculated from data of national BoHV1 surveys from 1994-2005 and provided minimum, maximum and most likely estimates (median) for a beta pert distribution. A worst case scenario was assumed, no decrease in *HP* was considered (BSSF ≥1).

♣ Commission Decision of 15 July 2004 implementing Council Directive 64/432/EEC as regards additional guarantees for intra-Community trade in bovine animals relating to infectious bovine rhinotracheitis and the approval of the eradication programmes presented by certain Member States

Infection via direct import

Only animals from countries with comparable sanitary conditions as in Switzerland were admitted for direct import (Fig. 1). This import pathway included no serological testing, therefore the probability of importing infected animals was p(D+). Data from previous surveys in the country of origin were used to estimate the expected residual *HP* as described above. Otherwise standard requirements of EU regulations were used as a basis to define the *HP* beta distribution ($N \geq 2300$, $s = 0$).

Based on the prevalence of infection in imported animals (*prev*) and the number of animals imported (*n*), the probabilities of selecting 1 or 2 or 3…to *x* infected animals (*m*) for import were calculated. The total number of imported infected animals (m_{total}) from a specific country or the whole import pathway was obtained by Eq. 2:

$$\mathrm{m}\,total = \sum_{m \geq 0}^{n} \binom{n}{m} prev^{m} * (1 - prev)^{n-m} * m \tag{2}$$

where *prev* = sampled value for *HP* * sampled value for *whp*
whp = within herd prevalence

Cattle are imported throughout the whole year. The disease transmission potential of infected import animals needed therefore to be adjusted ($BSSF_{adj}$).The remaining time within the domestic population until the next survey was considered by a time weighting factor ($tw_{x)}$ on a monthly basis. Origin and average numbers and of monthly imported animals over five years were used to determine the monthly import probability *p(imp)*:

$$BSSF_{adj} = BSSF * tw_x * p(imp)_{t=x} \tag{3}$$

The expected total number of infected animals of Eq. 2 was then multiplied with each monthly $BSSF_{adj}$ to consider the spread until the next survey accurately. The sum of all monthly estimates provided the total number of infected animals through direct import. It was assumed that each infected animal would result in one infected domestic herd (worst case scenario).

Infection via indirect import

This import pathway was applied for cattle originating from countries or regions having a lower sanitary status, thus considered not officially free from BoHV1 (Fig. 1). Animals of the indirect pathway were supposed to be kept a certain time in isolation facilities in groups and to undergo two serological testing rounds (Fig. 1). In consequence the probability that at least one animal was infected in the group at the end was expressed as p(> 1D+¦T--).

Only animals that tested negative in the first testing round were admissible for importation. The probability to introduce a certain number *x* of infected animals (*m*) into each isolation facility of group size (*g*) was given by the probability of selecting animals having a false negative test result p(D+¦T-) in the exporting country. Therefore test sensitivity (Se_1) was included additionally:

$$p(m = x) = \binom{g}{m} \left\{ (prev * (1 - Se_1))^{m} * \left(1 - (prev * (1 - Se_1))\right) \right\}^{g-m} \tag{4}$$

In view of the required minimum isolation period, the model accounted for the risk of transmission of an undetected infection within the isolation group and detection by the second serological test before the group was admitted to the destination farms' cattle population. The number of potentially infected animals within the isolation group at the end (m_{end}) of the isolation facility period were obtained by inclusion of the transmission probability p(trans) and the number of infected animals within the group at start of the isolation (m_{start}). The probability that all animals of the isolation facility group were still test negative at the end of the period was further dependant on the test sensitivity (Se_2) and specificity (Sp_2) of the second testing round:

$$p(allD- \mid T--) = Sp_2^{\,g-m_{end}} * \left\{ \left(\frac{m_{end}}{g} * (1 - Se_2) \right)^{m_{end}} \right\} \quad (5)$$

In a final step, the resulting number of infected groups was summed up and then multiplied by $BSSF_{adj}$, as described in the module "direct import". In the indirect import pathway, one infected group was supposed to be delivered to one farm and infect one domestic herd.

Pre-survey prevalence and pre-survey probability of freedom

In each of the 5000 iterations of the model, the expected number of infected herds of every module were summed up to a final number of infected herds before the next survey. The resulting pre-survey prevalence in the domestic population was expressed as a probability distribution. With regard to the internationally required *HP*-threshold level and the defined confidence, the pre-SPF from disease was derived directly from the *HP*'s cumulative probability distribution as the proportion of the sampled values that lied beyond the threshold. Starting from the overall level of confidence required (γ) and pre-SPF estimate, the required confidence level *x* (1 - type-I error) for the consecutive survey can be calculated by Eq.6 of (Hadorn et al., 2002):

$$x = \frac{(\gamma - preSPF)}{(1 - preSPF)} \quad (6)$$

RESULTS

The baseline model and sensitivity analysis

The rank correlation of input parameters showed that the pre-survey prevalence and hence the pre-SPF was most correlated with the remaining base line *HP* within Switzerland (r=0.78) and less strongly with values of BSSF (r=0.27). Infection through direct import was most correlated with the *HP* in the country of origin (r=0.79) and to a similar extent number of imported animals and the *whp* (r=0.40 and 0.37, respectively). Estimates of infection caused by indirect import was only slightly dependant on the number of imported animals (r=0.27). The impact of BSSF-value on both, direct and indirect import module outcomes, was moderate (r<0.1). The residual domestic *HP* accounted for 98.08% of the pre-survey prevalence estimate in average, whereas the mean contribution of the module direct import was 1.65% and 0.26% of the module indirect import.

Figure 2 presents the shift of the predicted model pre-survey prevalence distribution in relation to an increasing residual *HP*. Step-wise increments of the residual *HP* distribution were

generated by increasing numbers of positive samples (s = 0-5) in combination with a locked sample size (N=2400).

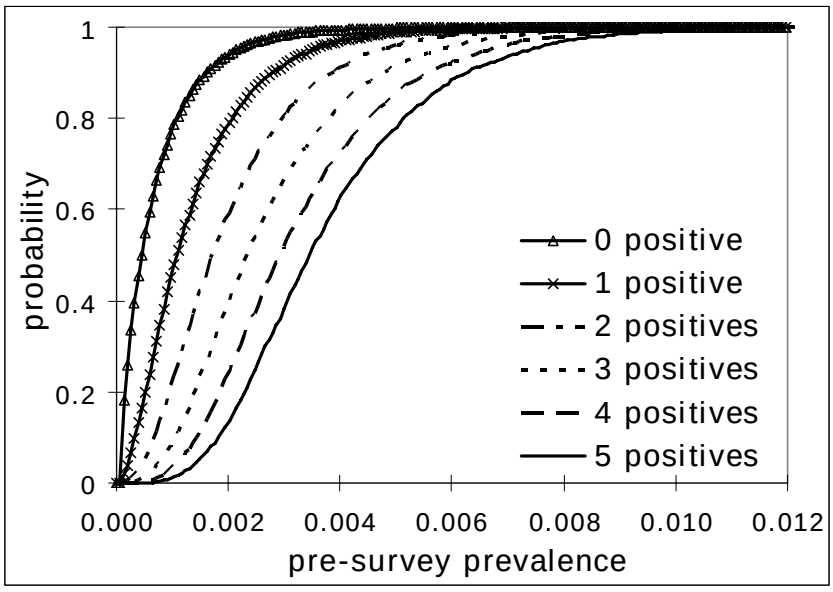

Fig. 2 Effect of increasing residual herd prevalence on the pre-survey prevalence estimate prior to the consecutive survey. Pre-survey herd prevalence was estimated using cumulative herd prevalence beta-distributions defined by a fixed sample size and increasing positive samples

Calculated BSSF values ranged form 0.53 to 4.76 (median 1.03). The cumulative pre-survey prevalence and the corresponding pre-SPF had an almost linear relationship with BSSF. Each one-unit-increase in BSSF reduced the baseline pre-SPF by 10%.

The model predicted a mean of 0.16 newly infected domestic herds per 1000 directly imported animals. In the case of indirect import, the restrictive import requirements limited the introduction of infected groups such that 1000 indirectly imported animals resulted in a mean of 0.026 infected herds. If the same scenarios were applied for indirect and direct import pathways, namely that each infected animal (instead of an infected group) lead to an additional infected domestic herd, the estimate of the module direct import increased by a factor of 2.8.

The module "direct import" was applied with an increasing *whp* using values from 0.1 to 1.0 in steps of 0.1. A *whp* of 1.0 would maximally implicate 1.94 additional infected herds (mean 0.22) assuming n=700 directly imported animals. The application of sampled *whp* values, ranging from 0.1 to 1.0 returned a maximum of 1.38 additional infected herds.

Application on Swiss BoHV1 survey data

The inputs and comparative results for the deterministic and its corresponding stochastic model output are listed in Table 2. Most of the loss in the pre-SPF of the deterministic model was explained by direct import of animals. In the new model, the estimation of the residual *HP* is including infection from both the domestic and the foreign cattle population. Under these conditions, the remaining domestic *HP* and the previous survey outcome, gained much more importance. In consequence, important pre-SPF differences for the years 2003 and 2006 were observed. These years were preceded by surveys that detected a positive domestic herd. In the opposite case, if the previous survey outcome was negative (confidence level of freedom from

infection achieved), the stochastic model predicted significantly higher pre-SPF compared to the estimate from the deterministic model.

Table 2 Detailed information on BoHV1 surveys conducted or planned from 2002-2007 in Swiss cattle and pre-survey probability of freedom estimates using deterministic and stochastic model approaches. Sample sizes of previous surveys in the stochastic model: (SSS) standard sample size calculation or (RBS) sample size calculation as described by Eq. 6

Input values	2002	2003	2004	2005	2006[a]	2007[a]
Population size (number of cattle herds)	50,591	48,231	48,790	46,574	46,574	46,300
Allowed threshold herd level prevalence	0.001	0.001	0.001	0.002[b]	0.002	0.002
Number of positive herds detected in previous survey	0	1	0	0	1	0
Sample size of previous survey RBS	2,300	2,150	2,820	1,188	1,385	1,395
Sample size of previous survey SSS	4,650	4,650	4,650	2,325	2,325	2,325
n direct imports from BoHV-1-free countries	[c]	[c]	[c]	155	976	552
n indirect imports from BoHV-1-infected countries	500[d]	8[e]	496	1145	388	658
n indirect imports, eradication of BoHV-1 accomplished but not officially free					345	680
Remaining pre-SPF from the last survey, deterministic model	0.8910	0.9094	0.8372	0.8947	0.8201	0.8618
Remaining pre-SPF from the last survey, stochastic model (RBS)	0.9328	0.5635	0.8022	0.9545	0.6413	0.8758
Remaining pre-SPF from the last survey, stochastic model (SSS)	0.9314	0.7732	0.9314	0.9841	0.7948	0.9365

[a] planned surveys that are not carried out yet
[b] adaptation of Swiss regulation to European animal disease surveillance regulation
[c] Swiss regulation on cattle import changed in July 2004, specific distinction between animal imports originating from EU countries officially free or not free from certain diseases
[d] Due e) qualitative risk-assessment only. The import number was adjusted to n=500 for the model input to obtain comparable results with the qualitative estimate
[e] Import stop due to foot and mouth disease outbreaks in Europe 2001

DISCUSSION

The flexible structure of the stochastic model allows to include various additional domestic or external risk factors, such as short term animal movements or infection via livestock commodities. Such factors could be incorporated directly as additional numbers of infected herds to the summary module or if necessary to be included as separate spreadsheet models. Numbers and causes of infected herds or animals are usually available from statutory case reporting or diagnostic laboratory records.

For BoHV1 data and surveys it could be demonstrated, how to account for various factors affecting the health status and disease dynamics of a national cattle population between two surveys to substantiate freedom from infection. The high influence of residual domestic *HP* on the pre-SPF was not surprising (see Fig. 2). First, the adoption of risk-based national surveys presumably shifted the mean value of the assumed residual *HP* beta-distribution to higher values

due to a smaller sample size. Observed *HP* from Swiss statutory case reporting suggested lower *HP* (range: 0 - $2.57*10^{-4}$). Secondly, model outputs with standard sample sizes indicated a significant increase in pre-SPF exceeding the estimates of the deterministic model. Thirdly the current national surveillance programmes might be less random than most countries admit. Countries might intuitively include subpopulations with an increased risk of infection. These facts were considered to be supporting evidence that the domestic *HP* in the model is overestimating the true situation. However, data are currently lacking to adjust for the biases mentioned above.

With the introduction of a novel spread factor explicit transmission modelling in terms of a SIR model was avoided. Further, BSSF was adjusted for seasonality of import and categories of export countries. It reflected the complex dynamics of cattle populations and the trading patterns between two surveys. The BSSF validation suggested realistic values if compared to reports from other BoHV1-free countries or regions. Reporting from Denmark, Bolzano and Austria indicated that spread of infection between herds was very limited, usually 0-1 secondary cases (Salman et al., 2003).

Compared to the previous deterministic model, where a preliminary fix margin for the potential spread between surveys was included, the calculated pre-SPF of the stochastic model reflected more realistic disease dynamics. The model highlighted the need not to underestimate the potential of the residual infections within the domestic population of a country or region officially free from the disease. Especially when a country has to deal with rapidly spreading infections that are characterized by latent phases or that might show only mild to in-apparent clinical signs.

In conclusion, the new model is a suitable tool to determine risk-based sample sizes for repeated surveys to substantiate claims of freedom on contagious diseases. With this model, a generic tool becomes available as it can be adapted to the changing conditions either in the importing and exporting countries or the disease dynamics. It is therefore applicable to the risk-based survey design of a wide range of infectious diseases.

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APPENDIX

Appendix 1: Input values or distributions of epidemiological parameters used to model the import risk and domestic spread of BHV-1 in Switzerland between annual serological surveys.

Parameter	Values or distribution as used in @RISK simulation	Reference
Population size	See Table 2	Swiss animal movement database
Sample size last survey	See Table 2	(Reist and Sievi, 2004)
Number of herds tested positive	See Table 2	(Reist and Sievi, 2004)
Threshold herd prevalence	0.1-0.2% [a]	64/432/ECC
Between survey spread factor (BSSF)	=RiskPert(1;1.01;4.75)	derived as explained in the text
Number and seasonality of animals imports	records per month and country	Swiss Fed. Custom Statistics
Herd prevalence countries not BoHV1 free	=RiskUniform(0.1;0.3)	(Boelaert et al., 2005; Teuffert et al., 2003)
Herd prevalence countries BoHV1 free	=RiskBeta(1;2301)	2001/292/EG, 2003/467/EG
Within herd prevalence countries not free	=RiskUniform(0.1;0.5) [b]	(Hage et al., 2003; Straub, 2001)
Within herd prevalence countries free	=RiskUniform(0.1;0.9) [b]	(Kofer et al., 1999; Nylin et al., 1998; Salman et al., 2003)
Test sensitivity 1	=RiskUniform(0.97;0.99)	(Boelaert et al., 2005; Isa et al., 2003)
Test specificity 1	=RiskUniform(0.95;1)	
Isolation facility group size	= 7	(Brülisauer et al., 2003)
Transmission probability	=RiskUniform(0.25;0.35)	(Knopf et al., 2004)
Test sensitivity 2	=RiskUniform(0.2;0.9175)	(Knopf et al., 2004)
Test specificity 2	= 1 [c]	

[a] changes in regulation see Table 2
[b] dependant on vaccination policy and coverage or eradication programme strategies
[c] any positive screening test result would be subject to confirmatory testing

EPIDEMIOLOGICAL TOOLS 2

TEST VALIDATION OF A COMMERCIAL ELISA TO DETECT PARATUBERCULOSIS IN DAIRY HERDS OF SOUTHERN CHILE

G. VAN SCHAIK*, F. HARO, A. MELLA AND J. KRUZE

SUMMARY

In Chile, *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) was isolated on several occasions and clinical cases were reported. Nevertheless, diagnostic tests were not yet validated. The objective of the study was to validate a commercial ELISA to detect *Map* shedding dairy cows in management conditions, prevalence and infection states existing in Southern Chile with different statistical approaches.

Blood and faeces were collected from 1,333 lactating cows in 27 dairy herds (both large commercial and smallholder dairy farms) between September 2003 and August 2004. Within the herds up to a maximum of 100 dairy cows were selected based on age (≥3 years old) and, if present, clinical signs. In herds with less than 100 cows, all cows ≥3 years old were sampled. Blood samples were tested using a commercial ELISA kit (IDEXX Laboratories, Inc.). Faecal samples were cultured on Herrold's Egg Yolk Medium (HEYM). Logistic regression as well as latent class models (i.e. maximum likelihood (ML)) methods and Bayesian inference) were used to determine the optimal cut-off value and the validity of the ELISA.

Map was cultured from 54 (4.1%) cows and 10 (37.0%) herds, which were all large, commercial dairy herds. The optimal ELISA cut-off values obtained with the logistic regression method were only influenced by prevalence. The higher the prevalence in a herd the more cows are shedding at lower ELISA S/P values. Thus, it is important to have information about the prevalence in a herd in order to make a correct interpretation of the ELISA results. The ML model did not converge as a result of empty cells in the cross tabulations. In the Bayesian model the Se and Sp of the ELISA were 26% (95% CI 18-35%) and 98.5% (95%CI 97.4-99.4%), respectively. For faecal culture the Se was 54% (95% CI 46-62%) and the Sp was 100% (95% CI 99.9-100%) Interestingly, the prevalence in the smallholder dairy farms was estimated to be 8% even though there were no faecal culture positive cows detected in those herds. There was no significant correlation between the two tests. The advantage of Bayesian inference is that the Se and Sp of both tests are obtained in one model relative to the (latent) true disease status, the model can handle small datasets and empty cells and the estimates can be corrected for the correlation between tests when the tests are not conditionally independent. Therefore, Bayesian analysis is the preferred method for *Map* that lacks a gold-standard and usually has low cow-level prevalence.

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INTRODUCTION

The Southern regions are the main milk-producing area in Chile, with 66% of Chilean milk delivered to milk processing plants and 80% of dairy herds. Approximately 84% of the 11,000 dairy herds in Southern Chile are considered ‘‘smallholder’’ dairy farms. These are subsistence farmers that produce <100,000 kg of milk per year. Cattle graze outside all-year round and are fed little or no concentrates. Milk usually is collected by hand and transported to a local co-operative milk collection centre where it is added to milk of other farms and cooled in a large refrigerated tank. The medium to large commercial dairy herds in Southern Chile often have more than 200 dairy cows that are grazed all-year round but are kept inside during the nights in winter. On these medium-to-large dairy farms, progress is rapid and husbandry mimics that adopted by the USA and Europe.

Little was known about the status of herds for *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) in Chile. However, clinical cases were reported and *Map* was isolated on several occasions (Kruze et al., 2000; Kruze et al., 2001). Nevertheless, diagnostic tests were not yet validated in Chile. For successful control programs for *Map* it is essential to have reliable diagnostics and appropriate management measures in place (Benedictus *et al*., 2000). The ELISA test is the most widely used test, because it is simple, fast to do and cheap. Culture of faecal samples is commonly used as the reference test for *Map* because of the high specificity (~100%) and reasonable sensitivity (~50%) (Whitlock et al., 2000). Several validation studies have been carried out with the IDEXX ELISA on panels with a known infection status (Dargatz et al., 2001; Collins et al., 2005). The drawback of the validation on specific panels is that these may not be representative of the population in which the test is going to be used (Greiner and Gardner, 2000). In a field validation, the performance of an assay should be monitored for validity in the target population and heterogeneity of the validity amongst subpopulations (Jacobson, 1998).

The sensitivity (Se) and specificity (Sp) of a test can be obtained with several statistical methods. When a perfect reference test (gold-standard) is available the Se and Sp of the test can be estimated directly. However, there is no gold-standard available for paratuberculosis. Hui and Walter (1980) developed a maximum likelihood (ML) method to estimate the Se and Sp in which two tests were simultaneously applied to individuals in two populations with different prevalences of disease. Pouillot et al. (2002) developed an online programme (TAGS) that can be used to solve these ML models. Use of Bayesian modelling to assess the accuracy of screening tests has increased recently in veterinary medicine (Gardner, 2002). Diagnostic-test evaluation is particularly suited to the Bayesian framework because prior scientific information about the sensitivities and specificities of the tests and prior information about the prevalences of the sampled populations can be incorporated.

The objective of the study was to validate a commercial ELISA to detect *Map* shedding dairy cows in management conditions, prevalence and infection states existing in Southern Chile with different statistical approaches.

MATERIALS AND METHODS

Farms

In Chile, no official records existed of the herd-status for *Map*. Herds were therefore selected based on the presence or absence of clinical evidence for the disease and, if available, previous test results. In addition, herds were selected that were spread over the region and representative for the management systems in Southern Chile (i.e. both large commercial and smallholder dairy farms). Within the herds up to a maximum of 100 dairy cows were selected based on age (≥3 years old) and, if present, clinical signs. In herds with less than 100 cows, all cows ≥3 years old were sampled. A brief questionnaire was administered to obtain information about the herd management relevant for introduction and transmission of *Map*.

Tests

Blood and faeces were collected from 1,333 lactating cows in 27 dairy herds between September 2003 and August 2004. Blood samples were collected in 10 ml vacutainer tubes and the sera frozen at -20°C until tested using a commercial ELISA kit (IDEXX Laboratories, Inc.). Duplicate samples were assayed following manufacturer recommendations and the S/P ratio for each sample calculated using an automated ELISA reader (IDEXX). Rectal faecal samples were collected using individual polyethylene sleeves, transported to the lab, and cultured within 24h on Herrold's Egg Yolk Medium (HEYM) and mycobactin J (3 tubes) and HEYM without mycobactin J (1 tube). Prior to culture, 2g of each fecal sample was suspended in 35 ml HPC solution and incubated at 37°C overnight. It was then centrifuged and the pellet re-suspended in an antibiotic solution containing nalidixic acid, vancomycin, and amphotericin B, and again incubated at 37°C overnight. A 0.15 ml aliquot of each suspension was used to inoculate all HEYM tubes which were incubated at 37°C for 16 weeks. Colonies resembling *Map* and showing mycobactin-dependence were tested by IS*900* PCR.

Analyses

Logistic regression as well as latent class models (i.e. maximum likelihood methods and Bayesian inference) were used to determine the optimal cut-off value and the validity of the ELISA. The logistic regression model regressed the ELISA S/P value, herd size, age of the cow and the prevalence of shedders on the faecal culture result of a cow. The optimal cut-off values were based on the probability to be faecal culture positive and a cow with a probability over 50% was considered infected (Van Schaik et al., 2005).

In the latent class methods, two populations that were expected to differ in prevalence were distinguished: medium-to-large commercial herds and smallholder dairy herds. The hypothesis was that the large commercial herds would have a higher prevalence than the smallholder herds. In the latent class models the ELISA results were interpreted with the cut-off value recommended by the manufacturer.

In the ML approach the tests are assumed conditionally independent and accuracy of each test is the same in both populations. For each population test results are cross-classified in 2×2 tables and each table thus provides three degrees of freedom (d.f.) for estimation. Formulas for the ML estimation are available in the "TAGS" programme by Pouillot et al. (2002).

For the Bayesian analysis the freeware program WinBUGS was used (Spiegelhalter et al., 1996). In general, the Bayesian approach to inference about, for example, the Se of a test

combines prior information about the Se (through the prior Pr(Se)) with the data (through the likelihood Pr(data |Se)) to obtain the posterior distribution of the Se, Pr(Se|data). Then, one can use the mean or median of this posterior distribution as an estimate of the Se. A model that assumes conditional independence as well as a model that assumes conditional dependence were used to obtain estimates for the prevalence in both populations and the Se and Sp of both the ELISA and faecal culture. More details about the models used are described elsewhere (Branscum et al., 2005).

The prior distributions that were used in the analyses are shown in Fig. A of the Appendix. In Table 1 the parameters of the prior distributions and the source are provided. The correlation between the ELISA and faecal culture was assumed to be zero (Nielsen et al., 2002), which was modelled by assuming identical priors for the test Se and Sp given the outcome of the first test (T_1) (i.e. for the correlation between the sensitivities $Pr(T_2+|T_1+, D+)=Pr(T_2+|T_1-, D+)$). In the sensitivity analysis, the priors for the prevalence were changed to uniform distributions between 0 and 1.

Table 1. The parameters and the source of the prior distributions for the Bayesian analysis of test accuracy and prevalence in the south of Chile.

Parameter	Distribution	Source
Prevalence in small herds	Beta(1.4;74)	Communications with the farmers indicated that the *Map* prevalence was very low because they had never seen clinical cases in their herd nor had their veterinarians.
Prevalence in large herds	Beta(10;100)	Communications with the farmers indicated that the *Map* prevalence was fairly high based on a regular occurrence of clinical cows and/or previous test results
Sensitivity ELISA	Beta(2.3;2.3)	Dargatz et al., 2001 ; Collins et al., 2005
Sensitivity Faecal Culture	Beta(51;51)	Whitlock et al., 2000
Specificity ELISA	Beta(81.5;3.7)	Dargatz et al., 2001 ; Collins et al., 2005
Specificity Faecal Culture	Beta(10,000;1)	Whitlock et al., 2000

Convergence of the model was investigated by observing the autocorrelation, the quantiles of the estimates for stability across iterations, and by running parallel chains with different initial values and investigating the Brooks-Gelman-Rubin convergence statistic (Spiegelhalter et al., 1996).

RESULTS

Descriptive results

Map was cultured from 54 (4.1%) cows and 10 (37.0%) herds, which were all large, commercial dairy herds. In smallholder farms *Map* was not isolated, even though there was a trend that smallholders purchased cattle more often than large herds (P=0.1; Table 2). Smallholders had on average 28 cows (SD 18) that produced about 3,777 kg milk per lactation (SD 885), while the large, commercial farms had an average herd size of 307 cows (SD 252)

that produced 6,658 kg per lactation (SD 2,166). Smallholders more often had a dual purpose breed (53% of the herds) than the large herds of which 90% had Holstein(-cross) cows. Calves were fed colostrum solely from their dams in 71% of the smallholder herds and 10% of the large herds. Other potential risk factors for introduction or spread of *Map* were not significant.

Table 2. Prevalence of management factors related to introduction and spread of *Mycobacterium avium* subsp. *paratuberculosis* in 17 smallholder and 10 large dairy herds in the south of Chile.

Management factors	Smallholder dairy herds (n= 17)	Commercial dairy herds (n= 10)	
Dual purpose breed (%)	53	10	*
Purchase of cattle in the previous 12 months (%)	35	0	
Purchase of cattle in the previous 5 years (%)	82	50	
Calves get only colostrum from their dam (%)	71	10	**
Calves are fed milk replacer (%)	35	60	
The sick pen is also used for calving (%)	59	50	
There is direct contact between adult cows and heifers (%)	29	40	
Adult cows and heifers are housed together (%)	12	10	
Heifers are grazed on pastures that are contaminated with faeces from adult cows (%)	41	20	
Heifers have access to water contaminated with faeces from adult cows (%)	47	20	

* P<0.05
** P<0.01

Cut-off value

At the cut-off value recommended by the manufacturer, ELISA Se and Sp relative to faecal culture were 38.9% (95% CI: 25.9% - 51.9%) and 94.9% (95%CI: 93.7% - 96.1%), respectively. The Se varied from 24.3% (95% CI: 10.5 – 38.2%) for the low shedders (<10 colony forming units (CFU)/g), 58.3% (95% CI: 30.4 – 86.2%) for the moderate shedders (10-300 CFU/g) to 100% in the heavy shedders (>300 CFU/g). Age, herd size, test date and farm were not related to the probability of a positive faecal culture result. Only the ELISA SP value and the prevalence were positively associated with the probability of a positive faecal culture result (Table 3).

The three lines in Fig. 1 show the probability that at a certain ELISA value a cow is culture positive in, from left to right, high, medium, and low prevalence herds. The higher the prevalence in a herd the more cows are shedding at lower ELISA SP values. For example, at ELISA S/P value 0.9 about 68%, ~30% and ~5% of the cows are faecal culture positive in high, moderate and low prevalence herds, respectively.

Table 3. Results of the logistic regression for faecal culture results and ELISA result (S/P value) and three levels of prevalence in 27 dairy herds in Southern Chile (n=1,333 cows)

Variables	Estimate	SE	OR	*P*
Intercept	- 6.00	0.56		<.01
S/P-value (ELISA)	3.25	0.46	25.8	<.01
Low prevalence (≤2%)	0.00	0.00	1.0	-
Moderate prevalence (2-10%)	1.94	0.69	7.0	<.01
High prevalence (≥10%)	3.75	0.57	42.5	<.01

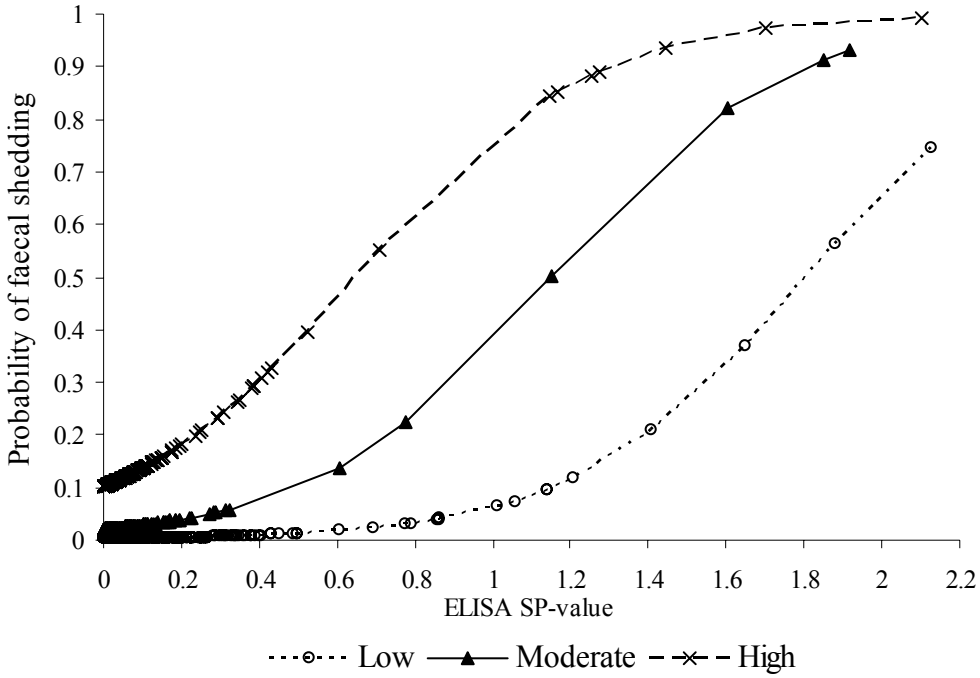

Fig. 1 Probability of having a positive faecal culture conditional on the ELISA value (S/P) in high (≥10%), moderate (2-10%) and low (≤2%) prevalence herds.

Test accuracy

In Table 4, the cross-tabulation for the results of the two tests for the two populations (smallholders and large herds) is shown. When the ML model was used, the estimations of Se and Sp obtained for the ELISA test were the same as those calculated from the two-by-two tables relative to faecal culture because the model considered faecal culture as the gold-standard (SE=SP=100%) as a result of empty cells in the cross tabulations (results not shown).

Table 4. Cross-tabulation of the ELISA and faecal culture results in smallholder dairy farms and large dairy farms in the south of Chile.

Smallholders	FC+	FC-	N
ELISA+	0	30	30
ELISA-	0	432	432
Total	0	462	462

Large herds	FC+	FC-	N
ELISA+	21	35	56
ELISA-	33	782	815
	54	817	871

In the Bayesian model the Se and Sp of the ELISA and faecal culture were fairly different from the other methods (Table 5; Appendix Fig. B). The estimates for faecal culture were as expected, although the SE was fairly high (54%). The Se of the ELISA was a lot lower than expected at 26% and the Sp slightly higher at 98.5%. Interestingly, the prevalence in the smallholder dairy farms was estimated to be 8% even though there were no faecal culture positive cows detected in those herds. There was no significant correlation between the two tests as indicated by a correlation for the Se of -0.1 (95%CI -0.4-0.2) and for the Sp of 0.003 (95%CI -0.0003-0.01). Correcting for the correlation did not significantly influence the parameter estimates (results not shown).

The sensitivity analysis resulted in slightly higher estimates for the prevalence in the smallholder herds (10% (95%CI 7-15%)) and lower estimates for the Se of the ELISA (25% (17-33%)) and faecal culture (50% (95%CI 41-59%)). The convergence of the Bayesian models was investigated and except from autocorrelation in the specificity estimates as a result of the narrow distributions there were no indications that the model did not convergence.

Table 5. Bayesian inferences about sensitivity, specificity and prevalence for an ELISA and faecal culture to detect *Mycobacterium avium* subsp. *paratuberculosis* in two populations of dairy herds in the south of Chile.

Parameters	Mean	SD	2.5% quantile	Median	97.5% quantile
Prevalence in small herds	8.29	1.49	5.63	8.20	11.43
Prevalence in large herds	12.18	1.62	9.21	12.12	15.53
Sensitivity ELISA	25.94	4.46	17.74	25.80	35.03
Sensitivity Faecal Culture	53.91	4.26	45.62	53.90	62.28
Specificity ELISA	98.48	0.52	97.38	98.51	99.42
Specificity Faecal Culture	99.99	0.10	99.96	99.99	100.00

DISCUSSION

The optimal ELISA cut-off values obtained with the logistic regression method were only influenced by prevalence. The higher the prevalence in a herd the more cows are shedding at lower ELISA S/P values. Thus, it is important to have information about the prevalence in a herd in order to make a correct interpretation of the ELISA results.

The advantage of latent class models relative to frequentist methods is that the Se and Sp of both tests are obtained in one model relative to the (latent) true disease status. Thus, the latent class methods are specifically useful for a disease like paratuberculosis for which no perfect reference test is available. The ML model could not be fitted to the data because there were empty cells in the two-by-two tables and the Sp estimate for the reference test was close to 1. In a Bayesian model, unlike in a ML model, sparse data and empty cells can be handled. Furthermore, the estimates can be corrected for the correlation between tests when the tests are not conditionally independent. Therefore, a Bayesian analysis was the preferred method for this study.

Fairly low estimates were obtained for the Se and Sp of the ELISA, which was in accordance with other studies (Dargatz et al., 2001; Collins et al., 2005). The estimates for the Se and Sp of faecal culture were also as expected (Whitlock et al., 2000). However, the prevalence in

smallholder dairy farms was considerably higher than expected. Even though the farmers said that they had never seen cows with clinical signs and all culture results were negative, the Bayesian model predicted a cow-level prevalence of 8% in the smallholder farms, which is not significantly different from that in the large, commercial herds. A prior was used for the prevalence that contained the prior assumption that the prevalence would be below 2% with 95% certainty. When, in the sensitivity analysis, the prior for the prevalence was changed to a uniform distribution between 0 and 1, the prevalence estimate for the smallholders increased slightly to 10%. The fairly small difference in the estimates for the prevalences in the two populations may bias estimates for the other parameters (Se and Sp). Toft et al. (2005) investigated the influence of the difference in prevalence between the populations on the validity of the estimates from the Bayesian model. They concluded that the larger the difference, the more precise the estimates (smaller standard errors). Greiner and Dekker (2005) discuss the low herd sensitivity in small herds with a low prevalence. Thus, another reason that no faecal shedders were found in smallholder farms may be the low herd sensitivity of faecal culture in herds with only one shedding cow. The lack of diseased animals in the populations (54 faecal culture positives) imposed a problem when estimating the Se of the tests. The estimated standard errors are based on large-sample theory, thus for small samples these estimates become questionable. Toft et al. (2005) also showed that the lack of constant sensitivity of a test between populations introduced bias towards the estimate supported by the population with the highest disease prevalence (in this case, the large herds), which may explain the increased prevalence in the smallholder herds. However, apart from the prevalence in smallholder dairy farms, the estimates for the test accuracy were in comparison with other validation studies (Whitlock et al., 2000; Dargatz et al., 2001; Collins et al., 2005).

The reason for the difference in the Se of the tests between the populations may be that the cows in smallholder herds were infected and showed an antibody response but were less likely to become a faecal shedder because they were exposed to lower doses of the bacteria or because their resistance was better. The smallholder herds mainly consisted of dual-purpose cattle with a low milk production that may have better opportunities to resist an infection with paratuberculosis than the higher producing Holstein cows in the commercial herds. Jakobsen et al. (2000) also found differences between breeds, but they also concluded that the apparent breed effect may be confounded by the effect of herd. Many of the smallholder dairy farms did not take biosecurity measures for paratuberculosis. They regularly purchased cattle and they often kept the calves with the cows and did not feed milk replacer, which would favour a high prevalence of paratuberculosis. However, the smallholder herds did feed their calves colostrums from their mother, while in 90% of the larger herds the calves may have been more at risk because they were fed pooled colostrums. *Map* can only be successfully eradicated in an economic way by a combination of test-and-cull programs and proper hygienic measures at the herd-level (Groenendaal et al., 2002). Thus, to stop further spread of *Map* the Chilean farmers need to adopt good management practices with respect to calving, and calf and heifer rearing. The study was an important incentive for *Map* control in Chile. The validated tests can be used to test and cull infected cows and monitor the success of measures on herd and regional level to restrict spread of *Map*. More research is necessary to the management of the herds in Southern Chile with respect to *Map* and to the differences in prevalence in smallholder and large, commercial dairy herds.

ACKNOWLEDGEMENTS

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APPENDIX

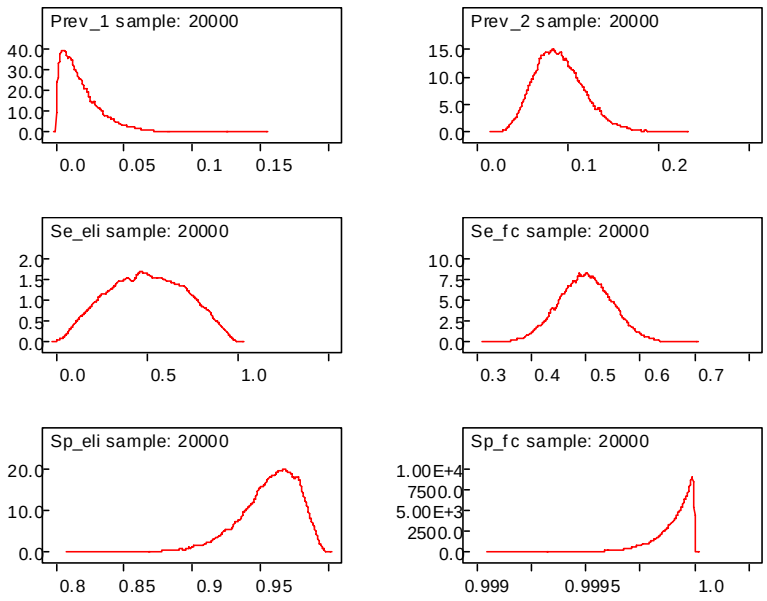

Fig. A The prior distributions for the sensitivity (Se), specificity (Sp) of the ELISA (eli) and faecal culture (fc), and the prevalence (Prev_1=prevalence in smallholders, Prev_2= prevalence in large herds) for the Bayesian analysis of dairy herds in Southern Chile.

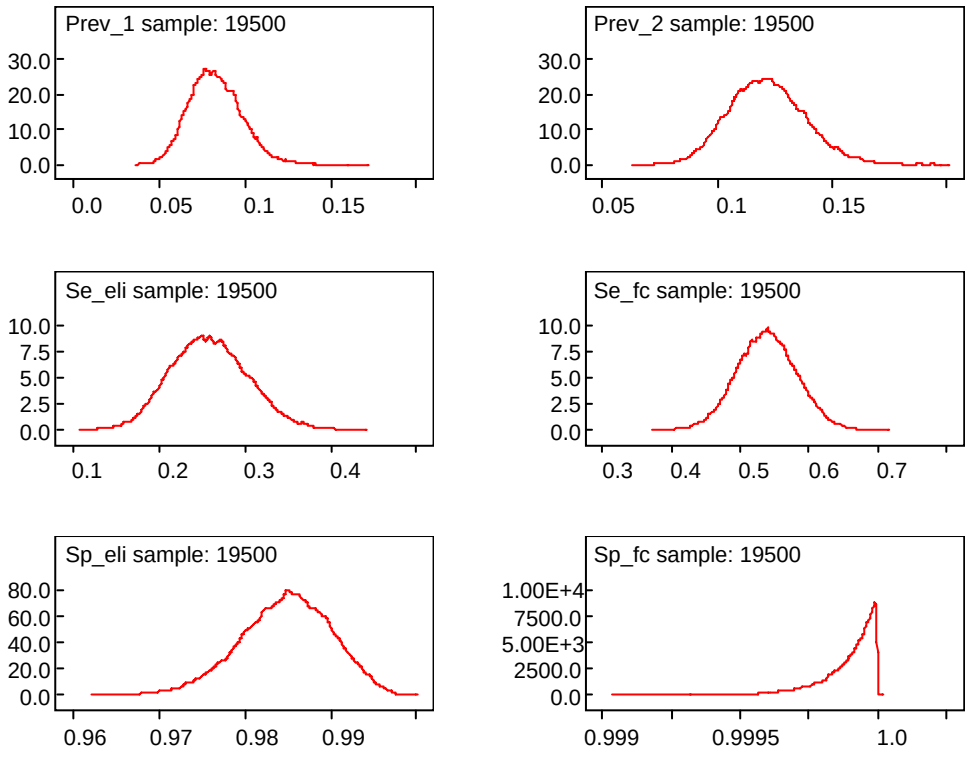

Fig. B The posterior distributions for the sensitivity (Se), specificity (Sp) of the ELISA (eli) and faecal culture (fc), and the prevalence (Prev_1=prevalence in smallholders, Prev_2= prevalence in large herds) from the Bayesian analysis of dairy herds in Southern Chile.

DATA QUALITY ASSESSMENT: COMPARISON OF RECORDED AND CONTEMPORARY DATA FOR FARM PREMISES AND STOCK NUMBERS IN CUMBRIA, 2001

N. HONHOLD* AND N.M. TAYLOR

SUMMARY

Epidemiological data analysis is increasingly carried out using large datasets obtained from centralised, often government, databases containing national or regional denominator data. This highlights the vital issue of data quality assurance; how accurately do the data reflect the real situation? To know this requires ground proofing, but this stage often has to be neglected for reasons of time and/or resources.

The foot and mouth epidemic of 2001 provided an opportunity to assess the quality of the data that were centrally available. In Cumbria, all farms were identified during the outbreak and enquiries made as to whether they had livestock. Where livestock were present, the species and how many of each were present was recorded. These field records have been compared to the data available from the Defra Disease Control System (DCS) database in 2001.

Field visits identified an extra 16% of premises compared to DCS. Although DCS correctly identified 89% of the farms that were found to have stock, almost 20% of farms in Cumbria were shown in DCS as not stocked when they in fact had stock, often substantial numbers of cattle or sheep.

Cattle and sheep numbers recorded in both DCS and the field records were assigned to herd and flock size classes. For cattle, there was agreement between field and DCS herd size classes in 60% of premises and disagreement of two or more herd class sizes in 13% of premises. Overall, there were almost 25% more cattle found in field visits than shown in the DCS data. For sheep, there was agreement in flock size class in around 50% of premises and disagreement by two or more flock size classes in 25% of premises. The total numbers of sheep recorded in DCS and found in the field were similar, however sheep were found on 50% more premises than indicated in DCS and 20% of premises had more than 50 sheep although DCS indicated that none were present. For dairy farms, 18% identified as such in DCS were found to be non-milk producing premises, mostly beef. There were 30% more dairy farms found to have sheep than was indicated in DCS, probably as a result of seasonal sheep movements.

This comparison of centrally held data and field records indicated a substantial disagreement between the two in terms of premises, types of stock present and numbers of stock present despite the DCS data being relatively recent. Conclusions drawn from the use of the central

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database would need to be treated with caution. This paper highlights that, even when data quality is assumed to be good (e.g. recent census data), testing of that assumption can reveal inadequacies in the data that could affect analyses made from it. There is clearly a requirement for ground proofing of all data even in situations where they are regarded as being sufficiently reliable for administrative purposes.

INTRODUCTION

It is increasingly common in veterinary epidemiology that research is being undertaken using large datasets obtained from centralised, often government, databases containing national or regional denominator data. This means that the epidemiologist is at least one step removed from the data from which conclusions are being drawn. The outputs from such work are regarded as scientifically valid and may even be used to inform policy formulation. But, as with any analytical work, the results can be no more reliable than the data used to undertake the analysis. The famous aphorism 'garbage in, garbage out' is an extreme example of this problem. This highlights the vital issue of data quality assurance; how far can or should this type of data be trusted? There is a need to be able to assess its accuracy.

The solution to this is to always ground prove the data. This involves checking a representative sample of the stored data against the actual situation in the field at the time of the analysis. However, in veterinary epidemiology, this seems to be rarely carried out, probably because of limitations of resources (human, financial and temporal); adding this additional phase to a study may make it harder to obtain funding and will certainly make it impossible to provide a rapid answer. There are therefore strong disincentives to undertaking this work.

The relative importance of this will depend on how accurately the data reflect the reality in the field at the time the work is being used for, which in turn depends on a combination of the initial quality of the data and the time that has elapsed since it was collected.

Great Britain (GB) is acknowledged to have generally accurate data on livestock holdings and population, amongst the best in the world. All keepers of ruminants and pigs must register that they keep these livestock with the Department for the Environment, Food and Rural Affairs (Defra) which issues a unique premises identifier and record both this and the address in the VetNet database. The GB government collects data on livestock numbers annually for the Agricultural Census. This covers the whole country and all species and is collected by the Office for National Statistics.

At the start of the FMD epidemic in GB in 2001, these two sets of data were amalgamated into the Disease Control System (DCS) database. Extensive use was made of these data at the start of and during the outbreak, for disease control purposes, mathematical modelling and research. During the epidemic, additional premises were added to the DCS system either because they were an epidemiologically separate part of an already known County-Parish-Holding (CPH) number or because they were unknown premises. These premises can be clearly identified in the DCS system.

In addition, during the FMD outbreak in 2001, field observations were recorded for all premises in Cumbria. These were most often during visits by veterinary staff, either as the result of a cull, or in the case on farms left with susceptible stock, during the final phase of demonstrating freedom from disease. In a minority of cases, the records were as the result of a telephone enquiry, but any premises with stock was visited. In all cases, stock numbers were

recorded. This provides a second and separate set of data which was held locally but not entered into the national central database and against which the latter can be compared retrospectively.

To summarise this complex and sometimes confusing picture, initial DCS data in 2001 consisted of the VetNet premises list of February 2001 combined with the agricultural census data from June 2000. It was at this point that a ban on animal movements was introduced. During the epidemic, extra premises were added to the premises list in DCS. Stock records were produced for all premises from visits by Defra staff. However, these data were not added to the DCS data in numerical form at the time, although they were sometimes added as comments in a text box.

MATERIALS AND METHODS

In GB, livestock owners must report to Defra that they keep livestock and the premises on which the stock are kept are then identified with a CPH code in the format nn/nnn/nnnn. The first two numbers indicate the county (e.g. 08 in the case of Cumbria) and the second part is a three number code that identifies the parish of that county within which the premises is located. The third part is the holding number, in many cases a serial number starting from n001. There are several 'series' of holding codes starting with different numbers. During the outbreak, two new series of Holding numbers were created to allow the registration of premises that were found to not be present in the VetNet records that were used to create the initial DCS database. Table 1 outlines the different H code series that were in use during the outbreak.

Table 1.Use of the holding number series in Cumbria to indicate premises type

Holding Series	**Used for**
0nnn	Permanent holding number for private premises with any livestock.
5nnn	Temporary holding number series used before the outbreak. Issued when new ownership of the holding is notified by the farmer and used until permanent number is issued.
60nn	Emergency numbers issued during FMD outbreak. May be blocks of land away from the main premises (sometimes referred to as 'land at' or 'field[s] at') but also contains many additional farms (i.e. with farm buildings) and a substantial number of single main premises.
65nn	
7nnn	Used for landless livestock keepers who rented land or buildings on a short term or informal basis.
8nnn	Used for shows and markets. Also for some local authority premises.
9nnn	A temporary holding code, replaced by the 5nnn series several years before 2001. These should have been replaced well before the outbreak but some had become permanent.

The key point is that any holding with a holding code commencing with 6 was one which had been identified and added to the database during the outbreak.

A download was made from DCS on 22-Oct-01 for all recorded holdings in Cumbria which contained all the original premises records and agricultural census numbers and also the premises added during the outbreak. This provided the overall record of numbers of CPH codes, both at the start and end of the outbreak. The last cull took place in Cumbria on 15 October 2001 (a 'slaughter on suspicion' (SOS)) and the majority of contacts made with farms until that point had been for direct control purposes i.e. related to the presence of infection, as opposed to post epidemic sero-surveillance, much of which took place after this date or post-epidemic livestock movement licensing.

There are essentially two sources of information as to whether a farm had stock or not and the types and numbers.

a) The Agricultural Census data incorporated into DCS. These figures are recorded in June of the year of the census i.e. the figures used in February 2001 were from the census of June 2000. As noted above, the download made from DCS on 22-Oct-01 has been used as the starting point for premises. The census data included a separate field for dairy cattle and this was the record used to classify whether a premises was recorded as a dairy farm in DCS. For each farm, there was therefore a record of dairy cattle, other cattle and small ruminants (the recorded numbers of sheep and goats have been added together).

b) Records of visits made by field staff to all premises in Cumbria during the outbreak. Farms which had culls (infected premises (IP), slaughter on suspicion (SOS) and dangerous contact (DC)) were obviously visited and numbers killed recorded. All those in the protection (3km) and surveillance (10km) zones (also referred to as PZ and SZ) were visited. All of these were visited during the outbreak and stock numbers recorded. By the end of the outbreak, these covered most of the county. At the end of the epidemic, all premises outside the SZ were contacted in order to determine if they had sheep. Stock numbers were recorded for these premises also.

These records were amalgamated to produce a record for each CPH with a visit by field staff for whatever purpose. This list was then checked against the DCS download. This led to the identification of almost 1800 CPHs being recorded in DCS but with no field visit records. Specific enquiries were made of these using the DCS contact data. Most of these were redundant CPHs (i.e. livestock were no longer kept) or those which had been superseded (i.e. a new code had been issued for the same premises, often when a new owner took over the premises), but they were contacted to be sure a complete dataset existed. Where stock was present, these were recorded and added to the list of stocked premises.

From these field records, a list of stocked and non-stocked premises was produced. For the stocked premises, the following stock numbers were used to produce a farm population. Where an IP or SOS cull had taken place, it was assumed that all susceptible stock had been killed and the population was taken as the number recorded as having been killed, to which were added any sheep killed in the 3km cull (see below). Where a farm had been slaughtered as a DC, either through tracing or as a contiguous premises, it was again assumed that all stock had been slaughtered at this date and these numbers were used to give the farm population, again with any 3km cull sheep included. Where the only cull recorded on the farm was a 3km cull of sheep, it was assumed that all sheep had been removed and this was taken as the population of sheep on the farm. This was not always the case so sheep numbers will be underestimated in some cases. The cattle population on these farms was derived from the maximum number of cattle recorded in the field visit records. Where no cull had taken place on a premises, the cattle and sheep

populations were taken as the maximum numbers of cattle and sheep found during field visits. Note that pigs were not included in this work. This is because there are few pigs in Cumbria and none of the IPs in the county involved this species.

For the lists of premises in the DCS download of 22-Oct-01, agricultural census and field livestock numbers were combined in a single database to allow direct comparison. For all of the premises in the list, the CPH series and whether the farm had cattle or small ruminants in the agricultural census or in field records was analysed. This gives a general impression of the overall accuracy of the DCS data compared to field records.

For more detailed comparisons, only data from the epidemic in north Cumbria has been included. The Cumbrian epidemic can be divided into several parts on the basis of spatial and temporal separation. The early phase of the epidemic took place in the northern part Cumbria and was the most intense phase of the epidemic and contains 668 infected premises (IPs), around one third of the whole GB outbreak. This part of the epidemic lasted from 28 February to 21 June 2001. The IPs were clustered closely together and the 3km SZs created a confluent geographical area . Details of these sub-divisions are described in Taylor et al. (2004). Because detailed analysis has been undertaken by the authors for the outbreak in this area due to specific epidemiological factors and the lack of automatic contiguous culling, a more detailed comparison of stock present, species, type and numbers in DCS data and field records for all premises within 3km of an infected premises (IP) in this part of the epidemic was carried out. The details of this detailed comparison are reported here.

For all premises within 3km of a north Cumbrian IP, the presence or absence of cattle and small ruminants was noted in both the DCS data and field records. For those that had cattle, whether the cattle present were ‘dairy’ or ‘other’ was recorded (when both were present the farm was classed as a dairy farm). The field data had several possible markers of whether a farm was a dairy farm. These included milk tanker collection records, whether Defra dairy engineers carried out work on a milk machine and the records of dairy cows being culled. From these flags and for both the DCS data and field records, each premises was identified as one of 6 farm types: dairy, dairy and sheep, beef, beef and sheep, sheep only, no cattle or sheep

For each premises within 3km of these IPs, for both the DCS data and field records, the numbers of cattle and small ruminants recorded in the census data and in the field records were compared by forming herd/flock size groups. For cattle these were 0, 1-50, 51-100, 101-200, 201-300 and >300. For small ruminants these were 0, 1-50, 51-200, 201-500 and >500.

RESULTS

1) Comparison of number of premises in VetNet before epidemic with premises found to exist: all Cumbria

In the download of 22-Oct-01 there were a total of 9,956 premises in Cumbria. These were divided by H code as shown in table 2. Before the outbreak started there were around 8,500 holdings in the VetNet system which were transferred to DCS. This increased by 1,400 during the outbreak, about 16%. Therefore the DCS initially contained 86% of holdings in Cumbria. This will be a biased estimate as some of those added will have been ‘land at’ or ‘fields at’. However, there were around 400 of these types of premises, so the majority that had been added were more substantial holdings.

Table 2. Numbers and percentages of premises in Cumbria in the DCS system on 22-Oct-01 by holding series

Holding series	Number	%
0nnn	7,826	78.6%
5nnn	40	0.4%
60nn	396	4.0%
65nn	1,007	10.1%
7nnn	300	3.0%
8nnn	171	1.7%
9nnn	216	2.2%
Total	9,956	100.0%

2) Record of presence of livestock: Stocked vs. Non-stocked: all Cumbria

Table 3 shows the comparison of stocked and non-stocked premises in the DCS data and field records for Cumbria.

Table 3. Comparison of numbers of stocked and unstocked premises in DCS data and field records in Cumbria

	DCS data	Field records
Stocked	5,250	6,439
Not-Stocked	4,709	3,520
Total	9,959	9,959

There was an overall agreement of 75.7% between the two sets of data. The major difference was that field records showed stock on many premises that DCS showed to be unstocked. Of the premises DCS data indicated were stocked, the field records show stock on 88.5%. Of the premises DCS data indicated had no cattle or sheep, the field records showed stock on 38.5% .

3) Farm type in north Cumbria

Table 4 shows the comparison of farm types in census data in DCS download on 22-Oct-01 and field records. There is agreement on farm type in 1,497 premises, just over 50%. The major differences are in the numbers of dairy farms which had sheep and in premises which DCS data indicated had no stock, in particular where only sheep were present in the field records but also for beef and beef/sheep farms. Another clear difference is in the number of dairy farms of any type. DCS data indicates around 950, but the field records show only 850.

Table 4. Comparison of farm types in census data in DCS download on 22-Oct-01 and field records

	Field record farm type						
DCS data Farm type	Dairy	Dairy and Sheep	Beef	Beef and Sheep	Sheep	No Cattle or Sheep	Total
Dairy	155	230	21	29	8	5	448
Dairy and Sheep	16	376	8	84	15	7	506
Beef	5	3	96	72	10	4	190
Beef and Sheep	1	22	24	533	50	6	636
Sheep	0	1	5	44	249	41	340
No Cattle or Sheep	27	20	163	141	301	88	740
Total	204	652	317	903	633	151	2860

4) Cattle population and herd sizes in North Cumbria

For the 2,860 premises within 3km of the IPs in the north Cumbrian cluster, the cattle numbers recorded in the DCS database and found during field visits have been compared. Table 5 shows the descriptive statistics for the overall population.

Table 5. Descriptive statistics for cattle

	DCS data	Field records
N	2,860	2,860
Sum	281,069	341,888
Mean	98.3	119.5
SD	128.8	146.3
Minimum	0.0	0.0
1st Quartile	0.0	0.0
Median	45.0	68.0
3rd Quartile	165.0	194.8
Maximum	1,593.0	2,250.0

Both means and medians are similar, though more weight should be placed on the medians because the distribution is clearly non-symmetrical. There are considerably greater numbers of cattle recorded in the field data as compared with the census data, almost 25% more. The reason(s) for this are not clear, unless it relates to calving patterns in dairy cattle. Whatever the cause, it indicates that there is considerable seasonal variation.

Table 6 shows the herd size classes recorded in the DCS database and field records for the premises in north Cumbria.

Table 6. Herd size class for census data and field records for cattle

		Field records						
		None	≤50	51-100	101-200	201-300	>300	Total
DCS data	None	680	191	73	83	30	23	1080
	≤50	45	267	48	17	6	1	384
	51-100	26	39	162	77	10	6	320
	101-200	26	14	37	337	104	25	543
	201-300	3	6	3	31	181	89	313
	>300	7	2	2	6	26	177	313
	Total	787	519	325	551	357	321	2860

There is agreement in the herd class for 1704 of the 2860 premises, around 60%. In around 13%, the disagreement is two size classes or more. Mostly, the field data show a larger herd size than the census data. DCS (census based) showed 1,080 premises with no cattle, whereas only 680 really had no cattle. There are over 200 premises for which the census data show no cattle present but the field data show significant numbers of cattle (>50) were present.

5) Sheep population and flock sizes in north Cumbria

For the 2,860 premises within 3km of the IPs in the north Cumbrian cluster, the sheep numbers recorded in the DCS database and found during field visits have been compared. Table 7 shows the descriptive statistics for the overall population. The total sheep population in the census and field data is very similar. This is not a coincidence. The Census data are collected in June when sheep populations are at their maximum and although many of the field records will have come from the late March to mid-May period of the 3km cull, lambing is mostly over by this period. Although the mean values are similar, the median values are clearly different. Given the non-symmetrical distribution of flock size, the median is more instructive. This figure is very different between the two datasets

Table 7.Descriptive statistics for sheep

	DCS DATA	FIELD RECORDS
N	2,860	2,860
Sum	882,153	868,908
Mean	308.5	303.8
SD	689.6	518.8
Minimum	0.0	0.0
1st Quartile	0.0	2.0
Median	3.0	108.0
3rd Quartile	327.0	390.0
Maximum	13,237	6,754

Table 8 shows the flock size classes recorded in the DCS database and field records for the premises in north Cumbria.

Table 8. Comparison of DCS data and field record flock size classes

		Field records					
		None	≤50	51-200	201-500	>500	Total
DCS data	None	563	243	281	180	111	1378
	≤50	52	171	55	19	4	301
	51-200	21	31	172	63	16	303
	201-500	14	15	85	148	62	324
	>500	15	7	31	138	363	554
	Total	665	467	624	548	556	2860

There is agreement on herd size class in 1,417 cases, around 50%. There is disagreement by two or more herd size classes in 714 cases, 25% of the time. In most cases, the field data show a larger herd size than the DCS census data. There is a particularly high number of premises for which the census data show an absence of sheep that the field data show had sheep present. The census data suggested that 1480 CPHs had sheep, whereas the field data identified 2195 CPHs with sheep.

6) Herd and flock sizes in 6nnn and non-6nnn holdings in north Cumbria

The 6nnn holding code premises were added during the outbreak. The cattle herd and sheep flock sizes were compared in these and the non-6nnn holding series premises (Tables 9 and 10).

Table 9. Cattle herd sizes in 6nnn series holding in north Cumbria

	None	≤50	51-100	101-200	201-300	>300	Total
6nnn	232	57	38	43	15	11	396
Non-6nnn	555	462	287	508	342	310	2464
Total	787	519	325	551	357	321	2860

Table 10.Sheep flock sizes in 6nnn series holding in north Cumbria

	None	≤50	51-200	201-500	>500	Total
6nnn	153	110	66	32	35	396
Non-6nnn	512	357	558	516	521	2464
Total	665	467	624	548	556	2860

This shows that whilst cattle and sheep flocks on 6nnn series premises tended to be smaller than on other premises, they were by no means all small hobby farmers. There were >50 cattle on 27% of the 6nnn holdings and >50 sheep on 33% and these included some substantial herds and flocks.

DISCUSSION

The initial DCS data, imported from VetNet at the start of the outbreak in 2001, were considerably incomplete in terms of numbers of premises in Cumbria overall including the north

Cumbria area when compared to the field records as is evidenced by the numbers of new premises added during the outbreak.

The reasons for creating emergency codes during the epidemic were largely epidemiological. They were for premises which were either previously unknown or were an epidemiologically separate set of livestock on geographically distinct land run under a previously known CPH. This could be a completely separate farm or a grouping of "off-fields". For example, one north Cumbrian IP had a single CPH initially, but this actually comprised 4 separate farms (with their own buildings) and two large separate blocks of land. The main problem is that there was no strict definition of what a CPH consisted of. It could refer to several enterprises sharing the same location, or to one enterprise spanning several locations which could be widely spread. The address registered was even sometimes in a different part of Great Britain. Whilst this flexibility in the definition of a CPH had utility from an administrative point of view, it was a significant problem from an epidemiological point of view.

For both cattle and sheep, but particularly sheep, there were significant inaccuracies in the DCS data (derived from the Agricultural census of June 2000) for stock types and numbers present on farms. The DCS data significantly underestimated the number of farms on which cattle and in particular, sheep were present. Where DCS did record stock as being present, the numbers of stock were not accurate enough to use as a substitute for the field data. This was true for larger (epidemiologically more significant) herds and flocks and also for the smaller groups of livestock.

Field records were not used to update the agricultural census data in DCS during the outbreak in 2001. In some cases it was entered in a text field, but this was not available within DCS for analysis. There were no extra fields in DCS at that time that were accessible to local staff to which these data could be added in numerical form.

Given that the field records are contemporary and that the data on farm type and stock numbers was specifically gathered for disease control purposes, these data must be regarded as the most accurate representation of the actual situation on the ground at the time of the outbreak. The census data are a poor substitute for these data and may result in inaccurate results. Census data can only ever be a snapshot at one particular point in the farming season. They are collected for the purposes of providing an aggregated overview of livestock populations in GB rather than for detailed analysis of stock locations and the type and size of individual farms. The June 2000 census data will not have accurately reflected the type of numbers of stock in the winter months, particularly in counties such as Cumbria where many hill sheep are over wintered in lowland farms, notably dairy farms. Lambing and calving seasons will also affect stock numbers at various times of the year.

There will always be some differences between field data and that held in central databases. The question is, how great are these differences and might they materially affect any analysis and the conclusions that may be drawn from it. This will depend on several factors:

- the coverage of the original data collection exercise and any subsequent updating processes will have a direct impact on the utility of the data.

- the longer the time period between data collection and use, the more likely it will be that changes will have occurred.

The results of this study suggest that there were significant differences which may have been expected to have affected the validity of studies based on data extracted from the datasets. Table 11 is the authors' qualitative assessment of the relative quality of the data in DCS and in field records. The field records are undoubtedly somewhat inaccurate, but the fact that they were collected directly and at the time of the outbreak should mean that they are more accurate than the centrally held data that were available in 2001 which was collected remotely and some time before the centrally held data.

Table 11. Comparison of data quality in different sources

	DCS at start of outbreak	Field records
CPH List	Poor	Good
Farm location	Poor	Adequate
Farm type	Poor	Good
Cattle present	Adequate	Good
Cattle population	Poor	Adequate
Sheep present	Poor	Good
Sheep population	Very poor	Adequate

The outcome of this study may seem to be critical of the quality of the farm premises and livestock population data held in GB in 2001. However, given that it is unlikely that many countries had or have better data, it must not be taken as anything other than a rare opportunity to undertake such an evaluation and an indication that ground proofing is necessary even for seemingly complete and recently compiled centrally held databases.

Any analysis which relies on secondary data not directly collected for the purposes of the study should contain either a ground proving phase or a statement of how data quality has been checked by the researchers. This is not a novel concept by any means in other fields, but is one which the authors believe needs to be reinforced in the area of veterinary epidemiology. Funding agencies should require that the quality of secondary data be checked and assured. The corollary of this is that any study that does not contain this phase should be treated with caution.

Since 2001, and partly as a result of the experiences during the FMD epidemic, Defra has established the Veterinary Surveillance Strategy which seeks, amongst other goals, to address the issue of data quality. It is planned within this to (i) quality assure (i.e. fully describe, including limitations) animal population data to make it easier to assess its fitness for different policy purposes, and to work with providers to improve it, and (ii) to validate samples of it whenever possible through capture of such data when visits are made to livestock premises for other purposes.

When ground proofing is carried out, it will undoubtedly always show that there is not 100% agreement between the two data sources. But how good is good enough? Whereabouts should the line be drawn? Indeed, should any sort of arbitrary line be drawn? Instinctively, this would seem wrong and yet it is done routinely with regard to statistical confidence, the magical, standard but arbitrary 95%. The authors are not attempting to state that data which are 95% accurate should be sought, nor prescribe the methods for checking data quality, but would suggest that the veterinary epidemiology community debate this issue with the intention of agreeing guidelines, if only as a requirement for a data quality assurance statement in papers which use data not generated by the authors.

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USE OF SOCIAL NETWORK ANALSYIS TO CHARACTERIZE THE PATTERN OF ANIMAL MOVEMENTS IN THE INITIAL PHASES OF THE 2001 FOOT-AND-MOUTH DISEASE EPIDEMIC IN THE UK

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SUMMARY

Some recorded movements of cattle and sheep during the initial phase of the 2001 Foot-and-Mouth Disease (FMD) outbreak in the UK, before the ban on animal movements was imposed, are analysed descriptively using Social Network Analysis (SNA) techniques. The phenomenon of diffusion within the network is investigated using some of the estimated parameters of the nodes. With the data available, a directed dichotomized network with 653 nodes and 797 links was analyzed. Relative betweenness, k-neighbours, structural equivalence and bi-components were able to identify key actors in the evolution of the initial phases of the FMD outbreak, such as markets, dealers and farms with atypical movement patterns. Sparse connectivity and the presence of few very central nodes or hubs produce a maximal outbreak dimension of 28-33% of the network assuming that the transmission of infection was the result of contact between holdings through animal movements.

INTRODUCTION

The 2001 Foot-and-Mouth Disease (FMD) epidemic in the UK is probably one of the best documented major livestock epidemics. 2030 agricultural holdings were declared infected during the course of the outbreak. Various control strategies were applied during the outbreak including a ban on animal movements, the implementation of surveillance and biosecurity measures and the culling of all livestock on infected premises (IPs) and farms that had 'dangerous contacts' with IPs or were contiguous to IPs. The economic losses associated with the epidemic have been estimated at £2.4 – 4.1 billion (The Countryside Agency, 2001) with an estimated reduction in the GDP of 0.2% in 2001 (Thompson et al., 2002).

Movement restrictions were imposed, as per EU policy, following the report of suspected FMD in pigs in an abattoir in Essex on 19th February. One day later Foot-and-Mouth Disease Virus (FMDV) was confirmed ,Knowles et al., 2001) and on 23rd February the whole of Great Britain was considered a controlled area, and the national ban on animal movements in and out of all premises, including slaughterhouses and markets throughout the country, was announced (Anderson, 2002). Thus, there was a time window between the estimated date of infection of the index case (on a pig farm in Northumberland) and the actual implementation of restrictions, during which FMDV was present in the country, and farming activities, including animal

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movements, remained unaltered. It has been suggested that the spread of virus between farms through the movement of animals before the implementation of control measures played a major role in the dissemination of the virus to different areas within the UK and became a major determinant of the extent of the outbreak (Ferguson et al., 2001; Kao, 2002).

The aim of this study is to explore recorded movements of cattle and sheep during the initial phase of the 2001 FMD outbreak in the UK, before the ban on animal movements was imposed, and to describe the network of these movements and quantify the relative position of its members using Social Network Analysis (SNA) techniques. The phenomenon of diffusion within the network is investigated using some of the estimated parameters of the nodes based on certain theoretical assumptions. The implications of the movement structure are discussed in the context of the investigation and control of an infectious disease epidemic in a livestock population.

MATERIALS AND METHODS

Study population

The index case and origin of the 2001 FMD epidemic is considered to have been on a pig finishing unit at Burnside Farm, Heddon on the Wall, Northumberland (England). FMD was confirmed on these premises on 20 February at 6:30 pm, and the National Animal Movement Restriction was announced at 5 pm of the 23 February, although animals in transit were allowed to continue their journeys. For a total of 80 premises, infection was estimated to have occurred on or before February 23rd 2001 (DEFRA FMD data warehouse). If animal movement had a significant role in spreading the virus throughout the country, this is most likely to be when it occurred. The nodes and the links of the network have been defined based on the following criterion: 'the first 80 infected premises and all holdings connected by animal movement/s to them between 6-2-2001 and 23-2-2001'. This corresponds to the period between the estimated infection date of the index case and the enforcement of the animal movement ban.

Data sources

The information gathered by the State Veterinary Service (SVS) during the outbreak was organized into a large relational database (DEFRA FMD data warehouse). Premises infected within the time window of interest were identified from one of the datasets included in the data warehouse, which included the estimated date of infection for each IP.

Cattle movement data were retrieved from the Cattle Tracing System (CTS). Holdings of origin and destination were identified for all cattle moved to or from any of the first 80 IPs between February 6th and February 23rd included. Sheep movements were recorded from the intelligence gathered by the Veterinary Officers during the field visits to suspect holdings, as collated in the DEFRA FMD data warehouse. A systematic search for information on animal movements available in selected databases of the DEFRA FMD data warehouse was conducted.

The DEFRA FMD data warehouse was constructed using several data sources (9 different databases), but the most important for this study is the Carlisle Epidemiology Database and, specifically, three of its tables: Species Clinical Signs Table (epi_speciesclinicalsigns), Tracings Table (epi_tracings) and Infected Premises Description Table (epivla_ipdescription). Two data fields were comprehensively reviewed to gather sheep movement data from them. The

information recorded in those fields named 'history' and 'clinical details' was often incomplete. Additionally, most of the information recorded in the database is truncated, probably due to word limit constraints at some stage during the data processing. Field veterinarians involved in the efforts to control the disease were contacted to assess the completeness and accuracy of the available animal movement data.

Analysis

A directed (A is connected to B but B may/may not be connected to A) dichotomized (multiple movements or more than one animal per movement are considered a single link) network was developed with all cattle and sheep movements as described above.

Relative betweenness of each node was calculated according to the equation for directed networks proposed by Gould (1987). Relative betweenness is defined as the frequency with which a node falls between pairs of other nodes on the geodesic (shortest) path connecting them, divided by the maximum value it can take in the network (Freeman, 1978-79).

A component of a network is the maximal connected sub-network without a cutpoint (de Nooy et al., 2005). Cutpoints, articulated points or cut-vertices are defined as nodes whose deletion increases the number of components in the network. The bi-component is a component without a cutpoint. The components of the network have been explored by identifying the bi-components of minimum size 3 once the network has been symmetrized.

The relationship between structural equivalence of the nodes and types of holdings was tested by calculating the dissimilarity matrix between all pairs of nodes based on Euclidean distances. Clusters which are structurally equivalent (having identical ties with themselves, each other, and all other nodes) (de Nooy et al., 2005) were detected using hierarchical clustering. This technique groups nodes in the network into subsets so that nodes within a subset are relatively similar to each other (Wasserman et al., 1994). Finally, the holdings in each cluster were described and the association between cluster and type of holding was tested by fitting a logistic regression model.

Diffusion in the network

To illustrate the reachability of the infection if a node in the network were selected randomly as containing the index case, the number of k<=2 and k<=8 neighbours for every node was calculated, and the distribution of premises according to this parameter was described. The diffusion curve was drawn for different type of nodes

Social Network Analysis was performed using Pajek 1.06 (Batagelj & Mrvar, 2005) and the statistical analysis with STATA 8.0 (StataCorp. 2003. Stata Statistical Software: Release 8. College Station, TX: StataCorp LP).

RESULTS

The network and betweenness

The network with all available cattle and sheep movements contains 653 nodes and 797 links among nodes (Fig. 1). It is a weakly connected network with an average of 1.22 links per node.

6.77% of the recorded movements (54) are of only sheep and 93.23% (744) are of only cattle. Only one movement between the same locations was of cattle and sheep.

The network contains 100 IPs (15.31%) and 553 non-IPs (84.69%). Of the first 80 IPs, only 53 appear to have had movements during the study period and consequently, are nodes of the network. There was no movement recorded for 27 of them. 47 holdings were connected to the above-mentioned 53 IPs, and became infected during the outbreak but not during the time window of the network (30 of them during March and 17 during April). The distribution of holding types among network members is: 526 farms (80.55%), 35 markets (5.36%), 15 dealers (2.3%), 8 abattoirs (1.23%), 4 hauliers (0.61%) and 65 others (9.95%), for which type could not be ascertained (9.95%) The 10 nodes with the highest betweenness are 3 farms, 4 markets and 3 dealers, located in 9 different counties. Betweenness, degree and k<=2 neighbours for the ten holdings with the highest betweenness are presented in Table 1, and 6 of them were declared IPs during the outbreak.

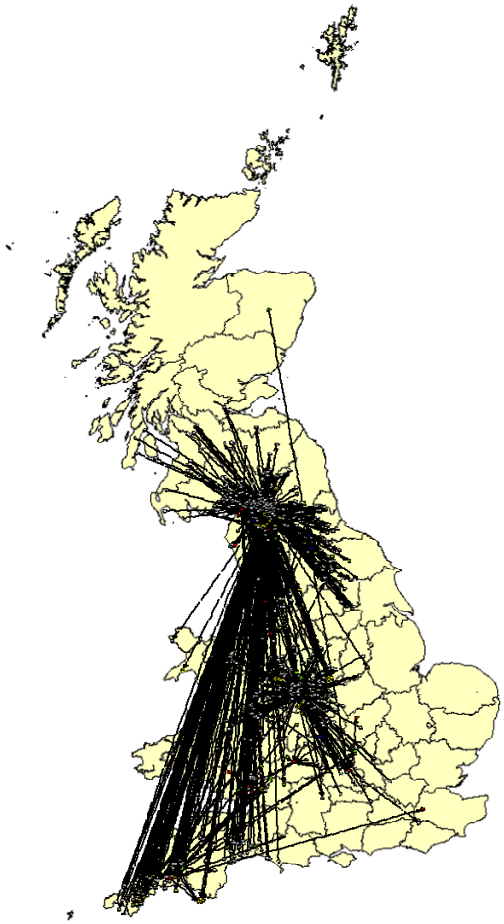

Fig. 1 Visualisation of the network.

Table 1. Betweenness centrality and secondary outcomes of the network. Data for the 10 holdings with highest betweenness is shown

Node	IP	Type	Betweenness	Outdegree	Indegree	All-links	% k-neighbours (k<=2)
11	Yes	M	0.23	167	295	462	28.48
10	Yes	F	0.087	22	149	171	3.52
77	No	M	0.038	2	2	4	28.02
478	No	M	0.038	2	2	4	28.02
4	Yes	D	0.026	23	41	64	26.18
1	Yes	D	0.016	6	2	8	1.99
159	No	F	0.005	2	1	3	28.02
508	No	M	0.005	3	1	4	0.76
2	Yes	D	0.004	4	1	5	0.76
501	Yes	F	0.004	3	4	7	0.612

K-neighbours

The average number of k-1 neighbours or outdegree per node is 1.2 (median=1, range 0-163). The average number of k-2 neighbours per node is 78.69 (median=22, range: 0-182). Based on the number of k<=2 neighbours for every node, the network contains three distinctive groups of holdings (Fig. 2):

- Group 1: 141 nodes (21.6%) have no k<=2 neighbours in the network.
- Group 2: 220 (33.7%) nodes have up to 7% of the network as k<= 2 neighbours in the network
- Group 3: 292 (44.7%) nodes have more than 24% of the network as k<=2 neighbours.

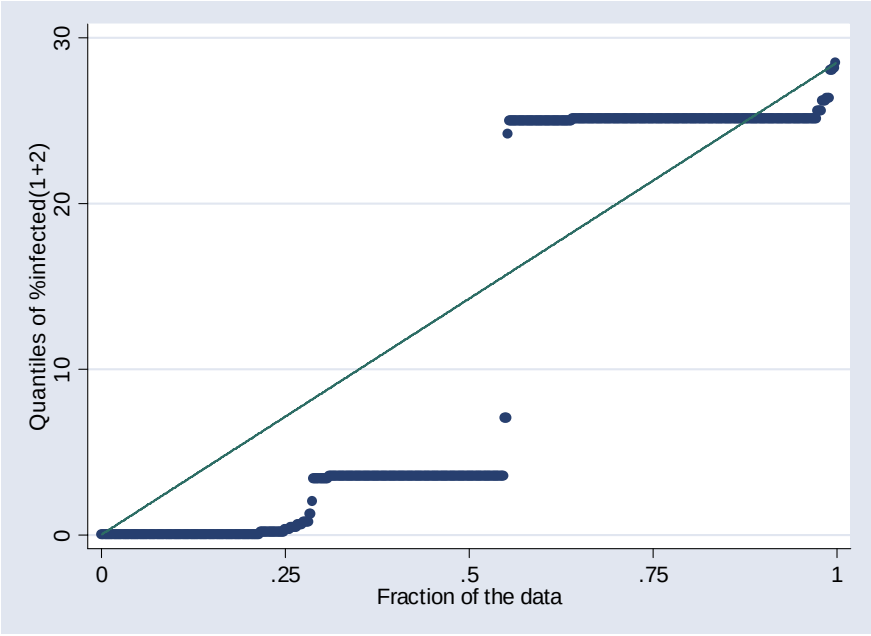

Fig. 2 Percentage of infected nodes from every other node assuming infection to a k<=2 neighbours

For k<=8, the group 2 is reduced to the 6.3% (41) of the network because 179 nodes belong now to group 3 that increases up to 72.1% (471). The most central node would produce a maximal outbreak dimension of 33% of the total network with a sudden increase of transmission at k<=2. The diffusion curve shows a logistic S-shape (Fig. 3). Marginal nodes as in group 2 produce a shift to the right of the diffusion curve whereas most of the nodes in group 3 lose the S-shape curve and have no diffusion power whatsoever.

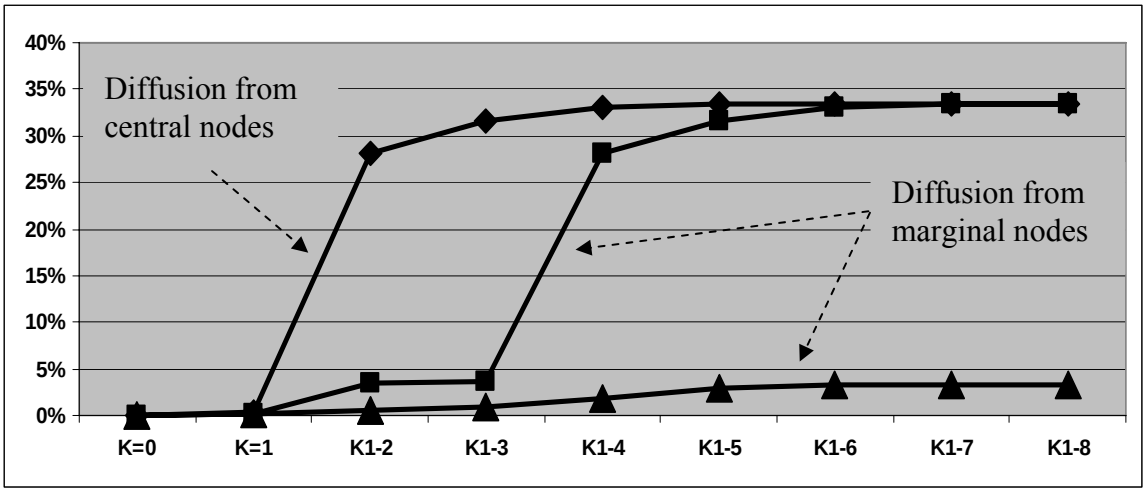

Fig. 3 Diffusion curve for three nodes randomly selected from each of the three types of nodes based on their proportion of k-neighbours.

Bi-components

Three bi-components of minimum size 3 and two cutpoints have been identified in the network. The first bi-component contains 2 nodes and is linked by a cutpoint 17th in the relative betweenness list. It is the sheep dealer linked to the index case of the Devon cluster in the South West of England. The second bi-component has 46 nodes linked to the rest of the network through the top node of the network, the one with the highest betweenness, identified as the Longtown market in Cumbria. The third bi-component contains 15 nodes and is linked to both cutpoints. The other 588 nodes (90%) do not belong to any bi-component, meaning that they are all linked to one of the two cutpoints and that, removing the link, they become isolated in the network.

Structural equivalence

Three hierarchical clusters (1-3) have been selected with 308, 215 and 130 nodes, respectively. Clusters 2 and 3 are internally very similar and contain mostly farms, 98.14% and 99.23% respectively (Fig. 4). Only 5 non-farm holdings are included in these two clusters. However cluster 1 contains almost all the non-farm holdings and 186 farms (60.39%). The odds of being a farm in cluster 2 and 3 are 34 and 84 times higher respectively than in cluster 1 (P<0.0001).

The distribution of the nodes by cluster is shown in Fig. 4 and the number of connections between and within clusters is displayed in Fig. 5 by the shrunken network with the clusters presented as nodes. Two different typologies of farms can be identified: one corresponds to clusters 2 and 3 where farms had only movements to holdings in cluster 1 and no movements

within the cluster. A second group of farms, those in cluster 1, have links either to other farms in cluster 1, 2 and 3 or to markets, dealers, etc. (Fig. 4). Farms in cluster 1 appear to be more similar in their movement patterns to non-farm holdings than to farms in clusters 2 and 3. All farms with betweenness greater than 0 are in cluster 1. The shrunken network contains 215 movements from cluster 2 to cluster 1, 130 from cluster 3 to cluster 1 and 452 within cluster 1.

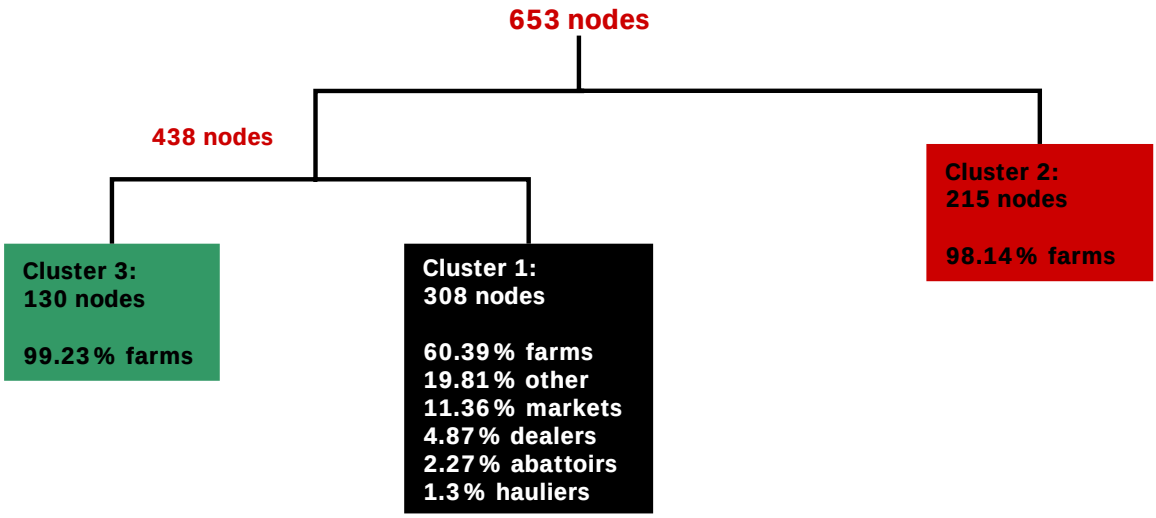

Fig. 4 Section of the dendogram obtained from the hierarchical clustering of the network.

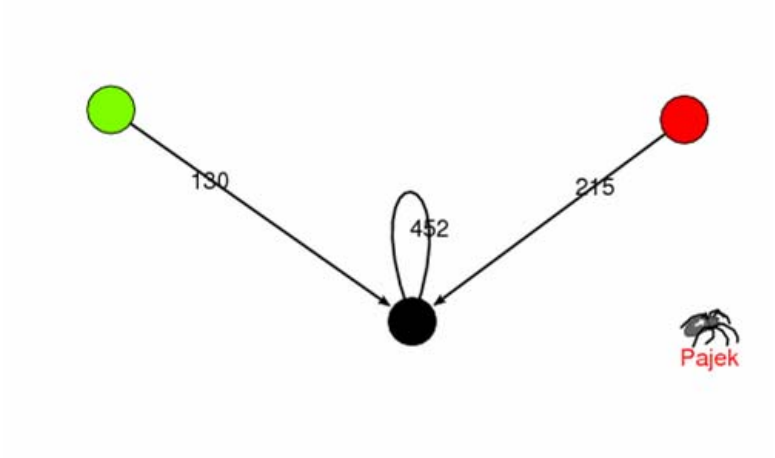

Fig. 5 Shrunken network according to the hierarchical clustering.
Figures are number of movements between and within clusters.

DISCUSSION

The data

Movement data were obtained from the CTS (cattle) and by extracting intelligence information gathered by Veterinary Officers during field investigations of suspect cases (cattle and sheep). The latter, available from the DEFRA FMD data warehouse and subject to limitations, was used to collect data about the sheep movements included in the network. Specific guidelines for the field investigation of FMD suspect cases were not issued until March,

resulting in inconsistent and unsystematic recording of events during the visits. Lack of a standard procedure for data collection during this phase of the outbreak raises questions about the completeness of the data. On the other hand, during this initial phase of the outbreak, all field investigations were conducted by Veterinary Officers of the SVS, and their records are thought to have been more thorough and complete than those collected by others in later phases (Nick Taylor, personal communication). In addition to this limitation, not all information recorded in the field has been made available through the DEFRA FMD data warehouse. During the process of retrieving the available movement data, it became evident that data in the areas of interest had been truncated for a number of the 80 IPs in the study population. After communication with the database managers it was concluded that not all tracing information was transferred to the DEFRA FMD data warehouse databases as only (probably incomplete) sheep movement data were stored in it. Cattle movement data were less affected due to the existence of a second and more reliable data source (CTS).

The probably incomplete sheep movement available in the DERA FMD data warehouse suggests that it is very likely to miss sheep movements when generating the network, contradicting the views of the some consulted field veterinarians. It is unlikely that only sheep were moved to or from the first 80 IPs during the study period, as 26 of them were found to have cattle as the infected species at the time of restriction. Despite the efforts to collate centrally all available data on the outbreak, the retrievable information contained there has considerable limitations for quantitative analysis.

The incompleteness of the only available source of sheep movement data and the criterion chosen to define the members and links of the network may have introduced bias into the analysis, restricting the authors' ability to draw conclusions. However, the results of the analysis still demonstrate the value of network analysis for identifying different typologies of movement patterns and key actors in the diffusion of infection.

Centrality measures

Betweenness provides an indication of the extent to which a node facilitates the flow through the links amongst members of the network (Borgatti, 1995). In this study, it is assumed that the transmission of infection is determined by the contact between holdings through animals moved from one to another. If a node with high betweenness is removed from the network, the speed of transmission between randomly selected nodes of the network decreases and the spread of infection within the network as a whole is reduced. Betweenness measures the extent to which a particular node lies 'between' the various other nodes in the network (Scott, 2000). It is reasonable to expect markets to have high betweenness because they determine the flow of animals between farms, abattoirs and other markets. Abattoirs should have null betweenness because they are end-points or terminal nodes in the network, with hauliers and dealers somewhere in between. However, the a-priori information of the betweenness of a farm is quite uncertain as this depends on the pattern of movements, type of farm, husbandry system, etc.

Consistent with the conceptual definition of betweenness, there are 4 markets and 3 dealers among the top ten betweenness nodes. The node with the highest betweenness is Longtown market in Cumbria, which had a pivotal role in the dissemination of FMD in the initial stage of the epidemic. One of the three dealers in the top ten handled the index case of the Devon cluster in the South West of England. The other two dealers are from Dumfriesshire County in South Scotland, and Herefordshire in the Midlands. Dealers may have a low number of connections in the network (low degree) but at the same time, they can have a high betweenness because they

are linked to other high-betweenness holdings. There are 3 farms among the 10 premises with highest betweenness. In fact, the holding with the second highest betweenness is a cattle farm that played an important role in the transmission of FMD in the Midlands and is linked to the Northampton market (8th in the list) in Central England. The other two are in Devonshire and in Dumfriesshire. These 'dangerous' farms have also been identified using the hierarchical clustering approach, corresponding to the second typology of farms, members of cluster 1. The existence of individual farms with high betweenness and clustered with more central non-farm holdings suggests that not only markets and dealers, but also this type of farm, which is difficult to detect '*a-priori*', can have a key role in the dissemination by animal movements of the infection in livestock populations. Such farms appear to have a movement pattern more like that of a market or dealer, and pose a high risk of spreading infection within the network.

With the data available for developing the network in this study, calculation of betweenness and the hierarchical clustering method are able to detect these critical actors in the evolution of the FMD outbreak in its initial phase, which have already been detected by previous studies (Ferguson et al., 2001: Mansley et al., 2003).

Infections generally do not flow through the shortest path in networks, and may be routed randomly at a junction (Borgatti, 1995 & 2005). The use of betweenness is still valid in the network as a measure of the amount of network flow in infected animals. To overcome the conceptual weakness of betweenness for explaining the random flow of virus within the network, it was combined with the k-neighbour parameter, which considers all available paths between two nodes.

Among those nodes in Table 1, three of the four markets have high betweenness and a high proportion of k<=2 neighbours. The fourth has high betweenness and a low proportion of k<=2 neighbours. In contrast, the farm identified as node 159 has a relatively low betweenness but a high proportion of k<=2 neighbours. Only two movements off were recorded for this farm, but one of them was to a high-betweenness market. Although the flow of the infection within the network would not be affected by the position of this farm, if this farm contained the index case, infection would reach 28% of the network. Node 10, which is a farm with the second highest betweenness has only one k=2 neighbour which is connected to a total of 160 other nodes through paths of k=3. In this example, it is clear that the cut-off path distance chosen (k<=2) does not give us full information of the centrality of this node and its potential for transmission. However, if the cut-off point is extended, the majority of nodes tend to be linked at relatively short distances, typified as the 'small-world' phenomenon (Watts & Storgatz, 1998; Scott, 2000). Increasing the distance for the characteristics of the flow of infection in the network, and hence the time window, is unlikely to be more informative. In this example, k<=2 neighbours does not provide us with full information on the potential diffusion power of this node, which is better defined together with its betweenness. The increase of the path distance to k<=8 transfers a number of nodes from group 2 to 3 since the number of nodes linked at that distance equally increases. However the outbreak size in the network rises from 28 to only 33% of the network.

Betweenness is computed for every node via the shortest possible path between two other nodes. At junctions, there is equal probability of taking either route. However the assumption is that infection flows in the network from node to node by transfer of an animal via every single directed link. Once an infected animal is moved to another holding, this becomes infected, and every time animals move to another holding, these become infected as well. Thus the combination of both parameters provides better insight into the transmission ability of a node. If the movement from a node to its neighbours were avoided, the hypothetical 'control' of the

spread within the network would vary according to the centrality measure of the node. The number of k-neighbours reflects unequivocally the spread of the infection through the network provided that any animal moved from an infected node results in the infection of all linked nodes with a maximum k distance and that all animals moved to other nodes are infectious. Betweenness adds a time-factor to the spread of the infection in the network, especially if the holding containing the index case has a high centrality or is connected to a high-betweenness holding.

Bi-components

The presence of three small-size bi-components containing only 10% of the nodes confirms the sparse connectivity of the network. Members of a bi-component always will be connected to each other and only to another bi-component through a cutpoint. The cutpoints are two important actors in the initial spread of the outbreak. They are bottlenecks that control the flow from one part to of the network to another. In the network in this study, the detected cutpoints have a geographical connotation whereby the sheep dealer is the cutpoint between the bi-component located in the North, mostly in Cumbria County, and the bi-component with the majority of the nodes located in Devon and Cornwall counties, in the South West of England. The algorithm used to detect bi-components does not consider the direction of the edges between nodes. It assumes flow either way between two nodes. This contradicts the principle of the network in this study where the movement has only one direction, and hence the bi-components are not totally connected. Despite this factor, the cutpoint still controls the flow between components and is of paramount importance to understanding the diffusion within the network.

The position of a cutpoint is critical as there cannot be communication, flow or diffusion between components if the cutpoint is deleted (Wasserman et al., 1994). The detection of bi-components and cutpoints in this network does not reveal any unexpected actor playing the broker role between self-connected areas of the network. Moreover the components are of small size and only contain a small fraction of the network. The market and the dealer detected as cutpoints in the analysis could be considered to be the 'usual suspects' in the diffusion of infection. However, it is unlikely that a farm could play such a role. If the analysis of a real-time network of animal movements during an outbreak detected a cutpoint identified as a farm, this should be a primary target for containment and investigation. It would be an important diffuser if the components or areas of the network linked by the cutpoints were large enough or strategically located for disease control purposes.

Diffusion

Diffusion is a special case of brokerage with a time dimension (De Nooy et al., 2005). Something, in this case FMDV, is passed on through movement of animals from one node to another in the course of time. It is assumed that the links are instrumental to the diffusion process as they are channels of contagion. Thus, the structure of the network is relevant to the diffusion process.

Applying a theoretical framework, the number of k<=2 neighbours for every node has been arbitrarily considered as a measure of the reachability of the infection, if each node contained the index case of the outbreak. In using k <= 2 as a cut-off, it is assumed that an IP would infect by animal contacts only those premises to which it is linked through a path distance of 1 or 2 animal movements. The average spread of the outbreak within the network would affect only

one in eight holdings. The average proportion of k<=2 neighbours is 12.2%. For k<=8, the average proportion doubles to 24.2%, which could be considered as the ability of this network to spread the infection from a randomly selected node. However, this is a summary measure from the three distinctive groups of nodes as above described (Fig. 2).

The structure of the network has an impact on the diffusion process. The connectivity distribution in the network and the presence of hubs explains the logistic spread from a central node and the small scale of the outbreak. If it is assumed that the most central node contained the index case, the maximal outbreak dimension would be only 33% of the total network. When different types of holdings are introduced in a directed network, the effect of the scale-free structure is diluted because many nodes are found with no movements off (abattoirs, farms, etc.), altering the outdegree distribution as in the form of a power-law decay.

The nodes in group 1 present a diffusion curve with the typical logistic S-shape, characteristic of a chain reaction. It means that the ties are important to the diffusion process. However it is the opposite for nodes in group 3 which do not have any transmission potential and if infected, could spread infection by transmission mechanisms other than animal movements off.

Of the 80 first infected IPs, 27 had no movement on or off recorded in the data sources available for this study. The remaining 53 appear in the network. An additional 47 holdings of those present in the network became infected once the movement ban was already in place. It is assumed that these holdings became infected not due to the arrival of infected animals, but by other factors likely to have been important in later stages (aerial spread, non-animal infected contacts, fomites, etc.) and because of the ease with which FMDV can spread (Gibbens & Wilesmith, 2002). The potential outbreak dimension of the network was never fulfilled. Only 53 IPs (8%) appear in it, hence some of the movements described in the network did not produce a successful transmission. If the real movement pattern among members of the network is used to explain the spread of a disease, the main underlying assumption is that the transmission occurs due only to existing directed links in the network, which is not always so. The dynamics of infectious diseases would be better modeled by combining the actual contact network of animal populations and other traditional parameters based on distance, densities, etc.

The main limitation of SNA and its application to animal movements (considered here as the link between elements) is the necessary condition of having a discrete number of elements in the network to study all relations (if possible) between its members. A closed population (a complete network) is a rare characteristic for farm animals as all movements within a country would need to be included (Christley et al., 2005) and imports/exports discarded. When the relations between farms are based on animal movements, the network expands every time a new farm is connected. In the network in this study, the missing data cannot be quantified nor can the impact of their absence on the conclusions of this study and its validity be assessed. The criteria for selecting nodes and their links determine the characteristics of the network and the centrality of its members. If the criterion is not epidemiologically grounded, the analysis might lead to wrong inferences in relation to the role of some actors in the outbreak, and to missing others with a more important role.

The lack of accurate movement data was one of the main obstacles when fighting the disease in the initial stages (Gibbens et al., 2001). As a result of this, The Animal Movement Licensing System (2001) was established in September 2001 to record movements of sheep, pigs and goats. The Cattle Tracing System was already in place and all cattle movements during the study

period had to be notified to the British Cattle Movement System (BCMS). Both systems are being improved and more processed data can be extracted from them for analysis. The development of templates to collect field intelligence in a structured way during outbreak investigations could facilitate the analysis during and after an outbreak. The development of movement networks in real time is possible in the UK and can provide outbreak investigators with information that can be used to define more cost-effective targeted control interventions. By exploring and analysing movement networks, pockets of undetected disease could be identified and controlled before it spreads. The detection of farms with high centrality and non-farming movement patterns critical for the diffusion process as described in this study could be a valuable component of early warning systems and contingency plans in countries with centralized animal movement records once markets, dealers and abattoirs have been contained.

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INTENSIVE PRODUCTION

ESTIMATION OF INFECTION TRANSMISSION PARAMETERS FOR *SALMONELLA* DUBLIN IN YOUNG DAIRY CALVES

L.R. NIELSEN*, B.H.P. VAN DEN BORNE AND G. VAN SCHAIK

SUMMARY

Field data were used to estimate number of days with faecal excretion of *Salmonella* Dublin and time to seroconversion in infected calves below the age of 180 days. Based on these estimates, the calves in four endemically infected dairy herds were grouped into infection states: Susceptible (S), infectious (I) and resistant/recovered (R). The infection groups and the number of new infections per week were used to estimate the transmission parameter β with a generalized linear model. From β, the reproduction ratio R at steady state and the basic reproduction ratio R_0 could be estimated for each herd. The R_0 denotes the average number of new infections caused by one infectious individual that is introduced into a fully susceptible population. R_0 ranged from 1.1 to 2.5 in the study herds, but with very wide confidence intervals. Data were too limited to show possible significant differences in the parameters between the study herds.

INTRODUCTION

Salmonella Dublin is a cause of concern in the cattle industry, because it is a zoonosis causing severe invasive infections in humans and because it causes economic and welfare losses in infected herds (Peters, 1985; Helms et al., 2003). The infection has a tendency to become endemic in many cattle herds in Denmark. When attempting to control *Salmonella* Dublin infections in such dairy herds it is critical to intervene in the calf barn where the infection spreads readily. However, little is known about the infection dynamics of *Salmonella* Dublin in calf barns of endemically infected herds, because most information comes from outbreak situations. Knowledge about the basic reproduction number, R_0, is useful for modelling the infection and the effect of potential intervention strategies. The reproduction number, R, at steady state, i.e. when the infection has become endemic, is always close to 1, meaning that on average every individual that becomes infected succeeds in transmitting the infection to one other individual during its' infectious period. However, R_0 may still be higher than 1, meaning that when one infectious animal is introduced into a fully susceptible population on average more than one animal will become infected and thus an outbreak is likely to occur. In an endemically infected herd the infection can die out, or a new outbreak may occur and the size of this outbreak is positively related to the proportion of susceptible individuals in the herd (Anderson & May, 1991). This is supported by varying clinical signs over time and fluctuating

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seroprevalence of *Salmonella* Dublin in infected herds that makes it reasonable to assume that even in endemically infected herds, smaller outbreaks are likely to occur intermittently.

The aims of the study were to 1) estimate characteristics of the infectious periods and serological response to infection in calves below 180 days of age, 2) to illustrate fluctuations in prevalence of the infection groups S (susceptible), I (infectious) and R (recovered or resistant) over time and 3) to estimate the transmission parameters, β, R and R_0 for *Salmonella* Dublin spreading among young calves (<6 months) in four Danish dairy herds with long term infection on the premises.

MATERIALS AND METHODS

The estimates were obtained by the use of field data collected in Denmark in 2001-2002 and a generalised linear model relating the number of new infections to the proportion of susceptible and infectious animals per week. The data was collected as part of a large project known as 'the Kongeåproject' through which previous knowledge of the four study herds was gathered (Andersen et al., 2000). These four herds were included in the study because they had several *Salmonella* Dublin positive cultures over a period of at least one year. They were therefore considered endemically infected with the bacteria. Clinical signs of salmonellosis were not obvious in these herds before the study period began. The sample collection was organised so that all calves that were born in the study period (a total of 88 calves) were sampled every 3-4 days for the first four weeks after birth and then once per week. All neighbouring calves in the same barn areas were sampled once per week. In total 181 calves were sampled in the study period. The number of calves varied between 16 and 69 per herd. Calves were sampled between 1-27 times each, on average 9.4 (SD=7.2) times. Every sample event involved collection of an un-stabilised blood sample from the jugular vein and a rectally collected faecal sample. It was attempted to collect a minimum of 25 g of faecal matter at each sampling. However, this often proved difficult in the very young calves. Blood samples were transported to the Veterinary Department of Steins Laboratory in Ladelund for detection of antibodies directed against *Salmonella* Dublin lipopolysaccharide (LPS) as described below.

Bacteriology

Faecal samples were cultured in the above mentioned laboratory for presence of salmonella bacteria by a conventional method described and evaluated elsewhere (Nielsen et al., 2004). The sensitivity of the faecal culture method has been estimated to be between 6% and 32% depending on the age of the animal and whether pooling of samples was used before individual follow-up. In the present study all faecal samples were cultured individually and the calves were very young, so the sensitivity was close to the highest obtainable, probably around 25-30% (Richardson & Fawcett, 1973). All salmonella positive isolates were typed at the Institute for Food and Veterinary Research in Copenhagen and the specificity was, therefore, assumed to be 100%.

ELISA

Blood samples were analysed for presence of antibodies directed against *Salmonella* Dublin O-antigen based LPS using an enzyme-linked immunosorbent assay (ELISA) that has been described in detail and evaluated elsewhere (Nielsen & Ersbøll, 2004; Nielsen et al., 2004). An ODC%-value, which is a background corrected ratio of the test sample optical density (OD) to a known positive reference sample, was calculated for each sample as follows:

$$ODC\% = \frac{\left(\overline{OD}_{sample} - \overline{OD}_{neg\ ref}\right)}{\left(\overline{OD}_{pos\ ref} - \overline{OD}_{neg\ ref}\right)} * 100\% \quad (1)$$

where $\overline{OD}_{sample}$ is the mean value of two test wells, $\overline{OD}_{neg\ ref}$ and $\overline{OD}_{pos\ ref}$ are the mean values of four reference wells in the ELISA plates, respectively. The sensitivity of the serum ELISA at the cut-off value used in the present study (25 ODC%) was approximately 40-46% and the specificity 89-98% for animals between 0-99 days of age. For calves from 100 days and older the sensitivity was estimated to be 82-85% and the specificity 88-97%. The reason for the low sensitivity in calves younger than 11-12 weeks of age is most likely due to a poor capability to produce antibodies by this age group of calves. This was documented in another study and should be taken into account when determining the infection groups from this data material (Da Roden et al., 1992). The non-optimal specificity may be due to maternal antibodies in this age group. Seroconversion was defined as at least a doubling of the ODC% to above 30 between two sample events. These criteria are based on a mix of results from previous studies and practical experience with the ELISA (Robertsson et al., 1982; Nielsen, 2003).

Infection status of the calves

To analyse data for transmission parameters it is essential to determine the infection status (susceptible (S), infectious (I) and recovered/resistant (R)) of all animals during the sampling period. This status was determined for all participating calves per week by both faecal shedding and by serology. In the absence of reasonable sensitivity of the bacteriological culture method serology offers another way to determine the infection status (Veling et al., 2000).

Calves were given a susceptible (S) status at sampling when there was no bacterial growth in the faecal samples and the ODC% was below 25. An infectious (I) status was assigned from the day that calves had a positive bacteriological culture and 17 days onwards. This average period was estimated from the data from culture positive calves (see the results section). Additionally, calves were assigned to infection group "I" based on seroconversion. The infectious period was set to start 36 days prior to the recorded date of seroconversion and 17 days onwards from that date if the calf was below the age of 100 days at time of seroconversion.

Seroconversion in calves older than 100 days lead the infectious period to be estimated to begin 14 days prior to seroconversion and the infectious period would be set to be shorter (12 days). An infectious period was followed by a recovered (R) period for 14 days unless new infection occurred within 14 days. In that case, the calf was defined to be continuously infectious. A resistant period (R) was also assigned to calves that had an ODC% above 25 and were not culture positive. This could for example be newborn calves with maternally derived antibodies or calves that continued to have high antibody levels beyond the designated 14 days recovered period following an infectious period.

Because calves older than one month were sampled on a weekly basis, the time-step for the analyses was a week. Therefore, calves that were sampled twice weekly were assigned the same status for the whole week. When calves changed from S to I and when it recovered (went from I to R), the whole week was assigned I. When calves were losing their maternal immunity (from R to S), the whole week was defined S. New infections were defined each time a calf became infectious after a susceptible period. Examples of infection groups for two calves are shown in Fig. 1A and 1B.

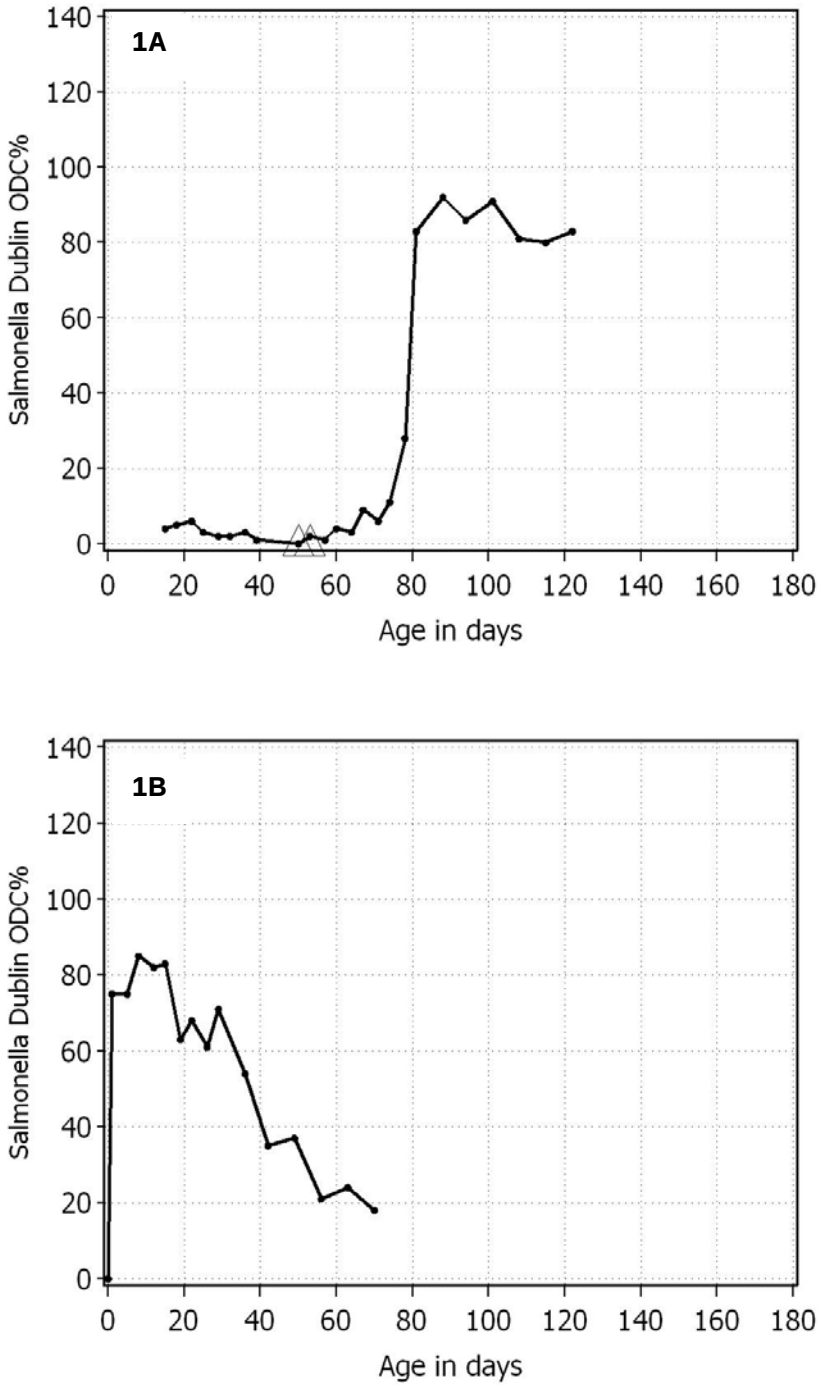


Fig. 1 Antibody measurements (ELISA) (lines), faecal shedding (Δ=positive for *Salmonella* Dublin) and infection status (S, I or R) in two calves. The calf in 1A was defined S from day 17 to day 52, then I until 71 days of age and finally R from 71 to 121 days of age at which point it left the calf barn. The calf in Fig. 1B was defined R from birth to 55 days of age and then S for the remainder of the sampling of that calf. The first sample was precolostral and therefore at 0 ODC%. After uptake of maternal antibodies the ODC% rose to very high levels.

Statistical analysis

To estimate the transmission parameter, β, the framework of a simple SIR-model of transmission of *Salmonella* Dublin between calves was used. The model is illustrated in Fig. 2. Homogeneous mixing of the calves was assumed. Calves were considered born into either the S or the R compartment depending on whether they received *Salmonella*-specific antibodies through colostrum. After an infectious period, calves were considered resistant for at least 14 days or until their antibody levels fell below a cut-off value of 25 ODC%.

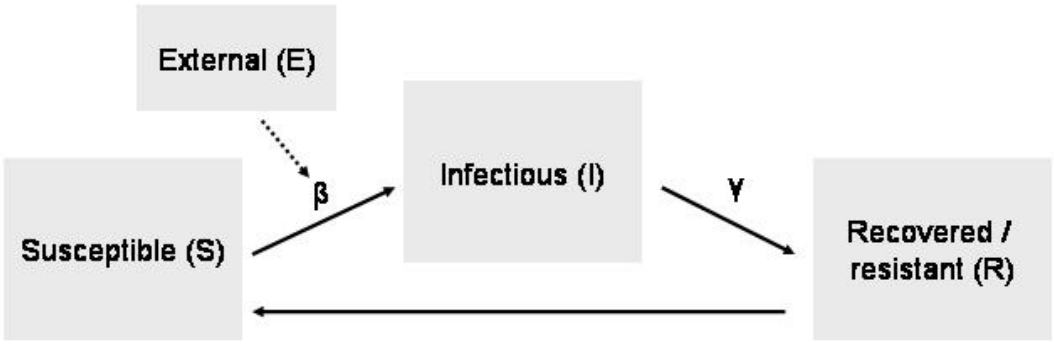


Fig. 2 Compartments and pathways in the SIR-model used for estimation of the *Salmonella* Dublin transmission parameter, β, in four endemically infected dairy herds. E is an external component that allows for new infections to occur due to environmental contamination and γ is the recovery rate.

New infections were assumed to occur at the rate estimated by the following formula:

$$\beta*(S*I+E)/N \quad (2)$$

where β is the infection rate (also called the transmission parameter), S the number of susceptible individuals present, I the number of infectious individuals present, E an external infectious component and N is the total number of animals present in the given time period (Geenen & Döpfer, 2005). According to this model, the number of new infections, C, in each time interval Δt is Poisson distributed and has an expected value:

$$E(C) = \beta \frac{SI\Delta t + E}{N} \quad (3)$$

Thus, log(β) could be estimated with a generalized linear model (GLM) using Proc Genmod in SAS®, version 9.1 (SAS Institute Inc., 2002) with response variable C, log(β(SIΔt+E)/N) as offset (in which Δt is one (week)), and a log link function. The external component (E) was added to correct for the potential infection pressure from the environment of the calves when no infectious calves were present. E was assumed to equal 0.001, which was a number small enough to not influence the β when infectious calves (Is) were present. To estimate the 95% confidence interval for log(β), the standard error (SE) was calculated as the two-sided confidence coefficient assuming a normally distributed variable multiplied by the SE from the model: ln(β) ± 1.96*SE. The overdispersion parameter was estimated from the scaled deviance statistic (McCullagh & Nelder, 1989). The overdispersion parameter allows for possible

dependence between grouped animals. Also, from a more practical point of view, it ensures that any lack-of-fit that remains after careful inspection and possible modification of the model, is reflected by larger standard errors and more conservative inference. An overdispersion parameter close to 1 indicates that the data follow a Poisson distribution.

The reproduction ratio (R_0) is the average number of secondary cases per week produced by one infected individual during the entire infectious period (Diekmann & Metz, 1990). R_0 was estimated by the following formula:

$$R_0 = e^{\log(\beta)} * 1/\gamma \quad (4)$$

where $1/\gamma$ is the estimated average infectious period in weeks.

RESULTS

Time of infectiousness and seroconversion

Based on the laboratory results of 19 calves that shed *Salmonella* Dublin in the study period, the average time of infectiousness (shedding of bacteria) was estimated to be 17 days (range 3-68 days) and the average time from onset of shedding to seroconversion in calves in this age group was estimated to be 36 days (range 11-67 days) (Table 1).

Table 1. Descriptive statistics for 19 calves that were faecal culture positive for *Salmonella* Dublin in four endemic Danish dairy herds

Variables	N	Mean	Std	Median	Min-Max
Age at start of infectious period (in days)	19	40	23	43	3-70
Infectious period (in days)	19	17	19	10	3-68
Age at seroconversion (in days)	10	75	15	76	52-100
Time from start of shedding to seroconversion	10	36	17	28	11-67

Transmission

The sizes of the infection groups S, I, R and the total number of animals per week in each of the four study herds are illustrated in Fig. 3.

Table 2 contains the results of the log-linear regression for the four herds as fixed effects and Table 3 contains the estimate across all four herds with a correction for repeated observations within the herds. The net reproduction number R was calculated as R_0*proportion of susceptible animals. The log-linear model with the four herds as fixed effects was overdispersed (scaled deviance of 4.8) and correction for over-dispersion resulted in larger confidence intervals around the R_0. The average infectious period in weeks that was used to estimate R_0 was 2.43 weeks (17 days/7 days per week).

Table 2. Transmission parameters for *Salmonella* Dublin in young calves in four Danish dairy herds based on an average infectious period of 17 days

	Log(β)	SE	P	R_0	95% CI	Proportion S	R
Herd 1	-0.83	1.26	0.51	1.06	0.09-12.51	55%	0.6
Herd 2	-0.02	0.63	0.98	2.39	0.69-8.20	37%	0.9
Herd 3	-0.35	0.89	0.59	1.72	0.30-9.86	54%	0.9
Herd 4	0.03	0.83	0.94	2.49	0.49-12.57	52%	1.3

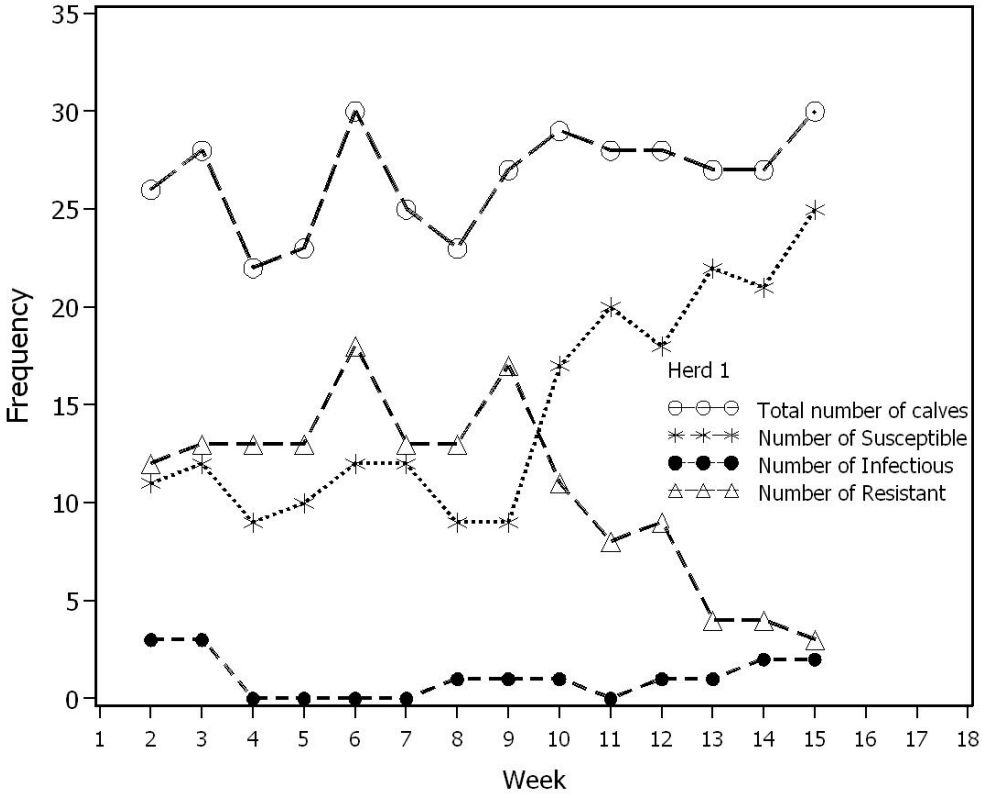
Table 3. Transmission parameters for *Salmonella* Dublin in young calves across four Danish dairy herds based on an average infectious period of 17 days

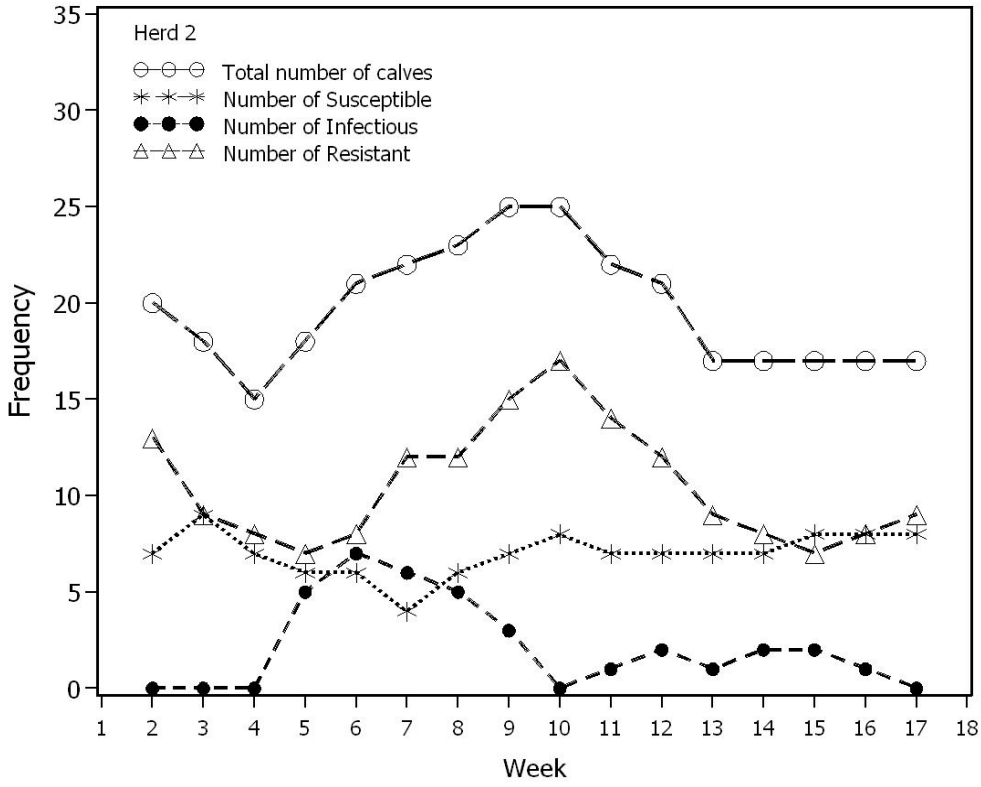
	Log(β)	SE	P	R_0	95% CI
Across herds	-0.21	0.13	0.11	1.96	1.51-2.55

Though the estimates appear to vary between the herds, the confidence limits are very wide and therefore significant difference between herds was not found. Figures 3a-d illustrate the fluctuations in the size of the different infection compartments in the model during the study period according to the data and the definitions. Herd 2 has a peak in infections, which one may consider a small outbreak among the calves in weeks 5-8. This is reflected in the R_0 estimate for this herd. Herd 4 appeared to have a similar outbreak in weeks 10-12, but the herd was very small and thus there were only few calves available for the model estimations resulting in a very wide confidence interval. Across herds, the R_0 estimate of *Salmonella* Dublin is significantly higher than 1, and the results indicate that upon introduction to a fully susceptible calf population an infectious calf would on average infect approximately two other calves and therefore be likely to cause an outbreak to occur.

DISCUSSION

The data set was unique in that all calves in four herds were sampled at least once a week for 12 weeks. The data were reasonably suitable to estimate the transmission rate of *Salmonella* Dublin based on the new cases in each time period in young calves, however, the data only covers calves up to 180 days of age. It would be preferable to be able to include several age groups or the entire herd; however, such data collection is extremely time-consuming an expensive, in particular if bacteriological culture needs to be performed on all samples. Since ELISA measurements do not give a very good indication of whether an animal is infectious, recovering from infection or a latent carrier, bacteriological culture is needed for this type of studies. On the other hand, it is known that conventional bacteriological culture also lacks sensitivity in cattle faecal samples and correct classification is therefore very difficult to obtain for this infection.





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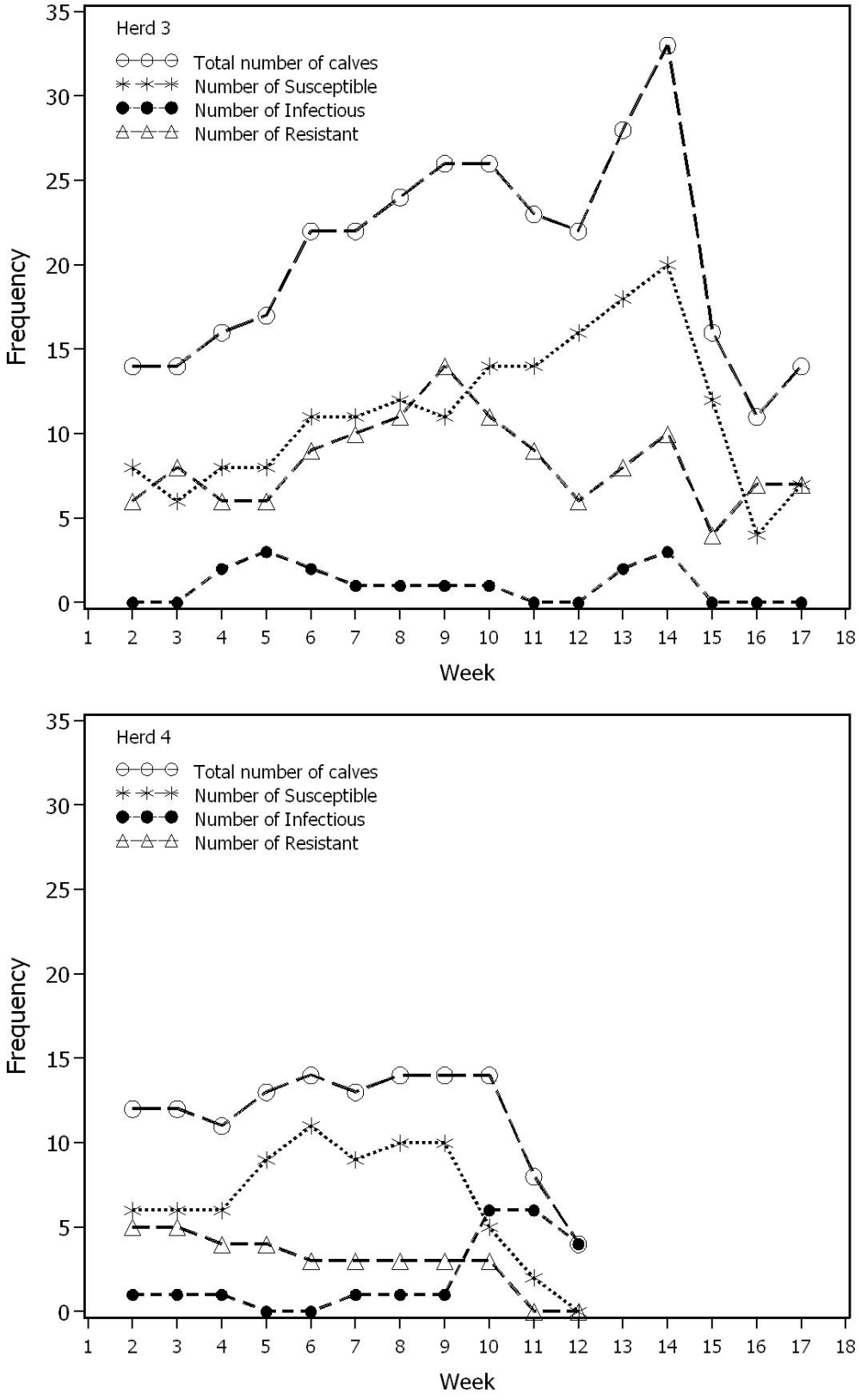

Fig. 3 The dynamics of the size of infection groups S, I, R and the total number of calves, N, in the population per week of the study period in four dairy herds. The large fluctuation in N is due to bull calves being sold from the herds around two weeks of age and movement of calves in groups between barn areas.

The time between each sampling should be as short as the average generation interval, i.e. the time from one animal becomes infectious to the time the second case from that animal becomes infectious. For Salmonella Dublin the generation interval is probably only between 3-7 days, which is why at least weekly samples are required.

The R_0 estimates around 2 indicated that *Salmonella* Dublin does not spread very rapidly through a susceptible population under management systems similar to the ones in these herds. Being an infection that primarily spreads via the faecal-to-oral route this makes sense. Direct contact between calves does not necessarily lead to transmission of infection, whereas a high contamination level of the environment from infectious calves in may lead to transmission. The number of calves per herd was too small to determine the differences between herds but there was an indication that in some herds *Salmonella* Dublin may spread faster than in others, and that smaller outbreaks occurred during some time periods. This may be due to hygienic measures in the herds, housing and management of the calves. Earlier studies on the risk factors for the spread of *Salmonella* Dublin confirm that herd management and co-infections with other diseases such as BVD and liver-fluke may aggravate an outbreak (Wray and Roeder, 1987; Veling et al., 2002).

The point estimate for the net reproduction ratio R was between to 0.6-1.3 which was expected because the herds were infected with *Salmonella* Dublin for several years and thus were in an endemic situation. However, as the data illustrated in Fig. 3 suggest that under endemic situations there may be fluctuations in the proportion of susceptible animals leading to the net reproduction ratio and thereby the transmission of bacteria between animals to increase periodically, whereas during other periods the herd immunity level would be sufficiently high that no or very little transmission of bacteria would occur.

The model fit to the data was not very good. The model was overdispersed, which indicates that there was less variation in the number of new infections than expected and the standard errors of the transmission rate had to be inflated to correct for this effect. The poor fit was probably a result of the fact that at times there were no infectious calves in the herd but new cases did occur (Fig.3). Therefore, an external component (E) was included in the model with a very low, constant value. The effect of this component was negligible in the situation that infectious calves were present.

The fact that new infections occurred without infectious calves present may be a result of environmental contamination from previously infectious calves as mentioned above or difficulty to properly define the infection status of a calf. The average time from onset of shedding to seroconversion in calves (36 days) was higher than that seen in adult cows (10-15 days). Few studies were available to aid in defining the infectious periods and recovery rates, and the studies that existed were based on clinical experiments, but they confirmed the time of infectiousness in this study (Robertsson, 1984). However, the individual variation in infectious periods and time of onset of infectiousness was not included in the analyses. The external component could also have been an environmental compartment related to the number of infectious animals in the lactating herd and survival of the bacteria in the environment (Wray et al. 1989). The next steps will be to include the parameters in a stochastic simulation model, in which the heterogeneity among calves and the infectiousness of other age groups in the herd can be included.

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ESTIMATED ECONOMIC LOSSES DUE TO *NEOSPORA CANINUM* INFECTION IN DAIRY HERDS WITH AND WITHOUT A HISTORY OF *NEOSPORA CANINUM* ASSOCIATED ABORTION EPIDEMICS

C.J.M. BARTELS*, H. HOGEVEEN, G. VAN SCHAIK, W. WOUDA AND TH. DIJKSTRA

SUMMARY

In this study, direct economic losses due to *Neospora caninum* infection, based on actual data from *N. caninum* seropositive reference herds and from herds that experienced an *N. caninum*- associated abortion epidemic, were calculated using a stochastic model with random elements. The results demonstrated that 76% of seropositive reference herds had no economic losses, whereas in the remaining 24% of herds, the economic losses went up to maximally €2000 per year. In epidemic abortion herds, economic losses continued after the actual event of the abortion epidemic for at least two more years with average costs of €50 per animal per 2 years. In both herd situations, highest losses were related to premature culling of seropositive cows and to a lesser extent to the effects of abortion (extended calving interval and age of first calving).

INTRODUCTION

Infection with *Neospora caninum* is a major cause of reproductive failure in cattle in many countries (Dubey, 2003), causing potentially considerable economic losses (Chi et al., 2002; Hogeveen & Van der Heijden, 2003). In addition to abortion, effects of bovine neosporosis may include stillbirths and neonatal mortality, early foetal death and resorption manifested as return to service, increased time to conception, increased culling, reduced milk production and reduced value of breeding stock (Trees et al., 1999). There have been studies investigating the effect of *N. caninum* serostatus on culling (Thurmond & Hietala, 1996; Cramer et al., 2002; Tiwari et al., 2005), milk production (Thurmond et al., 1997; Hernandez et al., 2001; Hobson et al., 2002; Romero et al., 2005) and reproductive performance (Jensen et al., 1999; López-Gatius et al., 2005; Romero et al., 2005) in dairy cattle. The results of these studies showed that the effect of *N. caninum* infection is not the same in different situations.

In various countries, among which The Netherlands, control strategies are being promoted (Dijkstra et al., 2005). To the authors' knowledge, these control strategies are not supported by some form of cost-benefit calculations. However, two studies have estimated the production losses due to *N. caninum* (Chi et al., 2002; Hogeveen & Van der Heijden, 2003). These studies were based on a normative model for both the epidemiological effects of *N. caninum* and the

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economic consequences of these effects. Therefore, the objective of the present study was to determine the direct economic losses of *N. caninum* infection related to culling, reproduction performance and milk production based on actual data from randomly selected *N. caninum* seropositive herds and from herds that experienced an abortion epidemic associated with *N. caninum*.

MATERIALS AND METHODS

Selection of herds and testing of animals

From a group of 108 randomly selected Dutch dairy herds of a previous study (Bartels et al., 2006a), all *N. caninum* seropositive herds (N=83) were included in the present study as the 'reference group'. No specific information on *N. caninum* abortion occurrence was available. Another group of 17 dairy herds was included in the study as 'epidemic abortion group'. Owners of these herds had contacted the Animal Health Service (AHS) between April 1997 and November 2000 because of epidemic abortion outbreaks (Wouda et al., 1999*)*. The first contact was between one month and one year after an abortion epidemic. Infection status for *N. caninum* was determined by detection of *N. caninum* antibodies using an AHS in-house ELISA-method (Wouda et al., 1998, Von Blumröder et al., 2004). The results of the ELISA-method were calculated as S/P ratio = {(optical density (OD) test sample - OD negative control)/(OD positive control - OD negative control)}. A cut-off S/P ratio of < 0.5 was defined as negative (N), an S/P ratio of 0.5-1.5 as low positive (LP), and an S/P ratio > 1.5 as high positive (HP).

Economic simulation model

A multi-process Monte Carlo stochastic simulation model was developed to estimate the economic losses of *N. caninum* infection and *N. caninum* associated abortions. The model was built in a spreadsheet (Microsoft Excel) with @Risk add-in software (Palisade Corporation, Newfield NY, USA). The basic process in the model is the animal (cow or pregnant heifer). For every iteration, each animal receives a number of characteristics (parity and with parity one or higher, milk production and calving interval) and a *N. caninum* infection status (Fig. 1). Depending on the *N. caninum* status, abortion might occur. Culling due to *N. caninum* might occur as a consequence of abortion or unrelated to abortion. The following direct economic losses were distinguished: premature culling, increased calving interval (for animals with a parity of one or higher), increased age at first calving (for pregnant heifers), additional inseminations, milk production losses and veterinary and diagnostic costs. Economic losses were calculated over a one-year period and three different herd infection states were distinguished: reference herds, herds in first the year following an abortion epidemic and herds in the second year following an abortion epidemic. Probabilities of events and consequences of events were dependent on the herd infection status. Within each iteration, multiple processes (animals) were run simultaneously to simulate a herd. Results of these individual processes were cumulated to determine the economic losses at the herd level.

Model input on cow characteristics and events

Based on data from the Dutch Dairy Cattle Improvement Organisation (NRS, Arnhem, The Netherlands), the milk production (kg per lactation) of a cow was based on a normal distribution with an average of 8,500 kg (sd 700 kg). The calving interval of a cow was based on a Pert distribution with a minimum, most likely and maximum value of 365, 400 and 450 days respectively. Based on the parity prevalence in the herds studied, parity of a cow had the

following probability: parity 0 (pregnant heifer): 18%; parity 1: 25%, parity 2: 18%, parity 3: 14%, parity 4: 10%, parity 5: 6% and parity ≥ 5: 9%.

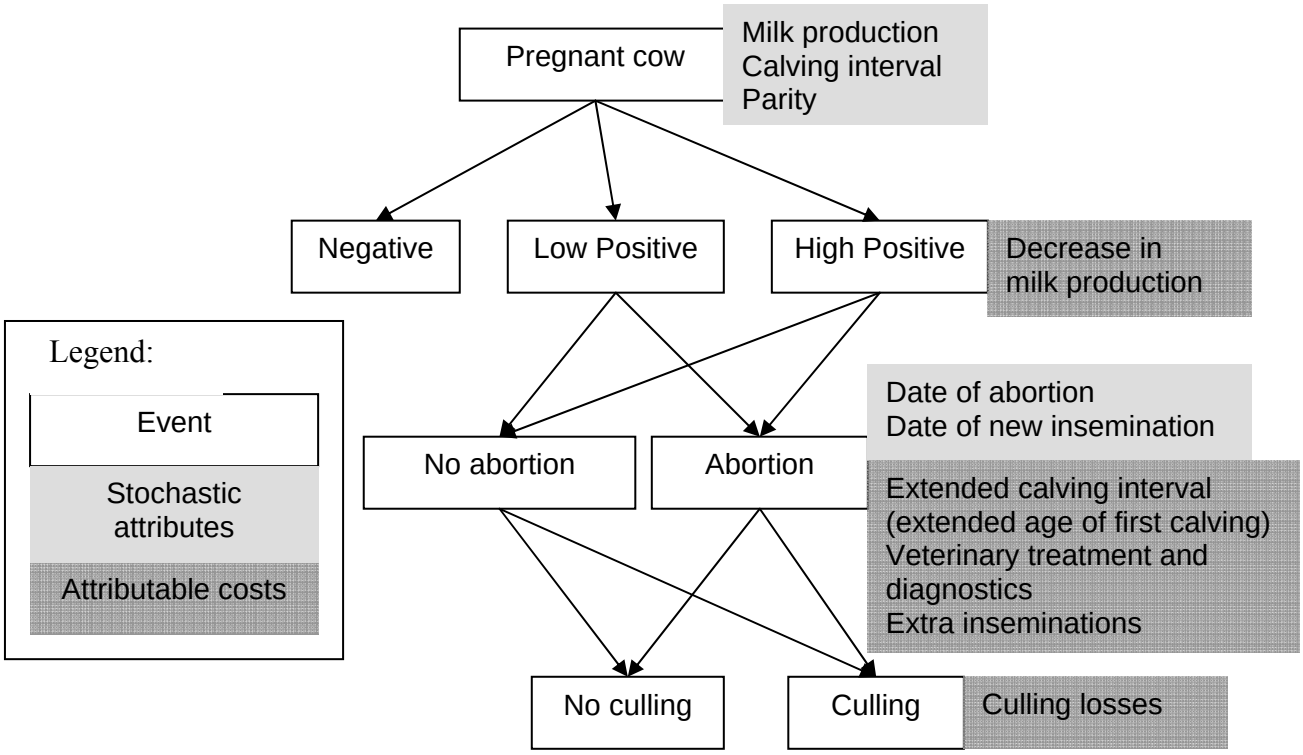

Fig. 1 Schematic overview of the stochastic model to estimate effects of *N. caninum* infection in Dutch dairy herds.

Events (Table 1) were modelled as discrete distributions and were based upon a previous study on the effects of *N. caninum* serostatus on culling, milk production and reproduction performance (Bartels et al., submitted). The prevalence of infection in reference herds (10.8%) was much lower than in epidemic abortion herds (47.0% and 34.4% in the 1st and 2nd year after abortion epidemic respectively). In reference herds, the proportion of HP cows was 40% of the seropositive cows, where as in epidemic abortion herds, HP cows consisted up to 75% of seropositive cows. In that study, significant effects of LP and HP animals compared to N animals were found for culling (all herd types), milk production (epidemic abortion herds in the 1st year after an abortion epidemic) and abortion (epidemic abortion herd types only). The effects of *N. caninum* have been quantified by relative measures such as hazard ratios for culling and odds ratios for abortion. However, the economic simulation model made use of probabilities of events occurring, given the cow status N, LP or HP. Using the model output, the simulation model probabilities have been calculated as follows. In reference herds, the culling data fitted a Cox Proportional Hazards model best. The base line hazard for culling (the probability of a N cow being culled on a particular day, given that it had not been culled up to that day) was multiplied by 365 to calculate the cumulative hazard for culling over a one-year period. Subsequently for HP cows, it was multiplied by the hazard ratio (1.6) found for HP cows. The hazard ratio for LP cows was not significant. In epidemic abortion herds, a parametric survival model with Weibull distribution and accelerated failure time fitted the data best. The cumulative hazards in the first and second year after the abortion epidemic were estimated by visual inspection of the cumulative hazard graph based on the model estimates ('cumhaz' after 'streg',

STATA/SE 8.2). For abortion, the base line abortion rate for N cows was calculated using the constant, $\beta 0$, from the logistic regression model as:

$$\text{Prob (abortion | N cow)} = \frac{1}{1 + e^{-\{\beta 0 + \beta jXj + \beta j * serostatus)}} \quad (1)$$

where $\beta_j X_j$ accounted for the estimated effect of parity and production level and β_j* *serostatus* accounted for the effect of serostatus. For LP and HP cows, the estimated parameters for abortion in LP and HP cows were added to Eq. 1 to calculate abortion rate in LP and HP cows respectively.

Table 1. Probabilities (in percentages) of cow characteristics and events for different herd infection status (seropositive reference herds, herds 1 year after an abortion epidemic and herds 2 years after an abortion epidemic) and different serostatus (N = negative, LP = Low Positive and HP = High Positive), used as input data `for the modelling of economic losses due to *N. caninum* in Dutch dairy herds.

Input data	Category	Serostatus	Herd infection status		
			Reference	Epidemic abortion 1st year	Epidemic abortion 2nd year
Within herd prevalence		N	89.2	53.0	65.6
		LP	6.2	14.0	8.7
		HP	4.6	33.0	25.7
Culling	Heifer	N	4.1	17.0	25.0
		LP	4.1*	25.1	27.7
		HP	4.1*	37.5	51.5
	Cow	N	24.0	5.1	12.8
		LP	24.0*	8.8	21.2
		HP	36.6	11.7	20.6
Abortion rate	Heifer	N	7.6	13.6	10.1
		LP + HP**	7.6*	26.9	16.0
	Cow	N	6.6	6.5	5.6
		LP + HP**	6.6*	14.2	9.1

* Probabilities for LP or HP were not statistically different from negative cows.
** Probabilities for LP and HP were not statistically different from one another.

Model input on economic consequences of N. caninum

Economical losses due to *N. caninum* caused by premature culling were estimated using a calculation of the retention pay-off. The retention pay-off is the difference in expected net revenue (in terms of present value) of the culled cow and a replacing heifer, corrected for the costs to buy or raise this replacing heifer. In the simulation model, different retention pay-off values were used based upon the production level and parity of the cow culled (Houben et al, 1994, updated for the Dutch market situation in 2004). Maximum retention pay-off was €1,332 for a cow in parity 3 with a relative production level of 120 % and the minimum retention pay-off was - €9 for a cow with a relative production level of 85 % and a parity of 3 or higher. The

costs for culling of a pregnant heifer were estimated to be €1,000 (normative; based upon the costs to raise a heifer). If a cow aborted and was not culled afterwards, abortion would result in a longer calving interval. This extension of the calving interval was estimated based on the simulated abortion date plus the number of days to a next simulated conception plus the duration of a full pregnancy. Results of Jalvingh and Dijkhuizen (1997, updated for Dutch market circumstances) were used to calculate the economic consequences of a non-optimal calving interval.

Table 2. Description of input variables used in the modelling of the economic losses due to *N. caninum* in Dutch dairy herds.

Variable	Distribution	Values	Reference
Costs for replacement of a culled cow (€)	constant	Retention pay-off	Houben et al., 1994, values updated for 2004.
Costs for replacement of a heifer (€)	constant	1000	Expert opinion
Stage of gestation (days) at abortion	pert	128 (min 128-max 260)	Bartels et al., submitted
Number of extra inseminations after abortion (cow and heifer)	discrete	Prob(1 ins) = 0.1 Prob(2 ins) = 0.8 Prob (3 ins) = 0.1	Expert opinion
Days to new conception after abortion (cow)	pert	71 (min 3-max 105)	Expert opinion
Days to new conception after abortion (heifer)	constant	50	Expert opinion
Costs for extra days to next calving (€)	constant	Optimized calving pattern	Jalvingh and Dijkhuizen, 1997, values updated for 2004
Costs for extra day to 1st calving (€)	constant	1.50	Expert opinion
Costs for additional insemination (€)	constant	18	Expert opinion
Costs of decreased milk production (€/kg)	constant	Milk prod. loss * costs of decreased milk production	Marginal costs to have more cows on the farm
Costs of vet consult (€per hour)	constant	100	Royal Dutch Society for Veterinary Medicine
Costs of laboratory investigations € for serology, € for post mortem	constant	 10 75	Animal Health Service Ltd.

Depending on the length of the calving interval, the costs of an extended calving interval varied from €0.30 to €1.15 per day. The economic losses due to *N. caninum* abortion were calculated by subtracting the damage of the initial calving interval from the damage of the extended calving interval. For pregnant heifers, a similar approach was followed, except that the time from abortion to new conception was taken as a constant. The economic damage of an increased calving age of the heifer was set as a normative value of €1.50 per day. The number of additional inseminations could be one, two or three (discrete distribution). The cost of one additional insemination was set to €18.

An effect on milk production was only found in HP cows in the 1st year after an abortion epidemic (Bartels et al., submitted). Although the effect was significant, the production loss in terms of kg milk per cow was not high (-0.32 (95%CI: 0.01-0.63) kg milk/day for the first 100 days in milk) (data not presented) and rounded off to 1% decrease in production. The economic

losses associated with decreased milk production, were calculated by multiplying the milk production losses with the costs of a decreased milk production. The latter costs (€0.07 per kg) were based upon the marginal costs of having more cows in order to fill the milk quota. It was assumed that there were no opportunity costs for additional labour and barn requirements and that the farmer had enough roughage available.

The costs for treatment, veterinary consult and laboratory investigation were based on a number of assumptions. As part of the monitoring of brucellosis-free status, Dutch farmers are obliged to test aborting animals serologically for brucellosis. Expenses for the veterinary visit and sampling of the cow are financed through a nationwide monitoring system. However, when 3 or more cows aborted, it was assumed that herdsmen wanted to have additional advice from the veterinarian (15 minutes at €100 per hour) on treatment and prevention with a likely outcome that more animals were tested and an aborted foetus was submitted for post mortem investigation.

Simulation and sensitivity analysis

Each simulation was run with 5,000 iterations with 65 simultaneous processes (simulating a herd seize of 65 animals). In a sensitivity analysis, baseline output resulting from default values was compared to output based on alternative values. For the sensitivity analysis, a farm in the 1st year after an abortion epidemic was used. The reason for this choice was that in this situation, most effects of *N. caninum* serostatus were incurred and this would provide the largest discriminating effect of the changed parameter. The following parameters were varied such that it was relevant for the Dutch situation: herd size (from 45 to 150 cows); probability of abortion in HP cows (increasing from 10 to 50% compared to LP cows); costs of culling a cow (from 50% to 150 % of the default costs) or a heifer (from 75 to 125% of the default costs); value of decreased milk production (from 170 to 210% of the default costs).

RESULTS

Reference herds with 65 cows had on average 7 seropositive animals (90% CI: 3-11). The mean economic losses of *N. caninum* infection in seropositive dairy herds were €117 per year, as an effect of premature culling of HP cows. However, no economic losses were present on 76% of dairy herds whereas in 5% of situations the costs were €1000 or higher. These costs were applied when the culled animal was a heifer and all costs made to raise the heifer were lost (results not presented). In the situation of an epidemic abortion herd, the number of seropositive animals was on average 31 (90% CI: 24-37) in the first year after an abortion epidemic. Mean economic losses in the first year were €2,053 (90% CI: €454 - €4,174) (Table 3). The highest costs were related to premature culling of *N. caninum* seropositive animals (€1,485 (90% CI: €863 – €3,317)) and extended calving interval or increased age at first calving (combined €376 (90% CI: €0 - €1,175)). The economic losses for reduced milk production were considerably less with €105 (90% CI: €70 - €140) while costs for treatment and diagnostics (€49 (90% CI: €0 – €235)) and extra inseminations for aborted cows (€38 (90% CI: €0 - €105)) were minor. In the second year after an abortion year, the mean number of seropositive animals in a herd of 65 cows was 22 (95% CI: 16-28). There was no longer an effect of *N. caninum* infection on milk production and effects on culling and abortion were less than in the first year after an abortion epidemic. Consequently, economic losses were €1,216 (90% CI: €0 – €2,924) primarily based on costs due to premature culling of seropositive animals. Thus, in addition to the costs at the time of an abortion epidemic, the mean economic losses in the two consecutive years after an

abortion epidemic amounted to €3,269 or €50 per animal in the herd. Premature culling of seropositive animals accounted for 78% of these costs, while the extended calving interval and age at 1st calving accounted for 16% of these costs. Reduced milk production (3%), treatment and diagnostics (2%) and extra inseminations (2%) contributed only little to the total costs.

Table 3. Economic losses (€) due to *N. caninum* infection in Dutch epidemic abortion herds in first and second year after an abortion epidemic.

	1st year after abortion epidemic			2nd year after abortion epidemic		
	5th percentile	Mean	95th percentile	5th percentile	Mean	95th percentile
Total	454	2,053	4,174	0	1,216	2,924
Premature culling	86	1,485	3,317	0	1,058	2,618
Extended calving interval	0	161	512	0	72	335
Extended age at 1st calving	0	215	663	0	63	334
Extra inseminations	0	38	105	0	16	60
Reduced milk production	0	104	140	0	0	0
Treatment and diagnoses	0	50	235	0	7	87

Sensitivity analysis

As a default, the herd consisted of 65 animals. When reducing or increasing the number of cows per herd, there was no effect on the average cost per animal. The 95% percentile costs reduced with increasing number of cattle in a herd (Table 4).

Table 4. Effects of changing base line input values on estimated economic losses due *N. caninum* infection in Dutch dairy herds in the first year after experiencing an abortion epidemic.

	Variation	Effect on mean	Effect on 95 percentile
Number of cows compared to 65	45	3%	+ 11%
	105	No effect	-18%
Costs for culling compared to default	-50%	-18%	-11%
	+50%	+18%	+13%
Value of culled heifer (compared to €1000)	-25%	-8%	-13%
	+25%	+10%	+13%
Increasing abortion risk for HP compared to LP cows	+10%	+1%	+2%
	+20%	+4%	+4%
	+50%	+14%	+10%
Costs for reduced milk production compared to €0.07/kg milk	+70%	+4%	+2%
	+115%	+6%	+3%

The greatest effects on economic losses were found when different assumptions on prices for animals that had to be culled prematurely and replacement costs for replacing heifers were considered. When the abortion risk in HP animals was increased compared to LP animals then the economic losses increased maximally 14% in case of 50% increased abortion risk in HP animals. The effect of changing prices for reduced milk production was small because milk production loss was a minor effect.

DISCUSSION

The objective of this study was to calculate economic losses due to *N. caninum* infection in dairy herds with and without a history of *N. caninum* associated epidemic abortions. The results demonstrated that for 76% of seropositive reference herds, there were no economic losses due to *N. caninum* infection. In the remaining 24% of herds, the economic losses ran up to maximally €2000 in the exceptional situation that two seropositive heifers were culled prematurely. For epidemic abortion herds, the economic losses continued after the actual event of the abortion epidemic for at least two more years. These costs were on average €50 per animal per 2 years and were in addition to the losses at the time of to the abortion epidemic (premature culling, prolonged calving interval and age of 1st calving, milk production losses, treatment and diagnosis).

The economic losses were calculated using a stochastic model. This kind of model provides the possibility to account for naturally existing variation. In addition to a mean outcome value, stochastic modelling provides information about the variation around a mean value (Dijkhuizen & Morris, 1997). The input data for the stochastic model were taken from a study on the effect of *N. caninum* infection in Dutch dairy farms (Bartels et al., submitted). In this study the effect of *N. caninum* infection on culling, reproductive performance and milk production was quantified based on actual data from these farms. There was no effect found of *N. caninum* serostatus on abortion, contrary to the fact that *N. caninum* infection is primarily an abortifacient agent. It was argued that abortion events were underestimated because these events were defined by recorded calving and insemination dates and not by notification of expulsion of a calf foetus. Most likely this underestimation was 'compensated' by the fact that HP cows were culled shortly after an abortion.

Two other studies (Chi et al., 2002; Hogeveen & Van der Heijden, 2003) have looked into the economic losses due to *N. caninum* based on previous studies and expert opinion. Chi et al. estimated the direct production losses (premature voluntary culling, loss of milk yield from abortion and abortion) and treatment costs (veterinary services, medication cost and extra farm labour cost) of *N. caninum* infection in the Maritime provinces of Canada at $2,304 (€1,921) annually per 50 cow herd. They used a partial budget model adapted from a spreadsheet suggested by Bennett (1999). Highest costs were associated with abortion and included an assumed 28% loss in milk yield for each aborting cow (Bennett et al., 1999). Hogeveen and Van der Heijden (2003) had estimated the average annual direct costs at €249 with a maximum of €5,604 for a herd with 50 lactating cows. Their estimates were based on cataloguing the economic effects of neosporosis on partial budgeting while the physiological effects of infection were based on literature information. In comparison with the results from the current study, Hogeveen and Van der Heijden (2003) used 3.5% milk loss in a seropositive cow, a higher percentage of premature culling after abortion and a normative value (180 days) for the extended calving interval after abortion. In the present study, using actual data as input for stochastic models of different herd situations, estimated economic losses due to *N. caninum* infection were

much smaller for the most common herd situation (i.e. seropositive herd with no history of an abortion epidemic).

The results of this study can be used as part of a cost benefit analysis on the control of *N. caninum* infection. Currently, control of *N. caninum* infection is focused on separating dogs from cattle, testing of aborting cows and keeping the within-herd seroprevalence low (Dijkstra et al., 2005). For the latter control measure, bulk milk testing has been evaluated (Bartels et al., 2005). In their study, Bartels et al. (2005) demonstrated that a negative bulk milk test result, for 85% of herds correctly predicted a within-herd seroprevalence below 15%. Regular testing of bulk milk might prove an useful monitoring tool, combining sampling ease with low costs for testing. A further study is needed to determine if the costs for regular bulk milk testing outweigh the potential economic losses in dairy herds. For herds experiencing an abortion epidemic, the results of the present study give better insight of the extent of direct costs following the abortion epidemic. For these farmers, knowledge of the potential economic losses will allow them to make better choices among various control options to reduce the effects of high seroprevalence in herds after an abortion epidemic (Dijkstra et al., 2005).

ACKNOWLEDGEMENTS

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A MODEL OF THE EMERGENCE OF INFECTIOUS PANCREATIC NECROSIS VIRUS IN SCOTTISH SALMON FARMS 1996-2003

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SUMMARY

Infectious pancreatic necrosis virus (IPNV) is a very widespread pathogen in the Scottish salmon farming industry. Because it was notifiable and subject of official controls, an extensive data set is available on the prevalence of IPNV. Combined with a well documented industry, these data have allowed the construction and testing of a model of the spread of IPNV between farm populations. This model uses a standard Susceptible – Infected (SI) epidemiological model to describe the spread of infection. This model is implemented in both freshwater and marine phases, with marine farms being supplied with smolts from one or more freshwater sites. There is no loss of infection from a population until it is harvested. The model indicates major reductions are required in transmission in both the freshwater and marine environments if IPNV is to be eradicated. Obtaining smolts from more than one source also increases marine infection pressure substantially. Even extensive improvements in marine biosecurity are ineffective on their own – while forming a vital part of a co-ordinated eradication strategy.

INTRODUCTION

Emerging diseases are diseases that spread to new areas or new species or express themselves with increased virulence in existing host populations (Krause 1998). Such diseases are increasingly reported from marine populations, partly due to environmental stresses such as pollution and climate change (Harvell et al., 1999). Aquaculture may create situations where disease emergence is enhanced and indeed many novel disease problems have been reported from cultured fish and shellfish (Murray & Peeler, 2005).

Epidemiological models are widely applied to the study of human health (Anderson and May, 1979) and in agriculture (Kao, 2002). However, although they have been applied to analysis of the general principles of disease spread in fish population (Reno, 1998) and to disease of fish in single populations such as experimental tanks (Smith et al., 2000, Ogut et al., 2005) or within a farm (Lotz et al., 2003, Revie et al., 2005) they do not appear to have been applied to the study of an epidemic spreading between fish farms.

The following paper describes the development and testing of a model of the spread of a virus through Scottish salmon farms using an extensive data set on the prevalence of infectious pancreatic necrosis virus (IPNV) obtained as part of an official pathogen monitoring programme (Anon, 2003). The model indicates that prevalence of the virus is most sensitive to controls on

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freshwater transmission, in spite of the relatively lower prevalence in freshwater farms. However, eradication would require a major improvement in controls in biosecurity in both freshwater and marine farms.

MATERIALS AND METHODS

Data have been collected on the presence of IPNV in Scotland as a result of a systematic surveillance program aimed at disease control and carried out by official fish health inspectors (Anon, 2003). Freshwater sites were sampled at least annually and marine farms were sample at least once every 2 years, in practice sampling often occurred more frequently. Typical samples were of 30 fish sampled in 6 pools of 5, although sample size varied. If any fish in a sample was positive the sample was considered positive. The data and its analysis are described by Murray et al., (2003), however here the data are extended to 2003 and freshwater sites used to hold broodstock are excluded as these are essentially marine fish held in freshwater.

The substantial quantity of data available allows assessment of the prevalence of IPNV in Scottish salmon farms. The dynamics of the fish farming industry are also well recorded (Stagg & Smith, 2002, Raynard et al., 2005). In such circumstances it is reasonable to use this information to build, constrain and test a model of the spread of IPNV through the industry.

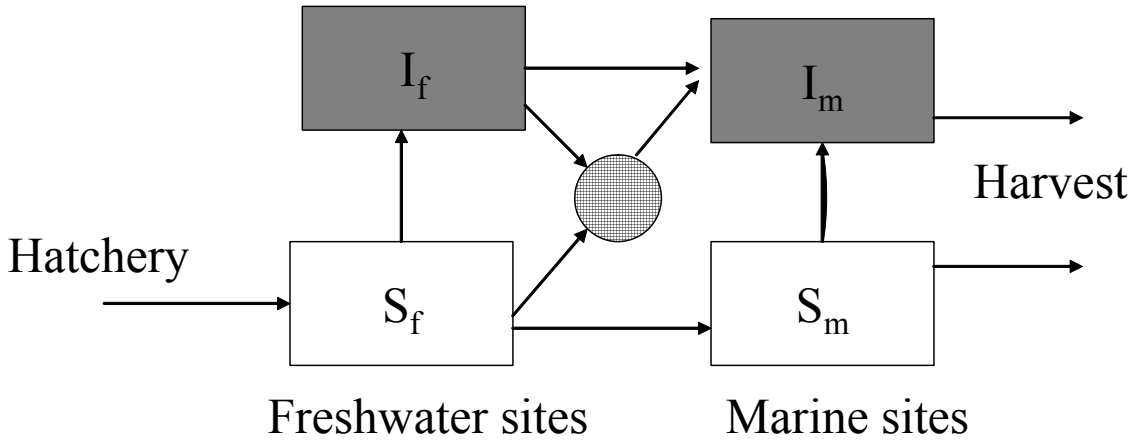

Fig. 1 Model structure used to simulate IPNV epidemiology in farmed Scottish salmon. I = infected, S = susceptible farm populations, f = freshwater and m = marine. Susceptible populations can become infected in either environment, and if marine sites receive smolts from both infected and uninfected populations they will be infected.

The model developed combines standard epidemiological approaches with the industry's structure (Fig. 1). In the model salmon spend 1 year in freshwater and are then transferred as juveniles (known as smolts) to seawater where they spend 18 months before being harvested (in practice these times may vary). Salmon may become infected with IPNV in either freshwater or seawater (Anon, 2003). Marine sites frequently receive smolts from more than 1 freshwater site (Raynard et al., 2005); in this simple model all marine sites use the same number of sources of smolts. If any of the freshwater sources supplying smolts is infected then the marine site is infected. Once infected, a population remains infected until it is harvested since infection may

persist in populations for years (Yamamoto, 1975). However, once a site is harvested and fallowed, a history of infection does not influence the likelihood of future infection. This is supported by a study showing a lack of a link between history of IPNV infection that is over 2 years old and recent IPN disease (Raynard et al., 2005), and by an unpublished analysis of infection persistence (Murray, unpublished). Young fish (fry) that input to the freshwater component of the model are assumed free of infection due to extensive broodstock testing (Anon, 2003).

The equations of the model are for freshwater:

$$dS_f/dt = s - m_y b_f S_f I_f - sS_f \quad (1)$$

$$dI_f/dt = m_y b_f S_f I_f - sI_f \quad (2)$$

Where s is the rate of input and output (=1/12 month^{-1}) and S_f and I_f are the proportions of the population that are in susceptible or infected farms. The transmission coefficient is b_f, however to allow for density dependent transmission with increased biomass (since I and S are proportions, valid I + S = 1) the parameter m_y is used (Eq. 3).

In a growing salmon population, IPNV transmission rate depends on whether this transmission is density independent (m_y = 1) or density dependent for which the following formula has been fitted from observations (Stagg & Smith, 2002):

$$m_y = g \times \ln(\text{year} - 1995) + 1 \quad (3)$$

The value of m_y depends on the fitted constant g, the best value for which is 0.388 for total Scottish farmed salmon production, but different regions have had different rates of growth. This logarithmic function has a value of 1 for 1996 (the start of the simulation) and indicates production is increasing, but at a slowing rate.

In marine sites a similar situation exists to freshwater, except that introduced (input) smolts can be of either infected or uninfected stocks, with the proportion X infected, dependent on a function of the proportion in the freshwater sites and the number of smolts used. The rate of input and of harvesting out is h (= 1/18 month^{-1}) while the marine transmission coefficient is b_m.

$$dS_m/dt = h(1 - X) - m_y b_m S_m I_m - hS_m \quad (4)$$

$$dI_m/dt = h\,X + m_y b_m S_m I_m - hI_m \quad (5)$$

The proportion of sites supplied with infected smolts X is:

$$X = I_f k/[1 + I_f(1 - k)] \quad (6)$$

X depends on the proportion of freshwater sites infected I_f and the number of smolt sources used by marine sites k, typically 2 to 3 (Raynard et al., 2005). Different model scenarios have been run using different assumed numbers of smolt sources.

The model's simulated prevalence is fitted to observations, first in freshwater (where prevalence is unaffected by the marine component), and then the marine component is fitted. In both cases only 1 parameter (b_f or b_m) and 1 initial value (the prevalence of infection I_f or I_m) are

altered to fit the model. Because only 2 factors are searched at a time it is possible to conduct a systematic investigation of parameter space until a minimum sum of squares difference between observed and simulated prevalence is found.

The result of this model fitting is thus values for the transmission coefficients in both the freshwater (b_f) and marine (b_m) environments. These can be used to find R_0 (the average number of secondary cases generated by the primary case) values (Reno, 1998) for both environments. For freshwater sites:

$$R_0 = m_y b_f / s \quad (7)$$

And for marine sites:

$$R_0 = m_y b_m / h \quad (8)$$

Since R must be reduced below 1, these parameters give insight into how far current policies are from bringing IPNV under control.

The R_0 value for the marine environment can be used to evaluate the role of multiple sources of smolts in the infection pressure of IPNV to marine farms. The value generated when k = 1 fits the model to observations by incorporating the effects of both transmission between marine sites and use of multiple sources into a single parameter, while if k is a more realistic value (2 or 3) the effect of multiple sources is explicitly simulated. Therefore the value of R_0 for k = 3 relative to that when k = 1 shows the proportion of total infection pressure that is due to transmission between marine sites, and hence the remainder is the proportion due to the use of multiple smolt sources.

RESULTS

IPNV has become increasingly prevalent in Scottish salmon farms in both freshwater and especially in marine environments. Prevalence is considerably higher in marine sites than in freshwater sites but has increased in both: 40% - 90% for marine sites and 5% - 25% for freshwater sites (Fig. 2).

The model has been fitted to the observations, as described in the methods, in both density dependent and density independent forms. The density dependent model produces a close fit to observations of marine and freshwater prevalence (Fig .2). The model over-estimates marine IPNV prevalence in 2000, this may be related to an epidemic of infectious salmon anaemia which was brought under control by improved biosecurity and culling and may have also improved IPNV control. The model is fitted to the entire data series and as a result of correcting for 2000 it underestimates some of the later values. The density independent model (not shown) provides a very similar fit to the density dependent model. Both versions of the model provide a good description of the observations.

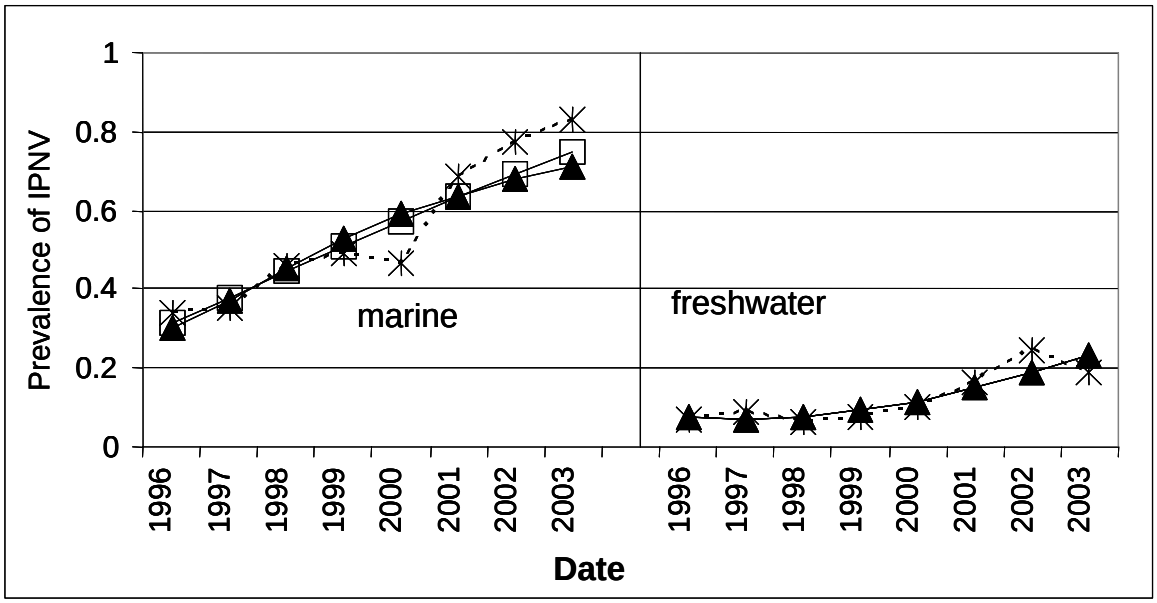

Fig. 2 Observed and density dependent simulated prevalence of IPNV in freshwater and marine environments. Stars: observations; triangles: k =1, squares: k =3 (simulations identical in freshwater) where k = number of freshwater sources of smolts

Marine prevalence can be fitted using different numbers of sources of smolts (1 and 3 shown in Fig. 2). The fit is similar and the optimum value of k cannot be distinguished based on model output. However, using other data on the numbers of smolt sources used by farms (Raynard et al., 2005), the different dynamics of the model under different values of k are used to analyse the role of multiple smolt sources in the current high prevalence of IPNV in the next few paragraphs.

An important outcome from the model for understanding the dynamics of IPNV is the R value (Reno, 1998). This parameter's value (Table 1) shows the extent to which transmission must be reduced to eradicate IPNV. The R value for the density dependent model has increasing values through the simulation as the farmed population increased.

Mean density dependent R values are quite similar to density independent R values, so both models indicate similar simulated transmission patterns. However, under the density dependent model R has increased as population has increased and so the final value is considerably higher. The implication of this elevated final R is that control is more difficult than indicated by density independent modelling.

Table 1. Model R values calculated for freshwater and marine water under assumption of 1, 2 or 3 freshwater sources of smolts (k) per marine site. DI = density independent, DD = density dependent, with initial (1996), mean, and final (2003) values shown

	Freshwater	Marine k = 1	Marine k = 2	Marine k = 3
DI R	1.41	2.13	1.75	1.45
DD 1996 R	0.86	1.40	1.17	0.98
DD mean R	1.31	2.14	1.77	1.48
DD 2003 R	1.57	2.55	2.12	1.77

In freshwater density independent R = 1.41, this means freshwater transmission would have to be reduced by 29% to eradicate freshwater IPNV. The mean density dependent R = 1.31, indicating a reduction of 24%. However, by 2003 R had increased to 1.57, indicating transmission would have to be cut by over a third (by 36%) to stamp out freshwater IPN and the required cut rises as population continues to rise under the density dependent model.

Marine R values are calculated for 3 different scenarios where k = 1, 2 or 3. When k = 1, b_m (and hence R) effectively incorporates both transmission between sites and the effect of the use of multiple smolt sources, given the rapid rise in IPNV prevalence the value of this is large (2.13 for density independent and 2.14 for mean density dependent values) or 2.55 for density dependent by 2003. However, if a typical marine farm is assumed to use 3 sources of smolts the values are lower at 1.45, 1.48 and 1.77 indicating cuts in marine transmission of 31% - 43% are required (if accompanied by eradication from freshwater). If the true number of sources of smolts is 2 the indicated cuts required for marine eradication are just over 50% of b_m by 2003. As in freshwater, the required cuts under the density dependent simulation rise with further increases in production.

If it is assumed a typical farm receives smolts from 3 (k = 3) sources (Raynard et al., 2005) then the mean marine R is decreased from 2.14 to 1.48 indicating about 30% of total infection pressure is due to use of multiple sources (and 70% transmission between marine sites). If k = 2 is more typical, 17% of total infection pressure is due to use of multiple sources. It is impossible to eradicate marine IPNV while IPNV exists in freshwater sites that supply the marine farms, but if freshwater IPNV were eradicated the value of k would be irrelevant. While IPNV does exist in freshwater, the use of multiple smolt sources adds significantly to infection pressure, even under the assumptions of low numbers of smolt sources used.

DISCUSSION

The availability of data has allowed the construction and testing of a model of IPNV's emergence to become a very widespread pathogen in Scottish marine salmon farms (90% farm level prevalence) and a common freshwater farms as well (25% farm level prevalence). This model uses simple epidemiological models and a model of the structure of the industry, with freshwater and marine production components, to describe the observed spread of IPNV infection. The simulation of observations is good, although an anomalously low prevalence following an ISA epidemic does distort the data in 2000.

Having obtained an understanding of the dynamics of infection implied by the model for conditions applying under the period of observations (1996-2003) this model can be projected to predict how the epidemic would continue to emerge under no change of policy (Fig. 3). The model is therefore run for a further 8 years (2004-2011), assuming the industry continues to grow at the rate determined by Eq. 3. Major changes in policy, specifically, cuts of transmission of about 50% are required, in both marine and freshwaters, to eradicate IPNV and that cutting the number of smolt sources would reduce marine infection pressure by about 30%; this is pressure in excess of marine inter-farm transmission which would still (for eradication) require a 50% cut even if the sources are cut to 1. The effects of such cuts singly or in combination are simulated.

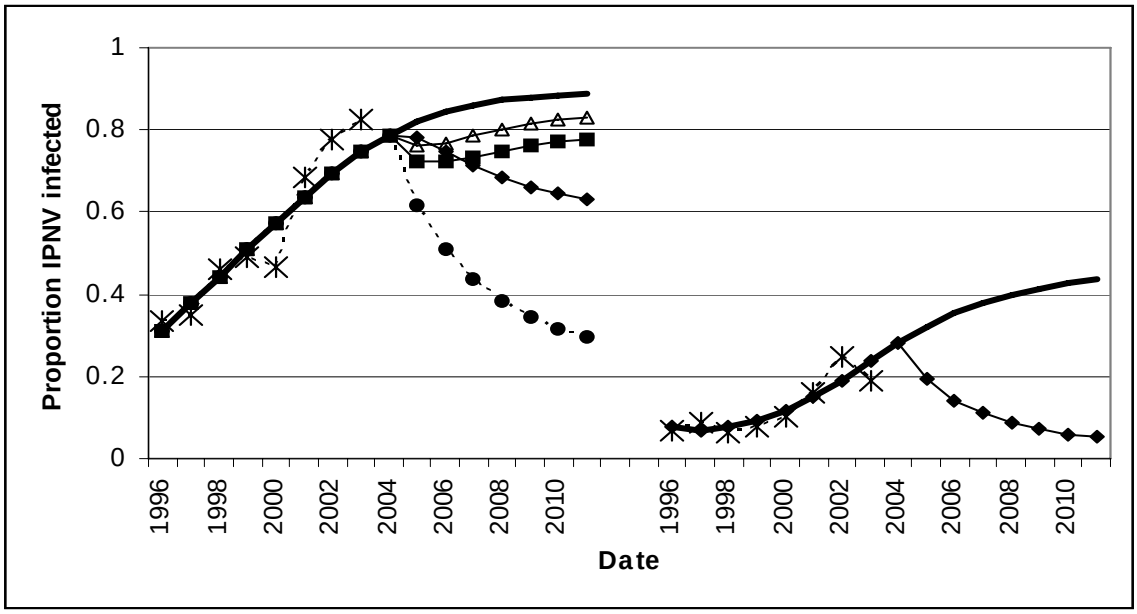

Fig. 3 Extension of simulations of density dependent IPNV prevalence over 8 years (2004-2011) based on maintenance of control policies and rates of expansion of production (solid line) or on implementation of controls based on 50% reduction in b_m (triangles), or 50% reduction in b_f (diamonds) or cutting k from 3 to 1 (squares) or combination of all three strategies (circles on dashed line).

The extended simulations indicate that marine IPNV prevalence will not increase much above the current very high level of about 90% over the next 8 years (Fig. 3). IPNV prevalence in freshwater farms is predicted to increase somewhat, to infect about 40-50% of sites, but this increase too becomes very slow by the end of the simulated period as infection approaches a dynamic equilibrium. In Shetland, where high IPNV prevalence occurred earlier than elsewhere, a similar equilibrium has already occurred with 90% prevalence in marine sites and 50-60% in freshwaters (Fig. 4). The freshwater equilibrium value is higher than predicted to occur elsewhere because the freshwater rate of spread is significantly more rapid in Shetland (Murray et al., 2003).

The modelling indicates that improved controls on marine transmission have only a minor effect on marine IPNV prevalence, because they are counteracted by rising prevalence in freshwater and hence increased input of already infected smolts to the marine farms. Similarly (and for the same reason), reducing the number of sources of smolts used has limited effectiveness. The only approach that has any effect in freshwater in this simulation is to control transmission in freshwater, but this approach also is far more effective at controlling marine prevalence than are direct controls based on marine biosecurity. When the controls are applied in combination the reduction in IPNV prevalence is dramatic, in spite of the rather ineffectual result of marine controls implemented on their own.

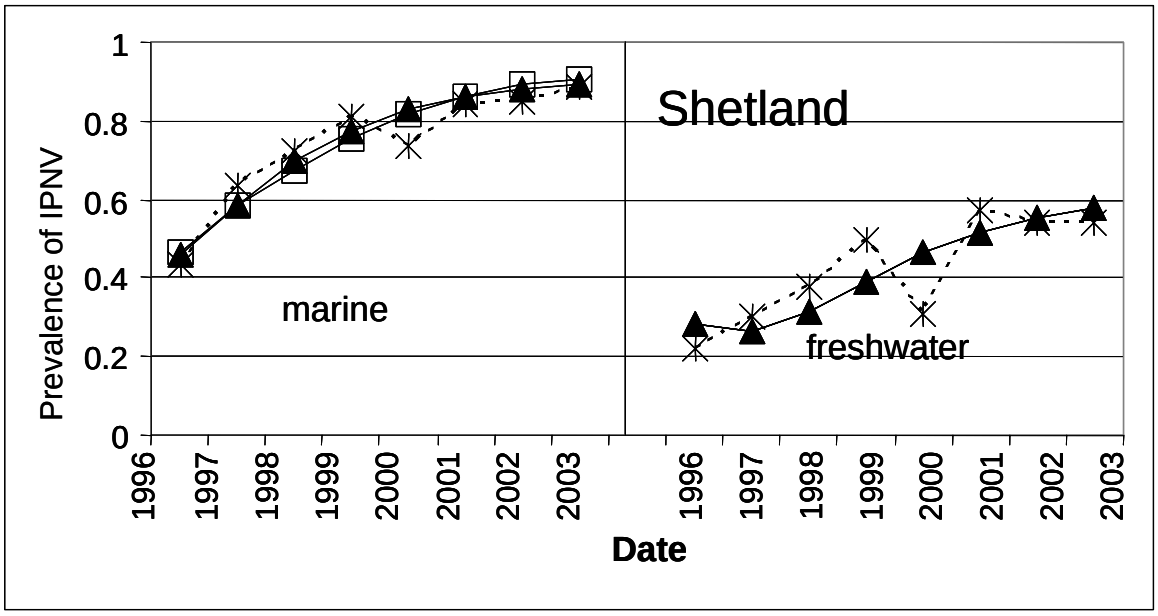

Fig. 4 Observed and simulated prevalence of IPNV in salmon farms sampled in the Shetlands (symbols as Fig. 2)

These predicted patterns under density independent simulations (not shown) are similar to the density dependent simulations shown, however predicted prevalences are lower and response to cuts more pronounced. This is because R is not increasing in density independent simulations and so is relatively smaller by 2003 and in the subsequent years. If transmission consists of a mixture of density dependent and density independent processes (or indeed is dominated by density independent processes) then slightly weaker controls may be effective at eradicating IPNV. However, the requirement for co-ordinated controls on freshwater and marine farms remains the same if IPNV were to be eradicated.

Given the very large improvements needed to eliminate IPNV, it seems unlikely that the effort required would be cost effective. An alternative method of elimination is the culling of infected stocks, again the high prevalence of IPNV in the Scottish industry makes this impracticable. Culling may be worthwhile in the initial stages of an epidemic and appears to have been successful in keeping Sweden IPNV free (Ariel & Olesen, 2002). An effective vaccine against IPNV would protect both vaccinated stocks and, by reducing infection pressure, unvaccinated stocks. Vaccines may interfere with eradication programs by masking infection. However in the absence of an effective eradication programme, development of improved vaccines and use of husbandry measures to prevent infection causing disease (Raynard et al., 2005) may be the most effective way to minimise impact of IPNV.

In the absence of effective control or eradication, Scottish authorities have been unable to legally enforce import controls against IPNV. Controls on internal movements are questionable when imports are not controlled and as a result the control policy based on movement restrictions has ceased. Weakening of controls may lead to an increase in the dynamic equilibrium value and hence rises in prevalence especially in freshwater. With this change in policy the immediate need for data on IPNV status has been lost and so sampling has been greatly reduced.

Revie et al. (2003) have simulated the prevalence of sea lice in Scottish salmon farms and has used this approach to look at the effectiveness of intervention strategies in the control of this parasitic copepod. Viral diseases affecting shrimps have also been modelled at the farm level, including Taura Syndrome Virus (Lotz et al., 2003). Modelling has also been used to analyse spread of disease in experimental tank situations, for example generating data on the effects of population density change within a population on IPNV (Smith et al., 2000) and furunculosis (Ogut et al., 2005) spread. In this simulation of spread between farms at regional and national scales the current model appears to be novel, at least with such a high quality of supporting data to constrain and validate the simulation.

The simple model used to describe IPNV can be applied to the modelling of other diseases between salmon farms, particularly ones causing infection in both the freshwater and marine environment (such as bacterial kidney disease or furunculosis). It can also be applied to pathogens only or largely present in the marine environment (such as ISA) or freshwater environment (such as *Gyrodactylus salaris*), indeed in such cases the model would be simplified by exclusion of one environment. The units might differ, for example for *G. salaris* the unit might be a river basin not an individual fish farm. The requirements are good data on infection and an understanding of the pathogens' different behaviours, such as reservoirs of infection, which may require modification of the model's structure.

ACKNOWLEDGEMENTS

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SPATIAL ANALYSIS

INVESTIGATING THE CLUSTER OF MYCOBACTERIUM BOVIS SPOLIGOTYPE 13 STRAINS IN THE SOUTHEAST OF ENGLAND

R. SOARES-MAGALHAES*, N.H. SMITH, J. DALE, K. GOVER, A. MITCHELL, J. MAWDSLEY AND N.G. STOKER

SUMMARY

The East Sussex county in the south-eastern part of England has been traditionally regarded as a 'hot-spot' for bovine tuberculosis, although adjacent counties are apparently free of the disease.

In this study a comprehensive epidemiological analysis of *Mycobacterium bovis* infections in cattle and badgers in East Sussex was performed by investigating the clonal relationship between isolates using three genotyping techniques. To understand the spatial distribution of clones of *M. bovis* spoligotype 13 amongst cattle and badgers in E. Sussex cluster analyses were conducted. In the case of cluster analysis for badgers a comprehensive map of badger setts for E. Sussex was used as an estimate of the badger population at risk.

INTRODUCTION

Mycobacterium bovis (*M. bovis*) is primarily the causative agent of bovine tuberculosis although it is able to infect a remarkable wide range of wild and domestic mammal hosts (Morris et al., 1994). Disease caused by *M. bovis* infection in cattle herds presents a major challenge for animal and human health because it is zoonotic (Grange & Yates, 1994).

In Great Britain and the Republic of Ireland Eurasian badgers (*Meles meles*) are recognised as the principal wildlife reservoir of the disease; several culling strategies to control the disease in this maintenance host have been used since the first link with bovine TB has been identified (Murhead & Burns, 1974; Donnelly et al., 2003). However, despite of the control policy based on regular tuberculin testing of national cattle, with compulsory slaughter of animals showing evidence of exposure, in the past two decades, the incidence of bovine TB in British herds has steadily increased (Krebs et al., 1997; Bourne et al., 2001).

Comprehensive epidemiological investigations of *M. bovis* infections in cattle are now possible using genotyping techniques such as spoligotyping and VNTR (Durr et al., 2000). These molecular techniques allow good discrimination between strains and assist in the systematic analysis of cattle herd breakdowns, by investigating the clonal relationship between

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isolates from cattle and associated wildlife (Durr et al., 2000; Haddad et al., 2001; Skuce & Neill, 2001; Goodchild et al., 2003).

Traditionally, TB incidence rates in cattle herds have been high in counties in the southwest part of England and Wales (Wilesmith, 1983). In the county of East Sussex, bovine TB cases where usually reported in the South Downs area; in 1986 the county was attested no longer endemic but a low risk infection coming from the badger population was assumed to be present (Wilesmith et al., 1986). Presently, cattle and badgers in E. Sussex are almost exclusively infected with *M. bovis* strains of spoligotype 13, and strains of this spoligotype are rarely recovered elsewhere in the UK. Furthermore, a unique chromosomal deletion has been identified in four strains of spoligotype 13 from E. Sussex (J. Inwald, personal communication).

To further understand the geographical localisation of *M. bovis* strains in the UK the frequency, distribution and links between cattle and badger *M. bovis* strains in E. Sussex and spoligotype 13 strains recovered outside E. Sussex were investigated. Data from the spoligotype database, the cattle tracing system (CTS) database, geographical information system (GIS) data and a comprehensive badger sett map from the Southeast of England was used. Molecular epidemiological data using genotyping techniques (such as VNTR and unique spoligotype 13 deletion analysis) have also been analysed for those strains.

MATERIALS AND METHODS

Mycobacterium bovis isolates

Mycobacterium bovis isolates recovered from East Sussex between April 1989 and October 2004, available at the Veterinary Laboratory Agency – Weybridge / TB research group laboratories, were primarily selected to be used in this study. The majority of isolates recovered from badgers were isolated after a localised culling operation performed during 1997. In addition, *Mycobacterium bovis* spoligotype 13 isolates recovered from counties other than East Sussex for the same study period were used.

Molecular epidemiological studies

Spacer Oligonucleotide typing (spoligotyping): This molecular method identifies polymorphism in the direct repeat (DR) region of *M. bovis* strains. The DR region is composed of multiple, virtually identical, 36-bp repeats interspersed with unique DNA spacer sequences of similar size. In spoligotyping, the DR region is amplified by PCR, followed by hybridisation of the labelled PCR-product to immobilised spacer oligonucleotides. Spoligotype patterns are recognised by the presence or absence of hybridisation signal. Spoligotype methodology was carried out according to Kamerbeek et al. (1997).

As part of the bovine tuberculosis control program, all cultured isolates of *M. bovis* from Great Britain are routinely submitted to the Veterinary Laboratory Agency (VLA, Weybridge, U.K.) for spoligotype analysis. The VLA spoligotype database presently holds typing information on over 20,000 *M. bovis* strains isolated from 1975 to 2004 (with 95% of the data for strains isolated since 1997).

Variable Number Tandem Repeats (VNTR): The VNTR typing system identifies polymorphism in the number of repeats at tandemly arranged repetitive DNA sequences similar to eukaryotic minisatellites. Six loci, named A to F, scattered through out the *M. bovis* genome

are used and amplified by PCR using a pair of primers to each loci. The VNTR genotype of a strain, representing the number of repeat elements at each locus, is presented as a series of six integers. However, VNTR D is known to contain a 24 bp deletion in one of the tandem repeats. The naming conversion at this locus reflects the presence of this deletion by a (*). Additionally, analysis using VNTR F can be complex because the locus contains a 79 bp tandem repeat and a 55 bp tandem repeat. Thus the potential sizes of the PCR products of the F locus are permutations of 79 bp and 55 bp tandem repeats, to a maximum of 5 copies to each of them. The PCR technique was applied according to the method of (Supply et al., 2001) but using a fluorescent based method on polyacrylamide gels and a semi-automated allele naming software (Genotyper® – Applied Biosystems).

Deletion analysis: A unique 1795 bp deletion affecting four genes (*Rv3403c-Rv3406*) was previous identified in strains of spoligotype 13 by microarray analysis. This deletion was absent from 9 of the most common spoligotypes found in Great Britain (ten strains of each spoligotype tested, J. Inwald, personal communication) and from spoligotype 13 strains from Ireland (twenty strains tested, J. Inwald, personal communication). The presence or absence of this deletion in a strain was identified by PCR using a pair of flanking primers. The product of the PCR reaction was analysed on agarose gels and compared with standard size markers.

Spatial epidemiological studies

The cattle tracing system (CTS) database records the movements of all cattle since June 2001. It also contains records from 1996 of the cattle passport scheme. The database was queried using standard methods. Georeferenced cattle holding location and numbers from East Sussex were collected from the TB50 and VetNet databases held by the Veterinary Laboratories Agency.

The badger sett map data used in this study was collected by E. D. Clements ('Clems') chief recorder of badger setts for the Mammal Society. The data was collected (on bicycle) between 1960 and 1990. The full map covers approximately 10000 km^2 in the southeast of England and records 1088 main setts. The map should be used as an indication of relative sett density. Main badger setts can remain in occupation for centuries (Roper, 1993) so it would be expected that the distribution remains the same - if it has not increased in number following badger protection in 1997. Anecdotal evidence (recent study in the Newhaven Valley site by T. J. Roper) suggests that all the setts shown on this map are still in occupation.

The data represented on this map also includes information about the bedrock geology of the sites. It was used to show that 87% of setts are in areas of Hastings beds or chalk (55% of the area) whereas only 13% of setts are found on clay soils (45% of total area). Roper (1993) observed that most of the setts recorded on clay are in fact in sandy outcrops too small to appear on the bedrock geological map.

Cluster analysis

Test for clustering and tests for detecting the most likely clusters of spoligotype 13 infection in cattle and badgers in East Sussex were performed. Georeferenced cattle holding CPH data and the 'Clems' badger sett map have been used as the host population at risk data. The 'Clems' map was georeferenced using ArcScan, a georeferencing tool from ArcGIS 9.0. A total of 706 badger setts belonging to E. Sussex were georeferenced enabling the construction of a badger sett kernel density surface.

Spatial first order properties of the distribution of spoligotype 13 infection in cattle and badgers in East Sussex County were investigated using kernel intensity functions for spoligotype 13 cases and the host population at risk. This enabled the construction of extraction maps that indicated the spatial relative risk (RR) of infection with spoligotype 13. Focused tests for clustering were performed using spatial scan statistics (Kulldorff & Nagarwalla, 1995) using SaTScan™ v.6. These aimed at the investigation of second order (local) properties of infection with spoligotype 13 for both cattle and badgers and indication of the most likely cluster of infection. An advantage provided by the spatial scan statistic is its ability to take into account the heterogeneous distribution of the population at risk.

For the focussed cluster analysis the special grid coordinates tool was used incorporating the centroid coordinates of the object in the extraction maps with the highest RR estimate. The centroid was estimated using the calculation (W+E)/2 and (S+N)/2, where W and E are the longitude of the most extreme western and eastern points of the spatial object, respectively and the S and N are the latitude coordinates of the most extreme southern and northern points, respectively.

The case population was assumed to follow a Bernoulli probability distribution and scanning windows of up to 50% of the population at risk were used to identify clusters of spoligotype 13. A likelihood ratio test was calculated for each window and the distribution of the likelihood ratio and its corresponding *P*-value was obtained by Monte-Carlo simulation, set at 999 iterations.

RESULTS

Spoligotype frequencies in E. Sussex

The frequency of each spoligotype identified amongst isolates from E. Sussex recovered between April 1989 and November 2003, and the associated animal host, is shown in Table 1.

Table 1. Spoligotype frequency and host for strains from East Sussex

	Frequency of isolates by host species		
Spoligotype	Cattle	Badgers	Other mammals [a]
13	87	60	
9	4	1	1
10			4
17	1	2	
25		1	

[a] primarily deer

More than one isolate was frequently taken from an outbreak of bovine TB in a cattle herd. Therefore, the 92 *M. bovis* strains recovered from E. Sussex cattle represent 35 herd breakdowns. The majority of cattle strains tested by spoligotyping are of spoligotype 13. Most badger spoligotypes in E. Sussex are also spoligotype 13. The majority (90%) were recovered in 1997 as part of a systematic badger culling operation. Cattle strains recovered prior to 1997 were from herds in close geographical proximity to this badger culling operation. Since 1997, the range of cattle infected with strains of spoligotype 13 has apparently expanded.

A cattle herd breakdown in 1990 gave a single isolate of spoligotype 9 and in 1991 a spoligotype 9 infected badger sett was found close by. Because spoligotype 9 isolates were recovered prior to 2001 no data was available on the CTS database. The spoligotype 17 strain from E. Sussex was recovered from an animal that had been imported from Gloucestershire where strains of this spoligotype are common.

It was found that the majority of cattle isolates of *M. bovis* (94%) from E. Sussex are of spoligotype 13 and the same strain is frequently recovered from badgers in the area.

VNTR types of strains in E. Sussex

VNTR types were obtained for 126 strains of *M. bovis* isolates from cattle and badgers in E. Sussex, shown in Table 2.

Table 2. Spoligotypes and VNTR types of strains isolated from cattle and badgers in East Sussex

Cattle			Badgers		
Spoligotype	VNTR type	Frequency	Spoligotype	VNTR type	Frequency
13			13		
	7353*33.1	63		7353*33.1	58
	8353*33.1	1			
17			17		
	7555*33.1	1		7655*33.1	1
9			25		
	6555*33.1	1		6554*23.1	1

All but one of the spoligotype 13 strains isolated from cattle from East Sussex share the same VNTR pattern (7353*33.1). The variant VNTR pattern (8353*33.1) is closely related. All badger isolates tested share the same common VNTR pattern found in cattle.

Deletion typing of strains in E. Sussex

A sample of strains of different spoligotypes isolated from cattle and badgers from E. Sussex were tested for the presence of spoligotype 13 specific deletion (Table 3).

Table 3. Presence of spoligotype 13 specific deletion in strains of *M. bovis* isolated from cattle and badgers in East Sussex

	Spoligotype				
	Cattle		Badgers		
Deletion	9	13	13	25	17
present		30	19	1	
absent	1				1

All spoligotype 13 strains tested from both cattle and badgers had this deletion. As expected, two strains of spoligotype 9 and 17 isolated in E. Sussex retain the region. However a single strain of spoligotype 25 apparently contains the spoligotype 13 specific deletion. This strain has

a typical VNTR type for strains of spoligotype 25 (6554*23.1), and has been re-spoligotyped several times.

It was found that most cattle and badgers in E. Sussex are sharing a single clone of *M. bovis* identified by spoligotype, VNTR pattern and a unique deletion.

Spoligotype 13 outside E. Sussex

Twelve strains with spoligotype 13 were recovered from cattle outside of East Sussex in five counties, shown in Table 4.

Table 4. Frequency and VNTR type of cattle and badger *M. bovis* isolates of spoligotype 13 outside E. Sussex

Host	County	Frequency	VNTR [a]
Badgers			
	West Sussex	1	7353*33.1
	Kent	1	NA
	Hampshire	1	NA
Cattle			
	Wiltshire (1990)	7	7356*33.1
	Wiltshire (1999)	1	7554*33.1
	Cornwall	1	7356*33.1
	West Sussex	1	7353*33.1
	Somerset	1	7353*33.1
	Buckinghamshire	1	7353*33.1

[a] The VNTR pattern of the East Sussex clone is 7353*33.1. NA: Not available

The strains recovered in Wiltshire were from two different breakdowns (seven strains in 1990 and one strain in 1999). The index cases from West Sussex and Buckinghamshire were born in East Sussex and share the same VNTR pattern and unique deletion as the E. Sussex clone. It has not been possible to identify a movement link by CTS between the outbreak in Somerset and E. Sussex, although this strain has the same VNTR pattern and bores the unique deletion as the E. Sussex clone.

It has not been possible to identify a movement link by CTS between the outbreaks in Wiltshire (1999) and E. Sussex; these strains bear a different VNTR pattern than the E. Sussex clone. Cattle tracing data is unavailable for the cattle breakdowns from Cornwall and Wiltshire (1990). Isolates from both these areas have a VNTR pattern different from the clone in E. Sussex. All cattle strains tested bore the unique spoligotype 13 deletion.

Three strains are available from badgers isolated in counties other than E. Sussex (see Table 4). The strain from West Sussex shared the VNTR pattern of E. Sussex strains; the other two strains were unavailable for analysis.

It was found that the cattle index cases from Buckinghamshire and West Sussex have been exported from E. Sussex as identified by VNTR type, deletion presence and CTS analysis; the East Sussex clone is rarely seen outside East Sussex despite the intense animal movement from E. Sussex as investigated by CTS.

Geographical distribution of Spoligotype 13 strains in E. Sussex

E. Sussex has 108 parishes. Of these, 18 parishes have a 1 yearly herd-testing interval for bovine tuberculosis, 13 parishes have a two yearly testing interval and 91 parishes are under a 4 yearly testing interval.

Between April 1989 and November 2003 herd breakdowns occurred in 14 different parishes located in the southern part of the county. From the ninety-two *M. bovis* isolates from cattle, 87 are Spoligotype 13 (Table 1). The geographical localisation of spoligotype 13 strains isolated from cattle and badgers in E. Sussex are shown in Fig. 1; all spoligotype 13 isolates are localised to 12 parishes in the southern E. Sussex parishes.

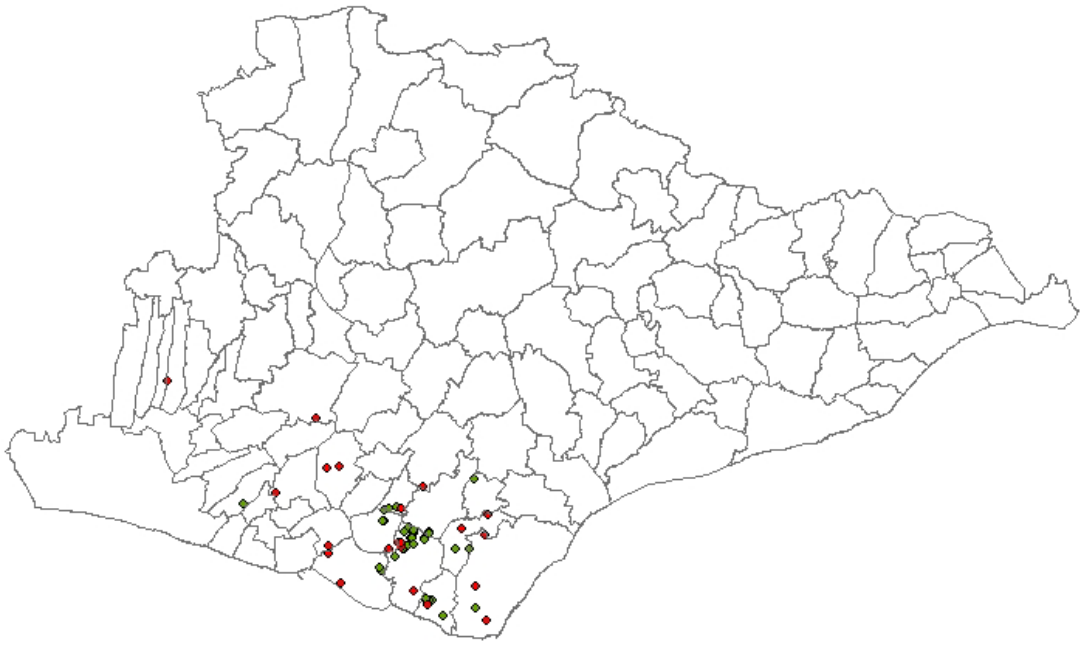

Fig. 1 Geographical distribution of herd breakdowns involving strains of spoligotype 13 (in dark grey) and badger isolates of spoligotype 13 reported between April 1989 and October 1997 (in light grey) [majority of badger isolates recovered in a systematic badger culling operation in 1997; parish boundaries are shown]

Cluster analysis of cattle and badger spoligotype 13 infections

Figure 2 shows the kernel density surface for East Sussex cattle holdings and the location of the most likely cluster of spoligotype 13 infections in cattle farms.

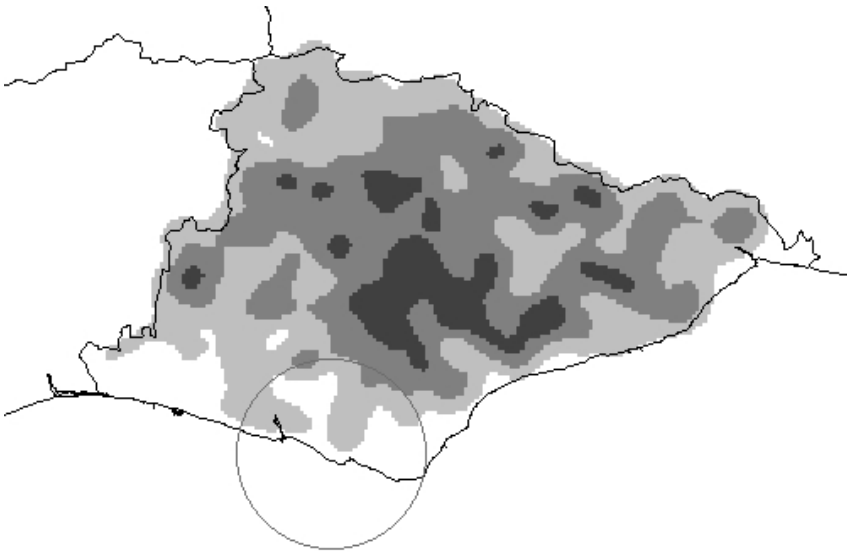

Fig. 2 Cattle holdings' kernel density surface and location of the most likely cluster of Spoligotype 13 infected holdings [Highest cattle holding density (number of holdings per km^2) represented by darkest colour; ranging steps: 0.00-1.23; 1.23-2.67; 2.67-4.45; 4.45- 7.01; most likely cluster of Spoligotype 13 infected holdings plotted as grey circle of radius of 8.1 km]

Using the spatial scan test based on a Bernoulli model the most likely cluster (P=0.001) of cattle spoligotype 13 infection identified during the study period includes 75 cases of spoligotype 13. This cluster coincided with the southern parishes of Seaford, Alfriston, Cuckmere Valley, Willingdon and Jevington, East Dean and Friston and Eastbourne, in East Sussex. The centre of this cluster was located in East Dean and Friston parish with a radius of 8.1 km covering the entire South Downs region of the County. The expected number of cases for that region – assuming that spoligotype 13 infection in cattle was Bernoulli distributed - was 6. No secondary clusters of cattle breakdowns were identified.

From Fig. 2 it can be seen that the incidence of bovine tuberculosis does not necessary correlate with cattle density as there is an area within the right quadrant of the cluster with low cattle holding density.

A badger sett map for the E. Sussex region has been formally georeferenced and a kernel density surface of badger setts has been produced, which is shown in Fig. 3. Visual examination of Fig. 3 suggests that cattle herd breakdowns involving spoligotype 13 correlate with an area of high badger sett density. It was found that East Sussex badger sett density, measured as the mean number of social groups per square kilometre per E. Sussex parish correlates more strongly (P=0.023; standard 95% confidence interval of 0.0005-0.006) with cattle spoligotype 13 herd breakdown than parish cattle density (measured as total animals per square kilometre per East Sussex parish; P>0.05). As shown by the kernel density surface of East Sussex badger setts there is an area of low badger sett density in a South-eastern to North-western strip to the north of the most likely cluster.

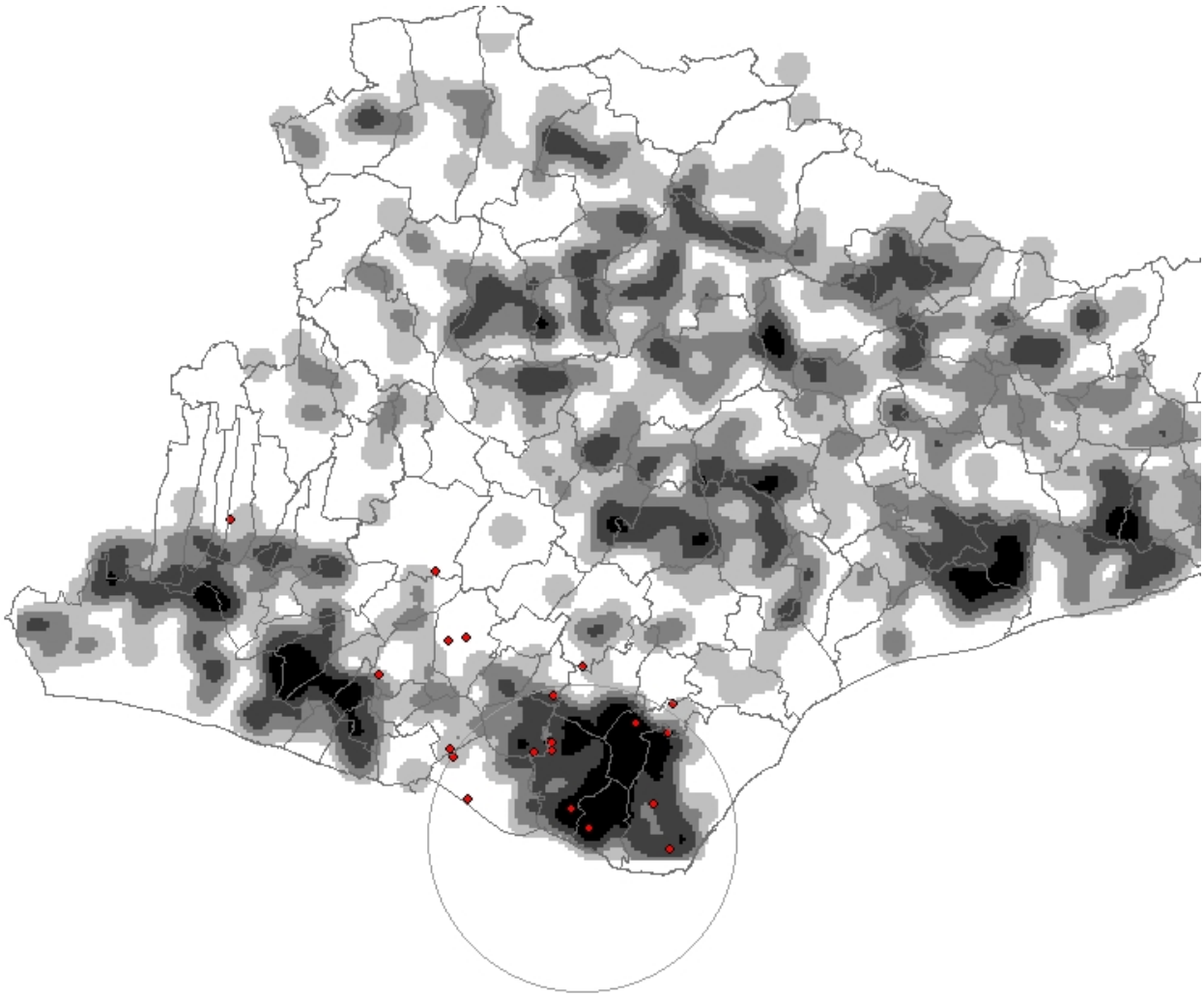

Fig. 3 Distribution of cattle spoligotype 13 breakdowns (dots) and badger sett kernel density surface [highest badger sett density (number of badger setts per km^2) represented by darkest colour; ranging steps: 0.00-0.17; 0.17-0.51; 0.51-0.89; 0.89-1.45; 1.45-2.76; most likely cluster of spoligotype 13 infected holdings plotted as grey circle of radius of 8.1 km]

DISCUSSION

In the present study, the East Sussex *M. bovis* spoligotype 13 cluster was investigated. From 1966 to 1975 most of the sources of infection to confirmed herd breakdowns in that county were either never disclosed or attributed to imported cattle from Ireland; as a result of the introduction of pre-export testing of Irish cattle, and the recognition in the mid-1970's of badgers as *M. bovis* reservoirs, most of herds breakdowns in East Sussex, between 1976 and 1984, were attributed to badgers (Wilesmith et al., 1986). The majority of cattle isolates of *M. bovis* (94%) from E. Sussex are of spoligotype 13 and the same strain is frequently recovered from badgers in the area. However, other spoligotypes other than spoligotype 13 were found in E. Sussex cattle breakdowns. The spoligotype 9 strain may represent transmission from cattle to badger since the badger isolate was picked up a year later in a badger culling operation. Alternatively, the spoligotype 17 strain was imported from Gloucestershire as investigated by CTS.

In 1986 it was believed that East Sussex was no longer endemic in what concerned *M. bovis* infections in cattle and it was assumed that a low risk of infection coming from the local badger population would still be present (Wilesmith et al. 1986). Further molecular epidemiological investigation of spoligotype 13 strains by VNTR and by unique deletion analysis has revealed that most cattle and badgers in E. Sussex are sharing a single clone of *M. bovis*. Infection may be moving from cattle to badgers (badgers as a spill-over host) or badgers may be the maintenance host and cattle the spillover host. However, one spoligotype 25 strain recovered from an E. Sussex badger will warrant further investigation; this strain shows a VNTR pattern characteristic of strains of spoligotype 25 but holds the spoligotype 13 unique deletion suggesting a recombinant strain.

CTS investigations to evaluate the extent of cattle exportation from E. Sussex has revealed that, between 1996 and 2003, about seventy thousand animals were born in E. Sussex and moved to other counties. Furthermore, each of those animals has made an average of 2.2 movements after being born in E. Sussex and exported elsewhere in the country. VNTR analysis identified a different spoligotype 13 clone in the western part of the country responsible for the breakdowns in Cornwall and Wiltshire (1990). It is also postulated that the breakdown in Wiltshire (1999) was caused by a third spoligotype clone since its VNTR pattern was distinctive from the latter. However, it was not possible to identify contact between the Somerset spoligotype 13 index case and E. Sussex; presumably this strain came from E. Sussex since it shares the same VNTR type and retains the deletion as all E. Sussex spoligotype 13 strains. It was found that East Sussex strains of *M. bovis* spoligotype 13 are rarely seen outside this county.

Since the 1970's over 1200 badger carcasses have been examined throughout E. Sussex; bovine TB positive carcasses have only been recovered from setts in the southern E. Sussex parishes. The badger sett density and the mean size of badger social group territory (43 square kilometres) in this county are considered high, especially in the South Downs region (Wilesmith et al., 1986), when compared to areas with persistent *M. bovis* infection in cattle (Barrow & Gallagher, 1981; Cheeseman et al., 1989; Clifton-Hadley et al., 1993). However, the isolates from badgers included in this study are not a representative sample of the badger TB infected population since 90% of the isolates described here were obtained in a single localised badger culling operation in 1997. This bias prevented a thorough cluster analysis of the spoligotype 13 infection for this host species. Nevertheless, the output data afforded by the focused cluster analysis of the cattle isolates shows a significant cluster (P=0.001) of cattle spoligotype 13 infection with centroid coordinates in East Dean and Friston parish and radius of 8.1 km. This finding together with the molecular epidemiological data show that indeed spoligotype 13 strains from cattle and badger are highly geographically clustered in the southern E. Sussex parishes. Previous studies have shown a positive association between badger sett density and cattle herd breakdowns (Wilesmith, 1983; Krebs et al., 1997). This study shows that in E. Sussex there is a correlation between parish badger sett density and spoligotype 13 cattle herd breakdowns (P<0.05; densities measured as the number of badger setts per square kilometre per E. Sussex parish). It could be argued that the incidence of bovine TB in the southern parishes of E. Sussex is due to the high density of cattle in that area and the absence of cattle in the rest of E. Sussex. No significant differences were found between the mean cattle density in breakdown parishes and non-breakdown parishes (P>0.05: densities measured as the number of cattle per kilometre square per E. Sussex parish).

Given the high cattle density in most of E. Sussex and the frequent movement of cattle within the county (as confirmed by CTS investigation), it is remarkable that strains of

spoligotype 13 are confined to a few southern parishes and have not spread throughout the county. However, if the spread of *M. bovis* strains with spoligotype 13 is determined more by badger-to-badger transmission than by cattle-to-cattle transmission, then any region of reduced badger sett density would introduce a natural barrier that would retard the spread of the spoligotype. It is possible that the low density of badger setts on the area localised north to the spoligotype 13 cluster, may significantly reduce the spread of bovine TB between badger social groups, halting the transmission of this spoligotype to cattle. It has previously been shown that significantly fewer badger setts are located on the clay than on chalk (Roper, 1993). Interestingly the parishes with a high incidence of spoligotype 13 herd breakdowns and a high density of badger setts are located on a region of chalk (The South Downs). These observations highlight the need for future geostatistical analyses which consider surface geology as a surrogate measure for badger sett density.

In which case, this study points out that *M. bovis* spoligotype 13 strains is highly clustered in the south parishes of East Sussex and that cattle and badger populations in the South Downs are sharing the same clone. Additionally, this study shows evidence that, despite the high cattle movements from E. Sussex, this clone has not seeded efficiently in cattle and badger populations outside E. Sussex when compared with other spoligotypes elsewhere in the country. Certainly it appears that understanding the factors that determine badger sett density may help to explain the geographical localisation of many spoligotypes of *M. bovis* in the UK.

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DYNAMICS OF THE SPACE-TIME INTERACTION IN THE MAIN GEOGRAPHICALLY-DEFINED CLUSTERS DURING THE 2001 FOOT-AND-MOUTH DISEASE EPIDEMIC IN THE UK

A. PICADO*, F.J. GUITIAN AND D.U. PFEIFFER

SUMMARY

During the 2001 Foot-and-Mouth Disease outbreak in the UK, the implementation of control measures was supported by models aiming to predict the behaviour of the infection in the whole country rather than at the local level. This paper explores the evolution of the space-time interaction in the four main geographically-defined clusters observed during the epidemic: Devon, Settle, South Penrith and Cumbria. For each of them, the space-time interaction is studied by means of the space-time K-function calculated for consecutive 20-day time windows.

The dynamics and characteristics of the space-time interaction varied significantly across local clusters, suggesting that the intensity of local spread and the effectiveness of control measures differed markedly between local outbreaks. These results suggest that real-time analysis of the space-time interaction can be a valuable decision support tool during the course of a livestock epidemic.

INTRODUCTION

Clustering of cases in space and time is considered to be an indicator of the infectious nature of a disease. More specifically, the pattern of space-time interaction has been used to describe the transmission characteristics of infectious processes both in human (Diggle et al., 1995; Zhao et al., 2002) and in animal populations (Norstrom et al., 1999; Sanchez et al., 2005; Wilesmith et al., 2003). The detection of space-time interaction has also been proposed as a tool for on-line disease surveillance (Diggle et al., 2004).

Several methods are available to test for space-time interaction (Kulldorff & Hjalmars, 1999; Ward & Carpenter, 2000a, 2000b). Amongst them, the space-time K-function (K(s,t)) (Diggle et al., 1995) has been successfully used in medical research, for example, to detect clusters of metabolic diseases such as type 1 diabetes (Zhao et al., 2002) and tumours in children (McNally et al., 2003; McNally et al., 2004). In the veterinary domain, K(s,t) was used to describe the space-time pattern of sheep scab outbreaks in the UK from 1973 to 1992 (French et al., 1999) and, more recently, of acute clinical infectious bursal disease in broiler flocks in Denmark (Sanchez et al., 2005). French et al. used the same methodology to detect clustering of equine grass sickness in the UK (French et al., 2005) and Wilesmith et al. (2003) used the K-

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function to describe the pattern of space-time interaction in two English counties during the 2001 Foot-and-Mouth Disease (FMD) epidemic.

During the 2001 FMD outbreak in the UK, decisions on the level of implementation of control strategies aimed at reducing local spread, such as the pre-emptive culling of farm livestock, were supported by simulations of the likely behaviour of the epidemic (Ferguson et al., 2001; Keeling et al., 2001; Morris et al., 2001). Different models were used and their predictions were in general accurate and robust, and the implementation of control policies informed by them was effective in achieving eradication of FMD by the end of September 2001. However, local differences in the behaviour of the infection existed from the start of the epidemic (Gibbens et al., 2001; Gibbens & Wilesmith, 2002). Structural differences including farm composition and agricultural activities (Kao, 2001; Wilesmith et al., 2003), differences in the level of implementation of control measures (Anderson, 2002; Gibbens et al., 2001; Gibbens & Wilesmith, 2002; Honhold et al., 2004a; 2004b; Thrusfield et al., 2005), temporal and climatic factors (Anderson, 2002; Gibbens & Wilesmith, 2002) have been proposed as potential reasons for the heterogeneous local behaviour of the epidemic. Subsequent studies that focused on high incidence areas reinforced these observations, and question the use of global models to establish local control measures (Honhold et al., 2004a; Honhold et al., 2004b; Taylor et al., 2004). A better understanding of the local transmission dynamics within a large outbreak could permit (1) the identification of local factors associated with FMD virus (FMDV) transmission (2) the improvement in the model's predictive capacities and (3) the design of tools to monitor the effectiveness of the control measures applied.

Different studies have approached the 2001 FMD outbreak in the UK from a local perspective (Honhold et al., 2004a; 2004b; Taylor et al., 2004; Wilesmith et al., 2003). In one of these studies (Wilesmith et al., 2003) the pattern of space-time interaction in pre-defined time periods was used to describe local differences observed between the two main local outbreaks observed during the course of the epidemic: Cumbria and Devon. Here the pattern of space-time interaction is assessed in the four main geographically separated outbreak clusters observed: Devon, Settle, South Penrith and Cumbria. The space-time K-function is applied to the sets of data that would have been available during the epidemic. The assessment of 'real-time' space-time interaction could have been used as an indirect measure of local spread and the effectiveness of control measures applied to reduce the short-range transmission during the epidemic.

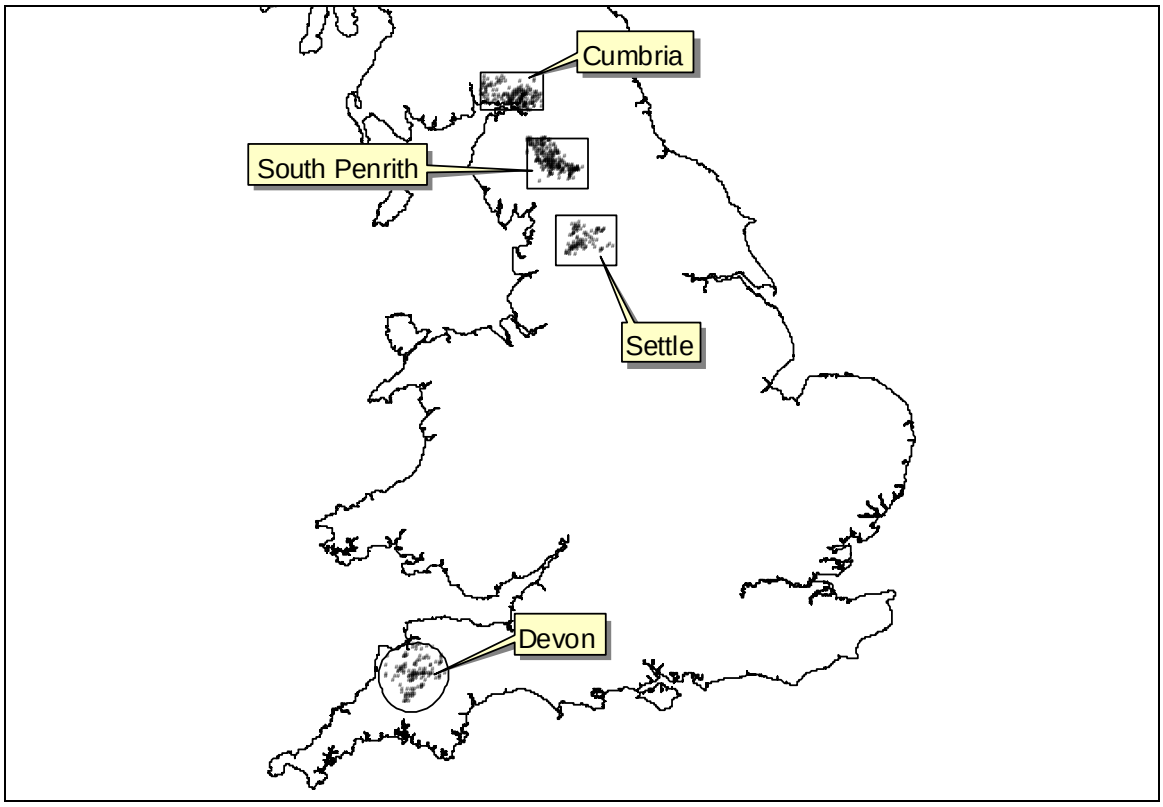

Fig. 1 Map of England with the 4 study areas: Cumbria, South Penrith, Settle and Devon (from North to South) represented. FMD Infected Premises in each of the study areas during the 2001 outbreak are presented as dots.

MATERIALS AND METHODS

Study population and data sources

The 4 main outbreak clusters (Cumbria, South Penrith, Settle and Devon) observed during the epidemic, and for which the dynamics of the infection have been suggested to differ considerably (Anderson, 2002; Gibbens et al., 2001; Gibbens & Wilesmith, 2002), were studied (Fig. 1). For each of the first 3 clusters a rectangular study area was defined subjectively by visual exploration of the distribution of infected premises (IPs). The study areas of Cumbria (1500 km^2), South Penrith (2000 km^2) and Settle (2000 km^2) registered 217, 273 and 102 IPs respectively. For the Devon cluster (2433 km^2) a zone of 25 km radius from the first 5 cases that occurred in the cluster was studied. The study area in Devon included 139 IPs.

Due to the rapid transmission of the FMDV within a farm, it is reasonable to consider a farm as an individual unit (Keeling et al., 2001). Geographical coordinates and number of individual animals on premises located in each of the study areas that had at least one animal of the species that are susceptible to FMDV infection (cattle, sheep, goats, pigs, deer) were retrieved from the agricultural census conducted in June 2000 by the Ministry of Agriculture, Fisheries and Food (MAFF) (MAFF, 2000). Specific epidemiological data related to the 2001 FMD epidemic for IPs in the study areas were obtained from the Department for Environment, Food and Rural Affairs (DEFRA) FMD epidemiology database, Disease Control System Database (DCSD)

(Gibbens et al., 2001). Premises were defined as infected with FMD when investigating officers from DEFRA observed clinical signs of FMD in susceptible animals or when, in pre-emptively culled farms, a positive laboratory result was obtained (Gibbens et al., 2001). The infection date in each IP, obtained from the DCSD, was an estimation based on clinical signs, FMD lesions observed, serology results or known effective contact with infected animals as described by Gibbens et al (Gibbens et al., 2001; Gibbens & Wilesmith, 2002). When the infection date was not available the "report date" recorded in DCSD (Gibbens et al., 2001) was used instead. The slaughter date for premises that were depopulated pre-emptively, or on suspicion but that were not confirmed as infected, was retrieved from the DCSD. It was assumed that depopulation made a holding non-susceptible to FMD and that there was no repopulation before the study period (14th February to 29th September 2001) finished.

For the analyses, data from the Agricultural census and the DCS Database were merged. In cases of discrepancy DCSD data, considered more recent and accurate, was used. The final database used included 7483 holdings with animals susceptible to FMD located in the 4 selected areas; 733 of them were declared IPs by the end of the study period.

Data analysis

The temporal pattern of the epidemic in each cluster was summarized by means of the hazard rate. The hazard rate of infection was calculated as the number of new IPs per week divided by the number of holding-weeks at risk (Fig. 2).

The space-time interaction among infected premises was visualised using the space-time K-function K(s,t) (Diggle et al., 1995) implemented through the SPLANCS library (Bivand & Gebhardt, 2000; Rowlingson & Diggle, 1993) in the statistical software R (http://www.r-project.org/). The space-time (K(s,t)) function is the cumulative number of events (IPs) within a distance (s) and time (t) of a randomly chosen IP divided by the expected number of IPs in a space-time box of size one area unit by one time unit (λ) (Diggle et al., 1995; Wilesmith et al., 2003). If there is no space-time interaction expect K(s,t) would be expected to equal K(t)K(s), where K(t) and K(s) are the spatial and temporal K-functions respectively. The function D(s,t) calculated as $D(s,t) = K(s,t) - K(t)K(s)$ allows us to study if IPs clustered in space are also close together in time. Finally, $D_0(s,t)$ representing the excess number of infected premises attributable to the time-space interaction was calculated as $D_0(s,t) = D(s,t) / K(t)K(s)$ (Diggle et al., 1995; Wilesmith et al., 2003). A maximum distance of 10 km and time separation of 21 days were used (Wilesmith et al., 2003). Ninety-nine Monte Carlo permutations were used to test for statistical significance at p=0.01 (Diggle et al., 1995). $D_0(s,t)$ was calculated for consecutive 20-day time windows to determine the space-time evolution of the FMD infection in each area. For statistically significant interactions ($P \leq 0.05$) in which the observed IPs were at least doubling the expected number ($D_0(s,t) > 1$) (Sanchez et al., 2005) the following aspects were analysed: limits in space and time representing the contagiousness in these two dimensions (Wilesmith et al., 2003) and maximum risk observed (maximum $D_0(s,t)$) per time window.

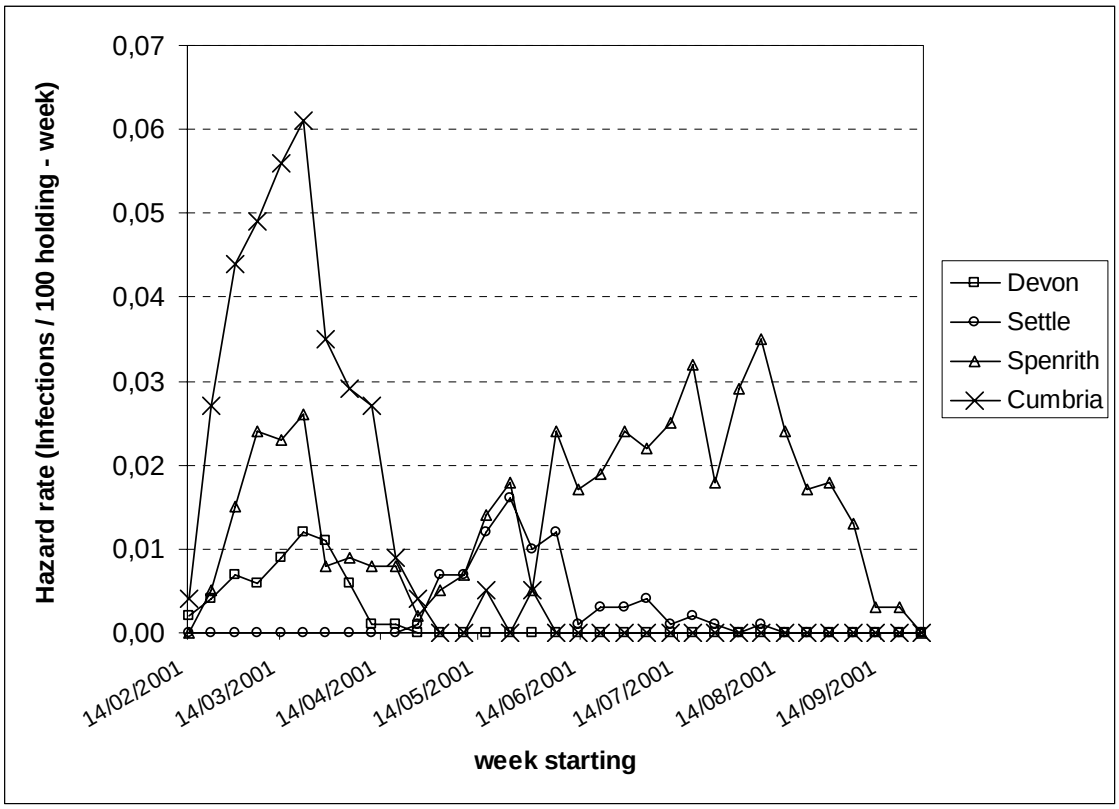

Fig. 2 Weekly hazard rate of infection by area calculated as the number of new infected holdings per week divided by the number of holding-weeks at risk

RESULTS

Figure 2 shows that the temporal behaviour of the FMDV infection was different between the different study areas. In Devon and Cumbria the epidemic started earlier than in the other two areas and presented a typical epidemic curve; a period of ascending hazard that peaked almost simultaneously in both areas, followed by a period of decreasing risk of infection. Finally FMDV was cleared from both areas during the tail of the epidemic. For the Settle area, the temporal pattern was similar but the outbreak had a late start and a long tail. Infections were observed first in the Settle area when the Devon and Cumbria outbreaks were already under control. Finally, the pattern observed in the South Penrith area was more suggestive of two consecutive epidemics than of a single one.

The space-time K-function results are presented as a series of 4 graphics per study area (Fig. 3 to 6) in which only the statistically significant interactions ($P \leq 0.05$) are shown. To assess the evolution of the space-time interaction throughout the epidemic, the extent of contagiousness in time and space, maximum $D_0(s,t)$ and the number of IPs per time window are presented.

Cumbria (Fig. 3) presented limited periods of space-time interaction. The most important interaction episode coincided with the period with the higher number of IPs, and had a predominant time component.

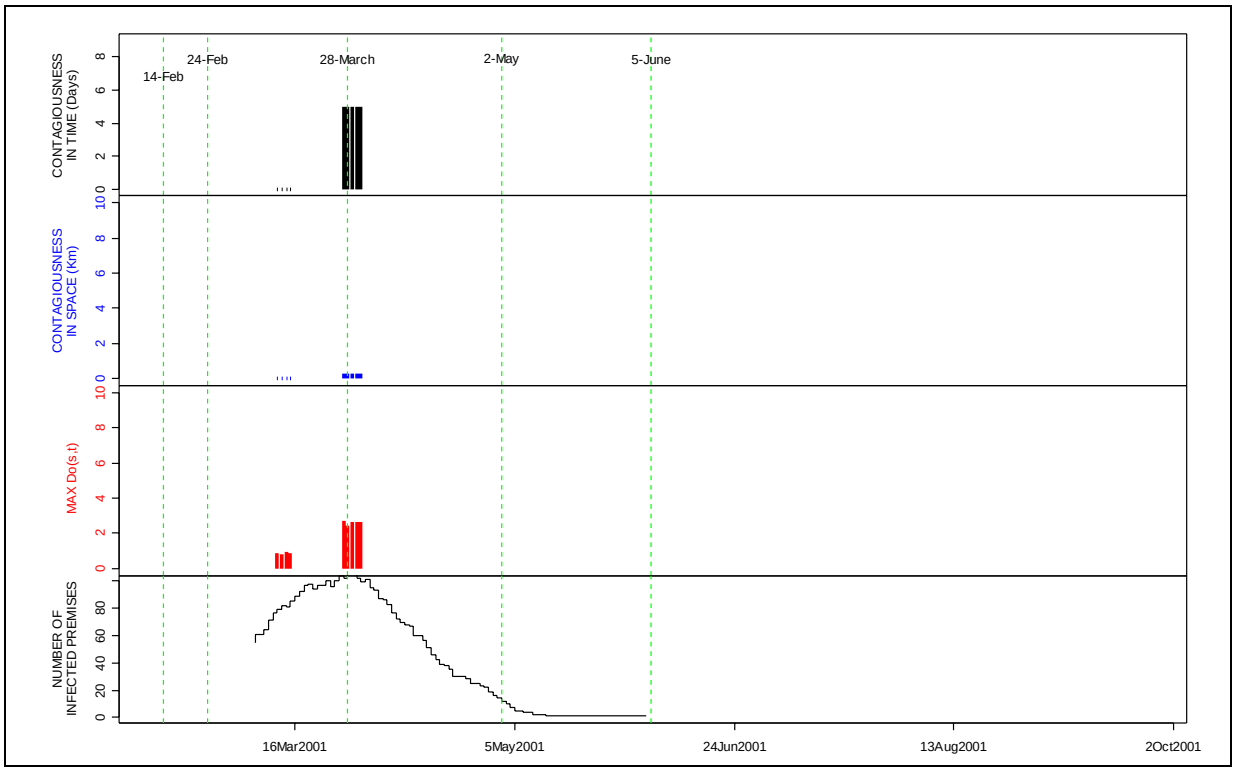

Fig. 3 Results of the space-time K-function for consecutive 20-day time windows in Cumbria [only results ($D_0(s,t)$) from statistically significant interactions ($P \leq 0.05$), limits in time and space, the maximum risk observed (maximum $D_0(s,t)$) and the number of IPs per (20 days) time window are presented]

Space-time interaction episodes in Devon (Fig. 4) were more scattered. They were not present at the start of the epidemic, but were more or less constant during the period when the number of IPs was increasing. The space-time interaction disappeared just before the number of IPs started to decrease.

In Settle there was an intensive and constant space-time interaction from the start of the epidemic until 15 June (Fig. 5). The space dimension decreased in importance throughout the epidemic and the time dimension was fairly constant until mid-June. The maximum risk recorded increased early June. From mid-June to the end of the epidemic, isolated but intensive episodes of space-time interaction were observed with an important spatial component.

DISCUSSION

The space-time K-function was applied to four selected areas, two of them already described by Wilesmith et al (2003). However the use of 20-day consecutive windows allowed a continuous evaluation of the space-time interaction and gave a picture of its evolution within the local cluster. This new analysis made it possible to describe the FMDV transmission characteristics throughout the epidemic. Changes in the pattern of space-time interaction can be interpreted as changes in the relative impact on the risk of infection of being close in time and space to an IP. These changes can be related to the effectiveness of control measures applied in the different areas during the outbreak.

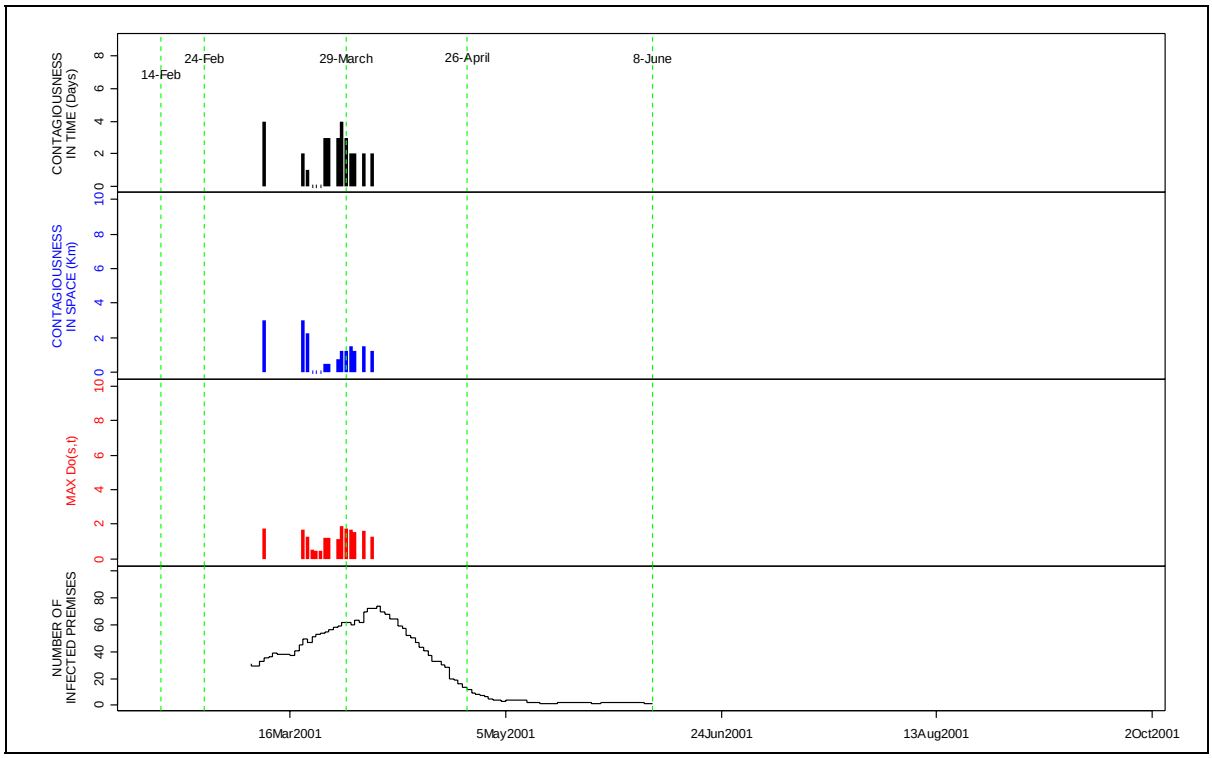

Fig. 4 Results of the space-time K-function for consecutive 20-day time windows in Devon [only results ($D_0(s,t)$) from statistically significant interactions ($P \leq 0.05$), limits in time and space, the maximum risk observed (maximum $D_0(s,t)$) and the number of IPs per (20 days) time window are presented]

South Penrith (Fig. 6) presented two phases of space-time interaction. The early phases were analogous to the situation in Cumbria and Devon: increasing numbers of IPs were overlapped by interaction episodes that disappeared when the number of IPs started to decline. Then, in a second phase, there were several periods of space-time interaction regularly distributed in time. The spatial and temporal components were high and constant. The maximum risk was more irregular and tended to increase reaching its maximum at the end of the epidemic.

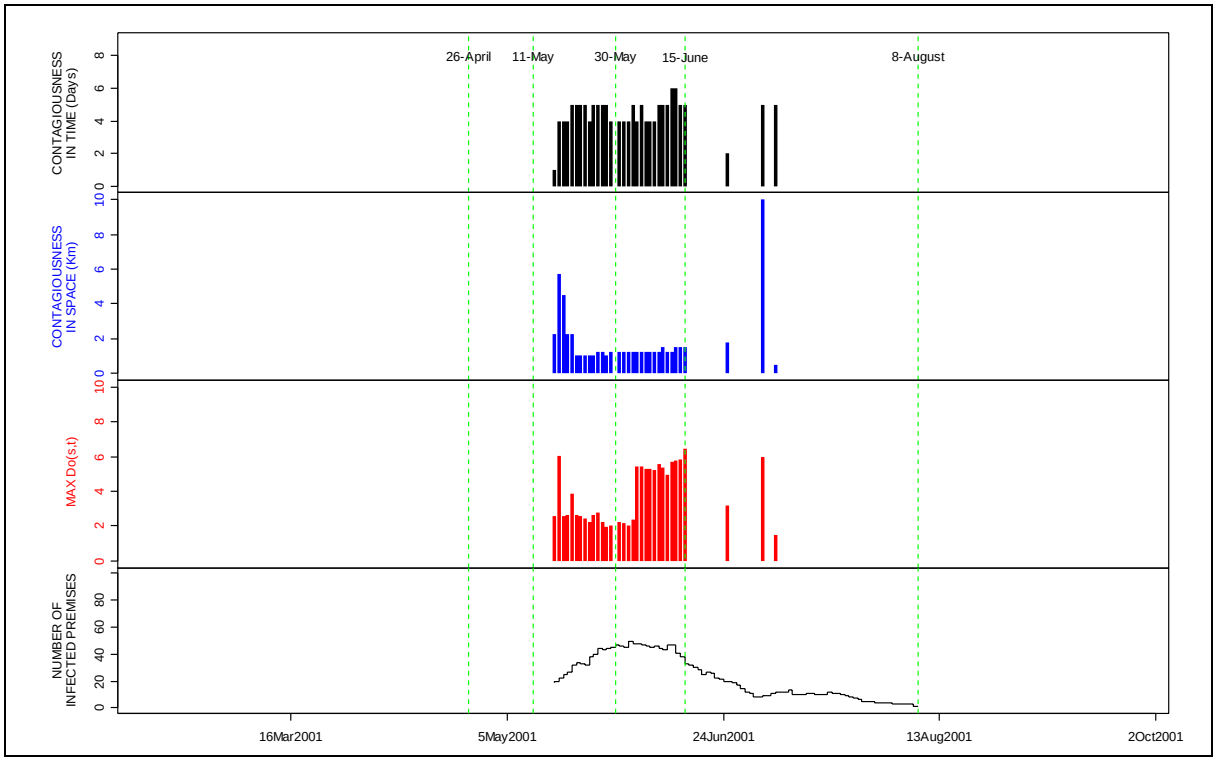

Fig. 5 Results of the space-time K-function for consecutive 20-day time windows in Settle [only results ($D_0(s,t)$) from statistically significant interactions ($P \leq 0.05$), limits in time and space, the maximum risk observed (maximum $D_0(s,t)$) and the number of IPs per (20 days) time window are presented]

In general, contagiousness in time and space differed between the study areas. Differences in the temporal and spatial patterns of transmission during the 2001 FMD epidemic between different areas in the UK have already been described elsewhere (Anderson, 2002; Gibbens et al., 2001). However, according to the results, the study areas of Cumbria and Devon presented similar disease transmission patterns. The space-time interaction was in general low, and disappeared after the peak of the epidemic when applied control measures seemed to be effective. Devon presented a predominant time component when the number of IPs per time window was maximum, suggesting difficulties in detecting and eliminating IPs by that time, as suggested by Wilesmith et al. (2003). In both areas the epidemic was under control by May (Fig. 3 and 4).

Settle experienced a relatively long outbreak (26th April to 8th August) despite control measures being already in place when the first infection in the area occurred in late April. The transmission pattern in this area was characterised by a constant space-time interaction during the first phase of the epidemic (Fig. 5). The predominance of the time component suggests that that virus transmission between farms was mainly due to failures to detect infection and cull livestock on IPs, which remained infectious for a long period of time (Wilesmith et al., 2003). $D_0(s,t)$ reached its maximum early in June when the number of IPs per time window was high and constant. Isolated episodes of space-time interaction due to transmission of FMDV by moving infected animals or contaminated material/people (spatial component predominant) may be associated with the long tail of the epidemic observed in this area (Wilesmith et al., 2003).

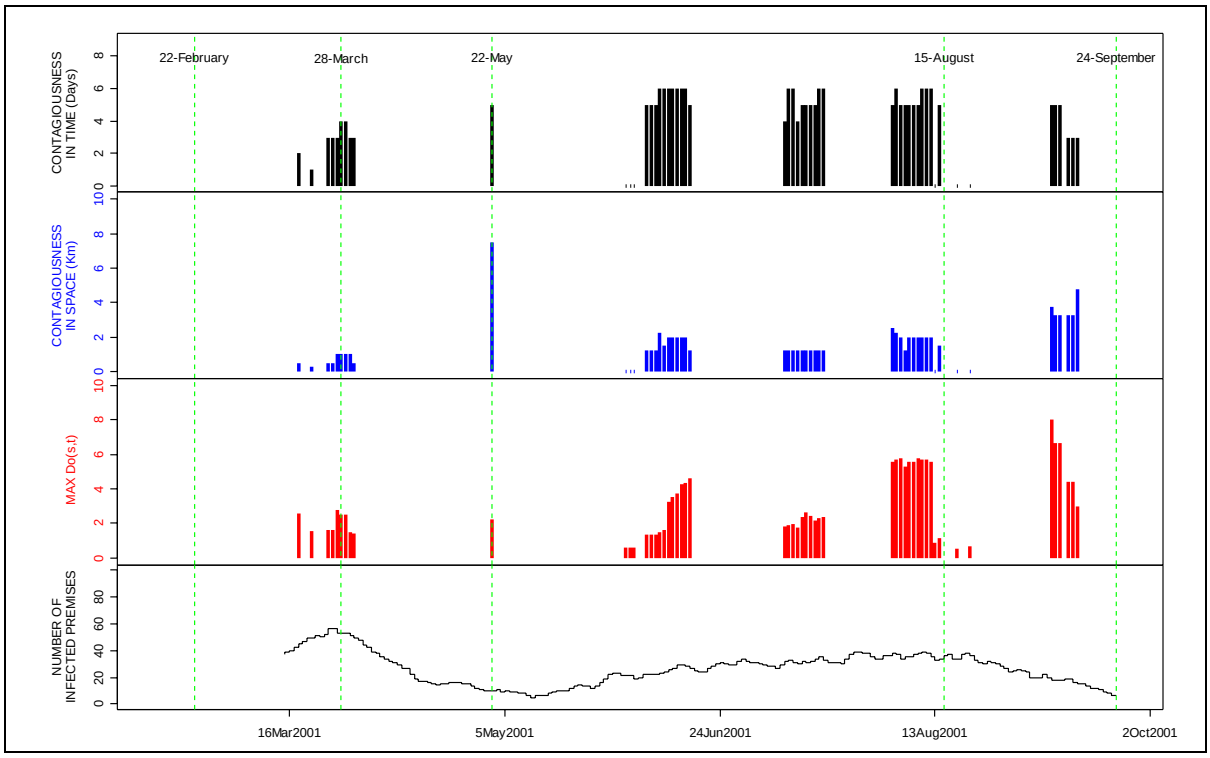

Fig. 6 Results of the space-time K-function for consecutive 20-day time windows in South Penrith [only results ($D_0(s,t)$) from statistically significant interactions ($P \leq 0.05$), limits in time and space, the maximum risk observed (maximum $D_0(s,t)$) and the number of IPs per (20 days) time window are presented]

The occurrence of new FMD cases in South Penrith until mid-September 2001 is directly related to the evolution of the space-time interaction in the area (Fig. 6). During the first phase of the epidemic, as in Cumbria and Devon, space-time interaction disappeared, suggesting that the implementation of control measures was effective at reducing local spread. In the second phase, regularly distributed interaction episodes with a predominant time component although the space component was constantly present and even increased in the last episodes, suggest that there was a regular but general failure of the control measures applied in the area. Wilesmith et al. (2003) suggest that the increased number of sheep holdings affected, where clinical signs are more difficult to detect, might be a cause of the pattern observed. Even when pre-emptive culling of livestock on farms was very intense (Taylor et al., 2004) and extra biosecurity measures were applied (Anderson, 2002), the local transmission of FMDV was not stopped and it remained in the area for a long period of time.

The use of the space-time K-function as a tool to assess the effectiveness of the control measures applied seems appropriate. Despite the limitation of it not being a multivariate analysis technique, since only proximity in time and space are considered, the results suggest that the real-time analysis of the space-time interaction can be a valuable decision support tool during the course of a livestock epidemic.

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UNDERSTANDING EPIDEMICS IN HETEROGENEOUS HABITATS: MODELS AND DATA OF CLASSICAL SWINE FEVER IN WILD BOARS

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SUMMARY

The dynamics of wildlife diseases are determined by processes at the level of host individuals and by the spatial structure of the host's habitat. An individual-based spatially explicit model of wild boar and Classical Swine Fever was applied to investigate the interaction of both. Disease persistence within a regional wild boar population was analysed with respect to availability and fragmentation of breeding habitat in that area. Beyond the decisive influence of host reaction patterns to the virus (i.e. virulence) it was found that regions with coherent wild boar habitat more likely to be related to persisting CSF outbreaks. If the same amount of wild boar habitat is scattered through the area, persistence will become less likely and, with low effective transmission rates, the mean duration of outbreaks decreases. However, if transmission of CSF in wild boar is more efficient, highly structured wild boar habitat might facilitate longer epidemics due to a stepwise passage of the infection. Recognising the importance of habitat structure and heterogeneous landscapes for the persistence of CSF in wild boar, it is intended to explore field data on CSF outbreaks in wild boar in order to understand the varying persistence patterns found in European databases.

INTRODUCTION

Classical Swine Fever (CSF) is a viral disease which has caused very serious economic losses in the European Union (EU). Classical Swine Fever virus infection occurs under natural conditions in domestic pigs and wild boar, *Sus scrofa*. Classical Swine Fever in wild boar is thought to be the main risk for CSF outbreaks in domestic pigs due to direct contact in some areas of extensive pig keeping (Laddomada et al., 1994; Zanardi et al., 2003) or to indirect contact through feeding of contaminated wild boar meat to domestic pigs (Wachendörfer et al., 1978; Teuffert et al., 1998; Laddomada, 2000). It is possible that the virus is circulating and perpetuated for years among some European wild boar populations, but it is yet unclear how the disease can remain in these foci (Laddomada et al., 1994; Fritzemeier et al., 1998; Kern et al., 1999; Fritzemeier et al., 2000).

Epidemiological databases comprise geographical data on CSF outbreaks in wild boars. This information contains valuable 'fingerprints' of the spatio-temporally spreading epidemic that it is important to understand (Thulke et al., 2005). A promising approach is to combine this visual knowledge about the epidemic with the feasibilities of rule-based simulation models (Grimm et al., 2005). Thus, an individual-based, spatially-explicit model to investigate the spread and

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persistence of Classical Swine Fever (CSF) in wild boar populations was recently developed (McCallum et al., 2001). The modelling technique enables the immediate adoption of existing knowledge on wild boar ecology and CSF epidemiology on either the individual or population level (Kramer-Schadt et al., 2005). However, in order to orient to the patterns of existing outbreak data, the model was implemented to work on GIS representations of existing geographies.

In the first step of this analysis, the interaction between individuals and the structure of landscapes in relation to disease spread and persistence was addressed. Dispersal and breeding site selection and/or spatial variations in the availability of breeding habitat are the most important population parameters responding to changes in landscape structure (Johnson et al., 1992; Wiegand et al., 1999). Therefore, the effects of these two factors on host-pathogen dynamics in the context of CSF in wild boar were analysed (Fig. 1).

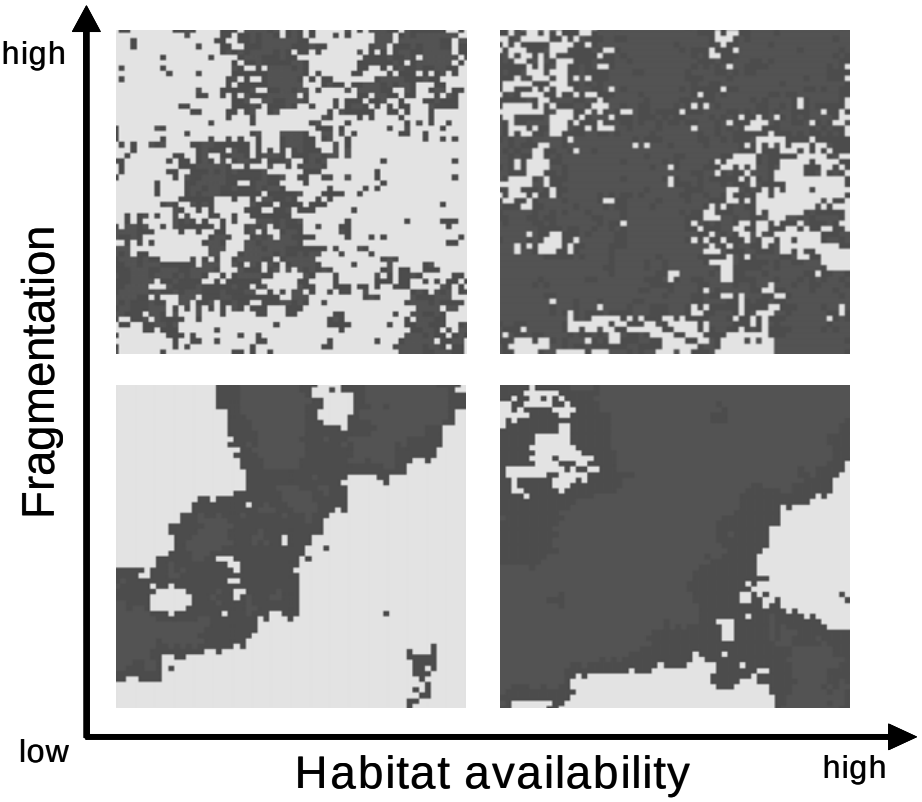

Fig. 4 Cross-design of the simulation study. The generation of virtual landscapes of different total capacity and aggregation of wild boar breeding habitat allows for relative analysis of the factors population density vs. habitat heterogeneity with respect to either the probability of persistence or the duration of the outbreak. (Grey cells = non-breeding habitat, black cells = breeding habitat)

MATERIALS AND METHODS

Modelling approach

The model is based on female boar groups within their home ranges of 4 km², the approximate mean home-range size for a variety of European wild boar populations (reviewed in Spitz and Janeau, 1990). The area of one female group's home-range within the landscape is

reflected by a grid cell. The habitat quality is attributed to each cell as breeding capacity of the home-range and varied between 0 and 4 (i.e. 0 = non-breeding habitat and 4 allows up to four females to breed). During simulation the habitat quality value of the home-range is important (a) for the number of females that are allowed to breed during one year and (b) by determining if last-year's sub-adult females split from the parental group due to overcrowding. In order to incorporate existing landscapes into the simulation, the habitat quality of a cell is determined from the vegetation coverage of the underlying map (Fernandez et al., 2006).

A fractal algorithm was used to distribute cells of varying habitat quality on the simulation area (Plotnick & Gardner, 1993; With et al., 1999; Wiegand et al., 1999). The generation of these virtual landscapes was intended to allow systematic investigation of the effects of landscape characteristics on the dynamics of a CSF outbreak (Peck, 2004). The stochastic landscape generation is driven by two parameters: (i) fragmentation, and (ii) coverage. With respect to the landscape characteristics, a cross-design study was performed (Fig. 1). The model starts start with a landscape of 60 by 60 cells, which reflects an area of 14,400 km². Six categories of landscapes were considered in the experiment (i.e. 3 fragmentation x 2 coverage values). However, for each repetition of the outbreak on the same landscape category, a new 60x60 landscape was created in advance. Thus, the analysis considered the effect of landscape issues and not only particular configurations. One thousand four hundred and forty (1440) habitat cells were initialised with individuals. The number of initial individuals per herd is proportional to the breeding capacity of the cell assuming an average ratio of [N breeding females]:[N wild boars] = 1:4. The age and sex attributes of the individuals were randomly drawn according to population census data found in the literature (Table 1). After a 'waiting time' of five years, to allow population structure to stabilise, an outbreak was seeded with one infected animal released randomly into the wild boar population.

The demographic parameters of the wild boar model were found in published data from populations in France, Italy, Poland and Germany. The data are based on long-term field studies of up to 10 years, mostly of hunted populations (Table 1). Parameters were fixed for the boar population dynamics according to these references. Parameters of the model that could not be set to a single value derived from the literature, i.e. the distribution function for many disease parameters or male group movement, were considered to be variable. In these instances a fixed value analysis was performed in between the upper and lower limit derived from literature or estimation (Table 2). The model was run for 40 replicates for each parameter combination. Time steps of one week were used, as this is approximately the incubation period of the disease (Artois et al., 2002). Thus, demographic variables were updated weekly for each individual (i.e. age).

Model rules of wild boar population ecology

Reproduction: Females reproduce once a year. The week of reproduction is assigned on the basis of a probability distribution resembling average monthly reproductive rates for wild boar populations in Europe, typically showing a maximum peak during April and May, and a minimum of almost 0 from October to December (Boitani et al., 1995; Table 1). Females older than 34 weeks of age are reproductive. The number of breeding females in each cell is limited by the breeding capacity value. Older females reproduce first, resembling the hierarchy in female groups. The number of piglets per female is drawn from a Gaussian distribution (Table 1).

Group split up: Female groups split up in the middle of the year (between week 17 and 30). A minimum of two yearling females (34-104 weeks age) disperse if the number of females in the cell exceeds the breeding capacity. Dispersing females search for non-occupied habitats to acquire a home range within a distance of 3 cells from the natal cell (i.e. 7 x 7 cell neighbourhood), representing a dispersal distance of 6 km (Truvé and Lemel, 2003). If no empty habitat is available, the females remain without breeding in their maternal cell, thus assuming return movements.

Table 1. Wild boar population demography parameters as used in the model.

	Symbol	Published values	Model parameter
Carrying capacity per home range [individuals]	CC	5-10 up to 40 [(1)]	5 breeding females
Maximum age [years]	Y_{max}	♀: 9 and ♂: 6 [(2)]; 11 [(3)]	11
Piglets per female [N]	N_{piglet}	3.2 ± 1.68 SD [(1)]; 1.5-4.5 [(3)] 4.95 ± 0.42 SE [(4)]; 6 ± 2 SD [(5)] 6.7 ± 2.1 SD [(6)]; 1.6 – 5.5 [(7)]	3.2, sd 1.68, range [0 – 10]
Survival rate piglets	SR_{piglet}	0.48 ± 0.37 SD [(1)]; 0.6 – 0.65 [(8)]	0.48, sd = 0.37, range [0.1 – 1.0]
Survival rate yearlings	$SR_{yearling}$	< 0.5 [(2)]; 0.26-0.47, 0.65 [(8)]	0.6
Survival rate adults	SR_{adult}	0.64 ± 0.24 SD [(1)]; ♀: 0.2 and ♂: 0.38 [(8)]	0.64, sd = 0.24, range [0.28 – 1.0]
Dispersal distances of subadult females	d_{fem}	4.5 up to 20 km [(9)]	max 9 km
Weekly dispersal distance male groups	d_{male}		range [2 – 12km]
Habitat preference	*HighHQ*		range [no, often, mostly]

[(1)](Focardi et al., 1996), [(2)](Stubbe et al., 1989), [(3)],[(4)](Boitani et al., 1995), [(5)](Ahmad et al., 1995), [(6)](Nahlik & Sandor, 2003), [(7)](Andrzejewski & Jezierski, 1978), [(8)](Gaillard et al., 1987), [(9)](Truvé and Lemel, 2003).

Male dispersal: The dispersal of male groups is modelled explicitly. In each time step (i.e. one week), all dispersing groups are assumed to move either with stepwise random direction or by orientating to the habitat gradient. Male groups are allowed to move into non-breeding habitat (habitat quality = 0). Male groups move d_{male} steps per week (i.e. 1, 3, or 6 cells) until they die or settle in free habitat. Preference to high quality habitat is determined by *HighHQ* giving the proportion of steps preferably taken towards good quality habitats if a decision is possible. Boar group movement is organised on a torus, thus males leaving the simulation area reappear but cleared from infection.

Mortality – The mortality rate of individuals is assigned according to survival rates reported in the literature (Table 1) for piglets (i.e. <34 weeks), yearlings and adults (>104 weeks). The baseline mortality parameter is assigned at the beginning of each year from the Gaussian distribution of the mean survival rates and their standard deviations (Table 1). In this way, the

environmental effect of "good" and "bad" years on mortality is accounted for (e.g. Jedrzejewska et al., 1997). If S_i is the random annual survival rate for the age class i in the year, the following:

$$m_i = 1 - \left(S_i\right)^{1/52} \quad (1)$$

was applied to determine the weekly mortality m_i with $S_i \sim$ GAUSSIAN(mean surv. $data_i$; std.dev. $data_i$). The individual in age class i dies according to random realisations of BINOMIAL(m_i) per time step.

Table 2. Epidemiological parameters of the model.

Parameter	*Symbol*	Values
Effective infection probability within herd	P_{inf_G}	0.005, 0.05, 0.25, 1
Effective infection probability between herd being the fraction of P_{inf_G}	P_{inf_N}	100%, 10%
Number of time steps, where piglet is protected by maternal antibodies	TS_{MA}	12, 48
Time of virus release	*SEASON*	April

Virulence category	T_{MAX}	X	P_{Trans}
High (long infections: No; immunes: few)	5	10	0.4
Medium (l.i.: some medium; i: yes)	15	5	0.7
Low (l.i.: majority very long; i: many)	30	1	0.9

Rules of CSF virus epidemiology

The epidemiological states 'susceptible', 'lethally infected' (including prenatally infected piglets), 'transiently infected' (i.e. virus shedding for 1 week and then latency for 3 further weeks) and 'immune' were distinguished (see Kramer-Schadt et al., 2005).

Infection: Infectious animals transmit the virus with infection probability P_{inf_G} (including contact and successful transmission) to other herd members and with infection probability P_{inf_N} to neighbouring groups, realised as a fraction of P_{inf_G} due to fewer contacts between animals of different cohorts (Table 2). After counting the number of infected animals within the group I_G and in neighbouring groups I_N, the probability P_{SI}, that a susceptible animal will be infected, is thus

$$P_{SI} = 1 - \left[\left(1 - P_{\inf\_G} \right)^{IG} * \left(1 - \left(P_{\inf\_G} / P_{\inf\_N} \right) \right)^{IN} \right] \quad (2)$$

Response to infection - Mortality probability of infected individuals is dependent on the virulence of the virus, i.e. its ability to kill its host, and other individual traits of the pig, such as age and health condition. For simplicity, outcomes of low, moderate or high 'virulence' will be referred to in the course of this paper. To reflect the different responses of individuals to an

infection, the probability that animals become infected only transiently P_{Trans}, depending on the age class of the pig, is defined with:

$$P_{Trans}Adult = (P_{Trans})^2,\ P_{Trans}Subadult = P_{Trans},\ \text{and}\ P_{Trans}Piglet = (P_{Trans})^{0.5} \quad (3)$$

In all other cases, animals are infected lethally. Individual survival is drawn from a survival curve of lethally infected animals on a population level using two form parameters (maximum survival time T_{MAX} and an exponent X), giving:

$$P_{SR} = \left(1 - \frac{t}{T_{MAX}}\right)^X \quad (4)$$

Thus, the proportion of acutely and chronically infected pigs is given by the exponent X, with high values of X resulting in many acutely infected, and low values of X resulting in many chronically infected, respectively. The parameter T_{MAX} then determines the maximum time span chronically infected pigs will survive. According to the basic idea of the conceptual model, high virulence results in less transient infections (i.e. eventually immune) and in the majority of lethal infections being acute (i.e. finishing within 4 weeks). Low virulence results in more transient infections and in many chronic courses of infection (i.e. long-term or persistent infection) (see Kramer-Schadt et al. (2005) for details). Discrimination of high, medium and low virulence is shown in Table 2.

Reproduction: When a pregnant sow is infected, 10/16 of her foeti are aborted, half of those that survive are persistently infected (PI) and the remainder are normal susceptible piglets. As PI piglets harbour lethal infections, they die in accordance with the above (T_{MAX}, X)-rule. If the reproducing sow was immune, then the piglets are born with maternal antibodies. These so-called partially protected piglets are set back to the status 'susceptible' after TS_{MA} time steps (Table 2).

Analysis of simulation output

For all ecological and epidemiological parameter combinations, the cross-designed simulation experiment generates repeated measurements over different landscapes (Figure 1). The effect of habitat availability (i.e. population density in simulation area) and habitat structure (i.e. fragmentation of the available habitat) were analysed. In order to describe the effect of landscape characteristics on the disease dynamics, the probability of persistence for about 10 years (i.e. 506 weeks post primary infection) was measured. In the case of self-limiting outbreaks, the main interest is in the mean duration of these outbreaks within the given landscape or habitat structure.

RESULTS

One thousand two hundred and ninety six (1296) parameter configurations were considered in the factorial design of the simulation experiment. In 72% of the parameter configurations, the probability of persistence was less then 10%. When analysing the simulation output using general linear models (GLM), the virulence parameter was the determining predictor for the

probability of persistence. The outcome underlines the importance of the virulence parameter for the persistence of the disease (Kramer-Schadt et al., 2005; Alban et al., 2005).

Cross tabulation of virulence vs. probability of persistence reveals that high virulence resulted in disease extinction in all simulations. Furthermore, none of the simulations with the medium virulent pathogen exhibited more then 70% of model repetitions with persisting disease. Therefore, the data were stratified with respect to the virulence attribute. The relationship between probabilities of persistence and habitat structure was analysed for the low virulence strata alone, and, changes in the mean duration of an outbreak when habitat was altered were analysed with respect to the parameter of high virulence.

Table 3. Results of GLM analysis referring to the probability of persistence with respect to the pathogen and habitat related variables.

Model parameter	Scaled estimate	AIC	ΔAIC
Intercept-only		21840	$>10^4$
Pathogen		10715	5
Virulence	**1.3**		
P_{inf_G}	-0.5		
P_{inf_N}	-0.1		
Habitat		10710	0
Availability (coverage)	0.1		
Structure (fragmentation)	-0.1		
Male dispersal			
Distance	not significant		
Orientation	not significant		

Probability of persistence: Higher fragmentation reduces the probability of persistence (Table 3). The effect is more prominent for low transmission dynamics (i.e. P_{inf_G}, P_{inf_N}). If the transmission is very probable, the simulated outbreak persisted in all runs and thus subsumes the effect of fragmentation (not shown).

After removing the dominant effect variables (i.e. virulence, probability of transmission), the impact of fragmentation becomes visible (Fig. 2) as indicated by the significant factor in the GLM. If the virus is characterised by low virulence (i.e. persisting *per se* through long lasting chronic infections), the probability of persistence increases in connected habitat. *Vice versa*, persistence of the simulated epidemic is less likely in more fragmented habitat and the effect is stronger if the habitat is only sparse. Further stratification does not produce valuable insight. Indeed, as indicated by the GLM analysis, the movement characteristics of the infection via dispersing male boar groups (i.e. distance and habitat preference) does not affect mean probability of persistence systematically.

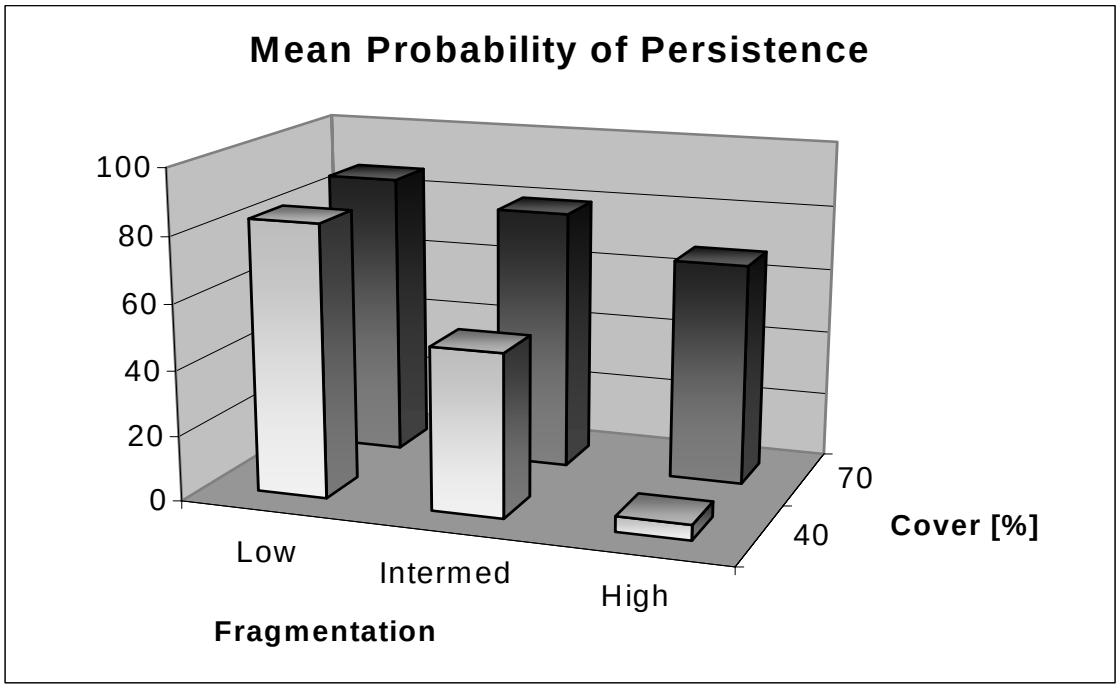

Fig. 5 The effect of fragmentation on the probability of persistence. The series depicts the changing probability of persistence with respect to increasing fragmentation (light bars or dark bars) and increasing coverage (light vs. dark bars). Highly fragmented habitat might reduce the probability of persistence dramatically, especially if habitat is sparse. (The effect is more pronounced if transmission is assumed not to be perfect, here $P_{inf\_G} = 0.05$.)

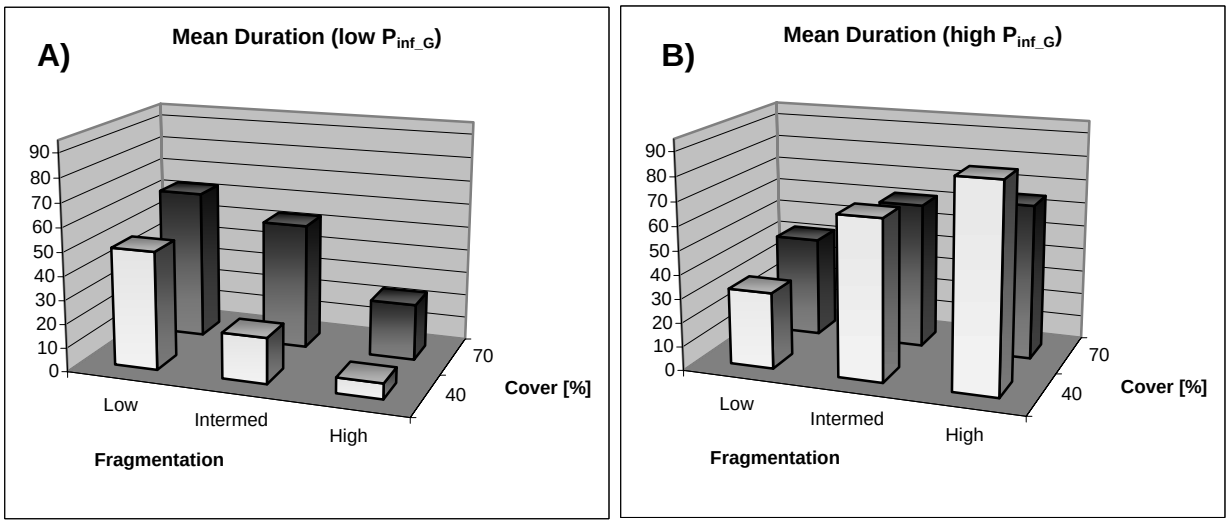

Fig. 6 The effect of fragmentation on the duration of an outbreak. The series depicts the changing duration of an outbreak with respect to increasing fragmentation (light bars or dark bars) and increasing coverage (light vs. dark bars). Highly fragmented habitat might either shorten the outbreak or prolong it, depending on the efficacy of transmission, especially if habitat is sparse. (A: less efficient transmission; B: more efficient transmission)

Mean duration: In the parameter sub-space where the simulated epidemic was not persistent at all (i.e. simulations with high virulence), the relationship between landscape variables and duration of the epidemic were considered. A decrease of the mean length of the outbreak was expected with increase in fragmentation, because loosely connected habitat would be expected to interrupt the spatial transmission chain and thus shorten the outbreak. The actual result strongly depends on the transmission dynamics assumed for the infection (i.e. P_{inf_G}, P_{inf_N}). Figure 3 shows the respective graphs for lower and higher transmission probability. If the transmission is relatively unlikely ($P_{inf\_G} < 0.1$), the expected relationship was found (Figure 3A). Increasing the fragmentation of the habitat proportion in the landscape reduces the mean duration of the outbreaks for any habitat availability value. However, when transmission is more efficient ($P_{inf\_G} > 0.1$) and hence, any susceptible individual might eventually become infected, the situation is reversed. Now, an increase in fragmentation prolongs the duration of the epidemic (Figure 3B). This is reasonable because the infection needs some 'handling time' to pass through the complete landscape configuration in addition to the basic length of time within a continuous habitat patch. Not surprisingly, the effect is less dominant when there are more habitats (i.e. coverage increased) which locally form larger coherent patches (see Fig. 1). The movement characteristics of the infection via dispersing male boar groups (i.e. distance and habitat preference) does not affect mean duration of the epidemics systematically.

In summary we found the following relations between landscape characteristic and persistence or duration of a CSF epidemic in wild boars:

1. If epidemiology supports persistence (i.e. low virulence), then fragmentation will counteract it.

2. If persistence is impossible, then more fragmented landscapes will still counteract the epidemic by shortening the duration of the outbreak. However, if transmission between individuals is efficient, then wild boar populations in more fragmented landscapes will suffer from longer epidemics due to time the infection needs for a stepwise passage through the varied habitat.

3. Habitat selection and movement length during dispersal of male groups were of minor importance. Rather, disease dynamics in resident boars and their neighbours are responsible for the persistence of the disease.

DISCUSSION

Most western populations of wild boars have remained free of CSF, and apparently only a limited number of populations in continental Europe have suffered disease outbreaks. It is still not known how the infection can be maintained in a focus at a relatively low rate. Classical Swine Fever foci in wild boars have never been reported to expand into an epidemic wave of infection on the continental scale, but seem rather limited in size, perhaps due to particular characteristics of the landscape that limit the movement of boars (Artois et al., 2002).

An individual based, spatially explicit simulation model was used for the spread and persistence of CSF in heterogeneous wild boar (*Sus scrofa*) populations. This model has been developed to support management efforts designed to prevent and eradicate disease outbreaks in real landscapes. The model integrates, at the individual level, the relevant aspects of wild boar society and spatial population dynamics on one side, and the disease transmission and its

outcome on the other. It simulates individual life history of wild boars sharing a territory with their family group and experiencing dispersal, reproduction and mortality depending on factors such as the age, the time of the year and the density of boars in the territory. The probability of disease persistence was investigated in relation to simulation scenarios of different wild boar densities and spatial heterogeneities driven by the landscape structure, and different courses of the disease.

The analysis of simulation results suggests that the driving factor for the dynamics of CSF is the response of individuals to an infection. Persistence is only possible when the probability of a chronic course of the disease is high and when the infection persists for a long time in some individuals, typically associated with low-virulent pathogens. For low-virulent disease, cycling of the epidemic for more then ten years within the simulation area was repeatedly observed. The proportion of model repetitions that persisted at least ten years were determined for each parameter combination and were searched for systematic changes when landscape features were altered in order to measure the impact of landscape structure. As expected, it was possible to determine a reduced probability of persistence when habitat structure became more fragmented. Furthermore, reducing the population density by removing half of all habitats reduced the probability of outbreaks lasting as long as ten years, particularly in fragmented habitat. This observation is plausible because continued cycling of the disease in the population requires continuous contact to the available susceptibles, which obviously might be distorted in more fragmented habitat structures.

A different response of individuals to the infection was assumed in the high virulence scenarios. The infected animals suffer heavily from the infection, only few are able to develop an effective antibody response, resulting in a low proportion of transient infections. The majority of the infected boars die quickly due to an acute infection. As one consequence of high virulence, the epidemic efficiently ‘consumes’ most susceptibles in an area and finally limits itself. Therefore, the set of simulation results associated to high virulence does not contain any parameter combination were the simulated outbreak last as long as ten years. To analyse the effect of landscape structure in this epidemiological scenario it is important, therefore, to refer to the duration of the epidemic. The mean length of the epidemic was calculated out of 40 model repetitions for each parameter combination and searched for systematic changes when landscape features were altered. Interestingly, the simulation results revealed strong dependence between how landscape attributes influence the length of the outbreak and the transmission probability (i.e. the assumed contact probability between individuals and the transmissibility during the contacts). With low transmission assumed (i.e. less then 10% chance between an infected and a susceptible individual over at most one week) fragmentation of habitat reduces the time horizon of an outbreak in the population. However, assuming higher transmission, the same outbreak will last longer in a more fragmented habitat compared to the coherent structure. This is reasonable because the final duration is determined by the time the infection needs to pass through all accessible parts of the habitat configuration. Furthermore, illustrating the advantage of the spatially explicit consideration, since the infection can only be spread stepwise (i.e. transmission, development of infectious course, next transmission), a more structured configuration will take longer for the infection to pass through compared to a ‘clumped’ one.

It can be concluded that spatial habitat heterogeneity influences the spatial structuring of populations and therefore the probability of effective infection transmission among individuals. These relationships critically influence the dynamics of CSF in wild boar populations and, in particular, the duration of outbreaks and the ultimate persistence of the disease. The insights gained from the simulation study are in good agreement with recent findings on disease spread

in heterogeneous host populations. For example, spatially structured host populations together with lower transmission rates between populations than within populations, have been shown to influence positively long-term persistence of the disease (Bolker & Grenfell, 1995). In this sense, this model suggests that regional wild boar populations can act as a long-term reservoir of CSF under certain combinations of disease virulence and spatial distribution of population abundance. The simulation results encourage the explicit consideration of spatial aspects of the host-virus dynamics to understand CSF in natural populations. Future steps must include the investigation of disease spread and persistence in relation to the wild boar habitat distribution in real landscapes. Its analysis in relation to patterns of disease spread and persistence observed in field studies will improve understanding of the relevant aspects in order to manage the disease better (Grimm et al., 2005).

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ECONOMICS 2

MULTI CRITERIA ANALYSIS OF ALTERNATIVE STRATEGIES TO CONTROL CONTAGIOUS ANIMAL DISEASES

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SUMMARY

Decision-making in controlling contagious animal diseases involves complex trade-offs between multiple objectives. An integral evaluation was performed to illustrate the potential support of evaluation techniques such as Multi Criteria Analysis (MCA) in choosing the control strategy that serves the general interest best.

The MCA was centred on the 3 high-level objectives of epidemiology, economics and social-ethics. The appraised control alternatives consisted of the basic EU strategy, a pre-emptive slaughter strategy, a protective vaccination strategy and a suppressive vaccination strategy. The approached stakeholders reflected the judgements of the European Chief Veterinary Officers, 'agricultural concerned' Europeans, and 'non –agricultural concerned' Europeans.

The preferences of the elicited stakeholder groups resulted in a surprisingly similar final ranking of control alternatives. Due to the balanced evaluation technique of the MCA overall differences between opposing stakeholders turned out to be not as great as they seemed in an unstructured, face to face meeting.

INTRODUCTION

Decision making in controlling contagious animal diseases is a complex, conflicting process, characterized by a mixture of epidemiological, economic and social-ethical value judgements. Different stakeholders will have different ideas about which strategy to choose. Their views may, for instance, represent the interests of the farming community, the commercially related industry, the animals, the consumer or the general citizen. This may create a situation of conflicting interests, as economic motives may prevail in the views of some, while animal or human welfare motives may be prominent in the view of others.

Application of a Multi Criteria Analysis (MCA) could support policy makers in choosing the control strategy that best meets all of these conflicting interests. MCA can be effective in increasing the understanding, acceptability and robustness of a decision problem. Although it is one of the most frequently applied tools within operations research and management science (Dodgson et al., 2000; Voogd, 1982), MCA methods are rarely applied in the management of animal disease control even though it generally improves the quality and transparency of the

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decision making process. The MCA study as described in this paper reflects the application of a MCA-framework to order the various contagious-animal-diseases control strategies according to the preferences of various stakeholders.

MATERIALS AND METHODS

Background to the presented MCA research

The presented MCA research was part of a large EU research project in which the consequences of outbreaks of contagious animal diseases were evaluated for various EU member states. Within this EU project, member state specific data were collected comprising demographic and livestock production data, epidemiological and economic data. These data were used as inputs in various modelling modules to obtain insight in the epidemiological and economic impact of outbreaks of contagious animal diseases. The results of these modelling studies along with the results of a detailed questionnaire to elicit the preferences of various stakeholders served as inputs of the presented MCA-framework (Huirne et al., 2005)

Definition of MCA

The general purpose of a MCA is to serve as an aid to thinking and decision-making, but not to take the decision. The MCA technique deals with complex problems that are characterized by any mixture of quantitative and qualitative objectives, by breaking the problem into more manageable pieces to allow data and judgements to be brought to bear on the pieces. Then the technique reassembles the pieces to present a coherent overall picture to decision makers (Voogd, 1982).

MCA establishes preferences between alternatives to an explicit set of objectives and measurable criteria to assess the extent to which the objectives have been achieved. A key feature of MCA is its emphasis on the judgement of the stakeholders involved, in establishing objectives and criteria and estimating the relative importance and weighting of each criterion.

There are many different MCA methods (Nijkamp et al., 1990). The principal difference between the main MCA methods is the way in which each alternative's performance across all criteria is aggregated to form an overall assessment. Two of the most applied MCA methods are the simple linear additive evaluation method and the concordance analysis method. The simple linear additive evaluation method combines the alternative's values into one overall value by multiplying the value score on each criterion by the weight of that criterion, followed by a summation of all those weighted scores (Dodgson et al., 2000; Voogd, 1982). This method is perhaps the simplest and most intuitive of all aggregation methods. However, the method is only suitable to aggregate scores within a corresponding measurement scale (quantitative or qualitative). The concordance analysis is an evaluation method in which the alternatives are ranked by means of their pairwise comparisons in relation to the defined criteria (Nijkamp et al., 1990). Due to the pairwise comparisons, this method is able to aggregate quantitative as well as qualitative scores into one overall evaluation value.

Both methods were used within the presented MCA study. A detailed description of the application of the methods is given in subsequent paragraphs.

Steps within MCA

The application of MCA involves eight steps as listed and described below:

1. Establish the decision context
2. Identify the alternatives to be appraised
3. Identify objectives and criteria
4. Score
5. Weight
6. Calculate overall value
7. Examine the results
8. Sensitivity analysis

1) Establish the decision context: Within this first step, the objective of the MCA should be clearly defined along with an identification of the key players or so-called stakeholders; i.e. decision makers as well as people who may be affected by the decision.

MCA is all about multiple conflicting objectives. There are ultimately trade-offs to be made. Nonetheless, in applying MCA it is important to identify a single high-level objective for which there will be sub-objectives. The aim of this MCA was to make best use of data currently available to support the decision on controlling contagious animal diseases as FMD, CSF and AI.

A key player or stakeholder is anyone who can make a useful and significant contribution to the MCA. Stakeholders are chosen to represent all the important perspectives on the subject of the analysis. One important perspective in the field of controlling contagious animal diseases is that of the final decision maker and the animal health authority to whom that person is accountable. Within this analysis the European Chief Veterinary Officers (CVO) were approached to express these governmental values by questionnaire responses. Those responses were given by a written questionnaire, so there was no interaction or exchange of information/experiences between the various participating CVOs.

Beside the group of CVOs, two other groups of European stakeholders were questioned for their judgements to reflect the general public opinion (viz. an agricultural interest group and a non-agricultural interest group).

2) Identify the alternatives to be appraised: The appraised alternatives per contagious animal disease consisted of the default EU measures (viz. stamping out of detected herds and installation of protection and surveillance zones) and one or more of the following additional control measures:

Pre = pre-emptive slaughter of neighbouring farms within a predefined radius around a detected farm. This measure results in a regaining of the disease free status (or removal of export bans) 3 months after culling the last detected animal.

Vac _kill = suppressive vaccination within a predefined radius around a detected farm. Vaccination is applied as a suppressive measure; all vaccinated animals will therefore be slaughtered as soon as the epidemic is under control. This measure results in a regaining of the disease free status 3 months after culling the last detected or vaccinated animal.

Vac _live = protective vaccination within a predefined radius around a detected farm. Vaccination is applied as a protective measure; all vaccinated animals will therefore stay on the farm after the epidemic is under control. This measure results in a regaining of the disease free status 6 months after culling the last detected animal.

3) Identify objectives and criteria: Assessing alternatives requires thought about the consequences of the alternatives, for strictly speaking it is the consequences that are being assessed not the alternatives themselves. Criteria and sub-criteria or indicators are the measures of performance by which the alternative control strategies are judged. Criteria are specific, measurable objectives. They are children of higher-level parent objectives, who themselves may be the children of even higher-level parent objectives.

This research is centred on three high-level objectives or main criteria, viz. epidemiology, economics, and social-ethics. Each criterion is broken down into lower level objectives or indicators to facilitate the scoring process. These clusters of indicators are as presented in Table 1.

In general, criteria and indicators are defined by help of the stakeholders in an iterative way. However, within the scope of this research, it was not possible to conduct such an extensive, iterative process. The definitions of criteria and indicators were therefore based on 1) the results of a former study in which Dutch stakeholders were interviewed by means of a Group Decision Room session to define the criteria by which animal control strategies should be evaluated (Huirne et al., 2002) and on 2) additional expert consultation.

4) Score: By determining criterion scores, attention should be paid to the measurement scale. In case of a quantitative scale the measurement unit is known, i.e. a quantity has been defined as a standard by which the magnitude of differences can be expressed. Measurement units are animals, farms, days, and so forth. The measurement unit of a qualitative measurement scale is unknown. Three qualitative measurement scales can be distinguished of which the ordinal scale contains most information, since the numbers of this scale give a rank order. However, this ranking order does not express the extent to which a choice-possibility is worse or better than any other choice possibility.

Even if the criterion scores have been determined on a quantitative measurement scale for all criteria, these scores are mutually incomparable since most of the measurement units will differ from each other. One criterion might be expressed in number of farms, whereas another criterion is measured in days. To make the various criterion scores comparable it is necessary to standardize them into one common measurement unit, by taking care that for each criterion the scores will get a range from 0 to 1. The method of standardization used for the scores in this study can be written as:

Standardized score i = (score i / maximum score)

or each score is divided by the highest score of the criterion concerned.

Related to standardization is the issue of the direction of the criterion scores. For some criteria a higher score implies a ‘better’ score, whereas for other criteria higher score implies a ’worse’ score. Each standardisation should therefore be accompanied by a consideration of the

direction of the scores. In this study the worst criterion score was given a standardized value of 0, whereas the best criterion score had a standardized values of 1.

Table 1. Overview main criteria and indicators, along with their average preference weights indicated by the CVOs of EU member states

Main criteria	Average CVO weight
Epidemiology	53
Economics	30
Social-ethics	17
Cluster of epidemiological indicators	
Duration	28
Number infected herds	25
Size affected region	19
Number destroyed animals	12
Number destroyed herds	12
Number destroyed non-farm animals	5
Cluster of economic indicators	
Direct farm losses	15
Consequential farm losses affected region	14
Consequential farm losses outside affected region	10
Losses other agricultural sectors	11
Losses non agricultural sectors	9
Organisation costs	11
Export restrictions EU markets	12
Export restrictions non- EU markets	9
Tax payer	9
Cluster of social-ethical indicators	
Efficacy	18
Socio-economic factors	12
Macro-economic factors	7
Commercially interested parties	8
Animal health	8
Animal welfare	7
Tourism	4
Non-farm animals	3
Human health	10
Governmental policy	8
Natural life-cycle	6
Food source	9

Criterion scores can be derived in many different ways. In this study the quantitative scores of the epidemiological and economic indicators were based on the results of stochastic

simulation modeling studies (Huirne et al., 2005). The presented MCA analyses were directed towards the 95 percentile values, assuming a risk-averse attitude with respect to the contagious animal disease control. The scores of qualitative social-ethical indicators were obtained by ranking the alternatives per criterion by its expected effectiveness. These effectiveness rankings were based on the insights obtained by the questionnaires, personal interviews and model studies.

5) Weight: A criterion's weight should depend on the range of difference in the criterion scores and on how much the stakeholders care about the difference. For instance, most stakeholders consider length of the epidemic an important decision criterion. However, when alternative strategies would result in an expected duration difference of only a few days, length would not longer be an important decision criterion. In this study, stakeholders were asked to express their judgements (= weights) on grounds of their subjective knowledge on possible ranges of criterion scores.

The weighting factors applied in this study were based on the results of a written questionnaire. By this questionnaire various groups of stakeholders expressed their judgements using comparative rating scales. Stakeholders had to make judgments of each indicator with direct reference to their judgments of the remaining indicators (Churchill, 1995), by dividing 100 points per cluster. This paper emphasizes the judgements of the CVOs. See for a further description of the questionnaire and its results Huirne et al., 2005.

6) Calculate overall value: By means of the simple linear additive evaluation method, the overall weighted scores of the three main criteria, epidemiology, economics and social ethics were obtained. In general the higher the overall value, the better the alternative control strategy scored within the concerned criterion.

However, the performed multi criteria evaluation was based on criteria, which were partially assessed on a quantitative scale as well as partially on a qualitative scale. To account for the specific characteristics of both measurement scales, a mixed data multi criteria technique was applied to determine an overall score per alternative. In this mixed data evaluation technique, which was a generalised form of the concordance analysis technique, differences in alternatives were expressed in a condensed way by means of paired comparisons. Standardized scores of each indicator were compared in pairs of the evaluated alternatives, resulting in so-called dominance scores. A positive score implies dominance of one strategy in relation to another while a negative value implies submission. A dominance measure of 0 implies an indifference between the strategies compared. By weighting these dominance scores per criteria, overall dominance scores of the three main criteria were obtained.

To compare the outcomes of the quantitative and qualitative dominance scores, the scores of the individual main criteria were standardized into the same unit. In this way the dominance scores of the quantitative criteria epidemiology and economics were comparable to the dominance score of the qualitative criterion social-ethics. By weighting these standardized dominance measures with the aggregated weights of the constituent criteria the overall dominance score per alternative was calculated, which represented the degree in which an alternative was better (or worse) than another alternative.

7) Examine the results: The aggregation of the dominance scores of the three main criteria (viz. epidemiology, economics and social-ethics) into one overall dominance score per alternative gives an indication of how much an alternative was preferred over another. These

overall dominance scores are also determinative in the overall ordering of the evaluated control strategies.

8) Sensitivity analysis: Sensitivity analysis provides a means of examining the extent to which the relative importance weights of each criterion/indicator makes any difference in the final results. Interest groups often differ in their views of the relative importance of the criteria (or weights) and of some scores, though weights are often the subject of more disagreement than scores. In this study special attention was given to the comparison between the ranking of alternatives based on the preferences expressed by the CVOs and the ranking based on the preferences expressed by the representatives of the general public.

Using the MCA model to examine how ranking of options might change under different weighting systems can show that for instance, two options always come out best, though their order may shift. If the differences between these best options under different weighting systems are rather small, accepting a second best option can be shown to be associated with little loss of overall benefit.

RESULTS

Weighting factors reflecting preferences of the CVOs

The response rate of the 25 CVOs on the written questionnaire was about 80% (i.e. 20 questionnaires). The averaged CVO weights for the three main criteria and their clusters of indicators are represented in Table 1.

With respect to the main criteria, the CVOs preferred the epidemiological criterion with an average relative weight of 53%. Corresponding average weights for the economic and social-ethical main criteria were 30% and 17%. Duration of the epidemic (28%) and the number of infected herds (25%) were regarded as the two most important epidemiological indicators. Differences between the relative weights of economic indicators were not as profound as the epidemiological indicators. Direct farm losses (15%) and consequential farm losses in affected region (14%) were regarded as the two most important economic indicators. Efficacy (18%) and social-economic factors (12%) were considered as the most important social ethical indicators (Table 1).

MCA application to evaluate three FMD control alternatives

This paragraph illustrates the overall MCA results based on the evaluation of three FMD control alternatives for an area within one of the studied EU member states, characterised as a net importing, densely populated livestock area.

1) Overall weighed scores main criteria: By means of the simple linear additive evaluation method, the overall weighted scores of the three main criteria, epidemiology, economics and social ethics were obtained as demonstrated by Table 2.

Based on the overall epidemiological score, the Pre strategy was preferred best, followed by the Vac_live strategy. The overall 0 score on the Vac_kill strategy indicates that – compared to the other 2 alternatives – Vac_kill scored worst on all epidemiological indicators. However, the efficiency with which this strategy controls an FMD epidemic is comparable with the efficiency of the Vac_live strategy. Due to the fact that the vaccinated animals are killed afterwards,

Vac_kill scored worst on all indicators involving number of destroyed herds or animals. These indicators, therefore, do not strictly reflect epidemiological efficiency; they also reflect a social-ethical element.

Table 2. Overall weighed scores per main criterion and FMD control alternative. Bold printed values reflect alternative with highest criterion score (=highest rank).

Criterion	Control alternative		
	Pre	Vac_live	Vac_kill
Epidemiology	**36**	27	0
Economics	58	53	**63**
Social-ethics	21	**55**	33

The ranking of the alternatives based on the economic criterion demonstrates that the Vac_kill strategy was preferred above the others. However differences in overall economic values among the alternatives were rather small, as reflected by the small difference in overall value between the first and second ranked alternatives (viz. 5 points). The economic ranking based on the MCA may differ from the economic ranking based on the result of adding all the losses to one overall economic value. By utilizing subjective weighting factors, the MCA ranking is not only accounting for the magnitude of the losses but also for, for instance, value judgements on topics as 'who is bearing the losses'.

From a social-ethical point of view, alternative Vac_live was evaluated to exceed the other 2 alternatives. With a difference of at least 22 points, Vac_kill was evaluated as the second best option.

Overall strategy value: Table 3 demonstrates the dominance scores of the three main criteria as a result of paired comparisons of the three FMD control alternatives. For instance, the fifth column of the table describes the results of the comparison between the Vac_live strategy and the Vac_kill strategy. As reflected by the positive scores, the Vac_live strategy dominated the Vacc_kill strategy on two of the three main criteria (viz. +5.19 on Epidemiology, +0.73 on Social-Ethics). However, regarding the Economic criterion, the Vac_live strategy was dominated by the Vac_kill strategy (economic dominance score = -0.57).

Table 3. Criteria dominance scores by paired comparisons of the evaluated FMD control alternatives (e.g. Pre/V_live = Pre strategy compared to the Vac_live strategy).

Criterion	Paired comparisons					
	Pre / V_live	Pre / V_kill	V_live / Pre	V_live / V_kill	V_kill / Pre	V_kill / V_live
Epidemiology	1.75	6.95	-1.75	5.19	-6.95	-5.19
Economics	0.28	-0.29	-0.28	-0.57	0.29	0.57
Social-ethics	-1.12	-0.39	1.12	0.73	0.39	-0.73
Total	0.92	6.26	-0.92	5.35	-6.26	-5.35

By aggregating the weighted dominance scores per criterion, the overall dominance scores of the three control alternatives were obtained According to these total dominance scores the Pre strategy was favoured over the other strategies; i.e. all total paired dominance scores were positive. The dominance difference with respect to the Vac_live strategy was, however, small (0.92). Vac_kill was completely dominated by the other strategies as reflected by its negative total dominance scores.

Ranking under different preferences or weighting systems

Beside the group of CVOs, an 'Agricultural concerned' interest group and a 'Non-agricultural concerned' interest group were questioned for their preferences. Table 4 summarizes the indicated preference weights for the main criteria among the three interest groups. This overview stresses the contrast in perspectives of the Non-agricultural interest group in comparison to the other interest groups.

Table 4. Criterion preference weights (%) per interest group

Interest group	Criterion		
	Epidemiology	Economics	Social-ethics
CVO	53	30	17
Agriculture	49	33	18
Non-Agriculture	50	15	35

An evaluation of the overall dominance scores based on the preference weights of these three interest groups makes it possible to examine differences in ranking of alternatives. Table 5 demonstrates - for instance - the interest group specific overall scores of AI control alternatives for an exporting, densely populated livestock area. Based on the preferences of the CVO and the Agricultural interest groups the Pre strategy was ranked first followed by the Vac-live strategy as second best alternative. From the Non-agricultural point of view, the ranking of these two alternatives was just the opposite. However, differences between first and second best alternatives were rather small. The loss of overall benefit associated with the acceptance of the second best alternative is highest for the Non-agricultural interest group (difference of 5.8).

Table 5. Overall dominance scores of AI control alternatives based on the criterion weights of the individual interest groups. Bold printed values reflect alternatives with highest scores (= highest rank).

Interest group	Control alternative		
	Pre	Vac_live	Vac_kill
CVO	**8.3**	7.4	-15.6
Agriculture	**8.2**	6.8	-15.0
Non-Agriculture	4.2	**10.0**	-14.2

DISCUSSION

Various MCAs were conducted within the EU project (Huirne et al, 2005) to evaluate the ranking of alternative strategies to control the contagious animal diseases as FMD, CSF and AI. Based on the judgement values of the CVOs, results showed a general tendency towards the

ranking of alternatives, which in most of the cases appeared to be independent of the evaluated disease (see for detailed information Huirne et al., 2005). This general tendency can be described as follows:

- From an epidemiological point of view, the Vac_live strategy was preferred as strategy to control epidemics of CSF or AI. For the control of FMD, the Pre strategy was preferred over the other alternatives. Vac_live was, however, the second best option.
- From an economic point of view, the default EU strategy (viz. stamping out of detected herds and installation of protection and surveillance zones) was ranked as best option for those situations where the EU strategy was evaluated as an effective control strategy. There was no unambiguous ranking of alternatives, which characterises the preference in the other situations (i.e. situations in which the EU strategy had a restricted control efficiency).
- From a social ethical point of view, the Vac_live strongly dominated the other control alternatives.
- From a multi criteria point of view, the Vac_live and EU strategies were generally preferred over the other control strategies for those areas where the livestock density was sparse to moderate. The Pre strategy was mostly preferred within the areas with a high livestock density, followed by the Vac_live strategy as second best option.

Difference in ranking between clusters of countries, comprising regions with comparable density and/or trade characteristics, were possibly underexposed due to the use of 'average' CVO judgements. Disaggregating the panel of CVOs into subgroups conform the density and trade characteristics of the country the CVOs represent, followed by an analysis per cluster would provide better insight into the possible presence of alternative rankings.

Individual CVOs - or in general – individual interest groups often differ in their views of the relative importance of the various criteria. Using the MCA framework to examine how ranking of alternatives might change under different preferences or weighting systems can show that, for instance, two alternatives always come out best. Their order, however, may shift. If the differences between these best alternatives under different weighting systems are rather small, accepting a second best option can be shown to be associated with little loss of overall benefit.

The criterion preferences of the 'Non-agricultural concerned' interest group differed from the other 2 elicited stakeholder groups (Table 4). Nevertheless, the final ranking of the AI control alternatives appeared surprisingly similar (Table 5). Generally, when opposing stakeholders discuss alternative options, they quickly focus on their differences of opinions, ignoring the effect of many criteria on which there is an agreement. The MCA technique provides a more balanced approach to ensure that all criteria enter the evaluation, with the result that overall differences are not as great as they seem in an unstructured, face-to-face meeting.

Based on the findings within the described study it can be concluded that the MCA technique is a suitable tool to assist the complex decision making process of controlling contagious animal diseases by providing structure to debates, ensuring quality conversations, documenting the process of analysing the decision, separating matters of fact from matters of judgement, making value judgments explicit, bringing judgements about trade-offs between conflicting objectives to the attention of decision makers, creating shared understanding about the issues, generating a sense of common purpose, and, gaining agreement.

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PRIORITISATION OF FOODBORNE PATHOGENS

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SUMMARY

Priority setting of pathogenic microorganisms in food requires consideration of many different aspects. This paper presents the disease burden and the costs-of-illness associated with four pathogens in the Netherlands that can (also) be transmitted by food. Data refer to all cases of illness, updated to the year 2004, where possible but irrespective of transmission route and will later be combined with estimates for the fraction transmitted by food. Expansion by other criteria and other pathogens is foreseen.

Among the investigated pathogens, *Campylobacter* spp. causes the largest disease burden of 1200 (range 800-1600) Disability Adjusted Life Years (DALYs) per year, followed by *Salmonella* spp. 600 (450-900) DALYs, noroviruses 400 (300-1000) DALYs and rotaviruses 380 (220-570) DALYs. Cost-of-illness (in million euros per year) caused by noroviruses 25 (15-42), rotaviruses 21 (12-37) and Campylobacter 20 (14-35) are similar, whereas the Salmonella-associated costs are lower at € 7 million (3-17) per year.

INTRODUCTION

Human health is threatened by a wide variety of pathogens transmitted by food. Effective and efficient policy-making on control, prevention and surveillance of these food borne pathogens requires focusing on the most relevant ones. Therefore, the need was expressed by Dutch decision makers to develop methods for prioritisation of existing and emerging zoonotic pathogens to provide an objective basis for decisions on future projects. The specific objective of this study was to develop a model that helps to establish the priority of community-acquired pathogenic microorganisms that can (also) be transmitted by food.

The priority setting of communicable diseases for surveillance has been studied in several countries; including Canada (Carter, 1991; Doherty, 2000) and the UK (Horby et al., 2001; Rushdy et al., 1998). Petersen et al. (1996) prioritized infectious agents transmissible to humans through consumption of undercooked beef whereby each infectious agent was numerically scored based on potential hazard and potential exposure. In 2002, Ross et al. (2002) described the development and use of a simple tool for semi-quantitative food safety risk assessment, which has utility for ranking and prioritizing risks from diverse sources. This tool was used to generate a risk ranking for 10 seafood hazard/product combinations (Sumner et al., 2002). In Canada, the Ontario Ministry of Agriculture and Food is developing a method of systematically

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ranking food-safety-risks to help prioritize the allocation of food safety resources (McNab, 2003). Results from this project are not yet available. In the USA, the Food Safety Research Consortium is developing decision tools for policy makers to better identify and prioritize opportunities to reduce-food-safety risks and allocate government resources accordingly. With the model, users can produce rankings by pathogens, by food, and by pathogen-food combination, according to five measures of the impact on public health: number of cases of illness, number of hospitalizations, number of deaths, monetary valuations of health outcomes, and loss of Quality Adjusted Life Years. The model uses a quantitative approach and quantifies the uncertainty of rankings and other results (Batz et al., confidential draft). Except for the last, all studies are based on a semi-quantitative approach, whereby the scores on several criteria are summed to an overall score. However, comparing the studies shows important differences between them with regard to the number of criteria used and the number of criteria per domain (i.e. disease burden, social costs, trends, response, perception, organization interest, exposure, infectivity). As a consequence, the overall score is dependent on the number of criteria per domain. Also, the arithmetic method for calculating the overall scores differs between the studies, which make them less comparable, and the attribution of scores to criteria to some extent is arbitrary. There is no objective basis to combine such highly divergent criteria on the same scale and then simply add up all scores. Another disadvantage of the attribution of scores to intervals is the loss of information. For example, if intervals for the incidence of illness are arbitrarily defined as 100-1000 and 1,000-10,000, then two diseases with incidences of 900 and 1,100 would carry different weights, whereas disease with incidence 1,100 and 9,000 would not. So, the validity of semi-quantitative studies is restricted, and it is difficult to develop consistent criteria and scores. Therefore, in the present study a quantitative approach is used, whereby quantifying also the uncertainty of the results caused by insufficient data.

Disease burden was considered in most of the above studies as an important domain. This is in line with general trends in public health research that increasingly present disease burden as a major tool for priority setting. Some examples are the Global Burden of Disease Study by WHO and the World Bank (1996) and the Dutch series of Public Health Forecast studies (1997). Accordingly, in the present study, disease burden will be used as primary criterion for the prioritization of food borne pathogens. Besides the burden of disease, the costs for the Dutch society of food borne infections are important as well. Therefore, in the present project the direct health care costs and direct non-health care costs as well as indirect non-health care costs associated with food borne infections and its sequelae are calculated. This is an on-going project and it is foreseen to expand the framework in the future with other criteria such as trends in incidences; per pathogen the attributable fraction to the different food products; and risk perception, although the latter one does not consist exclusively of objectively measurable characteristics.

MATERIALS AND METHODS

Selected pathogens

Four gastro-enteric pathogens that can (also) be transmitted by food, were selected: norovirus (NV), rotavirus (RV), thermophilic *Campylobacter* spp. and *Salmonella* spp. NV and RV were observed to be the most frequent pathogens of community-acquired gastroenteritis (GE) (de Wit et al., 2001a), and Campylobacter and Salmonella were the most frequently observed bacterial pathogens. All four pathogens result in acute GE, which in most cases is self-limiting within a few days to weeks. For few patients the disease is fatal. Apart from GE, fatal or

not, no other illnesses are known to be related to a NV infection or a RV infection in humans. Campylobacter and Salmonella infections, however, do result occasionally in complications. Reactive arthritis (ReA) is the most significant sequel occurring sporadically after Salmonella infections (Raybourne et al., 2003). ReA, Guillain-Barré syndrome (GBS), and inflammatory bowel disease (IBD) are the most significant sequelae occurring after campylobacteriosis (Mangen et al., 2005). GBS is a neurological disease frequently preceded by an acute infectious illness and affecting at least the motor, sensory, and autonomic nerves supplying the limbs. The term ReA describes an acute aseptic arthritis triggered by an infection elsewhere in the body. Crohn's disease and ulcerative colitis are collectively classified as IBD. IBD is characterized as chronic intestinal disorders of unknown etiology. For background information on the three illnesses see Mangen et al. (2005). The frequency of other post-infectious complications following a campylobacteriosis or a salmonellosis is low and was therefore disregarded in the current study. Although, most NV cases and RV cases are acquired in the community, both viruses are responsible for nosocomial infections. NV is a common source of nosocomial infections acquired during a stay in health-care institutions, such as hospitals, nursing homes and homes for elderly (van Duynhoven et al., 2005). RV is one of the major pathogens triggering nosocomial infections in paediatrics (de Wit et al., 2000). Programs to reduce foodborne pathogens in the population, - the aim of the current study -, however, would have no or only an indirect impact on nosocomial infections acquired in health-care institutions. Therefore in the current study only community-acquired NV and RV cases were considered.

It is planned to expand the model in the future for other (foodborne) pathogens and emerging zoonoses.

Outcome trees and incidence

In order to assess the burden of disease and the cost-of-illness (COI) for the various pathogens under study, the disease outcomes following each specific exposure and ingestion or infection had to be defined. Therefore for each pathogen, separate models of the disease process had to be collected or designed, resulting in outcome trees (see Fig. 1). Each block represents a health outcome and transition probabilities between all blocks must be established. In this study the incidence approach was used to estimate the disease burden and cost-of-illness.

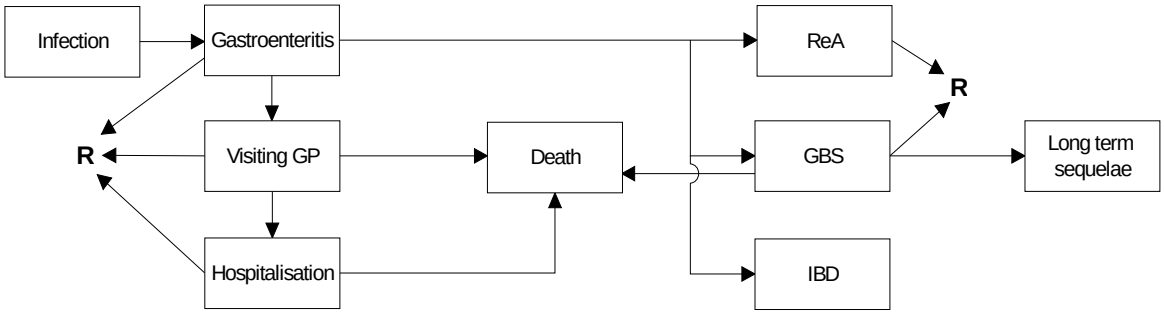

Fig.1 Outcome tree of Campylobacter-associated GE and sequelae (R = recovery).

Note: Campylobacter infections may result in GE and occasionally in sequelae such as ReA, GBS and IBD. Salmonella infections may result in GE and sporadically in ReA (no GBS and no IBD). NV and RV infections do result in GE, both fatal and non-fatal. No other sequelae are known.

The number of acute GE cases in the entire population and the number of cases visiting a GP, both hospitalized or not hospitalized, for NV, RV, Campylobacter and Salmonella

infections were based on SENSOR, a community based cohort study (de Wit et al., 2001a), and NIVEL, a GP based cohort study (de Wit et al., 2001b). Except for NV, incidences were extrapolated to 2004 following the trend observed in laboratory-confirmed cases (van Pelt et al., 2005). Age-distributions of RV and NV cases in entire population and at GP were based on de Wit et al. (2001a;b). Due to the low number of cases in SENSOR and NIVEL, the age-distribution of Campylobacter and Salmonella cases was based on the age-distribution observed within LSI the surveillance system of laboratory-confirmed cases diagnosed by regional public health laboratories (van Pelt et al., 2003). For some GE cases, an admission to hospital, mainly due to dehydration, is necessary. Based on studies from England/Wales (Lopman et al., 2003) and the US (Mead et al., 1999) it was assumed that minimum 0.22% of the NV cases in the population would be hospitalized, and most likely and maximum 0.33% of the NV cases in the population would be hospitalized, whereby using the NIVEL age-distribution as a proxy. By combining data from the National Disease Registry on hospitalizations for GE and laboratory surveillance data in a linear regression model, van Pelt et al. (2005) indirectly estimated the incidence of GE, and the proportion of hospitalizations attributable to RV infection, but only for children younger than five years for the years 1996 to 2004. Taking the average over these years and extrapolating by the proportion attributable to RV (most likely 54%; range 36%-66%), the number of hospitalized cases younger than five years was estimated. By subtracting the nosocomial RV cases, about 13% (de Wit et al., 2000), and extrapolating also for community-acquired hospitalized RV cases that were five years and older, about 5% (de Wit et al., 2000), the number of hospitalized RV cases was estimated. Of all laboratory-confirmed Campylobacter-associated GE cases, about 6,100 cases in 2004 (van Pelt et al., 2005), it was assumed that 9% would be admitted to hospital, according to a case-control study conducted in 2003 (Doorduyn et al., in prep.). The assumed age-distribution of Campylobacter-associated hospitalized GE cases was based on Doorduyn et al. (in prep.). Based on the Dutch National Disease Registry for hospitalization, van Pelt et al. (2005) reported for 2004 a total of 650 hospitalized salmonellosis cases (most likely estimate), with a range of 483 to 788 hospitalized cases in previous years. The assumed age-distribution of Salmonella-associated hospitalized GE cases was based on Doorduyn et al. (2006). In some rare cases GE might be fatal. A one year study of outbreaks of GE in the Netherlands showed that only residents (about 26%) of nursing homes or homes for the elderly died because of an NV infection (van Duynhoven et al., 2005), and nobody in all other settings. But Mead et al. (1999) estimated for the US that NV would be fatal in 0.001% of the entire population of ill patients. In the current study it was therefore assumed that most likely 0.001% of all NV infections would be fatal (range: 0% to 0.002%), mostly (about 95%) elderly persons. Based on Chang et al. (2003), it was estimated that 0.05% of community-acquired hospitalized RV cases that are younger than five years would be fatal. The number of fatal Campylobacter-associated GE cases and the number of Salmonella-associated GE cases was estimated based on Helms et al. (2003), who had estimated the relative excess mortality for patients with campylobacteriosis and salmonellosis, and using age-specific mortality risks from Statistics Netherlands and age-specific incidences as observed over the years within LSI (van Pelt et al., 2003 & 2005), the number of fatal Campylobacter-associated GE cases and the number of Salmonella-associated GE cases was estimated.

Mangen et al. (2005) had estimated in a previous study the number of Campylobacter-associated GBS cases, Campylobacter-associated ReA cases and Campylobacter-associated IBD cases, relying on Dutch and international published studies for GBS incidences; ReA incidences; and for IBD incidences. These estimates were used in the current study, updating ReA and IBD according to the estimated number of Campylobacter cases in 2004. Based on Hannu et al. (2002) it was assumed that 7% (6% - 9%) of the campylobacteriosis cases visiting a GP would

develop ReA. Whereas for Salmonella-associated ReA cases it was estimated that 8% (2.3% - 15%) of salmonellosis cases visiting a GP would develop ReA (Raybourne et al., 2003).

Disease burden

The different outcomes of infectious disease can be combined in one single measure, the Disability Adjusted Life Year (DALY), following the methodology proposed by Murray et al. (1997). The DALY methodology adds up the sum of years of life lost (YLL) and years lived with disability (YLD): DALY = YLL + YLD.

YLL is the number of years of life lost due to early mortality. YLL due to a specific disease in a specified population is calculated by summation of all fatal cases (n) due to the health outcomes (l) of that specific disease, each case multiplied by the expected individual life span (e) at the age of death. Thus:

$$YLL = \sum_{l} d_l \times e_l \tag{1}$$

In the current study the expected life span of fatal cases were derived from the standard life tables as reported by Statistics Netherlands (CBS, 2005).

YLD is the number of years lived with a disability, weighted with a factor between 0 and 1 for the severity of the disability. YLD is calculated by accumulation over all cases (n) and all health outcomes (l) of the product of the duration of the illness (t) and the severity weight (w) of a specific disease:

$$YLD = \sum_{l} n_l \times t_l \times w_l \tag{2}$$

The estimated burden of disease, attributable to one agent, is obtained by adding up all the health outcomes caused by this agent. The estimated number of DALYs with regard to the different illnesses is presented both discounted at a rate of 4% and not discounted. The used discount rate of 4% is in accordance with the Dutch guidelines for public health in 2004 (Oostenbrink et al., 2004)

The DALY uses explicit preference weights for health status. A major project had been carried out in the Netherlands to derive weights for 53 diseases of public health importance, involving the estimation of weights for 175 disease stages and/or severity levels (Melse et al., 1998). For GE and GBS severity weights, however, were based on Havelaar et al. (2000). The disability weights used in this study are available on request from the first author.

Estimates on the duration of the adverse health outcomes were derived from the results of earlier studies in the Netherlands (Havelaar et al., 2000; Mangen et al., 2005; Rockx et al., 2002), supplemented with the results of international studies (Food Standards Agency, 2000; Hannu et al., 2002; Roberts et al., 2003; Silverstein et al., 1999). More details are available on request from the first author.

Cost-of-illness

Besides the estimation of the burden of disease for the various pathogens under study, the associated costs-of-illness (COI) were estimated. COI were applied for each pathogen

separately, using the societal perspective. Following the guidelines of Oostenbrink et al. (2004), the COI was estimated, considering direct health care costs (DHC), direct non-health care costs (DNHC) and indirect non-health care costs (INHC), using Dutch cost estimates for the year 2004. In accordance with the guidelines, this study did not consider indirect health care costs, which would comprise the future savings in health care costs in the life years lost due to premature death. The estimated costs with regard to the different illnesses are presented both discounted at a rate of 4%, following Dutch guidelines (Oostenbrink et al., 2004), and not discounted.

The DHC category included valuation for medical services such as general practice (GP) consultations, specialists' consultations, hospitalisation, drugs, rehabilitation and other medical services. For each health outcomes (l) of that specific disease and for each specific medical service, the DHC related to a specific pathogen were estimated by multiplying the number of cases requiring health care service (m) by the required health care service units per case (p) and by the costs per health care service unit (mc). The formula for DHC for a specific pathogen with health outcomes l and for health care service i is, in basic notation:

$$DHC = \sum_{l} \sum_{i} m_i \times p_i \times mc_i \tag{3}$$

Travel costs of patients, costs for additional diapers, informal care and co-payments by patients, are some examples of DNHC. DNHC were estimated for each pathogen separately. For each health outcomes (l) of that specific disease and for each specific non-health care service j, the direct non-health care costs related to a specific pathogen were estimated by multiplying the number of cases requiring non-health care service (r) by the required non-health care service units per case (q) and by the costs per non-health care service unit (rc). The formula for DNHC for a specific pathogen with health outcomes l and for non-health care service j is, in basic notation:

$$DNHC = \sum_{l} \sum_{j} r_j \times q_j \times rc_j \tag{4}$$

INHC, which are defined as the value of production lost to society due to disease, were considered in the current study. Production losses could be the consequences of: a) temporary absence from work; b) permanent or long-term disability; and c) premature mortality. Productivity losses that occur due to sickness leave of sick individuals, and, where available, information on third persons taking care of patients were estimated. In this study the friction cost method to estimate INHC was applied. In this method, production losses are only considered for the period needed to replace a sick, invalid or dead worker, the so-called 'friction period' (Koopmanschap et al., 1992 & 1995). The friction cost method takes into account that in the economic processes a sick, invalid or dead person can and will be replaced after a period of adaptation (Koopmanschap et al., 1992). The length of the friction period depends on the situation on the labour market. A high unemployment rate generally allows fast replacement of a sick, invalid or dead person, whereas in the case of a low unemployment rate, on average more time is needed to find someone on the labour market that could fill in the position. It was assumed for the year 2004 a friction period of 154 days, similar to the friction period reported in Oostenbrink et al. (2004) for the year 2002.

The INHC for a specific pathogen were estimated for each health outcomes (l) of that specific disease and for each types of sickness leave (k) separately by multiplying the number of

cases with sickness leave (s) by the duration of sickness leave (u) by the wage costs (v) per day. The formula for INHC for a specific pathogen with health outcomes (l) and for each types of sickness leave k is, in basic notation:

$$INHC = \sum_{l} \sum_{k} s \times u \times v \tag{5}$$

To calculate the cost-of-illness for the different pathogens data on the number of cases per age group, the volumes for use of resources, and the actual economic costs of each of these items were needed. Information was needed per age group, because of differences in incidence and type of costs (e.g. sickness leave). Volumes for use of resources and sickness leave were derived using Dutch and international studies that were: de Wit et al. (2001a;b); Food Standards Agency (2000); Mangen et al. (2005); Oostenbrink et al. (2004); van den Brandhof et al. (2004); and van Pelt et al. (2003 & 2005), as well as some information from Statistics Netherlands . Actual economic costs were derived from Dutch publications, namely: CTG (2003); Mangen et al. (2005); Oostenbrink et al. (2004) and van den Brandhof et al. (2004), and if required, updated to 2004 using published price indices (Oostenbrink et al., 2004). Full details with respect to assumptions made will be made available on request.

Uncertainty

Due to restrictions in available resources it was decided to use for NV and RV in the current study for most uncertain parameters low values, most likely values and high values only. The low, most likely and high values might have been either, the estimated 5th, 50th and 95th percentile of a statistically uncertain parameter, respectively, or an optimistic, most likely and pessimistic parameter value for systematic uncertainty and/or uncertainty due to a lack of data and the use of data from expert consultations, respectively. Due to restrictions in available resources, variability as a frequency distribution was not explicitly modelled, but used instead a most likely value, which might have been either a point estimate, or where available, the mean of a variability distribution.

For Campylobacter and Salmonella, however, second-order stochastic simulation models were used. With the help of these models it was possible to explicitly and separately model variability and uncertainty. A detailed description of the models is given in Mangen et al. (2005) for the Campylobacter model. The Salmonella model is identical to the Campylobacter model, of course adapting the numbers and distributions with specific information on Salmonella-associated GE and sequelae, whereby omitting GBS and IBD. In order to account for uncertainty and variability, each model was run with 200 simulations and 2000 iterations per simulation. Given that variability is less important from a decision making point of view, it was chosen to present only the uncertainty around the most likely estimate in the burden (or cost) for these two pathogens in the summary tables and figures. The most likely estimate was always the median of estimated uncertainty, and the 5th and 95th percentile represent the attendant uncertainty. It has to be noted, that for each outcome individually the 5th, 50th and 95th percentile was estimated and presented, but these figures do not need to come from the same simulations. The sum of the medians is not necessary the median of the sum. Therefore adding up values in tables might give slightly different values than the totals shown in the table.

For Campylobacter-associated ReA and Salmonella-associated ReA, however, the uncertainty was too large, and scenario analysis was the only possible and sensible way to model and to analyze these model parameter uncertainties.

Scenario analysis

In the baseline it was assumed that 7% of the campylobacteriosis cases visiting a GP would develop ReA and 8% of salmonellosis cases visiting a GP would be at risk to develop ReA, respectively. However, given the uncertainty of this latter assumption, scenario analysis was applied. Assumptions made with respect to population at risk to develop Rea are summarized in Table 1 for the baseline and the two alternative scenarios.

Table 1. Assumed population at risk to develop Campylobacter-associated ReA and Salmonella-associated ReA, respectively, for the baseline and two alternative scenarios

	Population at risk to develop:	
	Campylobacter-associated ReA	Salmonella-associated ReA
Base	7% of campylobacteriosis cases visiting a GP	8% of salmonellosis cases visiting a GP
Scenario analysis I	7% of laboratory-confirmed campylobacteriosis cases	8% of laboratory-confirmed salmonellosis cases
Scenario analysis II	7% of campylobacteriosis cases in entire population	8% of salmonellosis cases in entire population

RESULTS

The incidence estimates for all four pathogens and sequelae, as used in the current study, is summarized in Table 2. The estimates correspond to a population of 16 million people in 2004.

In Fig. 2 the estimated disease burden, most likely estimates and uncertainty range, for the four pathogens, is shown, both undiscounted and discounted at 4%. Most likely estimates for YLD, YLL and DALYs of the four pathogens are summarized in Table 3.

The most likely estimates and the uncertainty range of total costs-of-illness for 2004 for the four pathogens are shown in Fig. 3, both undiscounted and discounted at 4%. Most likely estimates for DHC, DNHC, INHC and total costs of the four pathogens are summarized in Table 4 for the year 2004.

Scenario analysis

Scenario analysis was applied for Campylobacter-associated ReA and for Salmonella-associated ReA (see Table 1). The assumption made about what part of Campylobacter-associated GE cases (Salmonella-associated GE cases) would be at risk to develop ReA had a large impact on both, the estimated incidence numbers and the disease burden. The assumption, however, has little impact on the COI estimate due to the relatively low costs assumed per ReA case. For example by assuming that 7% (8%) of all ill persons, rather than 7% (8%) of all GE cases that visit a GP would be at risk to develop Campylobacter-associated ReA (Salmonella-associated ReA), the estimated total disease burden associated to Campylobacter would increase from an estimated total of 1,200 DALY to 2,200 DALY per year. For Salmonella this would be an increase from 590 DALY to 790 DALY per year. COI estimates would increase by less than € 0.4 million for Campylobacter and less than € 0.2 million for Salmonella.

Table 2. Incidence estimates (cases per year) for NV-associated GE, for RV-associated GE, for Campylobacter-associated GE and sequelae, and for Salmonella-associated GE and sequelae.

	Incidence estimate (cases per year)		
	Most likely	Low	High
Norovirus GE	470,000	360,000	645,000
No GP	460,000	350,000	625,000
GP	10,000	7,000	16,000
Hospitalisation	1,000	790	2,100
Fatal	5	0	13
Rotavirus GE	190,000	110,000	325,000
No GP	170,000	100,000	305,000
GP	11,000	6,900	17,000
Hospitalisation	3,000	2,000	3,700
Fatal	1.4	0.9	1.7
Campylobacter GE	59,000	25,000	140,000
No GP	45,000	19,000	110,000
GP	14,000	5,000	33,000
Hospitalization	570	500	650
Fatal	25	18	34
Campylobacter sequelae			
GBS	60	40	85
ReA	1,000	430	2,600
IBD	12	10	14
Salmonella GE	35,000	9,000	140,000
No GP	30,000	7,000	110,000
GP only	5,400	700	20,000
Hospitalisation	640	540	740
Fatal	39	34	42
Salmonella ReA	460	100	1,900

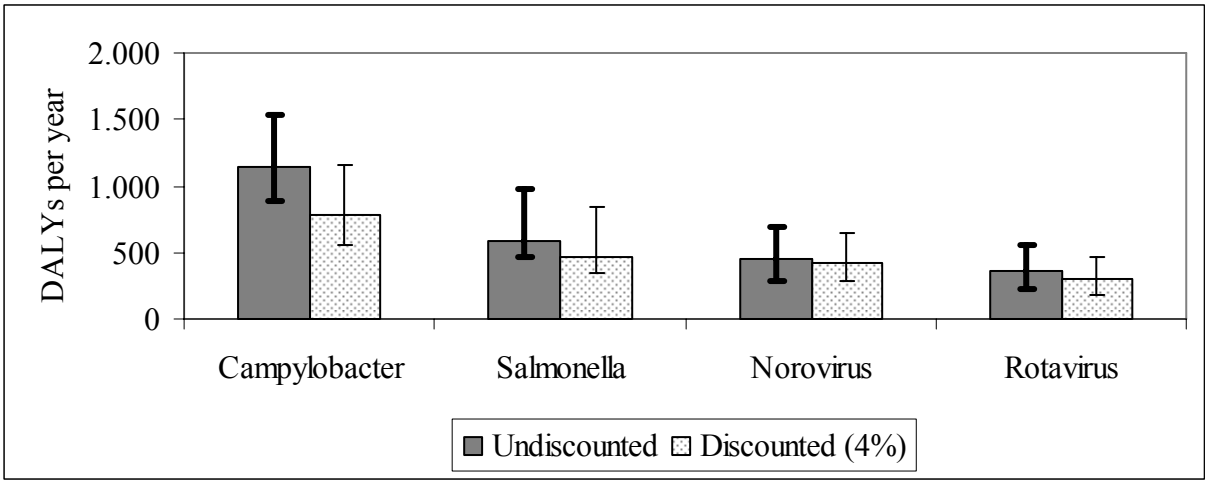

Fig. 2 Disease burden of infectious diseases that can (also) be transmitted by food. The figure shows the total disease burden associated with different pathogens, undiscounted and discounted at 4%, and the uncertainty around the most likely estimate.

Table 3. YLD, YLL and DALY estimates for 2004 (most likely estimates) of infectious diseases that can (also) be transmitted by food, total and GE only [a]

	Campylobacter		Salmonella		Norovirus	Rotavirus
	GE only	Sum	GE only	Sum	GE only	GE only
YLD	180	680	100	150	390	260
YLL	390	430	440	440	55	110
DALY	600	1200	550	590	450	370

[a] Summations do not tally up.

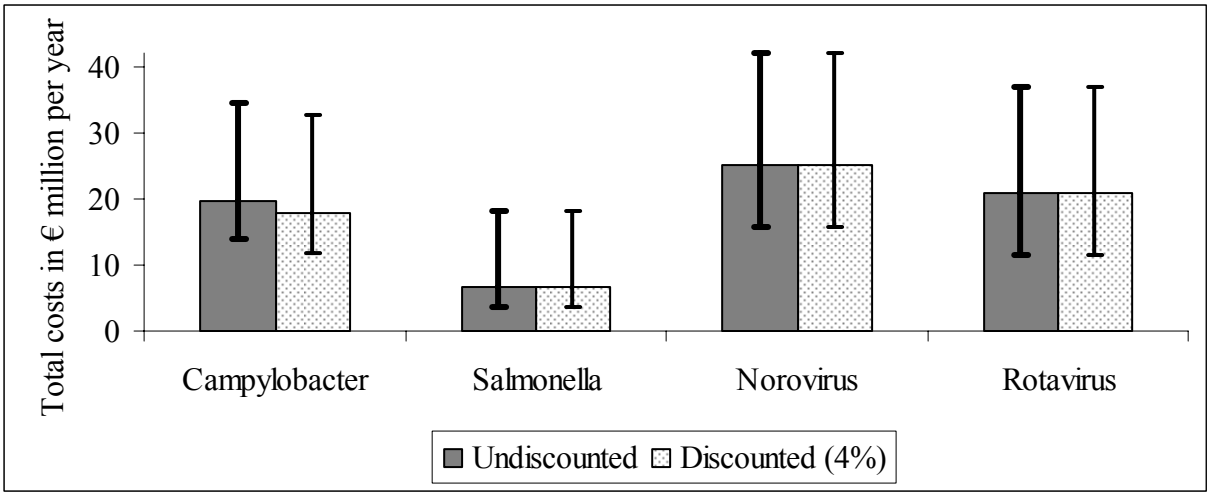

Fig. 3 Costs-of-illness of infectious diseases that can (also) be transmitted by food. The figure shows the total costs-of-illness associated with different pathogens, undiscounted and discounted at 4%, and the uncertainty around the most likely estimate for the year 2004.

Table 4. DHC, DNHC, INHC and total costs in € million for 2004 (most likely estimates) of infectious diseases that can (also) be transmitted by food, total and GE only [a]

	Campylobacter		Salmonella		Norovirus	Rotavirus
	GE only	Sum	GE only	Sum	GE only	GE only
DHC	2.1	8.6	2.5	2.5	1.8	6.0
DNHC	0.02	0.18	0.03	0.03	0.3	0.2
INHC	9.3	11.2	4.2	4.3	23.0	14.5
Total costs	11.4	19.6	6.7	6.8	25.2	20.7

[a] Summations do not tally up.

DISCUSSION

Priority setting is a complex process that involves consideration of many different factors and there is no generally accepted single process that will lead to unequivocal conclusions. It is therefore not expected, nor desirable, that a project on priority setting will lead to a single list of priorities. Rather, the process of priority setting should help to integrate complex information in a structured framework so that it is easily accessible to decision makers, and can easily be updated if new information becomes available. This paper presents one step in this direction, by

integrating a large body of evidence on the social impact of several infectious diseases that can (also) be transmitted by food into two indicators: disease burden and cost of illness. The choice for these indicators is based on experience from other projects on priority setting. In contrast to other studies on priority setting, a quantitative approach was chosen rather than a qualitative or ranking approach. One advantage of a quantitative approach is that the end result is less dependent on arbitrary choices such as the choice of the type and number of indicators. Also, all factors are weighed in proportion to their true values, instead of on some simplified scale. A disadvantage is that the process is very resource intensive, requiring careful consideration of a large volume of data while many data gaps may exist. Such data gaps result in uncertainties about the final results, but the quantitative approach also helps to prioritize among data needs and to identify key research questions.

Disease burden and cost of illness estimates are data based indicators of social impact of illness, but it must be realized that in order to complete the calculations, many choices must be made that include value judgments. In that sense, these estimates are not objective but are subjective and need to reflect societal values. The methods used to calculate both indicators are based on a large body of theoretical and practical studies and where possible existing guidelines were followed. These guidelines reflect choices made in the Netherlands, and may not be directly applicable in other countries. For example, in the Netherlands the productivity losses of fatalities are estimated using the friction method, assuming that after a certain period of time the deceased person will be replaced on the labour market. Other countries prefer to use the human capital method, in which all foregone earnings in an individual's life span are assigned to the cause of death.

Among the pathogens evaluated, NV and RV are the agents that cause most cases in the general population. Yet, the disease burden is somewhat lower than that of Salmonella and less than half of that of Campylobacter. This is related to the fact that most cases of viral gastroenteritis are mild, of relatively short duration, and have a low case-fatality ratio. About 97% of community-acquired NV cases and 93% of community-acquired RV cases recover without requesting medical services. Recently, complications of NV infections in hospitalised patients have been reported (Mattner et al., 2006), mainly affecting patients with underlying disease. Consideration of such complications is of importance for hospital hygiene, but is of limited significance from an overall public health point of view. In the case of bacteria triggered gastroenteritis, the request for medical services is far higher, namely more than 20% for Campylobacter, and about 15% for Salmonella. Also, in contrast to viral infections, bacterial gastroenteritis results in more and more serious sequelae. Often, sequelae associated with bacterial infections are long lasting and/or chronic, resulting in a considerable disease burden. When discounting, the disease burden of Campylobacter is affected most, because it includes a relatively important component of chronic residual disabilities of GBS and IBD.

Using COI as the indicator, the impact of viral gastroenteritis is greater than that of bacterial gastroenteritis. Salmonella, with an estimated € 6.8 million, has the lowest total COI of all four pathogens. In all cases, the INHC (mainly temporary absence from work) were much higher than the DHC. For chronic and long-lasting diseases, such as those associated with bacterial infections, the DHC do contribute significantly to the total cost and are often far higher than the estimated INHC for these chronic diseases by using the friction cost method. The relative importance of different cost categories differs per pathogen. For example, 26% of all costs related to RV infections were due to hospitalisation, or 80% of all DHC. In contrast, more than 90% of all costs related with NV infections were due to INHC (sickness leave). For Campylobacter, a high proportion (40%) of all costs are related to sequelae (and more than 70%

of all DHC). These results show that costs associated with foodborne pathogens may have an impact on very different sectors of the society, namely the public health sector, ill citizens and employers. The effects of discounting are limited because most costs relate to acute effects.

The cost estimates as presented in this paper are not exhaustive. In particular, outbreak related costs and the opportunity costs associated with sickness leave from unpaid work have not yet been considered. Also, costs related to nosocomial infections and preventive measures in hospitals and other health care institutions have not been included as they are not relevant for priority setting of foodborne pathogens in the community. Programs to reduce foodborne pathogens in the population, however, would have no or only an indirect impact on nosocomial infections acquired in health-care institutions. Therefore only community-acquired foodborne illnesses were considered in the current study. From other perspectives, e.g. vaccine evaluation, these costs may well be important. Furthermore, some costs such as those of reactive arthritis were underestimated because of a lack of information.

Although, this paper describes a project in progress, it allows the conclusion, that priority setting is a difficult task. The current study could show that: the severity grades considered (for example all cases or only cases searching medical services); the criteria chosen (e.g. COI or DALYs); and the perspective taken (for example the society (all costs) or the public health sector only (DHC)), are of great influence when setting priorities. Changing one of these factors, or giving a higher priority to one of them, might result in another priority setting of the four pathogens under study.

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2005	Bill Reilly: From TB to VTEC: The changing epidemiology of foodborne zoonoses	Simon More: Towards eradication of bovine tuberculosis in Ireland: A vritical review of progress
2004	Ulrich Kihm: BSE and the stable to table concept	Gary Smith: Spatial models of infectious disease in the USA: a crisis of conference and confidentiality
2003	Sir David Cox: The current state of statistical science	Ynte Schukken: Molecular and mathematical epidemiology of bovine mastitis
2002	George Gettinby: Informatics and epidemiology – the first 400 years	Bryan Grenfell: Deterministic and stochastic influences on the dynamics and control of infectious diseases
2001	Will Houston: Science politics and animal health policy: epidemiology in action	Mart de Jong: Design and analysis of transmission experiments
2000	Jim Scudamore: Surveillance – past, present and future	Dirk Pfeiffer: Spatial analysis – a new challenge for veterinary epidemiologists
1999	Aalt Dijkhuizen: The 1997/98 outbreak of classical swine fever in the Netherlands: lessons learned from an economic perspective	Mark Woolhouse: Understanding the epidemiology of scrapie
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- ☐ 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