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PLENARY PAPER

IMPROVING FARM ANIMAL HEALTH – UNDERSTANDING INFECTIOUS ENDEMIC DISEASE

L.E. GREEN*

SUMMARY

Endemic infectious diseases (e.g. mastitis, Johne's disease or tuberculosis in cattle, footrot in sheep, PRRS in pigs, macroparasite infections in all species) are common causes of disease in livestock. They affect health and well being and reduce the economic potential of each animal, herd and industry. The diseases create instability in farming systems and consequently reduce the likelihood of sustainable farming. Endemic diseases are necessarily complex; those that are less complex are generally already controlled. Not surprisingly, therefore, understanding control for each process has been a slow and we are far from understanding many endemic infectious diseases. To date pathogens and disease processes that are more understood have been studied in a pure or monodisciplinary environment. Endemic infections are too complex for such an approach and require a multidisciplinary environment. Ironically because of their complexity much of the basic monodisciplinary advances have not been applied to, or are not relevant to, endemic diseases because of the complexity. To improve animal health we need to understand endemic disease persistence, that is, the process that enables a pathogen to remain infectious until susceptible hosts arise in populations where the pathogen is already present. This process involves understanding of many physical science disciplines outside of epidemiology and includes many social science disciplines that influence decision making of farmers, countries and the international community. It serves to explain why many laudable control programmes have not been as successful as anticipated or have become less successful over time.

The aim of this paper is to provide an insight into mechanisms for disease persistence through heterogeneities in host and pathogen and use of the environment, together with the non-biological forces including economics, legislation and society, that influence persistence of certain diseases. Approaches to control of infectious disease are discussed and the non biological factors that influence control programmes are highlighted together with their role in successful disease control.

INTRODUCTION

Infection and disease

The key to improving animal health through control of infectious endemic disease is, to my mind, prevention rather than cure. It is worth highlighting from the start that there is a distinct and important difference between infection (an animal infected with a pathogen) and disease

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(clinical ill health from an infection) and this can be used to assist our understanding of infectious disease processes and to consider various approaches to disease control.

Population disease control

Populations can be considered at various strata: a herd or flock on a farm, a region, country, land mass or the global population and control can occur at any of these levels. Disease control may be implemented at any one of these strata by the farmer or regional, national or international governance and this will influence the type of control programme.

Endemic versus exotic disease

An infectious disease is endemic when it persists in a population. A pathogen is exotic when it is absent from a population either because it has never been introduced or because it has been eliminated from a population: global elimination is eradication. Once eliminated, all animals within the pathogen-eliminated group are susceptible and so elimination requires a permanent control strategy to prevent re-introduction unless global eradication occurs.

Pathogens themselves are not necessarily endemic or exotic. It is quite possible to have pathogens that are exotic in one location and endemic in another, e.g. bovine tuberculosis is endemic in the UK and Eire but exotic in many countries of mainland Europe, while foot and mouth disease is exotic from the UK but endemic in many parts of Asia. However, the nature of pathogens does vary. Pathogens with a short infectious period that cause high mortality in epidemic form and appear to persist less well (e.g. rinderpest) are more likely to be managed through population elimination. At the other extreme pathogens that on introduction to a naïve population take many years to make an obvious impact on health but by then are highly persistent (e.g. caseous lymphadenitis in sheep, Johne's disease in cattle) may be impossible to eliminate. These extremes in persistence highlight that optimum control strategies will vary by pathogen.

Historic population disease control

Each population carries with it not only its current endemic pathogens but also all the external influences that have influenced the current situation. For example, in GB, many pathogens were eliminated by the mid 1800s including Rinderpest, sheep pox, swine fever and rabies. Importation of animals for food from main land Europe was prohibited before 1840 and after this time there were legislative controls preventing the sale of diseased livestock or their products. The agricultural economy in GB has always been volatile and other external influences include subsidies and agricultural developments that have had a huge impact on animal health. These external influences have affected farming practice and the current location of livestock species, herd size, breeds used, genetic selections made, foods fed and current disease management are as a result of this complex history.

DISEASE PERSISTENCE

Persistence versus introduction

Examination of exotic diseases and their behaviour when an epidemic occurs provides information on introduction. Classic routes of introduction include introduction of an infectious animal or animal product or wind or fomite borne spread. Control of recently introduced exotic

diseases is focused on control of the route of introduction. That is, prevention of exposure of susceptible animals to infectious ones through movement restrictions and killing infectious individuals or herds. Whether this approach is successful will depend on whether this process is complete before the pathogen has started using mechanisms for persistence. These routes of introduction are similar for endemic diseases but we see them less clearly since a population is already infected.

Routes for persistence

It is quite likely that reintroduction of disease from infectious individuals (e.g. mastitis, footrot, BVD, IBR) is one strategy that presents as persistence. In this case there is a continuum of introductions (or reactivations). If this is the only route of persistence then these diseases may be controlled by managing risks for introduction. For most persistent diseases there are further complexities that enable a protracted infectious period so that the pathogen persists to infect new susceptibles in the population. Understanding mechanisms for persistence are vital for understanding disease management. This is the central theme for this paper.

Complexities of persistence

Epidemiologically infectious endemic diseases are defined as those which exist in a population with an effective reproduction number (R) of 1; that is, on average every infectious individual infects one susceptible individual over the infectious period and so the disease persists. When a disease enters a fully susceptible population the mechanism for spread that creates an epidemic is the first generation transmission of infection. Typically this will be by infectious individuals successfully contacting (i.e. transmitting) infection to susceptible individuals in the population. This is Ro, the number of infectious individuals that arise from one infectious individual over their lifetime infectious period in a susceptible population. Theoretically the population then becomes resistant, the pool of susceptibles falls to below a certain level and, without introduction of new susceptible individuals the pathogen dies out. R becomes less than 1. To drive R to less than 1 each infectious individual needs to infect less than 1 susceptible individual. We rarely see infections like this, because natural fade out means they are unlikely to be endemic diseases or because these are the ‘easier’ ones to eliminate and we have already managed these diseases through elimination.

The effort to remove endemic diseases is far greater than the theoretical threshold for elimination based on introduction. (Even for pathogens with no evidence of persistence this appears to be the case, e.g. in humans the generation time for infection is one month but it took 200 months to declare small pox eliminated after the last known case). This is because the introduction or invasion of a pathogen is not usually the only mechanism by which it persists in a population. Understanding persistence of pathogens is key to being able to assess whether elimination of a disease is possible and if it is not, then the pathogen specific mechanisms for persistence can direct control programmes.

Identifying the factors that enable persistence to occur is a disease by disease process and will vary with the host, pathogen or environment contributing to successful persistence. The more able a pathogen is to survive without the host, the greater the chances of persistence and the less chance of elimination.

Although we need to know the specifics for persistence for each endemic disease that we wish to control there are certain generic routes for persistence that we can consider that will

assist us in investigating the specifics. All are pathogen led but we see the pathogen 'using' manipulation of the host, the pathogen itself or its environment to persist.

Host heterogeneity in response to infection

All hosts are not equal and pathogens manipulate this host heterogeneity of variable genetic make up, age and sex differences to aid persistence. We have known for a long time that in macroparasitic infections that not all hosts are equal and some hosts are heavily infested and, if treated, the same individuals become heavily infested again. We can postulate from the above that for some microparasitic infections this mechanism is also likely. We know for example with JD that most of the shedding occurs from a few 'super shedders' in a herd. Similarly the persistently infected bovine in bovine viral diarrhoea (BVD) disease is the key source for infection to the herd.

Variable immune response by age

We see age related differences in either susceptibility to infection or disease development with many infectious diseases. E.g. JD, where cattle infected < 6m.o. are more likely to be successfully infected and diseased compared with cattle infected after this time and BVD where early embryonic infection leads to a totally different pattern of infection and disease compared with later infection (see below).

Variable genetic immune response

The host immune system can also be heterogenous. That is some hosts are naturally resistant, partially resistant or fully susceptible to disease. This is the idea behind genetic selection for hosts with heritable resistance to infection. Once concern with genetic selection for heritable resistance in hosts is that the speed of this process will inevitably be long and the speed at which pathogens change may be far shorter. It therefore seems probable that host genetic selection to prevent a specific disease is unlikely to be successful. We have seen a recent indication of this with the selection programme for scrapie (one of the slowest infectious diseases to manifest) and recent reports of scrapie in apparently genetically resistant sheep. Manipulating host genes to have generically 'better' resistance through an 'improved' less specific immune response may be more useful. An alternative genetic route for resistance may be host genetic heterozygosity in the genome that is not necessarily heritable but a convergence in species genetics e.g. the close genetic link between modern Holstein type cattle through the use of AI may lead to species genetic convergence and lack of heterozygosity. Thus a global population can become more and more uniformly susceptible to a disease. This may be explained by the co-evolution of pathogens and hosts, i.e. as one evolves, so does the other.

Variability in disease between sexes

We see sex differences in infection in particular with sexually transmitted diseases. Males may be symptom less carriers spreading infection while females become clinically diseased

Pathogen manipulation of the immune system to extend the infectious period

Pathogens can extend their infectious period by altering a host's immune response to infection. We see this in BVD where persistent infection occurs in individuals infected *in utero* before 120 days gestation when the pathogen is not recognised as foreign to the host. These PI cattle are infectious 'for life' compared with cattle infected after birth which are usually infectious for just a few days. Several models of BVD persistence indicate that it is these PI

cattle that prevent die out of BVD from a herd. They also contribute to introduction of BVD since PIs are generally young, and young animals are more likely to be purchased.

Infectious bovine rhinotracheitis (IBR), a herpes virus, persists through persistent infection of hosts and reactivation in certain individuals in a herd may be responsible for maintaining and transmitting infection to susceptible individuals.

An altered immune response may explain how vertical transmission may occur once (e.g. BVD) or several times (e.g. *Neospora caninum*) in a breeding female's lifetime. Vertical transmission is a very elegant route for increasing the infectious period.

Evasion of immune system

Some pathogens persist by partially evading the host immune system e.g. TB, JD, CLA. So apparently healthy but infectious individuals lead to persistence of the pathogen. Again, these individuals are the most likely to transmit infection between herds. This mechanism also affects detection of infected individuals since immune based diagnostic tests or PCR may fail to detect infected individuals.

Pathogen manipulation of the immune system to increase susceptibility

Another host immune response is immunity that wanes over time. Waning immunity shortens inter-epidemic period and so increases the probability of persistence because individuals become susceptible more rapidly than when life long immunity occurs when new susceptible individuals would be necessary to maintain infection. An extreme of this is observed in diseases where we use clinical signs to define disease (e.g. mastitis, diarrhoea, pneumonia) but we also see waning immunity in pathogen specific diseases or strains of pathogen (e.g. *S. aureus* mastitis, *E. coli* diarrhoea).

Multiple host species

Finally pathogens may remain infectious for longer through infecting more than one host species. This increases the probability of successful persistence in each host species since all the mechanisms for persistence within each host species can be used in each species. If infection of multiple host species is an adaptation for persistence then transmission between host species must occur.

Host population heterogeneity

Host population heterogeneity influences persistence as well as individual host heterogeneity.

Within a population (a herd)

One reason that the historical stamping out policy was successful for elimination of some diseases was that herd sizes were small. We still see this phenomenon in countries where e.g. BVD has been eliminated; it is far easier in countries with small average herd sizes. The increase in herd size increases the risk of persistence in endemic diseases because of the increased number of new introductions per farm. The practice of buying and selling of livestock may affect endemic disease prevalence on a farm. When the national disease prevalence is higher than that on farm A then purchasing stock randomly is a risk for raising disease prevalence on

farm A. Conversely if Farm A has higher than average prevalence selling is likely to lower disease prevalence on farm A.

The larger population size increases the probability that such an introduction is successful that the pool of susceptibles assists in maintaining pathogen.

Seasonal breeding aids persistence. A pool of susceptible individuals at one time of the year may act in a straight forward way to aid persistence but also may be a mechanism that pathogens use to persist by passing from others to offspring or by recrudescing from the environment e.g. nematodirus infection in sheep.

Between herd persistence

Just as several host species aids persistence, so proximity of host species aids persistence because close proximity assists in re-introduction of disease. The current situation in GB where dairy cattle tend to be on the west and pigs on the east of the country increases the density within each species and the likelihood that infection can 'jump' populations and find susceptible individuals, e.g. porcine respiratory and reproductive syndrome virus (PRRSV) in pigs. Where different strains are circulating or developing (e.g. FMD, influenza) between herd transmission of strains may result in multiple strains per herd and therefore aid persistence within a population.

Where herds are far apart then transmission of pathogens between herds may occur because of connectivity between populations.

Pathogen heterogeneity

Our definitions of disease may use a clinical sign where many pathogens may cause similar clinical signs e.g. mastitis. Defining mastitis as a clinical sign may be useful if we wish to minimise clinical mastitis or lower somatic cell count from any cause by attributing risks and therefore benefits to removal of risks. However, it is not useful if we wish to understand the pathogenesis of disease. We know mastitis is caused by many pathogen species. These can be grouped variously but one grouping may be contagious or environmental pathogens. Contagious pathogens are transmitted primarily from infected cattle to susceptible cattle, while environmental pathogens are transmitted primarily from the environment to susceptible cattle. The historical control measures described above were useful to control the dominant contagious pathogen *S. agalactia* initially, then *S. aureus* and other streptococci species and this left cattle susceptible to the environmental opportunists that are currently dominating in GB.

This case definition is a classification above the bacterial species level. Yet we know that there is more genetic variability in *E. coli* than the whole of the eukaryotic animal kingdom. Recent advances in molecular techniques (Smith et al., 2005; Zadoks et al., 2006) highlight that within single species of pathogen strain variation occurs and strains vary in their behaviour in hosts. Many molecular typing techniques are laboratory specific (Zadoks et al., 2006) which may create confusion but even using objective strain typing such as multilocus sequence typing (MLST) or genomics – reading the nucleic acids directly - is challenging in a quantitative setting because the variability is vast and defining a useful subgroup of a species of bacteria will be subjective. A case definition that is sensitive, specific and useful to understand persistence is required; this may not be easy to identify. As a result case definition varies between studies and studies are often not totally comparable.

Strain and species variability

Historically we have cultured bacteria and classified them by phenotypic and biochemical tests, this assumes that vertical inheritance of genetic material is the most important trait that classifies a bacteria. However, horizontal gene transfer occurs in all bacteria and the introduction of a new gene e.g. via a plasmid may change a bacteria from a non-pathogenic to a pathogenic state. In addition, mutations occur at each replication of a bacterium and these also create pathogen heterogeneity.

Strain variability may lead to heterogeneity in parasite transmission and may result in multiple dynamic processes. For example, some strains of *S. aureus, S. uberis* and *E. coli* are apparently more udder adapted than others. These differences mean that in herds where udder to udder transmission dominates that the udder adapted strains dominate but in a system where non udder transmission dominates the less adapted strains will dominate. Bacterial strains may therefore be in equilibrium with some strains more dominant than others i.e. within species strain competition. The competition is present because a change in equilibrium may occur and a different strain dominate when

- a new strain of pathogen is introduced to a population e.g. introduction of a host infected with a different strain (see above for large herd sizes)
- the host becomes resistant (e.g. vaccinated) to the dominant strain
- the environment changes and the dominant strain is less fit e.g. iron levels in milk alter

i.e. R ~ {all udder pathogens}

Environment

The ability of pathogens to persist outside the host varies. Those that are more able to survive in the environment usually create reservoirs. These must survive for longer than the period of infectiousness in the host to be of importance for persistence e.g. *M. paratuberculosis* subspecies *avium*. In this way these pathogens can persist in the absence of susceptible individuals. This behaviour has been overlooked for many diseases and our reliance on culture (see above) may have led us to under-rate the importance of the environment, since many pathogens may be viable but non-culturable.

Climate change has highlighted the importance of the environment and we see pathogens surviving for longer periods in a mild environment in the environment or in vectors.

Lack of understanding of the role of the environment or an assumption that the environment plays a similar role across countries may have affected our understanding of some pathogens e.g. *D. nodosus, C. pseudotuberculosis.*

Heterogeneity from control programmes

Successful control programmes will alter the host, pathogen or environmental risks for disease. Control creates another heterogeneity, possibly with unpredictable consequences. Mastitis in cattle is a good example of this. A clinical sign itself, in the 1950s most clinical mastitis in GB was caused by *S. agalactia*. By the 1960s *S. aureus* was the prevailing pathogen, now it is *E. coli* and *S. uberis*. The source of pathogens has changed from primarily transfer between cattle at milking to repeated introductions from the environment with some persistent

infection in hosts. We also hypothesised that vaccination against S. uberis may allow dominance of *S. aureus*.

These alterations are themselves perturbations in the whole process and can trigger new developments in persistence, e.g.

- use of antibacterials may lead to development of resistance among certain strains of the pathogen
- use of a vaccine may alter the competition between species or strains
- genetic selection of hosts may select for hosts that are less susceptible to one species or strain of pathogen but more susceptible to another
- restricting a population may lead to greater contact between hosts and more transmission of a pathogen

Modelling persistence

All of these heterogeneities require the classic susceptible-infectious-resistant (SIR) model, where homogeneity is assumed, to be modified. This has been done in some circumstances with age or sex, but less so with other heterogeneities because of the complexity that heterogeneity creates in these simple models.

Overall R_0 is often a weighted average of all individual strains of an organism, all routes of persistence and transmission and consequently developing control programmes based on an average R_0 may not lead to elimination. There will be sections of the host population (perhaps due to management or environmental circumstances) within which R_0 will be greater than the average.

Alternatively, Ro may not fully reflect the dynamics of infection maintenance in an endemic situation. There is theoretical evidence of this in models where Ro is reduced to a value less than 1 but disease persists. A backward bifurcation is seen with disease persistence when the 'naïve' Ro is less than 1. Only when management practices are introduced that further reduce transmission (and thereby lower the 'naive' Ro to a value below a next threshold) elimination occur. In reality, we are saying that if a model of an infectious process omits one (or more) route of persistence then elimination may be predicted but not achieved in reality. Practically we need to know all possible routes for persistence. This is difficult when endemic disease appears constant and either cohort studies or perturbation to a system may be required to observe all possible routes for persistence. A theoretical model may be useful to assess the sensitivity of a system to a theoretical mode of persistence.

This is the key issue to consider when developing control programmes and when addressing apparent failure of control programmes.

DISEASE CONTROL

The aim of a control programme is to minimise disease and introduce permanent economically feasible and societally acceptable management changes that keep disease minimised. We can control a disease by altering the balance of susceptible, infected or immune individuals in a population.

Creating susceptible populations

Culling and treating individuals leads to a large proportion of population susceptible (e.g. bTB culling policy, reduces infectiousness) and great care is required to reduce exposure to these susceptibles. This is the basis for mastitis control programmes and for most other programmes where there is no vaccine.

Creating infected but not diseased populations

It is disease that affected the health and well being of animals and therefore infected but not diseased individuals may be protected from disease. This is the basis of control with endemic stability.

Creating immune populations

This is the ideal option to control immunity, typically through vaccination. Necessarily the nature of pathogens that persist using poor or waning immunity mean that effective vaccines are not easy to develop and further control strategies may be necessary.

As a consequence of persistence, elimination and control of endemic diseases is far more complex than we may have once thought and may explain apparent failures of a previously successful control programme or explain why a new control programme is unsuccessful or less successful than predicted.

'Failure' to control- pathogen adaptation

A control programme may appear to 'fail' after many successful years because of host, pathogen or environmental changes as discussed above. Reasons include diagnostic tests losing sensitivity, the pathogen 'finds' alternative routes for persistence e.g. another host species (of concern for bTB). For endemic instability – the stable relationship between host and pathogen changes e.g. the pathogen is killed or highly susceptible / super shedder hosts introduced that allow pathogen to build up then instability. When resistance fails, vaccine failure may occur because of strain mutation or selection or invasion of an alternative species e.g. mastitis.

Control programmes may also fail from the start. We have to consider whether an appropriate approach to control is being used. For example, to control mastitis we have to control all of the current known pathogens and all strains within these species – our aim is control either through creating susceptible cattle or resistant cattle, to do this we assume that we can control all potential pathogens in the udder – can we? Do we know them all? Even if we control all the known current major pathogens, and assume that strain variation will not out compete that control are we certain that more pathogens are not waiting – there seems a potential list already – mycoplasmas, coagulase negative staphylococci (CNS), some corynebacteria. However the most stable cattle may be those infected but not clinically diseased.

Where control is implemented the disease process will change as controls are implemented and control programmes therefore need to be reviewed and modified. A good example of this is again mastitis. In the 1950s over 80% of mastitis in GB was caused by *S. agalactiae*. A control programme to reduce mastitis caused by *S. agalactia* was highly successful and clinical cases went from over 120 per 100 cows per year to about from over 70 / 100 cows / yr. However, whilst the amount of mastitis attributable to *S. agalactia* reduced, the amount of clinical mastitis overall did not plummet by the 80% that was expected. *S. aureus* and other Streptococcal spp.

became more dominant. In the 1960s a second control programme was proposed, the five-point plan. Once again this was very successful, reducing clinical cases to about 40 cases / 100 cows /yr. However, from the early 1980s onwards there was no further reduction in clinical mastitis; recent data suggest that the incidence is currently nearer to 50 cases / 100 cows / yr and that the dominant pathogens are *E. coli* and *S. uberis.* The control programme has changed the major source of infection from other cattle and contagious type pathogens to the environment and environmental pathogens.

Externalities

Just as the current endemic diseases in a population are a result of past external influences so the present control and future decisions on management of endemic disease are influenced by these same externalities. These are highlighted briefly below. Their relationship with disease control is complex and non-linear both initiating and preventing disease control, determining how, where and when disease control may occur. We ignore them at our peril.

Economics

Improved animal health is economically beneficial when the cost of the improved productivity adjusted for the cost of control is less than the cost of the disease. The improvement may be the cost of a vaccine, genetically resistant hosts, biosecurity, any of the above that is associated with elimination or control. The simple benefit is increased production of, for example, milk , meat or wool. The reality is far from simple. For many endemic diseases we do not have the information to estimate the costs associated with disease. We often have far less certainty about the costs associated with the changes required to eliminate / control a disease and have even less information again about the true benefits of control, e.g. the proportional reduction in disease. Farmers and their advisers have little information to make a complex decision. The likely improvement in health has to be fairly certain, or the costs quite obvious, for farmers to invest in change unless a farmer is a real risk taker, or has another income. Farmers also have many alternative routes to make money and may use these to find a 'better' economic return (Stott et al, yr) than the apparently simple one.

Subsidies

Society and politics influence farmer decisions e.g. in the UK until 2005 farmers received £300 for cattle >30m old under BSE regulations. Only when this stopped did one farmer working with us on lameness in dairy cows decide to change aspects of the farm environment. For him, the £300 per fallen cow was apparently sufficient to tolerate involuntary culling for lameness. Similarly, compensation for bTB affect farmer behaviour and attitude (Bennett ref). Some farmers change buying or selling practices and may 'use' the compensation to farm in an alternative way.

Legislation

Diseases controlled by statute may alter individual farmers and the farming community's attitude to its control. Since they may feel powerless to change events or that it is no longer their responsibility. This will affect attitudes to disease control. Legislation can also change animal demography and animal movements. A key factor in the spread of FMD in 2001 was that each year sheep were counted for subsidies over a period of approximately 6 weeks, with farmers declaring the number of sheep on their property on one day. The buying and selling of sheep occurred to maximise subsidies on farms.

Societal choice

Society has always influenced animal health by prioritising certain species e.g. cattle and diseases for control. Now, society influences animal health in many ways including ethical considerations and changes in acceptability of animal care. The best animal welfare is not the cheapest option for control of BVD. This is important information because it highlights that there is a cost for good welfare and society has conflicting desires, cheap food and good welfare.

Quality assurance schemes have been introduced for all major species of farmed animal. One impact of this is that farmers that do not keep animals to a standard to be within a QA scheme may make the rest of the population more susceptible to disease because e.g. the lowest health animals travel further to the few farmers or abattoirs that will accept animals outside the scheme.

There is now a move towards sustainable agriculture and integration of social and physical sciences to assist in this understanding. Multidisciplinary research with epidemiologists, ethologists, molecular biologists, population biologists, vets, geneticists, economists, political scientists, statisticians, mathematicians, social scientists may help to address how some of these externalities influence control of endemic diseases.

CONCLUSIONS

Endemic diseases are complex. Advances in understanding host and pathogen heterogeneities have increased our epidemiological understanding of additional complexity of these diseases and may help in the design of appropriate control strategies. When considering control of endemic diseases, the infection processes themselves as well as the influences of host, pathogen and environment, societal influences, attitudes to disease control through economic, legislative and social drivers should be taken into consideration.

For each endemic disease considered for control or elimination, a detailed knowledge of the infection process and mechanisms for persistence is essential. Control programmes must include routes for persistence as well as introduction of a disease and need to be re evaluated because the disease process changes because of control measures. We can improve animal health now if we use all the knowledge at our disposal and in the future if we target research questions at understanding persistence.

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FREEDOM FROM DISEASE

SAMPLE SIZES FOR DECLARING DISEASE FREEDOM WITH UNCERTAIN DIAGNOSTIC TEST PERFORMANCE AND PREVALENCE

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SUMMARY

Previous studies have proposed approaches to compute sample sizes for the evaluation of freedom from disease based on the evaluation of a threshold value for the prevalence, i.e. the presence of a disease in a population will only be detected if its prevalence exceeds an expected threshold value. These methods do not allow for a zero-prevalence when a disease is absent in a population.

We present a procedure to calculate sample sizes for the estimation of the probability of freedom from disease by allowing the event of zero prevalence and accounting for the uncertainty of the performance of the tests (*Se* & *Sp*) and the prevalence given the population is not free (*prev*). The procedure is an iterative process to estimate the smallest required sample size to obtain a predetermined probability of freedom of disease with a certain confidence and a minimal number of false positive test results. The procedure is illustrated by the case of obtaining a sufficient sample size for estimating freedom from classical swine fever in a wild boar population.

The proposed procedure may have a specific application in the surveillance of emerging diseases since they start with a desirable probability of freedom from disease and not with threshold prevalence. Moreover, the proposed methods may be very useful in the case where the dynamics of an emerging disease in a population are unknown.

INTRODUCTION

In 1995 the Agreement on the application of Sanitary and Phytosanitary methods (SPS agreement) of the World Trade Organization (WTO) laid down the current animal health-related rules for international trade. To facilitate international trade there has been an increasing need for countries to provide science-based evidence to support their claims to freedom from livestock diseases. One way to provide this evidence is by diagnosing the absence of a disease in animals in a survey sample. Cannon and Roe (1982) have proposed methods to compute sample sizes for the decision of freedom from disease based on the evaluation of a threshold value for the prevalence, i.e. the presence of a disease in a population will only be detected if its prevalence exceeds an expected threshold value. Cameron and Baldock (1998) proposed improved methods for estimating the confidence of freedom from a disease in a population

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taking the performance of the diagnostic test into account. Sometimes the degree of accuracy of the diagnostic methods is not clear and a distribution describing the probability of these values has to be determined, based on prior information from literature or own research. Therefore, Johnson et al. (2004) proposed a procedure to calculate the sample size for surveys to substantiate freedom from disease which takes the uncertainty about the diagnostic test performance and the threshold prevalence into account. Still, none of the earlier proposed methods allow for a zero-prevalence when a disease is absent in a population, which at the end is an essential criterion for claiming freedom from disease.

Mintiens et al. (2005) introduced the concept that a non-zero prevalence could only occur given that the population is not free from disease. In addition the authors developed the concept 'probability of freedom from disease' by using the probability of positive test results under uncertainty of presence of disease. The Bayesian inference theory was used to get the posterior probability distributions for parameters of interest such as probability of freedom from disease (*F*), prevalence given the population is not free (*prev*), sensitivity (*Se*) and specificity (*Sp*) of the diagnostic test. The probability for a positive test result was proposed to be expressed as in Eq.(1):

$$P(T^+ \mid F, prev, Se, Sp) = (1-F)(prev \times Se + (1-prev)(1-Sp)) + F(1-Sp) \qquad (1)$$

The purpose of the current work is to develop procedures to calculate sample sizes for the estimation of the probability of freedom from disease by allowing the event of zero prevalence and accounting for the uncertainty of the performance of the tests and the prevalence given the population is not free. An illustration of the procedure is carried out on the case of classical swine fever virus in a wild-boar population.

MATERIALS AND METHODS

Binomial distribution of positive test results

Taking a sample from a population of animals, when there is no certainty about the presence of infectious agents, implies drawing samples from two possible scenarios: i) from a population in which the disease is present or ii) from a population free from disease. Table 1 shows the distribution of cases for a diseased population and a disease-free population for a sample size *n*. In a diseased population (Table 1a) a number of $(z_1 + n - x - z_2)$ diseased animals would exist in a sample of size *n*, which divided by the total sample size *n* provides the proportion of infected animals or true prevalence. On the other hand for a non-diseased population (Table 1b), the total sample size *n* would correspond only to healthy animals. No cases are classified in the disease status but positive cases may result from the diagnostic test due to its imperfection to detect the true condition of the animal. So, when positive results are reported they would actually be false positives since the true status of the population is non-diseased. Then the amount $(x - z_1)$, number of false positives in a diseased population, would equal x in a non-disease population given that no truly positive cases are possible i.e. $z_1 = 0$.

Table1a. Cases according to disease status and test results for a diseased population.

	D^+	D^-	Total
T^+	z_1	$x - z_1$	x
T^-	$n - x - z_2$	z_2	$n - x$
Total	$z_1 + n - x - z_2$	$x - z_1 + z_2$	n

Table1b. Cases according to disease status and test results for a disease-free population

	D^+	D^-	Total
T^+	*0*	$x - z_1 = x$	x
T^-	*0*	$z_2 = n - x$	$n - x$
Total	*0*	n	n

where,

D^+ denotes disease status

D^- denotes healthy status (free from disease)

T^+ denotes positive test results

T^- denotes negative test results

n is the total sample size

x is the number of positive test results

z_1 is the number of positive test results when the animal is truly diseased, i.e. true positive test results.

z_2 is the number of negative test results when the animal is truly healthy, i.e. true negative test results

Based on the two scenarios illustrated in Table 1, the probability to get a positive test result can be expressed as (Eq.(2)):

$$P(T^+ \mid prev, Se, Sp) = \begin{cases} prev \times Se + (1 - prev) \times (1 - Sp) & \text{if the population is diseased} \\ 1 - Sp & \text{if the population is free from disease} \end{cases} \quad (2)$$

where,

prev is the prevalence (probability of disease) given the population is not free from disease

Se is the sensitivity of the test, the probability to detect a truly infected animal

Sp is the specificity of the test, the probability to detect a truly non-infected animal

Equation 2 gives the probability of getting a positive test result when one animal is randomly selected from the population. If a total number of n animals are sampled then the probability to obtain a particular number of x positive test results is characterized by the following binomial distributions (Eq.(3)).

$$x \sim \begin{cases} Bin\,(n,\ prev \times Se + (1 - prev) \times (1 - Sp)) & \text{if the population is diseased} \\ Bin\,(n,\ 1 - \mathrm{Sp}) & \text{if the population is disease free} \end{cases} \quad (3)$$

Since one of the features of the problem is the uncertainty of the accuracy of the diagnostic tests as well as of the prevalence given the population is diseased, and since the values of these indicators are needed to be known in advance specially for the computation of the probabilities of positive results, beta distributions are stated for these indicators. The parameters of the beta distributions might be taken from previous studies or obtained from some other reliable sources of information. A particular value for each indicator would be obtained by random selection from their corresponding beta distributions.

The expressions of the beta distributions are formulated as:

Sensitivity	$Se \sim Beta\,(a_{se}, b_{se})$	$a_{se} > 0, \quad b_{se} > 0$
Specificity	$Sp \sim Beta\,(a_{sp}, b_{sp})$	$a_{sp} > 0, \quad b_{sp} > 0$
Prevalence	$prev \sim Beta\,(a_{prev}, b_{prev})$	$a_{prev} > 0, \quad b_{prev} > 0$

Procedure to compute sample size for estimating the probability of freedom from disease

The proposed procedure consists of different steps to compute the smallest sample size n to obtain a desired probability of freedom from disease (F_0) with a certain level of confidence (p) and for a minimal number of allowed positive test results x_0.

Step 1: The procedure starts with the selection of a starting-value for n and x_0 and random values for *Se, Sp,* and *prev* from their known beta distributions.

Step 2: Given the sampled values in step 1, the probability distribution of positive test results can be obtained by using the binomial distributions formulated in Eq.(3). Figure 1 shows the two distribution curves, one for the case that the population is diseased, and the second for the case that the population is free from disease. The separation or proximity between the two curves depends on the values for *Se*, *Sp* and *prev* that were randomly selected in step 1. For instance, for a given sample size 500, let 0.71, 0.99, and 0.1 be the random sampled values from some pre-defined beta distributions for *Se*, *Sp,* and *prev* respectively, the probability curves for the different positive test results are clearly separated (Fig. 1a). For random values *Se*=0.76, *Sp*=0.98 and *prev*=0.02, the probability curves of positive tests are more overlapping (Fig. 1b).

Step 3: For a starting number of positive test results x_0 it is judged whether it would belong to the diseased or to the disease-free population. When the distribution curves are distant enough from each other, the corresponding population to the value of interest x_0 can easily be identified. However, when the curves overlap, x_0 falls in the common region of both curves and some criterion has to be considered to decide on which of the two populations the value x_0 belongs to.

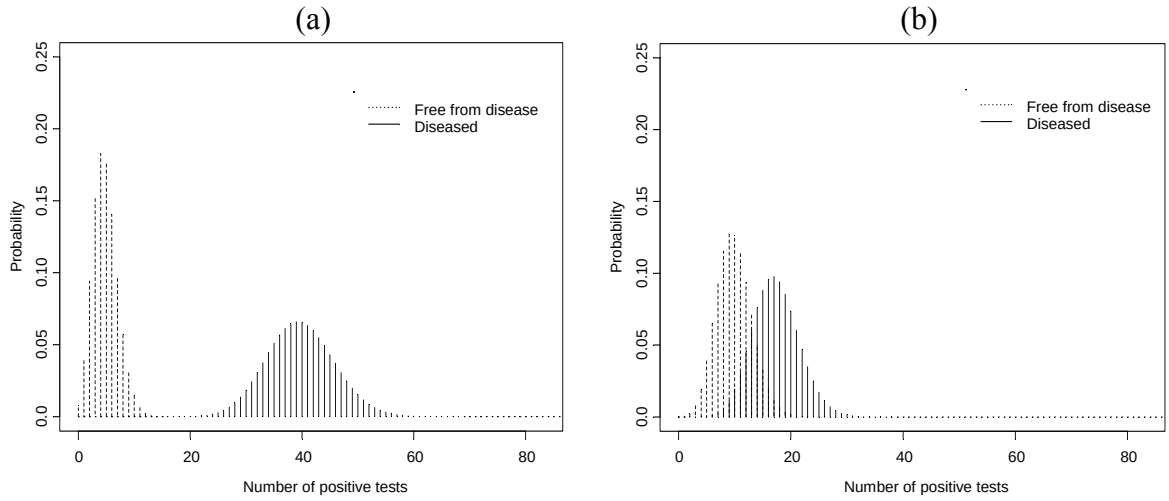

Fig. 1 Probability distribution of positive test results for diseased and disease-free population, for different sampled values of *Se, Sp,* and *prev.*

Here, the criterion will be based on the comparison of x_0 with the 0.05 quantile from a diseased population. If x_0 is less than or equal to the mentioned quantile, then x_0 would be labelled as coming from a non-diseased population, otherwise x_0 would be categorized as resulting from a diseased population. For example, let x_0 =50 be the number of positive tests of interest. Figures 2a and 2b are two possible drawings of the probability distribution curves of positive tests for any two different sets of sampled values of *Se*, *Sp* and *prev*. The 0.05 quantile for the diseased population is indicated with an up arrow at the bottom of each graph. In Fig. 2a, it can be noticed that x_0 =50 is less than the cited 0.05 quantile, which suggests that these 50 positive tests are a result from a disease-free population, whereas in Fig. 2b x_0 =50 is larger than the 0.05 quantile, which suggests that these positive results originate from an diseased population.

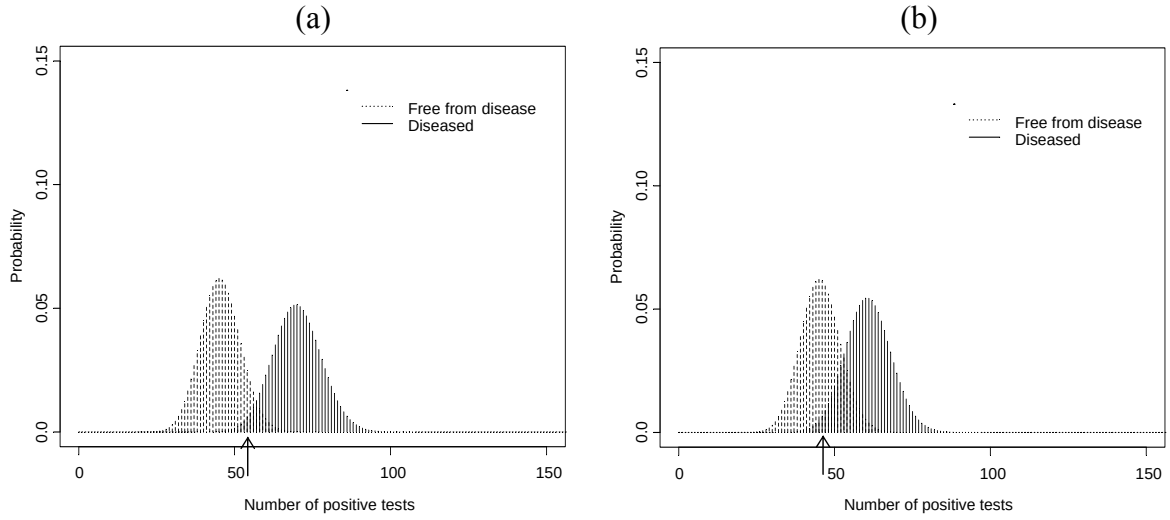

Fig. 2 Probability distribution of positive test results for diseased and disease-free populations for different sampled values of *Se, Sp,* and *prev.* Indication of the 0.05 quantile from the diseased population (up arrow).

Step 4: Since the magnitudes of *Se*, *Sp* and *prev* were sampled values from their predefined beta distributions, some other sets of samplings are necessary to verify the classification of x_0.

Therefore, new random samples for *Se*, *Sp* and *prev* are drawn for a finite number of times and by repeating steps 1 to 3 the label of x_0 (i.e. belongs to a diseased or non-diseased population) is registered at each iteration time.

Step 5: The proportion of times when x_0 is labelled as from a disease-free population is computed, which is the proportion of times that x_0 is less than the 0.05 quantile of the probability distribution from a diseased population. This proportion quantity would be an approximation of the probability of freedom from disease F when x_0 positive test results come out from a sample size n.

Step 6: Steps 1 to 5 are repeated for a finite number of times and the approximate probability of freedom from disease is registered at each iteration time. After a number of iterations the distribution of the approximated F can be obtained and the *(1-p)* quantile of F (hereafter denoted as qF) can be identified where p is the predetermined level of confidence for the fact that F is at least equal to qF, i.e. for a given sample size n (Eq.(4)):

$$P(F \geq qF \mid x_0) = p \tag{4}$$

Step 7: Let F_0 be a particular probability of freedom from disease to be achieved by the researcher. If in that case the qF, mentioned in step 6, is less than F_0, then the current initial sample size n needs to be increased and steps 1 to 6 are to be repeated until qF is at least equal to F_0. On the opposite, if qF is larger than F_0, then the initial sample size n can be decreased and steps 1 to 6 are to be repeated until qF is as close as possible (no lower) to F_0. In either case, the goal is to find the minimum sample size satisfying the condition $qF = F_0$. The acquired n will guarantee with probability p that the probability of freedom from disease in the population is at least equal to F_0 given x_0 positive test results.

Step 8: Another particular interest within this procedure is to find the smallest number of positive test results x_0 with the respective minimum sample size that accomplishes the criterion explained in step 7. Thus, in this case the steps from 1 to 7 should be performed iteratively for each possible number of positive test results by starting with $x_0 = 0$. The value for x_0 needs than needs to be increased until the first value x_0 with a minimum sample size different from zero that accomplishes the condition of $P(F \geq F_0 \mid x_0) = p$.

Illustration

The procedure was implemented in S-plus statistical software and was applied to the case of classical swine fever (CSF) virus in the East-Belgium wild-boar population, which had the suitable characteristics for the particular problems to be accounted by this procedure. Some evidence about the distribution of the prevalence and the sensitivity and specificity of the diagnostic methods for detection of CSF-virus in wild-boar population was described by Mintiens et al. (2005) under the form of beta distributions. Random values for *Se*, *Sp* and *prev* were sampled from these specific beta distributions in order to get an estimation of the probability of freedom from CSF-virus as well as to calculate appropriate sample sizes for estimation of the required probabilities. The sample sizes were computed for two preset values for the probability of freedom from disease (F_0=0.95, F_0=0.99) and two levels of confidence (p=0.95, p=0.99).

RESULTS

As an illustration to the procedure, the results of the sample size calculation for establishing the probability of freedom from disease at two pre-defined levels (F_0=0.95, F_0=0.99) and two levels of confidence (p=0.95, p=0.99) are given below. Mintiens et al. (2005) estimated the beta distributions of *Se* and *Sp* for a serological testing procedure to be *Beta(5.915, 1.966)* and *Beta(783.174, 9.994)* respectively. The beta distribution of the prevalence, given the population is infected (*prev*), was estimated to be *Beta(0.184, 1.207)*.

Table 2 presents the required samples sizes that were calculated using the procedure and using the prior knowledge about *Se, Sp* and *prev.* The sample sizes are stated for the different levels of F_0 and p and for different numbers of positive results x_0 that can be allowed. For all levels of F_0 and p, the minimum number for x_0 that can meet the criterion $P(F \geq qF | x_0)=p$ is 2.

Table 2. Minimum sample size to estimate probability of freedom from disease given the number of positive test results, and for different levels of confidence.

Probability of freedom from disease (F_0)	Confidence of estimation of F (p)	Number of positive tests (x_0)			
		2	5	7	10
0.95	0.95	300	730	1020	1400
	0.99	310	760	1080	1460
0.99	0.95	470	1120	1530	2040
	0.99	510	1170	1590	2100

DISCUSSION

The approach developed in this paper attempts to work out a procedure to calculate appropriate minimum sample size for the estimation of probability of freedom from disease when there is no certainty about the presence of disease or infectious agents in an animal population. The problem also comprises uncertainty about the diagnostic test performance i.e. sensitivity and specificity, and vagueness about the probability of disease, say prevalence, if the population is diseased.

To account for the aforesaid characteristics beta distributions are stated for the indicators of sensitivity, specificity and prevalence (given disease presence) to save the lack of knowledge about the accuracy of the tests as well as for the disease load in the population. These distributions may be determined based in evidence from previous studies or in the worst cases when no evidence is available a vague distribution Beta(1,1) can always be helpful. The method also allows for zero prevalence to take into consideration the fact that no sureness exists about the presence of disease, and this is the concept to be evaluated in the process of sample size calculation. Two populations are contrasted to assess whether the result of a fixed number of positive tests is actually due to a possible presence of disease or due to false positive results as a consequence of the inaccuracy of the diagnostic test. In the former case it is presumed that the result comes from a diseased population, in the latter case it is deduced that the result comes from a healthy population.

The comparison of the number of positive results with the 0.05 quantile from a diseased population helps to decide with a probability level of 95% that the population is free from disease, if the number of positive results is less than the reference quantile. The value of 0.05 aims the guarantee of a minimum margin of classification error, this quantile can be changed for any other desired level but it is not advisable to choose a value higher than 0.05 since it would mean to have higher probabilities of misclassification e.g. to say that the result belongs to a free from disease population when it actually comes from a disease population; on the contrary, levels lower than 0.05 will improve the possibility to be right in the decision about the correct interpretation of the result.

Given the uncertainty of the indicators (sensitivity, specificity, prevalence), the repetitive random sampling allows impartial selection of values to be used in the construction of the probability distributions for the number of positive cases, which consequently will give a fair estimation of the probability of freedom from disease.

In the application for the classical swine fever virus in wild-boars, the connection between probability of freedom from CSF-virus and sample size was obtained in two ways as demonstrated in the results, which shows in general increased sample sizes for higher levels of probabilities of freedom as well as for higher number of positive tests. It was also obtained that a maximum number of two reactors should be observed in a minimum sample size of 300 animals to state that there is a 0.95 probability that the probability of freedom from CSF-virus is larger than 0.95 in the population of wild-boars. This particular illustration of the method can be used as a guide to reproduce results for some other population of animals with similar characteristics and problems as the classical swine fever-virus in the wild-boar population.

The proposed method is applicable to the special case when the presence of disease in the population is not confirmed, thus the first task is to come up with the estimation of the probability of freedom from disease and then to look for a suitable sample size to achieve the probability of freedom of the researcher's interest. This evidently differs from other approaches where the presence of disease is confirmed and then the analyses is based on the sample size satisfying the condition of prevalence below a threshold value.

Furthermore, the method is intended for infinite population cases. The extension of the procedure for a finite population, by using the hyper-geometric instead of the binomial distribution for the probability of positive test results, could be a next challenge.

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CURRENT VALUE OF HISTORICAL AND ONGOING SURVEILLANCE FOR DISEASE FREEDOM: SURVEILLANCE FOR BOVINE JOHNE'S DISEASE IN WESTERN AUSTRALIA

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SUMMARY

Confidence in freedom from disease is generally derived from multiple sources of varied surveillance information, and typically this surveillance evidence has been accumulated over time. In the state of Western Australia (WA) the main surveillance evidence supporting Free Zone status in the national bovine Johne's disease (BJD) program comprises periodic surveys and the ongoing clinical diagnostic system. This paper illustrates a simple approach to current valuation of historical surveillance information, based on the calculated sensitivity of the surveillance processes, the time elapsed since the data were accumulated, and the probability of new introduction of disease into the population during that elapsed time. The probability that the WA cattle population was free from infection was estimated following each of 11 years, giving a median probability that the State was free of BJD (at a level 0.2% of herds and 2% of animals within herds) at the end of 2005 of 0.94.

INTRODUCTION

Under the World Trade Organisation's Agreement on the application of Sanitary and Phytosanitary Measures, barriers to trade based on animal health status must be substantiated by scientifically valid evidence. To facilitate export of animals or animal products, or to prevent their import from an infected country or zone, a country or zone should be able to demonstrate that particular diseases or agents of interest are not present. In practice, absence of infection from a population is assessed based on the negative outcomes over time of a surveillance system aimed at detecting infection if it were present, and the capacity of the population to keep infection out.

The results of random surveys of the population may be used to estimate the diagnostic sensitivity (*i.e.* the probability that infection would have been detected in the population if it were present at some agreed level, the design prevalence, P^*) of such surveys (Cannon & Roe, 1982; Cameron & Baldock, 1998), and the same may be done for other targeted or general surveillance system components (SSCs) (Cameron et al., 2003; Martin et al., in press). SSC-specific sensitivity estimates may be combined into an estimate of the sensitivity of the entire surveillance system (*SSe*) for the disease (Martin et al., in press). This estimate is valid at the

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time the surveillance was conducted, but its applicability declines over time, since historical evidence of "freedom" is not what is needed in assessing the population today.

Negative results from a surveillance system of known *SSe* may be used to estimate the probability that the population is free from infection at P^*, using Bayesian inference; but this calculation is intrinsically controversial given the need for an estimate of the prior probability that the population was infected at P^*, before the surveillance was conducted.

A method for deriving the prior probability of population freedom for a surveillance time period ($PriorPFree_{tp}$) from the posterior (post-surveillance) estimate for the previous time period ($PostPFree_{tp-1}$) and the probability of new introduction of infection into the population during the intervening time period ($PIntro_{tp}$) has been described by Martin et al. (in press). This paper reports on its application to BJD in WA.

Western Australian freedom from BJD

The State of Western Australia has been conducting surveillance for BJD for many years. The disease has been diagnosed on nine occasions during the last 50 years, each case being associated with the introduction of infected cattle from other States of Australia; all infected herds were destocked and the disease was eradicated in eight historical cases (most recently in 1994). In July 2006 the ninth introduction was detected, and an eradication program is currently under way. The analysis presented in this paper assumes that eradication will again be successful. In 1995 and 2001, two substantial surveys were conducted, targeting herds which have introduced animals from other States in which BJD is present, and in 2003 – 2004 a large number of animals were tested for BJD (some in 2003 and some in 2004) as part of an investigation following isolation of the causative organism from a flock of sheep. The other major surveillance component for BJD in WA is the clinical diagnostic system, which has been the source of all nine diagnoses in the State.

Measures taken by WA to reduce the probability of introduction of BJD into the State were, until 1998, certification of the source herd as having shown no signs of BJD for five years, and negative serum ELISA testing of all animals in the consignment. Since 1998 WA has only accepted cattle from source herds which have attained a specified status in the national market assurance program (CattleMAP), which is based on repeated negative tests of the whole herd to substantiate BJD-free status. CattleMAP has resulted in a substantial decrease in the probability of introduction of BJD into the State.

This paper presents an analysis of the WA surveillance system for BJD over the years 1995 – 2005, to give an estimate of the probability that the State was free of the disease (at P^*) at the end of that period. The method used for estimation of prior probability of infection allows quantitative valuation of historical surveillance results, use of negative findings from both the targeted surveys and the clinical diagnostic system, and robust estimation of the current probability of population freedom at P^*.

MATERIALS AND METHODS

Surveillance for BJD in WA has, over the last 11 years, comprised the ongoing clinical diagnostic system and periodic serological surveys. The surveys were conducted as follows.

1995 survey

A total of 7,294 animals were tested from 127 herds considered to be at high risk of acquiring BJD. These were herds which had imported animals from other States in which BJD was known to be present, during the period 1989 to 1993. Herds included were those importing cattle of dairy breeds, and those with multiple importations of beef breeds. Cattle tested were all imported individuals present at the time of testing, plus up to 100 in-contact WA-born animals over three years of age. Details of laboratory testing of blood and faeces are described by Ellis et al. (1998). Blood samples from all animals were tested with an absorbed ELISA for antibody. After any necessary follow-up testing to resolve the infection status of ELISA reactors (Ellis et al., 1998), all had negative outcomes.

2001 survey

This was identical in design to the 1995 survey, covering herds that imported animals during the period 1994 – 1998, and 5,597 animals from 92 herds were tested; all with negative outcomes.

Table 1. Nodes and sources of parameter estimates for Survey SSC model

Node	Node type	Source of input values	Branches
1. TRACE HERD	Risk category	P[a]: Herds processed R[b]: Expert opinion	Yes No
2. HERD TYPE	Risk category	P[a]: *Population*: Industry statistics *SSC*: herds processed R[b]: Expert opinion; outbreak history	Dairy Feedlot beef (FLBeef) Rangelands beef (RLBeef) Agricultural region beef (AgBeef)
3. HAS IMPORTED	Risk category	P[a]: *Population*: Importation records; industry statistics *SSC*: herds processed R[b]: Outbreak history	Yes No
4. HERD STATUS	Infection	Design prevalence	Infected Uninfected
5. ORIGIN OF ANIMAL	Risk category	P[a]: *Population*: Importation records; industry statistics *SSC*: animals processed R[b]: Outbreak history	Imported Not imported
6. ANIMAL STATUS	Infection	Design prevalence	Infected Uninfected
7. AGE	Detection category	Expert opinion	Under 3 years ≥3 years
8. ELISA RESULT	Detection	Literature; expert opinion	Positive Negative

[a] Proportions [b] Relative risks

2003 – 2004 survey

The cattle strain of *Mycobacterium avium paratuberculosis* was isolated from a single pooled faecal sample from a flock of sheep during surveillance for ovine Johne's disease in 2003. In the resulting investigation 3,914 cattle from 30 herds were tested using the same laboratory and follow-up procedures as used in the 1995 and 2001 surveys (Ellis et al., 1998), but in this hunt for a source of infection, every animal over two years of age was tested. All had negative outcomes (after follow-up testing to resolve the status of ELISA reactors).

Scenario tree models (Martin et al., in press) were developed for the two SSCs; the clinical diagnostic system (*Clinical* SSC) and the surveys (*Survey* SSC). These models were used to calculate the probability that each SSC would detect BJD if it were present in WA at specified design prevalences. A period of one year was selected as the appropriate surveillance time period for this slow moving disease of relatively minor economic importance. The surveys were each considered to apply to a single time period. The surveillance unit in each SSC was the individual bovine, and infection was considered to cluster (potentially) in herds, but not at any other grouping level in the population. The reference population for each of these SSCs is the cattle population of Western Australia, consisting of approximately 2,130,000 cattle (data from Australian Bureau of Statistics (ABS) agricultural census 2001) in approximately 4,000 herds.

The steps necessary for each animal to give a positive SSC outcome, represented by nodes in the scenario trees, are listed in Tables 1 & 2. Table 1 shows nodes in the *Survey* SSC. In the *Clinical* SSC tree, node 1 (TRACE HERD) was omitted since there were no properties traced from known sources of infection; nodes 2 – 4 were the same; node 5 (ORIGIN OF ANIMAL) was not included, so nodes 6 & 7 became 4 & 5; node 8 was replaced by the chain of detection nodes in Table 2.

Table 2. Detection nodes in the *Clinical* SSC scenario tree

Node	Node type	Source of input values	Branches
6. CLINICAL SIGNS (CS)	Detection category	Expert opinion; literature	Yes No
7. FARMER NOTICES ILLNESS	Detection	Expert opinion	Yes No
8. VET ATTENDS	Detection	Expert opinion	Yes No
9. SAMPLES SUBMITTED	Detection	Expert opinion	Yes No
10. LAB TESTS FOR JD	Detection	Expert opinion; laboratory records	Yes No
11. BJD DIAGNOSED	Detection	Literature	Yes No

Table 3a. Branch probabilities, proportions (of units processed) and relative risks used in modelling the *Clinical* and *Survey* SSCs.

Node	Limb(s)on which node is situated	Branch of node	Probability or proportion of units processed	Relative risk
TRACE HERD	n/a	Yes	survey-specific	10 [a]
HERD TYPE		Dairy	0.069 [b]	10
		FLBeef	0.008 [b]	1
		RLBeef	0.088 [b]	1
		Agbeef	0.836 [b]	1
HAS IMPORTED	HERD TYPE Dairy	Yes	0.05 [b]	10 [a]
	HERD TYPE FLBeef	Yes	0.01 [b]	10 [a]
	HERD TYPE RLBeef	Yes	0.01 [b]	10 [a]
	HERD TYPE Agbeef	Yes	0.025 [b]	10 [a]
HERD STATUS	All	Infected	0.002	
ORIGIN OF ANIMAL	All	Imported Not imported	Beta(4.125, 47.875)	(23, 38, 145) [c]
ANIMAL STATUS	All	Infected Uninfected	0.02	
AGE	All	Under 3	Pert(0.5,0.6,0.7)	
ELISA RESULT	AGE over 3	Positive	Pert(0.1,0.2,0.3)	
	AGE under 3	Positive	Pert(0.08,0.12,0.15)	
CLINICAL SIGNS	AGE over 3	Yes	Pert(0.1,0.2,0.4)	
	AGE under 3	Yes	Pert(0.005,0.015,0.03)	
LAB TESTS FOR BJD	AGE over 3; CS Yes	Yes	(0.63, 0.78, 0.95) [d]	
	AGE over 3; CS No	Yes	(0.02, 0.19, 0.48) [d]	
	AGE under 3; CS Yes	Yes	(0.03, 0.24, 0.58) [d]	
	AGE under 3; CS No	Yes	(0.02, 0.09, 0.18) [d]	
BJD DIAGNOSED	AGE over 3; CS Yes	Yes	0.7	
	AGE over 3; CS No	Yes	0.3	
	AGE under 3; CS Yes	Yes	0.7	
	AGE under 3; CS No	Yes	0.15	

[a] Risk relative to low risk branch, for which the relative risk therefore has a value of 1.
[b] WA population proportions, used for Clinical SSC. Proportions processed in the Survey SSC were survey-specific, and were derived from the herds processed.
[c] Complex distribution derived from outbreak data. Values shown are the 5%ile, mode and 95%ile of the resulting distribution (3,000 iterations).
[d] Complex distribution derived from opinion of multiple experts. Values shown are the minimum, mode and maximum of the resulting distribution (3,000 iterations).

Many branch probabilities, particularly for detection nodes (i.e. probabilities of sequential steps involved in the detection of an infected animal) could not be supported by data, and relied heavily on expert opinion (see table 1). Such opinion was gathered by telephone interview from up to four selected individuals with relevant expertise, including veterinarians (private

practitioners; government field officers; pathologists), farmers and BJD specialists. Within-herd age distributions were estimated by livestock production specialists for each herd type. Experts' individual estimates for each parameter estimated were summarised using appropriate probability distributions (to represent both uncertainty and variability) or point estimates, and the average of these was then used as the parameter estimate for the model.

Values used for branch probabilities, proportions and relative risks are as shown in Tables 3a and 3b, which list nodes from both trees.

Table 3b. Branch probabilities for the detection nodes FARMER NOTICES ILLNESS, VET ATTENDS and SAMPLES SUBMITTED in the *Clinical* SSC scenario tree

Limbs on which node is situated				
HERD TYPE	CLINICAL SIGNS	FARMER NOTICES ILLNESS	VET ATTENDS	SAMPLES SUBMITTED
Dairy	Yes	(0.75, 0.84, 0.88)[a]	(0.67, 0.72, 0.78)[a]	(0.89, 0.94, 0.99)[a]
FLBeef	Yes	1	0.5	(0.76, 0.90, 1.00)[a]
RLBeef	Yes	(0.13, 0.15, 0.17)[a]	0	(0.04, 0.51, 0.74)[a]
AgBeef	Yes	(0.81, 0.87, 0.94)[a]	(0.33, 0.47, 0.55)[a]	(0.90, 0.94, 0.99)[a]
Dairy	No	Pert(0.01, 0.05, 0.1)	(0.03, 0.07, 0.12)[a]	(0.00, 0.00, 0.20)[a]
FLBeef	No	Pert(0.01, 0.05, 0.1)	0.5	0.25
RLBeef	No	0.01	0.0001	0.25
AgBeef	No	0.03	Pert(0.1, 0.25, 0.5)	0.25

[a] Complex distribution derived from opinion of multiple experts. Values shown are the minimum, mode and maximum of the resulting distribution (3,000 iterations).

Two levels of design prevalence were used to account for clustering of BJD in herds. The among-herd design prevalence (P^*_H) was 0.002, and the within-herd design prevalence (P^*_U) was 0.02. The latter is that used in the Australian National Johne's Disease Program. The among-herd design prevalence was selected as a reasonable compromise between the exacting requirements of disease eradication programs and those of surveillance to support freedom from disease. The same design prevalence (0.2% of herds) is specified by the OIE (World Organization for Animal Health) in its surveillance standards for maintenance of free status for enzootic bovine leucosis, which is another slow moving disease of cattle.

Sensitivities of the *Survey* SSC for each survey (*CSe_Survey_1995*; *CSe_Survey_2001*; *CSe_Survey_2004*) and for the *Clinical* SSC (*CSe_Clinical*) were calculated allowing for grouping of animals within herds. In the *Clinical* SSC this required dividing the population into herds, for which purpose all herds in each HERD TYPE were assigned average numbers of cattle (derived from industry statistics from ABS), namely *Dairy* 464; *FLBeef* 1,418; *RLBeef* 3,587; *AgBeef* 210.

Clinical and *Survey* component sensitivities (for detection of clinical disease and seropositivity respectively) were assumed to be independent, and combined in each time period to give an overall surveillance system sensitivity for the time period (SSe_{tp}), using Eq(1):

$$SSe_{tp} = 1 - (1 - CSe\_Clinical_{tp}) \times (1 - CSe\_Survey_{tp}) \quad (1)$$

SSe is always estimated for a given P^*. As with diagnostic tests, sensitivity may be used, together with specificity and a prior estimate of the probability of infection being present (*PrioPInf*), to derive a posterior estimate of the probability of freedom, *PostPFree* (the negative predictive value of the test) (Martin et al., in press). Assuming perfect surveillance system specificity Eq(2) is used:

$$PostPFree_{tp} = \frac{1 - PriorPInf_{tp}}{1 - PriorPInf_{tp} \times SSe_{tp}} \quad (2)$$

For 1995 a neutral or uninformed *PriorPInf_1995* of 0.5 was used. The negative surveillance findings during 1995 then led to *PostPFree_1995* being greater than (1 – 0.5), or *PostPInf_1995* < *PriorPInf_1995*. At the end of 1996, with new negative surveillance findings represented by *SSe_1996*, there was residual "confidence" in the population being free of infection, based on *PostPFree_1995*.

The only reason for a decline in the probability that the population was free (at P^*) during 1996 was the possibility that infection (at P^*) might have been introduced into the population during the year. This could have happened in either of two ways: (a) it was introduced into an uninfected population; or (b) it was present at < P^* at the end of 1995 and spread to reach a prevalence > P^* during 1996. Assuming that the probability of introduction remains constant over time, and that the likely rate of spread of infection in the population remains constant over time, then the probability of (b) occurring is equal to the probability of (a) for a given time period, since the probability of (b) is actually the probability of (a) having occurred some constant number of time periods ago. In this analysis the annual probability of introduction had changed considerably over the period covered by the analysis, but there was no reason to suppose that the likely rate of spread of BJD in the WA cattle population would have changed. The probability that the prevalence of established BJD infection in WA would rise above P^* during a given time period ($tp = i$) was assumed to be equal to the probability of introduction and establishment of BJD during time period (i – 9). Nine years was taken as an appropriate interval because the likely minimum length of the transmission cycle of BJD in a cattle herd is around three years, and a period of three cycles for the prevalence to rise above the design prevalence was thought appropriate. The value of *PIntro* for 1987 was therefore used as the *offset PIntro* for 1996.

The probability of introduction of BJD into WA with imported cattle was estimated for each year using a stochastic quantitative risk assessment model, which was developed in 1996 for predictive purposes (Martin & Casey, 1996). For this analysis, the model was adapted to estimate the probability of BJD introduction with the cattle that were actually imported during each year, using retrospective estimates of the prevalence of BJD among cattle in other states of Australia. The mean probability of introduction for each year was the proportion of 3,000 iterations of the stochastic model in which one or more infected consignments was introduced into a breeding herd. Introductions of infected cattle for fattening and slaughter were ignored. These retrospective estimates of the probability of introduction are higher than predictive estimates made in 1996, largely due to recent reports (Jubb et al., 2004) of a much lower sensitivity of the serum ELISA (mode 20% for animals over three years of age) than the estimate used in the original model (mean 50%). The analysis of historical surveillance data calls for the probability of introduction and establishment ($PIntro_{tp}$), since this is the starting point for spread to exceed P^*. The probability of establishment (given introduction of an infected animal) is unknown. It clearly varies among cattle populations, and it is generally

assumed that the reason for WA having remained free from BJD historically is associated with a low probability of the infection becoming established in the State, following introduction. For this analysis a figure of 0.5 was used for this probability. The probability of introduction and establishment for a given year was therefore the modelled probability of introduction of an infected animal during that year multiplied by 0.5.

Allowing for the possibility that infection (at P^*) was present at the start of 1996 and it was also introduced during the year, the probability that the population was infected at P^* at the end of1996 was therefore (Eq(3)):

$$PriorPInf\_1996 = PostPInf\_1995 + PIntro\_1987 - (PostPInf\_1995 \times PIntro\_1987) \quad (3)$$

Equations 2 and 3 were applied to successive surveillance time periods.

RESULTS

Means of the output distributions of *CSe_Clinical*, *CSe_Survey* (1995, 2001 and 2004), *SSe*, *PIntro*, *PriorPInf* and *PostPFree* are presented in Table 4. Figure 1 shows the fluctuations over the 11 year period in median *SSe*, *PriorPfree*, *PostPFree*, and mean offset *PIntro*.

Table 4. BJD surveillance[a] in WA, 1995 - 2005: Yearly surveillance component sensitivities, probability of introduction and establishment, prior probability of population being infected and probability of population freedom at design prevalences of 0.2% of herds and 2% of animals within an infected herd.

Year (*tp*)	Survey *CSe*	Clinical *CSe*	Surveillance *SSe*	$PIntro_{tp-9}$	*PriorPInf*	*PostPFree*
1995	0.294	0.673	0.769	0.067	0.500	0.814
1996		0.673	0.673	0.065[b]	0.239	0.903
1997		0.673	0.673	0.065	0.156	0.939
1998		0.673	0.673	0.172	0.222	0.912
1999		0.673	0.673	0.243	0.310	0.870
2000		0.673	0.673	0.189	0.294	0.876
2001	0.211	0.673	0.742	0.231	0.326	0.886
2002		0.673	0.673	0.147	0.244	0.900
2003		0.673	0.673	0.230	0.307	0.870
2004	0.259	0.673	0.758	0.104	0.220	0.932
2005		0.673	0.673	0.125	0.185	0.928

[a] Means of output distributions from 1000 iterations of a stochastic model.
[b] Extrapolated from incomplete cattle importation data available at the time of analysis

PIntro varied considerably from year to year, depending on the number, type and sources of cattle brought into the State. In the 20 years analysed (1986 – 2005 inclusive) the highest mean estimated probability of introduction of one or more infected animals into a breeding herd in WA was 0.49 in 1990, and the lowest 0.02 in 2003. The mean annual probability for 1986 – 1998 inclusive was 0.31, while it was 0.04 for 1999 – 2005. The reasons for this were presented in the introduction. *PIntro* was lower in 1986 and 1998 than in subsequent years due to lower

numbers of cattle imported into the State. Data for 1987 were missing, so the average of 1986 and 1988 figures for the probability of introduction was used for this year (Table 4).

Estimated annual surveillance component sensitivity for the clinical diagnostic system (*CSe_Clinical*) was 0.67 (0.52; 0.81) (mean (5%ile; 95%ile) of output distribution), and this became the annual *SSe* for each year apart from those in which the surveys were conducted, when mean *SSe* was boosted to 0.77 in 1995, 0.74 in 2001 and 0.76 in 2004.

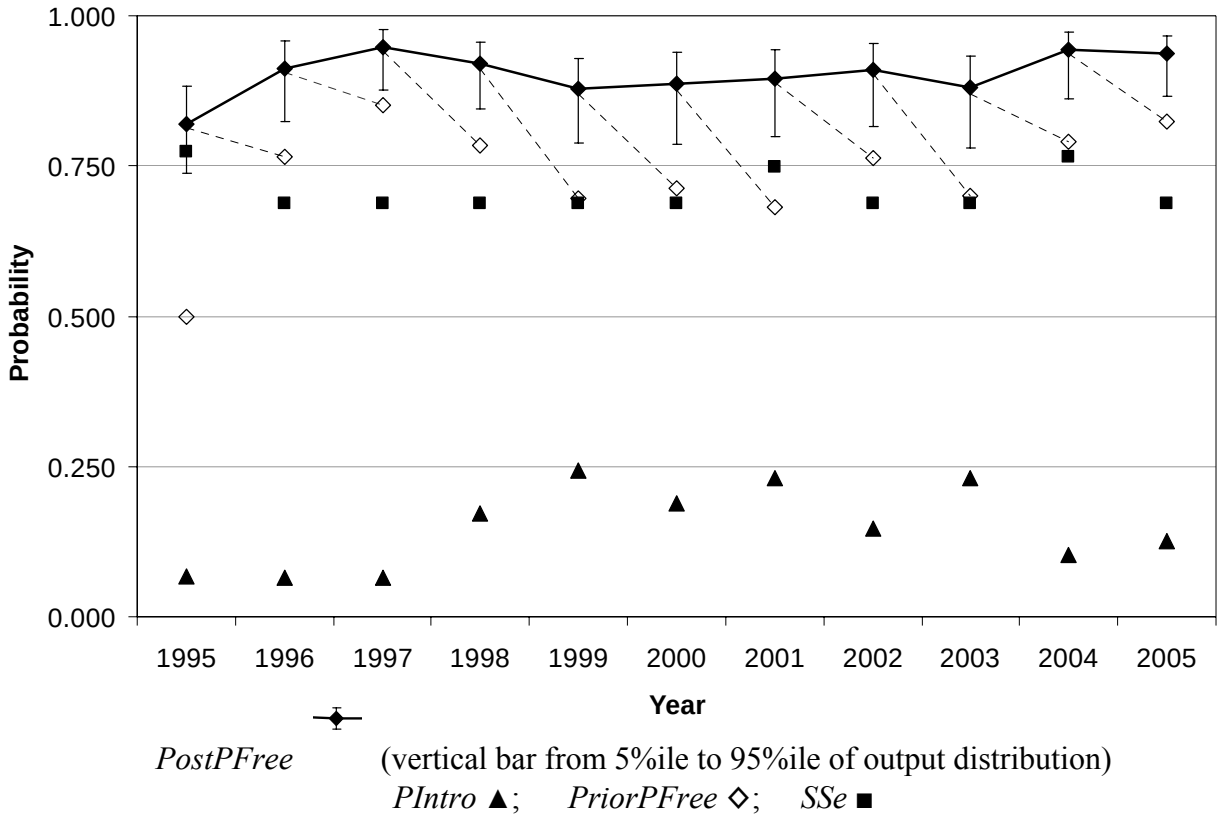

Fig. 1 Probability of WA freedom from BJD over time, combined surveillance sensitivity, and annual probability of introduction and establishment 9 years previously (1986 – 2006) – medians of output distributions from stochastic models (mean for *PIntro*)

The estimated posterior probability of WA cattle being free of BJD (at P^*) for 2005 was 0.93 (0.87; 0.97). Over the 11 years analysed it reached a peak in 1997 of 0.94 (0.88; 0.98) and a low point in the year with highest offset *PIntro* (1999) of 0.87 (0.79; 0.93).

DISCUSSION

The outbreak of BJD in WA which was detected in July 2006 clearly means that the State is not free of the disease until such time as it has been eradicated. The rules of the national Johne's program lay down procedures for dealing with an outbreak of BJD in a Free Zone, and these are being followed at the time of writing.

In the absence of any reasons for supposing otherwise, the annual sensitivity of the clinical diagnostic system was assumed to be constant over the 11 years analysed. In practice this is a reasonable assumption, since the minor fluctuations in population numbers and attitudes and

practices of farmers and vets do not lead to significant changes in *CSe_Clinical*. The one area where dramatic changes would affect the *CSe_Clinical* is in the laboratory; a new battery of tests with very high sensitivity would raise it significantly; this is because the sensitivity of the laboratory testing applies to all infected, sampled animals in the population. On the other hand, changing the attitude of *AgBeef* farmers to calling the vet when they see a cow with diarrhoea will affect the probability of a positive outcome only for a subgroup of the population; *AgBeef* cattle with clinical signs.

The end result (*PostPFree_2005*) is not sensitive to the length of the surveillance time period, since sequential time periods are all incorporated. Clearly a shorter time period results in lower *CSe*s and lower *PIntro* for each time period, but overall the amount of surveillance information and the likelihood of disease having been introduced is not affected substantially by the size of the temporal slices into which it is divided.

The current probability that a population is free from disease at P^* is determined using an accumulation of all the surveillance evidence available, and the value of this evidence is constantly being eroded by the probability of new introductions of disease into the population. In the absence of overwhelming surveillance evidence for absence of disease (i.e. *SSe* very close to 1) the most influential factor affecting perceived and actual probability of freedom is the ongoing probability of disease being introduced into the population. In the case of BJD, a disease which spreads slowly, it would have had to have been introduced and to have become established several years ago for it to be becoming visible today. Hence, the input to which *PostPFree_2005* is most sensitive is the offset *PIntro* (for 1996 and the immediately preceding years). The (inverse) relationship between offset *PIntro* and *PostPFree* is apparent in Fig 1. The number of time periods by which *PIntro* should be offset in this analysis might be better estimated from a model of potential BJD spread in WA.

PostPFree_2005 is also sensitive to the selected values for P^*, through the effect of P^* on *SSe*. Since a high value for P^*_H (eg 0.01) can result in values for *SSe* in excess of 0.99 (in this case, *SSe* for 2005 would be 0.99, and for 2001, a survey year, it would be 0.999), the negative effect of *PIntro* on the prior probability of population freedom is then overwhelmed by the surveillance evidence supporting freedom in the Bayesian updating of the probability of freedom at the high P^*.

SSe may also be influenced by the values specified for relative risks of different population subgroups being infected, in the scenario tree models. However, *PostPFree_2005* is remarkably insensitive to changes in relative risks applied at any of the three risk nodes in the SSC models, again reflecting the relative importances of a moderate ongoing surveillance system sensitivity and a substantial ongoing probability of new introductions of disease. If the ongoing probability of introduction and establishment can be reduced to a very low level, this will allow confidence in the population being free from disease to accumulate steadily over time. This mimics qualitative, subjective assessments of a population's disease status.

In summary, this procedure effectively accumulates historic and ongoing surveillance evidence towards current "confidence" in freedom, discounting its value appropriately over time; a process which models and parallels the usual practice of subjective, qualitative assessment. This process also provides a transparent and appropriate mechanism for estimating a prior probability of population freedom based on past surveillance evidence for the absence of disease.

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SPATIAL EPIDEMIOLOGY

DISTANCES BETWEEN NEIGHBOURING HERDS OF RELEVANCE FOR LOCAL SPREAD OF *SALMONELLA* DUBLIN BETWEEN CATTLE HERDS IN DENMARK

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SUMMARY

A spatial generalised linear mixed model was developed to estimate the range of spatial correlation between dairy herds with positive *Salmonella* Dublin herd status. Herd status was a binary outcome of high/low antibody levels to *Salmonella* Dublin bulk-tank milk samples collected from all dairy herds for surveillance purposes. The spatial correlation between herds was modelled using an exponential decay function for eight different regions in Denmark. The analyses were performed on data from one year after initiation of the *Salmonella* Dublin surveillance program (4th quarter of 2003) and two years later (4th quarter of 2005), where the national prevalence had decreased from 21.7% to 16.6%. The range of spatial correlation was 2-17 km in 2003 and 2-6 km in 2005 with fairly large standard errors in regions with low prevalence. There was no obvious association between the range of spatial correlation and the density of herds in the different regions.

INTRODUCTION

The most commonly found *Salmonella* serotype in Danish cattle is *Salmonella enterica* subspecies *enterica* serovar Dublin (*Salmonella* Dublin). It is known to cause higher case mortality ratio in human patients than other types of *Salmonella* more commonly found in humans (Helms et al., 2003). Furthermore, *Salmonella* Dublin causes economic and welfare losses in infected cattle herds. Therefore, a national surveillance program for *Salmonella* Dublin was initiated in Denmark in October 2002 in order to monitor the number of infected herds and lower the number of new infections by limiting trade between infected and non-infected herds. The latter was encouraged by allowing the *Salmonella* Dublin status to be publicly available on the internet and on health certificates accompanying animals when moved between herds or markets (Nielsen et al., 2006a).

In the surveillance program, all dairy herds are tested regularly, i.e. bulk-tank milk samples are collected automatically through the obligatory milk quality recording scheme approximately at quarterly intervals within the year. Their test status is based on antibody measurements in four consecutive bulk-tank milk samples (Warnick et al., 2006). Non-dairy herds consist of very mixed types of herds ranging from very small hobby herds with low trade activity to large slaughter calf production sites that purchase bull calves from many dairy herds. The non-dairy

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herds are assigned infection status according to blood samples collected either at slaughter or sometimes by owner requested sampling of the herd (Nielsen et al., 2003).

In the 4th quarter of 2003 (2005), the national seroprevalence among 7,237 (5,694) dairy herds was 21.7% (16.6%) and among 18,967 (18,442) non-dairy herds it was 3.3% (3.7%). However, more than 20% of the non-dairy herds did not have enough samples collected to evaluate the status of the herd. These were generally very small herds with very few animal movements, i.e. low risk herds regarding *Salmonella* Dublin infection. The distribution of seropositive herds is very regional with high prevalence regions strongly associated with the cattle herd density in the country (Fig. 1).

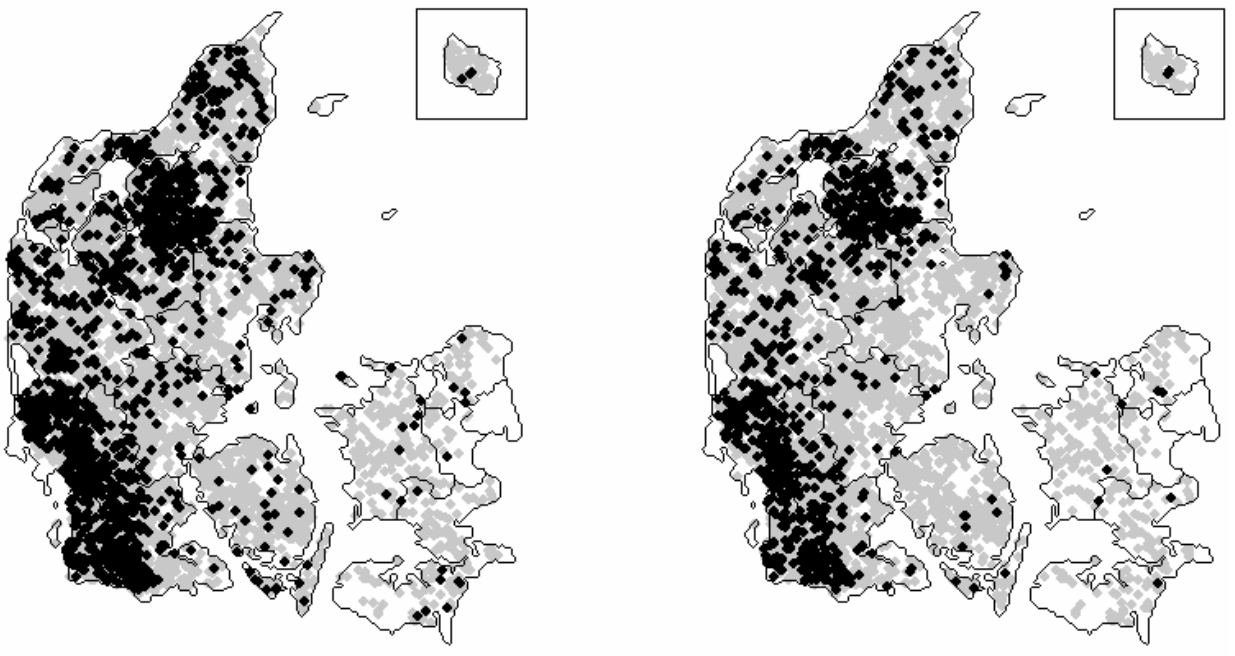

Fig. 1 Distribution of *Salmonella* Dublin-seropositive dairy herds in Denmark in the 4th quarter of 2003 (left map) and 2005 (right map). Black dots point to herds with antibody levels above the cut-off values and grey dots point to herds below the cut-off values used in the national surveillance programme.

A number of risk factor studies have indicated that in addition to transmission through trade or movement of animals between herds, local spread of *Salmonella* in cattle is important (Vaessen et al., 1998; van Schaik et al., 2002; Nielsen et al., 2006b). In some studies, it was shown that contact over fences, sharing of pastures and manure spreading equipment were risk factors for local spread of the infection, but the actual distance of spread was not estimated. In Nielsen et al. (2006b), it was shown that having cattle herds with a high antibody status for *Salmonella* Dublin within a radius of 2 km was a significant risk factor for becoming infected, but the modes of infection were not explored and other distances than 2 km were not tested in that study. Thus, the distance of local spread and possible causes of differences in the distance of local spread are still unknown. The information is important for defining protective zones around outbreak herds during eradication trials and control campaigns. To the farmers, protection of their own herds against *Salmonella* infection will become increasingly important as case-by-case tracing of sources of infection in humans will be used more widely.

The objective of this study was to estimate the range of spatial correlation between neighbouring dairy herds of relevance for local spread of *Salmonella* Dublin. Data from the Danish Cattle Database including *Salmonella* Dublin status of the herds, coordinates and risk factors such as herd size, purchase and regional location in Denmark was used to estimate range of spatial correlation in different regions of the country with varying prevalence of *Salmonella* Dublin and varying herd density.

The range of spatial correlation can be estimated based on geostatistical principles. Geostatistics is a part of spatial statistics concerned with continuous spatial variation (Krige, 1951; Journel & Huijbregts, 1978; Isaaks & Srivastava, 1989; Cressie, 1991). The basic idea in geostatistics is that measurements at locations close together tend to be more alike than measurements at locations farther apart. In classical geostatistics, the spatial variation in a process is modelled using a semivariogram describing the variation between locations as a function of the distance between the locations. As the separation distance between locations increases, the variation generally increases. At a certain distance, an increase in the separation distance no longer causes a corresponding increase in the variation. This distance is called the range of influence and is an estimate of the average distance between locations where locations no longer are correlated with respect to a certain measure such as infection status of the herds.

MATERIALS AND METHODS

Selection of herds and recordings from the Danish Cattle Database

All dairy herds from beginning of 2003 (N=7,237) to the end of 2005 (N=5,694) were included in the study. For all herds the following variables were collected or derived from the Danish Cattle Database: Unique herd ID-number, *Salmonella* Dublin ELISA measurements on bulk-tank milk samples, date of bulk-tank milk sampling, region (8 regions with 2 larger islands and the peninsula, Jutland, divided into 6 regions, Fig. 2), *Salmonella* Dublin apparent prevalence in the region, herd size (total number of cattle), number of herds within a 5 km radius, UTM-coordinates and purchase of animals in the preceding quarter of the year (YQ) from seropositive herds (yes, no).

Due to small sample sizes for a number of smaller islands that were left out of the analyses, the total number of dairy herds used in the analysis was 7,003 in 2003 and 5,515 in 2005, respectively.

Definition of herd infection status

For the analyses, all dairy herds had a *Salmonella* Dublin classification status (positive, negative) assigned to each YQ in 2003-2005 based on the last four consecutive bulk-tank milk samples for dairy herds. A positive classification status was given if the four bulk-tank milk moving average ODC% was <25 and no increase of more than 20 ODC% was found when comparing the most recent measurement to the average of the three previous measurements. If a herd was classified more than once during the same YQ, because more than one bulk-tank milk sample was collected in that YQ, the status for that YQ was defined as the worst infection status. If a herd was not assigned a status in one YQ due to lack of samples in that YQ, the YQ was assigned the same status as the preceding YQ. When herds ceased milk-production they were assigned a herd status in the same YQ when production stopped, but not after that.

Fig. 2 Map of Denmark with the eight regions used in the analyses. Two regions were larger islands (Zealand and Funen) and six regions were formed by dividing the peninsula, Jutland. Other areas (smaller islands) were not included in the analyses due to too few observations.

Estimating the range of spatial correlation using a spatial generalized linear mixed model

Conventional geostatistical analyses are based on an assumption of a Gaussian spatial stochastic process $S(x_i)$ based on observations $Y_i=S(x_i)+Z_i$ at sampling locations x_i, where Z_i are independent zero-mean Gaussian random variables. In the present study the outcome was the binary outcome *Salmonella* Dublin herd status. Therefore, to estimate the range of correlation in space we used a spatial generalized linear mixed model. In this analysis, the spatial components and the parameters of potential risk factors were estimated simultaneously. The spatial component in the model measured the correlation in *Salmonella* Dublin herd status between herds as a function of the spatial distance between herds. This approach has been described by Diggle et al. (1998) as model-based geostatistics.

Let Y_{ij} be a binary outcome corresponding to having a positive *Salmonella* Dublin herd status in herd i, $i=1...N$, with value 1 and 0 otherwise.

$$Logit(p_{ij}) = \mu + \beta X_{ij} + s_j \quad (1)$$

where p_{ij} is the probability for a positive *Salmonella* Dublin herd status in herd i, $i=1...N$, X_{ij} is a covariate and β is the corresponding parameter estimate.

Let $S = (s_1, s_2, ..., s_N)$ be a vector of spatial effects of neighbouring herds. The distribution of S is given as $S \sim N(0,R)$ with R_{kl} defined as a function of the distances d_{kl} between the herds at location s_k and s_l. We assume an isotropic spatial process with R_{kl} given as $R_{kl} = \sigma^2 \rho_{kl}$, with an exponential correlation function $\rho_{kl} = exp(-\varphi\, d_{kl})$, with Euclidian distances $d_{kl} = ||s_k - s_l||$. The exponential correlation function ρ_{kl} reaches zero asymptotically, with the practical range of spatial correlation defined as the distance where the correlation is 5%. The spatial range of correlation was estimated as $3/\varphi$, defined as the distance beyond which the correlation between neighbouring herds is below 5%. The analysis was performed using the GLIMMIX macro in SAS (Statistical Analysis System, version 9.1). The range of spatial correlation can be interpreted as the range of influence in geostatistics (Isaaks & Srivastava, 1989). Estimates were obtained for 8 regions for two YQs (4th YQ of 2003 and 4th YQ of 2005, respectively).

Estimating the range of spatial correlation using an iterative approach in a generalised linear model and semivariogram estimation

Concurrent estimation of the spatial parameters and the effect of potential risk factors using the spatial generalised linear mixed model is an attractive choice. However, a few problems arose. The main problem was lack of convergence for a few regions, resulting in no parameter estimates. Furthermore, visual inspection of the semivariogram is not possible using the spatial generalised linear mixed model. Visual inspection of the semivariogram is important in order to evaluate model fit, e.g. inclusion of a nugget effect. Therefore, an alternative iterative approach was used alternating between 1) estimating potential risk factors and risk-factor adjusted residuals and 2) fitting a semivariogram based on the adjusted residuals (Cressie, 1991). Deviance residuals were used (McCullagh & Nelder, 1989). Estimates were obtained for 8 larger regions for two YQs (4th YQ of 2003 and 4th YQ of 2005, respectively). The iterative procedure used was as follows:

1) Initial estimates of the deviance residuals were obtained adjusted by potential risk factors. The generalised linear model

$$Logit(p_i) = \mu + \beta X_i \quad (2)$$

was used where p_i is the probability for a positive *Salmonella* Dublin herd status in herd i, $i=1...N$, X_i is a covariate and β is the corresponding parameter estimate. The deviance residuals r_i were calculated as

$$r_i = -2(y_i \log(\pi_i/(1-\pi_i)) - \log(1-\pi_i)) \quad (3)$$

where y_i is the binary outcome *Salmonella* Dublin herd status (positive, negative) for herd i, $i=1...N$ and π_i is the estimated probability for having a positive *Salmonella* Dublin herd status.

2) The empirical semivariogram $\gamma^*(h)$ was estimated as

$$\gamma^*(h) = \tfrac{1}{2} \Sigma(r_m - r_n)^2/N(h) \quad (4)$$

where r_m and r_n are deviance residuals for herds m and n at locations x_m and x_n, separated by the Euclidian distance h, $h=||x_n - x_m||$ and $N(h)$ is the number of pairs of herds in distance h. The spatial parameters were estimated in a semivariogram model, $\gamma(h)$ using non-linear regression. An exponential semivariogram model was used given by

$$\gamma(h) = c_0 + c\ (1 - exp(-h/a)) \quad (5)$$

with the three spatial parameters: nugget effect, c_0, partial sill, c, and range of influence, a. The practical range of influence was estimated as $a'=3a$.

3) A second generalised linear mixed model with fixed spatial parameters was used to obtain a new set of deviance residuals.

4) A second empirical semivariogram was estimated and an exponential semivariogram model was fitted estimating a new set of spatial parameters (range of influence, nugget and partial sill).

After 2 iterations, only minor changes in the spatial parameter estimates were observed.

The empirical semivariogram was calculated using a programme coded in the SAS programming language (Statistical Analysis System, version 9.1), and the exponential semivariogram model was fitted using non-linear regression (PROC NLIN in SAS, Statistical Analysis System, version 9.1).

RESULTS

The number of dairy herds decreased from 7,237 herds in the 4th quarter of 2003 to 5,695 herds in the 4th quarter in 2005. In the same period, the prevalence of dairy herds with a positive *Salmonella* Dublin herd status decreased from 21.7% in the 4th quarter of 2003 to 16.6% in the 4th quarter of 2005. In 2003, 7.0% of all dairy herds purchased animals from herds with a positive *Salmonella* Dublin herd status in the preceding quarter. In the 4th quarter of 2005 this number had decreased to 3.6%. Descriptive statistics are given in Tables 1 and 2. The regions used for the analyses are shown on a map (Fig. 2).

Due to a limited number of herds with purchase from herds with a positive *Salmonella* Dublin herd status, purchase could not be included as a risk factor in the analyses. Thus, the spatial parameters were initially estimated using the spatial generalised linear mixed model with herd size as the only risk factor included. The spatial parameter estimates were verified using the iterative approach. The estimated range of spatial correlation and corresponding standard error for each region is given in Table 3 based on the iterative approach. The standard error is large for most of the regions compared to the estimate of the range of spatial correlation. The semivariograms (Fig. 3) also show a large non-spatial variation (nugget effect) compared to the spatial variation (partial sill).

Table 1. Descriptive statistics of *Salmonella* Dublin herd status for the 4th quarter of 2003 and 2005 and stratified by possible risk factors for dairy herds in Denmark.

		Salmonella Dublin herd status 2003				
			Positive		Negative	
Variable	Level	N	N	(%)	N	(%)
Overall		7,003	1,548	(22.1)	5,455	(77.9)
Purchase from positive herds[1]	Yes	497	228	(45.9)	269	(54.1)
	No	6,506	1,320	(20.3)	5,186	(79.7)
Region	Zealand	326	16	(4.9)	310	(95.1)
	Funen	418	22	(5.3)	396	(94.7)
	NJN	554	85	(15.3)	469	(84.7)
	NJS	791	320	(40.5)	471	(59.5)
	NJW	1,115	189	(17.0)	926	(83.0)
	WJ	1,257	305	(24.3)	952	(75.7)
	EJ	1,055	108	(10.2)	947	(89.8)
	SJ	1,487	503	(33.8)	984	(66.2)
		Salmonella Dublin herd status 2005				
Overall		5,515	937	(17.0)	4,578	(83.0)
Purchase from positive herds[1]	Yes	206	118	(57.3)	88	(42.7)
	No	5,309	819	(15.4)	4,490	(84.6)
Region	Zealand	253	7	(2.8)	246	(97.2)
	Funen	318	7	(2.2)	311	(97.8)
	NJN	455	47	(10.3)	408	(89.7)
	NJS	613	222	(36.2)	391	(63.8)
	NJW	873	120	(13.8)	753	(86.3)
	WJ	998	184	(18.4)	814	(81.6)
	EJ	809	46	(5.7)	763	(94.3)
	SJ	1,196	304	(25.4)	892	(74.6)

[1]Purchase from herds with a positive *Salmonella* Dublin herd status in the previous quarter

Table 2. Descriptive statistics of herd size and number of dairy herds per km^2 stratified by *Salmonella* Dublin herd status for the 4th quarter of 2003 and 2005 in Denmark.

	Salmonella Dublin herd status			
	Positive		Negative	
	Median	Q_1-Q_3	Median	Q_1-Q_3
Year 2003				
Herd size	198	135-263	151	101-216
Number of herds per km^2	0.23	0.20-0.31	0.22	0.18-0.28
Year 2005				
Herd size	233	163-298	174	110-244
Number of herds per km^2	0.14	0.10-0.18	0.13	0.09-0.17

Table 3. Estimates of range of spatial correlation (km) between herds with a positive *Salmonella* Dublin herd status in the 4th quarter of 2003 and 2005 for dairy herds in Denmark. Results are based on the iterative approach.

	Range of spatial correlation (km)			
	2003		2005	
Region	Estimate	SE	Estimate	SE
Zealand	NA	-	NA	-
Funen	NA	-	NA	-
NJN	17.0	5.9	NA	-
NJS	11.5	3.1	5.2	1.6
NJW	3.4	2.4	2.7	1.6
WJ	2.3	3.2	5.3	5.2
EJ	5.7	4.7	NA	-
SJ	5.8	1.7	2.4	2.6

NA: Not analysed due to a limited sample size and/or a limited number of herds with a positive *Salmonella* Dublin herd status

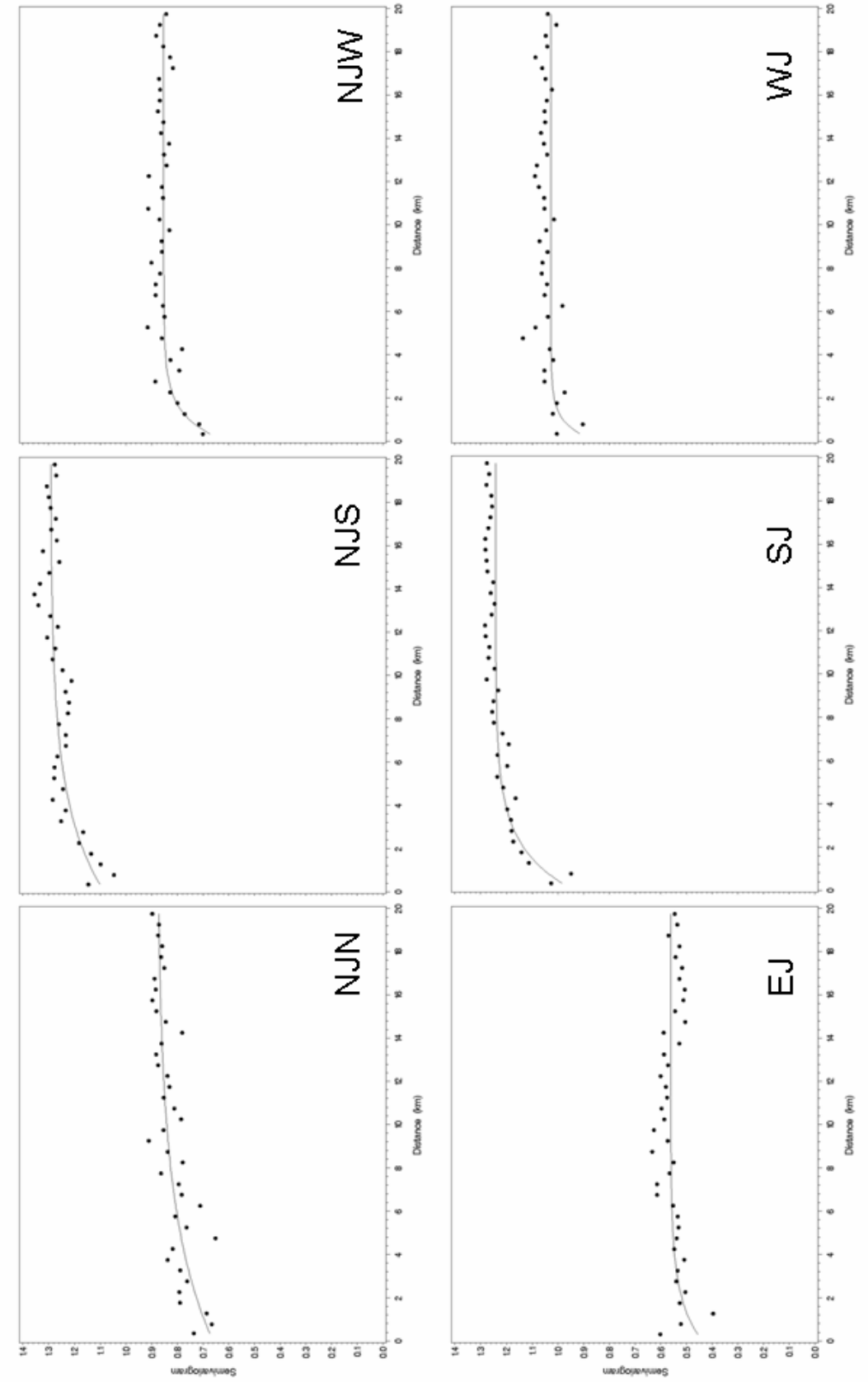

Fig. 3 Empirical (•) and fitted (—) semivariograms for the six regions in Jutland in 2003

DISCUSSION

The range of spatial correlation for *Salmonella* Dublin infection is of interest to decision makers in surveillance and control programmes and to farmers who want to improve biosecurity on the farm premises. However, the range of influence of infected herds on their neighbours is not easily determined. Other than direct spread of bacteria through moving animals, it is most likely affected by many factors not only related to distance, but also to type of landscape, roads, wildlife, climate, cattle density etc. because *Salmonella* bacteria most frequently would be transferred from one herd to another in faecal matter on animals, people, equipment, dirt, vehicles, etc. In this study, it was not possible to include such other factors, but it was attempted to estimate the range of influence of neighbouring herd status for *Salmonella* Dublin based on data from a national surveillance programme. The estimates varied between 2.3 to 17.0 km in 2003 and 2.4 to 5.3 km in 2005. The standard errors were generally high indicating that the estimates are not very precise and should be interpreted with caution. However, there was a tendency towards smaller ranges of influence in 2005 compared to 2003. In the same period, the prevalence went down from an overall prevalence of 22.1% to 17.0% test positive dairy herds in the tested regions with big differences in prevalence and change in prevalence between regions. It could be hypothesized that the range of spatial correlation could influence these changes in prevalence together with other factors. Apparently, the herd density was not associated with the range of spatial correlation. For instance, Eastern Jutland (EJ) which is a scarcely cattle populated area had approximately the same range of spatial correlation (approximately 6 km in 2003) as Southern Jutland (SJ) which is one of the most densely cattle populated areas in Denmark. The range of spatial correlation in the Northern and the Southern part of Northern Jutland (NJN and NJS) was both above 10 km. These estimates are higher than expected. For instance, Nielsen et al. (2006b) assumed a radius of 2 km to be a reasonable range within which spread between neighbours could occur.

One reason the estimates may be imprecise is that the herd status was determined using antibody measurements. This classification is not perfect. In a study by Warnick et al. (2006) it was estimated that the positive predictive value of the classification scheme used in this study was approximately 80% meaning that around 20% of those herds classified as positive would in fact not be infectious to their neighbours. The negative predictive value was approximately 99% meaning that around 1% of those herds classified as negative in this study could be infectious to their neighbours.

Another reason the estimates of the range of spatial correlation may be imprecise is the sample size (i.e. number of herds), especially the number of herds with a positive *Salmonella* Dublin herd status. When the prevalence of test positive dairy herds was below 10%, difficulties with convergence and large standard error of spatial parameter estimates occurred. More research is needed regarding the impact of sample size on …. when dealing with spatial generalised linear models.

In the present study, we have performed the analyses using the binary outcome *Salmonella* Dublin herd status based on four consecutive bulk-tank milk samples. An alternative is to use the actual *Salmonella* Dublin ELISA results in the bulk-tank milk samples. However, the ELISA results generally vary a lot between repeated samplings in the same herd. We initially performed the analysis estimating the range of spatial correlation using the ELISA results as a continuous measure (data not shown), so that the range of spatial correlation could be estimated directly using the semivariogram. However, due to normal variation in ELISA results, the spatial variation (spatial correlation) was small compared to the random non-spatial variation. The

range of spatial correlation was estimated with a large uncertainty or sometimes even impossible to estimate due to convergence problems in the analyses. Graham et al. (2005) performed spatial analyses of *Salmonella* Typhimurium ELISA signal-to-noise ratio, which is a continuous outcome of the ELISA. However, they also estimated a relatively small spatial variation. Therefore, we decided to use the classification scheme used in the surveillance programme where the ELISA results were used to classify dairy herds as *Salmonella* Dublin antibody-positive or negative.

Two different methods have been used to estimate the range of spatial correlation. Using the spatial generalised linear mixed model is attractive as the spatial parameters are estimated simultaneously with non-spatial risk factors. However, convergence problems were seen in a couple of regions. The alternative iterative approach estimates the spatial parameters based on residuals adjusted by non-spatial risk factors. This iterative approach has been described by Cressie (1991). The iterative approach is convenient as it is possible to visually inspect the semivariogram and evaluate model fit.

In conclusion, the range of spatial correlation for *Salmonella* Dublin could not be estimated with high precision based on the available data. The range of spatial correlation appears to be between 2-17 km depending on regional prevalence and probably other factors that could not be evaluated in the present study.

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SPATIAL ANALYSIS OF RESULTS FROM A NATIONAL BULK MILK SURVEY OF *NEOSPORA CANINUM* IN SWEDISH DAIRY HERDS

J. FRÖSSLING*, A. LINDBERG, A. NØDTVEDT AND C. BJÖRKMAN

SUMMARY

In this study, the national herd prevalence and spatial distribution of *Neospora caninum* infected dairy herds in Sweden were investigated. The investigation was based on a bulk milk survey comprising samples from 2978 herds. Test-positive herds were found in all parts of Sweden and the overall prevalence of test-positive herds was 8.3% (CI: 7.3-9.3). Presence of spatial autocorrelation was tested using the Moran's *I* test. Possible clusters of test-positive herds were identified by applying the LISA test statistic and the spatial scan statistic. Analysis based on data aggregated by postal code areas as well as analysis based on exact coordinates identified significant clusters of high prevalence in the middle parts of Sweden and low prevalence in the southern parts. This was not expected considering the results from other European studies of *N. caninum* in cattle. However, the findings are supported by the distribution of previously known case herds.

INTRODUCTION

The protozoan parasite *Neospora caninum* is of significance mainly because of its ability to cause abortion and persistent infection in cattle. Although the full nature of the parasite-host-interaction is still unclear, it is known that infection may be vertical, i.e. by transmission of tachyzoites from cow to offspring during pregnancy, or horizontal, by ingestion of oocysts shed by a main host (Dubey, 2003). So far, identified main hosts are dog and coyote (McAllister et al., 1998; Gondim et al., 2004).

Presence of *N. caninum* has been demonstrated in cattle populations worldwide but the estimated prevalence of cattle infected with *N. caninum* varies considerably between herds, regions and countries. In a European study, the herd prevalence in the south and middle of Sweden was estimated as 16%, whereas 49%, 63% and 76% of investigated dairy herds in Germany, Spain and The Netherlands had at least one seropositive cow (Bartels et al., 2006). The reason for the regional differences is only partially known, but dog density and climatic factors have been suggested as explanations (Schares et al., 2003; Rinaldi et al., 2005). Presence of coyotes has also been found to be a risk factor (Barling et al., 2000). In Sweden, serological surveys have shown that the prevalence of *N. caninum* is 0.5% in dogs (Björkman et al., 1994) and 2% in individual dairy cattle (Björkman et al., 2000). From bulk milk screenings of herds in the south and middle of Sweden the herd prevalence in this region has been estimated to approximately 20% (unpublished data).

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The aim of this study was to estimate the national prevalence and the general spatial distribution of *N. caninum* infected Swedish dairy herds based on bulk milk testing. This was the first *N. caninum* survey of its kind in Sweden. Therefore, possible geographical patterns, as well as the presence of specific infection clusters, were of interest. The geographical representativeness of the study sample was also subject to investigation, as this was considered essential for the interpretation of results.

MATERIALS AND METHODS

Study sample

Samples were collected in 2002 within the mandatory control/surveillance programmes for enzootic bovine leucosis and infectious bovine rhinotracheitis. In total, the study sample was a selection of bulk milk samples (n=2978) corresponding to approximately 30% of all dairy herds present in the country this year. Samples were selected at the laboratory by convenience sampling, i.e. not through a strictly random procedure, and not under control of the investigators.

Diagnostic test

An iscom ELISA was used to demonstrate presence of specific antibodies to *N. caninum* (Björkman et al., 1997; Frössling et al., 2003). When applied to bulk milk samples from Swedish dairy herds, this test has been estimated to have a diagnostic sensitivity of ~50% (CI: 21-79) and a specificity of ~81% (CI: 72-89) (Frössling et al., 2006). These estimates refer to the ability to detect 2 or more test-positive cows within a herd.

Location data

The locations of herds in the study sample and all dairy herds in Sweden were specified by three digit postal codes (n=276) and exact coordinates. Postal codes and coordinates were retrieved from the databases of the Swedish Dairy Association (study sample: 2002, background population: 2006) and the Swedish Board of Agriculture (2006), respectively. The geographical distribution of herds and possible clustering were investigated by utilizing both data aggregated by postal code area and point data. Applicable postal codes and coordinates were available for 2944 and 2580 of the 2978 herds in the study sample, respectively. In the background population of 2006, postal codes were available for all herds while exact coordinates were available for 7361 out of 8288 herds. The herd density of the background population per postal code area is presented in Fig. 1.

Spatial analysis

The *N. caninum* prevalence was defined as the proportion of test-positive herds of the total number of sampled herds and was calculated for each postal code area. Due to differences in precision in estimates for each area, the prevalences were also adjusted by empirical Bayes smoothing, i.e. adjusted towards the overall mean. By applying a first-order contiguity rook spatial weight, estimates were also adjusted considering the estimates from neighbouring areas (Bailey & Gatrell, 1995). Presence of spatial autocorrelation was tested using the Moran's *I* test for global spatial autocorrelation using the same contiguity weight matrix as above (Assuncao & Reis, 1999). To minimize the effect of missing data and prevent "islands" in the maps, postal code areas were first transformed into Thiessen polygons. For presentation purposes, outcomes

were later converted back to normal format. In order to classify areas by type of possible spatial correlation, a local indicator of spatial association (LISA) test statistic was also applied (Anselin, 1995). Considering the variance instability due to difference in sample size between areas, the calculations were based on the empirical Bayes method. The number of permutations used was 9999. All smoothing and testing for spatial associations of area-aggregated data was performed using the GeoDa software version 0.9.5-i5 (https://geoda.uiuc.edu/).

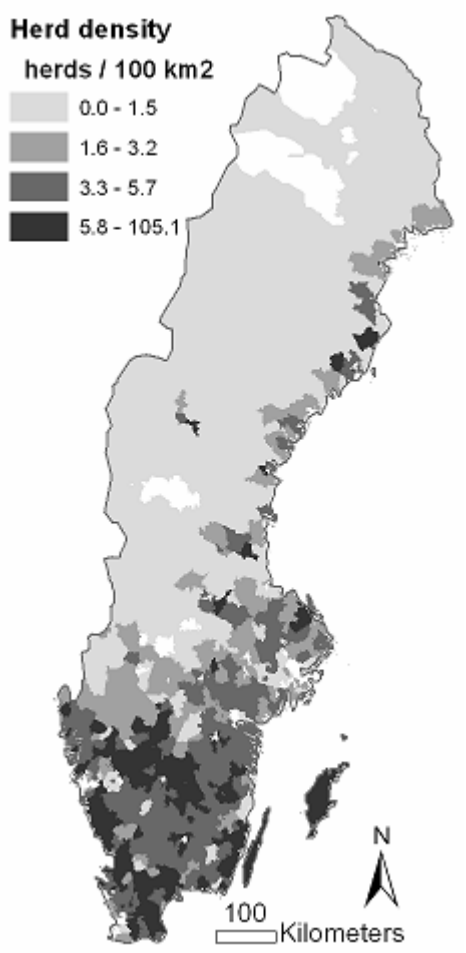

Fig. 1 The population of Swedish dairy herds presented as density by postal code area (2006)

Clustering of test-positive herds was also investigated based on location determined by exact coordinates and using the spatial scan statistic (Kulldorff, M. and Information Management Services, Inc. SaTScan™ version 7.0, www.satscan.org, 2006). The testing was performed using a Bernoulli model (Kulldorff, 1997) where the test-positive herds were considered cases and test-negative herds were considered controls. The maximum cluster sizes tested were 50%, 20% and 10% of the population at risk and both circular and elliptic cluster shapes were applied. Significance of clusters was tested using Monte Carlo hypothesis testing (9999 permutations).

The geographical representativeness of the study sample was investigated by calculating the number of sampled herds compared to the total number of dairy herds present in each postal code area. Presence of spatial associations was tested using GeoDa as described above.

Data management, basic statistics and creation of map shape-files was performed using Stata Statistical Software release 9.2 (Stata Corp., College Station, TX) and ArcView version 9.1 (ESRI Inc., Redlands, California, USA).

RESULTS

Herds that tested positive for *N. caninum* (n=247) were found in both the southern, middle and northern parts of the country. However, differences in the distribution of positive herds were apparent. The overall prevalence was 8.3% (CI: 7.3-9.3), but a slightly higher prevalence was predominately found in the middle parts of Sweden (Fig. 2). This tendency was more obvious when prevalence estimates were adjusted by empirical Bayes smoothing, especially when the spatial weight matrix was applied.

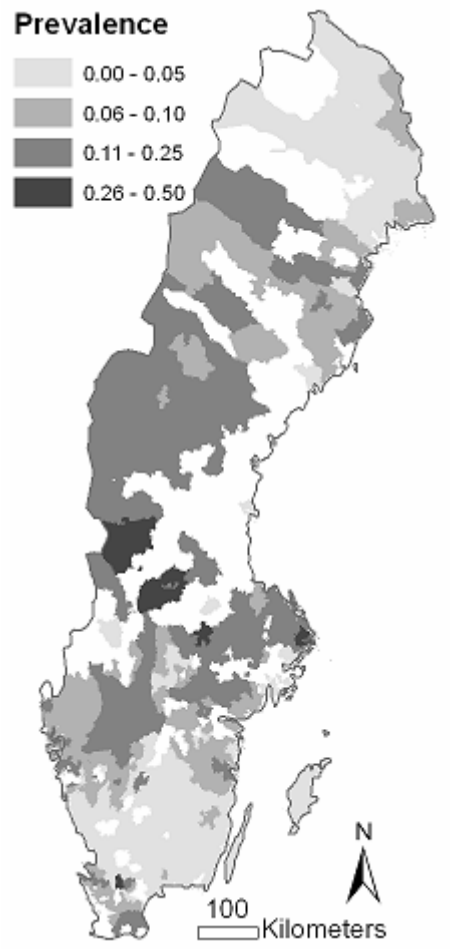

Fig. 2 The prevalence of *Neospora caninum* positive dairy herds in Sweden by postal code area (N=2944, n=247, 2002). Estimates are adjusted by empirical Bayes smoothing applying a spatial weight matrix. White areas are areas where information is missing.

The Moran's *I* test indicated that spatial autocorrelation was present (I=0.12, p=0.004) and local spatial correlation tests based on both postal code data and coordinate data identified a high prevalence cluster in the middle of Sweden and a low prevalence cluster in the south of Sweden. However, when applying the LISA test, not all high or low prevalence areas were

significant at the p=0.01 level (Fig. 3a). One positive and one negative significant cluster were found in most analysis runs when using the spatial scan statistic. Although size, shape and exact location of clusters differed depending on settings, all positive clusters were located in the middle parts of Sweden while all negative clusters were located in the south. The setting with elliptic clusters and a maximum cluster size of 20% of the population at risk yielded the most informative outcome, which is presented in Fig. 3b. The identified low-risk cluster in the south included 486 herds and had 12 observed cases versus 42 expected (relative risk=0.25, p=0.0002) while the high-risk cluster in the middle of Sweden included 490 herds and had 80 observed cases versus 42 expected (relative risk=2.43, p=0.0002).

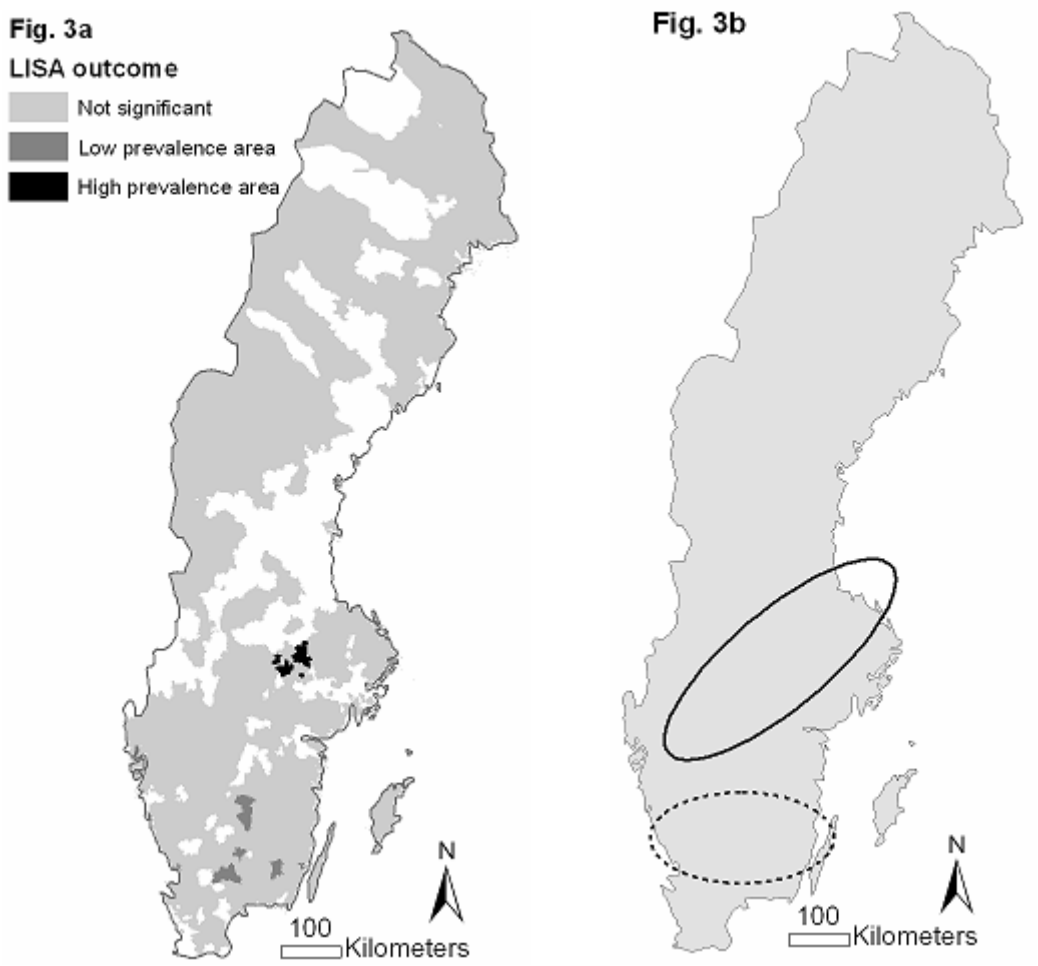

Fig. 3a Postal code areas in Sweden identified by a local indicator of spatial association (LISA) test statistic as clusters of high and low prevalence (significance level p=0.01) of *Neospora caninum* test-positive dairy herds (2002). Areas were transformed into Thiessen polygons before analysis and then converted back to normal format for presentation purposes. White areas are areas where information is missing.

Fig. 3b Clusters with a significantly higher (solid line) or lower prevalence (dashed line) of *Neospora caninum* test-positive dairy herds than expected (Sweden, 2002). Cluster analysis was based on exact coordinates and performed using the spatial scan statistic with a maximum cluster size of 20% of the population at risk.

Some postal code areas (n=125, 31%) were missing in the selected sample of bulk milk samples. In particular, the middle east areas and some southern areas were underrepresented in this material. On the other hand, a very high proportion of herds was sampled in the very north and in the western coastal areas of the south-middle of Sweden (Fig. 4). These differences in sampling density were significant when investigated using cluster analysis (p<0.01 in the LISA test statistic).

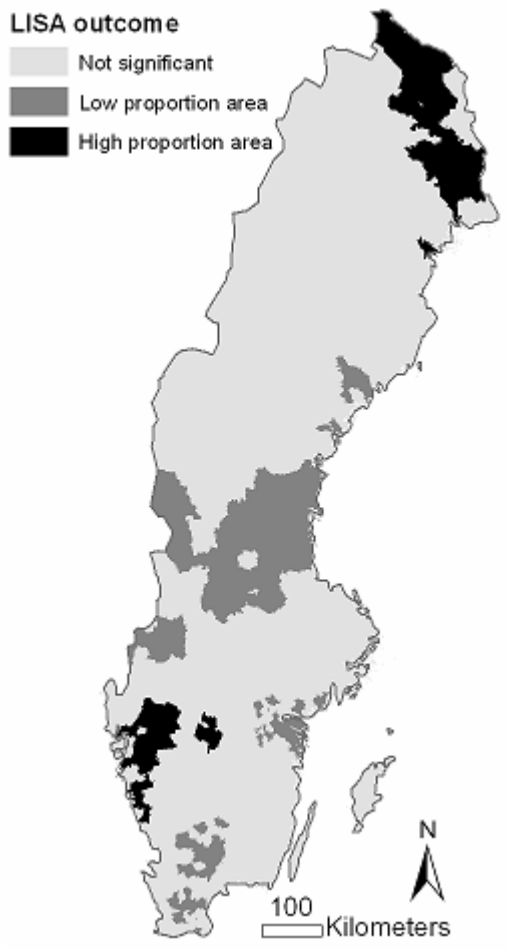

Fig. 4 Regions that were underrepresented or overrepresented in the present study sample of dairy herds that was used to investigate the spatial distribution of *Neospora caninum* test-positive herds in Sweden. The study sample (n=2944) was selected in 2002 and was compared to the background population of dairy herds in Sweden 2006 (n=8288). Geographical representativeness was investigated by calculating a local indicator of spatial association (LISA) test statistic, significance level p=0.01

DISCUSSION

In this study, both visual inspection and exploratory spatial analyses of data indicate that there is a higher prevalence of *N. caninum* infected herds in the middle parts and a lower prevalence in the south of Sweden. The overall prevalence of infected herds was lower than previous estimates, which on the other hand were limited to the south middle regions. Because the previous estimate of diagnostic sensitivity of the test used was based on a small number of

herds (and the confidence interval consequently was very wide) it is difficult to estimate the true prevalence of infection. Still, it is likely that the proportion of herds with *N. caninum* infected individuals is higher than the estimated 8.3%.

Although the geographical distribution of test positive herds roughly coincides with the distribution of known case herds (data not shown), it was not expected that the south would have a significantly lower prevalence of *N. caninum*. Other studies have shown that, with few exceptions, the prevalence of *N. caninum* is higher in the southern parts of Europe compared to the northern parts (Hemphill & Gottstein, 2000; Bartels et al., 2006). There are also indications that within regions, prevalence is higher in areas with higher temperatures in spring and summer (Schares et al., 2003; Rinaldi et al., 2005). In addition, herd sizes are larger and the herd density is higher in the south of Sweden. These are demographic features that are often considered risk factors for a higher prevalence/incidence of infectious diseases.

The small number of studies that have investigated possible effects on the spatial distribution of *N. caninum* infection in cattle have shown a significant effect of dog density and vegetation profile. Further analyses of the presented data to investigate the effect of such covariates are underway. One factor of special interest is the distribution of the Scandinavian wolf population. Although few in numbers, breeding pairs of wolves are reported to be located in the middle parts of Sweden, which in this material is identified as a high *N. caninum* prevalence area.

A national survey to investigate the prevalence of *N. caninum* infected beef cattle in Sweden has recently been started. A comparison of the spatial distribution of infected beef herds and the results from the present study will be of interest when interpreting the effects of environmental factors on prevalence. In the case of bovine *N. caninum* infection in Swedish cattle, vertical transmission is considered to be the most important route of infection and in other countries live animal trade is the most significant factor in the between-herd spread of the infection. However, live animal trade in Sweden is limited in comparison with other European countries. In particular, trade between dairy and beef herds is not common. If spatial distributions of infected herds are similar for the dairy and the beef cattle populations, this would indicate the presence of common, perhaps environmental, risk factors of importance.

The date of origin differed for some of the information used in this study. As *N. caninum* causes a chronic infection which, by nature, spreads relatively slowly, these differences are probably of minor significance. It could be expected that more of the herds in the study sample would have been present in the data if an earlier version of the databases could have been obtained. On the other hand, only few years ago coordinate locations were only available for a limited number of farms.

Possible edge effects were not considered in this analysis. However, since the major parts of Sweden's borders are either coastline or very sparsely-populated mountain areas, it is likely that outcomes from the cluster analyses are not influenced at all by edge effects and that estimates from spatial smoothing were only affected to a limited extent.

One objective of this study was to investigate the geographical representativeness of the study sample. It was found that, compared to the background population, a disproportionately large number of samples had been selected from the very north and middle west parts of the country. On the other hand, many postal code areas in the middle east areas, where a large number of dairy herds are located, were not at all represented in the study sample. Also, too few

samples had been selected from areas in the south. This adds uncertainty to the interpretation of some of the results. Closeness to the high prevalence middle area could indicate that the middle east region also has a relatively high number of infected herds. This region was indeed included in, or was close to, some of the significant clusters of cases identified by cluster analysis based on exact coordinates. There is a fairly large proportion of previously known case herds in this area, which supports the credibility of this finding.

Although data was missing for some regions, it can be concluded that there are geographical differences in the distribution of *N. caninum* infected dairy herds in Sweden and that the overall tendency is that prevalence is higher in the middle parts of the country and lower in the southern parts. This information adds to the current knowledge of *N. caninum* infection in Swedish cattle and can be used to inform future epidemiological studies of this parasite.

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FIELD EPIDEMIOLOGY

QUANTIFIED ESTIMATES OF RISK FACTORS FOR POST-WEANING MORTALITY OF WEANER MERINO SHEEP IN SOUTHEASTERN AUSTRALIA

A.J.D. CAMPBELL*, A.L. VIZARD AND J.W.A. LARSEN

SUMMARY

Death of Merino sheep in the first year after weaning is a major, largely overlooked production and welfare problem in the Australian wool industry. Current weaner management recommendations are vague and do not quantify expected changes in mortality risk.

Uni- and multivariate analyses of the survival records of spring-born Merino sheep between weaning and ~13 months old from nine Merino flocks in southeastern Australia from 1996–2002 (n = 3655) were performed. Bodyweight at weaning, average seasonal growth rate and month of shearing were associated with mortality (all $P < 0.05$). Females had a smaller mortality risk than males. Shearing in March, May or June, but not December or July, increased the risk of death two- to four-fold, compared to unshorn weaners ($P < 0.01$).

The strong association between growth rate and mortality suggests that modest supplementary feeding at critical times of the year, particularly if it targeted the lightweight weaners in a flock, could efficiently and cost-effectively improve survival, regardless of the cause of death.

INTRODUCTION

Despite few direct investigations of its extent or prevalence, considerable mortality amongst Merino sheep in the first year after weaning ('weaners') commonly occurs, constituting a widespread problem on Australian woolgrowing farms. Deaths of 10–25%, or more, have been recorded in flocks managed under commercial conditions in a wide variety of environments in Western Australia, South Australia, Victoria and Queensland (McDonald, 1975; Mulholland et al., 1986; Barton & McCausland, 1987; Cobon et al., 1990; Allworth, 1994; Harris & Nowara, 1995; Fogarty et al., 2005). These deaths occur in apparently healthy weaner flocks and are not restricted to particular weaning ages or seasonal conditions. Excessive weaner mortality was recently estimated to cost the industry about $AUD100 million annually (Sackett, 2006) and, by any standard, represents an unacceptable animal welfare practice in the production of Australian wool. This study presents quantified estimates of risk factors for weaner mortality from analyses of several years' survival records from Merino weaner flocks in the Western District of Victoria.

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MATERIALS AND METHODS

Data from two field trials spanning 1996 to 2002, inclusive, were analysed. One trial was a central progeny test, managed under guidelines provided by the Australian Merino Sire Evaluation Association (Casey et al., 1995) and the other compared the effect of different shearing times on wool and livestock production of weaners and adult ewes. All of the weaners in both trials were born on the one farm, which was 15 km west of Geelong, Victoria, but the last cohort of weaners in the shearing trial was moved to another farm, 20 km to the northwest, when they were five months old. The average annual rainfall of both farms was 520 mm. All weaners within each trial were managed similarly, apart from shearing time in the second trial. According to commercial best practice for the district, lambing started in September (spring) and continued for five weeks. Marking and mulesing was performed seven weeks after the start of lambing, when they were also tagged with individually numbered eartags replicated in each ear. Lambs were weaned 12–13 weeks after the start of lambing.

Weaner deaths occurring during the post-weaning period ('PWP', between weaning and 12–15 months of age) were analysed. A weaner was recorded as having died if it were found dead, or remained missing at two or more consecutive musters and was not found elsewhere on the farm. Weaners were weighed at weaning and then regularly throughout the PWP.

Mortality rates ('MR', Eq. 1) during the PWP were calculated for weaners in each birth-year cohort, assuming that deaths occurred at the midpoint of the period between observations (Rothman, 1986). Mortality rate ratios between groups of weaners in a cohort (e.g. bodyweight quintiles or sex) were calculated ('MRR', Eq. 2) and were combined across cohorts using the Mantel-Haenszel method (Rothman, 1986). The attributable fraction ('AF', Eq. 3) and population attributable fraction ('PAF', Eq. 4) of deaths for weaners in a group were calculated (Rothman, 1986), and a multivariate Cox survival analysis was also performed, specifying clustering at the cohort level (Hosmer & Lemeshow, 1999).

$$\text{MR} = \text{number of deaths} \div \text{total number of sheep-days at risk} \quad (1)$$

$$\text{MRR} = \text{MR}_{\text{group}} \div \text{MR}_{\text{reference group}} \quad (2)$$

$$\text{AF} = (\text{MR}_{\text{group}} - \text{MR}_{\text{not in group}}) \div \text{MR}_{\text{group}} \quad (3)$$

$$\text{PAF} = (\text{MR}_{\text{all weaners}} - \text{MR}_{\text{not in group}}) \div \text{MR}_{\text{all weaners}} \quad (4)$$

The effect of different shearing times on weaner mortality was only analysed in the data from the shearing time trial (n = 2036). In that trial, lambs were allocated at weaning to six different shearing times, namely December (at weaning), March, May, June, July or unshorn throughout the PWP. They all grazed the same paddock and were otherwise managed identically.

RESULTS

The median length of the PWP was 332 (range 288–371) days, with a median of 96 (31–238) days between observations. In total, records from 3655 weaners in nine cohorts over seven

years were examined. Overall, 520 (14.2%) weaners died, representing a mortality rate of 15 deaths/1000 weaners/month. The smallest cohort mortality was 4.5% and the greatest was 26.8%. The death rate in all but one of the cohorts was greatest early in the PWP and decreased with time from weaning (Fig. 1).

Univariate analyses

The average weaning weight of the cohorts was between 15 and 19 kg and the lightest weaners in a cohort were approximately 8 kg lighter than the average in all cohorts. When mortality rate ratios were combined across cohorts, weaners in the lightest and second-lightest weaning weight quintiles in any year experienced 3.5 (95% CI 2.9–4.1) and 1.5 (1.1–2.0) times the risk of death, respectively, of weaners in the middle weight quintile (Table 1). Weaners in the middle and heavier weight quintiles all had statistically similar risks of death. These relative risks were the same across cohorts and were independent of the lambs' actual weaning weights. The attributable fraction of mortality amongst weaners in the lightest weight quintile was 0.71 (0.65–0.76) and the population attributable fraction of this weight group was 0.31. Male weaners experienced 1.4 (1.1–1.6) times the risk of death of females. Shearing in March or May increased mortality risk by 1.6 (1.0–2.6) and 3.2 (1.3–7.6) times, respectively, compared to weaners not shorn in those months but shearing in December, June or July did not cause a statistically significant increase in risk of death.

Multivariate analyses

The changes in risk associated with weaning weight, growth rate, sex and shearing time from the multivariate analysis are shown in Table 2. Increasing weaning weight by 4 kg reduced risk of death throughout the PWP by about one to two thirds, depending on absolute bodyweight, with the biggest effect at light weights. Increasing average growth rate during the five months after weaning (i.e., summer–autumn) substantially reduced mortality, particularly at low growth rates. Avoiding shearing in March, May or June reduced risk of death at these times by approximately 75%, although mortality risk following shearing in March was not reduced if summer–autumn growth rates were small.

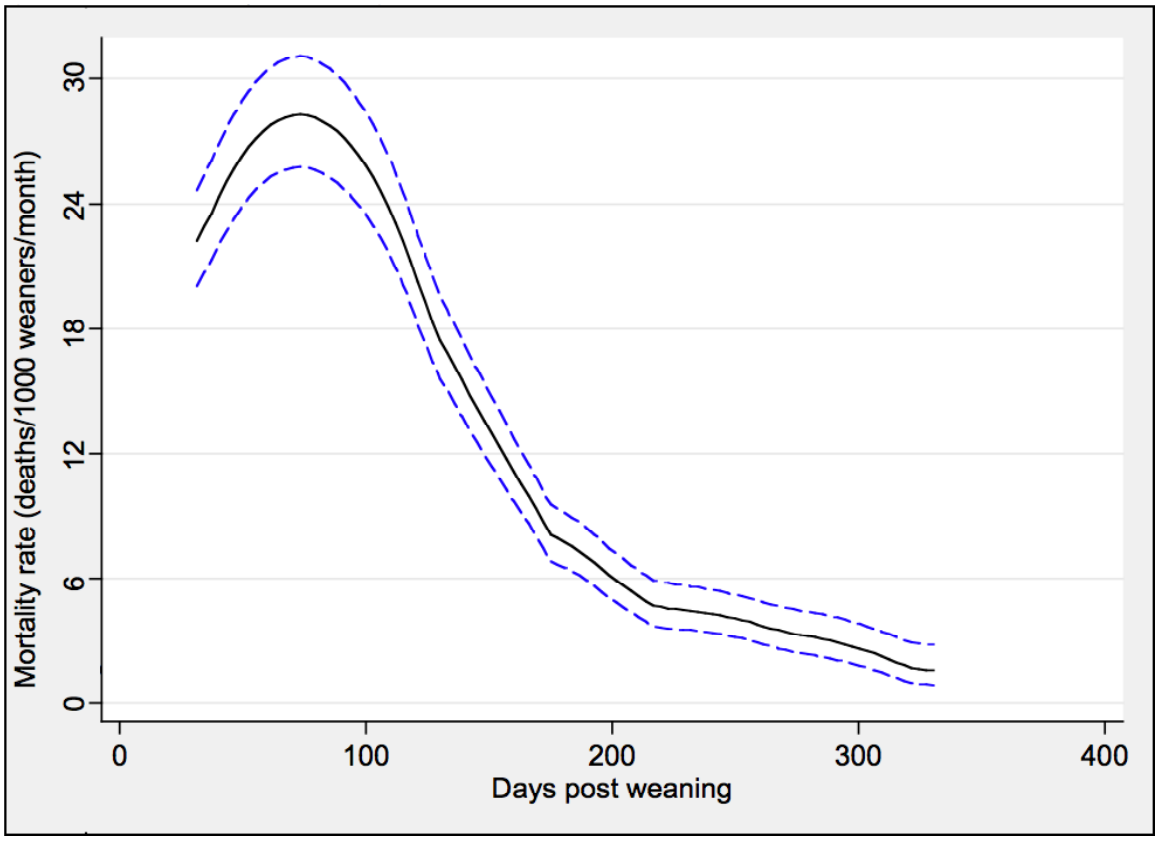

Fig. 1 Smoothed graph of weaner mortality rate during the post-weaning period, combined over all birth-year cohorts (dashed line indicates 95% confidence interval)

Table 1. Total mortality, mortality rate (deaths/1000 weaners/month), and mortality rate ratio, relative to middle quintile, of weaners in each weaning weight quintile throughout the PWP (N.B. weaning weight not recorded for 16 weaners)

Weaning Weight Quintile	Number		% Mortality	Mortality rate (95% CI)	Mortality rate ratio (95% CI)
	Died	Total			
1	224	758	29.6	35.7 (31.3–40.7)	3.5 (2.7–4.6)
2	109	738	14.8	15.6 (12.9–18.8)	1.5 (1.1–2.0)
3	79	723	10.9	11.1 (8.9–13.8)	Ref
4	57	744	7.7	7.6 (5.9–9.9)	0.7 (0.5–1.0)
5	50	676	7.4	7.2 (5.5–9.6)	0.7 (0.5–1.0)
Total	519	3639	14.3	14.9 (13.7–16.2)	

Table 2. Decreases in mortality risk associated with changes in weaning weight, growth rate, sex and shearing time

Change in Parameter	Risk Decrease
Weaning weight (kg)	**[a]
8→12	66%
12→16	53%
16→20	45%
20→24	38%
Growth rate over summer–autumn (kg/month)	**
0.25→0.5	74%
0.5→0.75	36%
0.75→1.0	20%
1.0→1.25	13%
1.25→1.5	9%
Growth rate over July–October +0.5 kg/month, if weaning weight (kg) was:	*
8	62%
12	54%
16	48%
20	43%
Females vs. males	38% **
Unshorn vs. shorn in:	
March[b]	48–76% **
May	74% **
June	77% **

[a] **: $P < 0.01$; * $P < 0.05$

[b] Smaller decrease at lower growth rates

DISCUSSION

In southern Australia, spring is the preferred lambing time in woolgrowing enterprises because it matches the annual pattern of pasture production with sheep nutritional requirements, permitting more sheep to be carried on the farm and optimising farm profitability (Foot & Vizard, 1993). Despite its advantages, a consequence of spring lambing is that weaners frequently graze poor quality, dry pastures during summer and early autumn that often provide insufficient protein or energy to meet the growth requirements of young sheep (Allden, 1982). In the current study, significant mortality occurred amongst weaner sheep that were run under commercial conditions on what was considered a well-managed, highly profitable woolgrowing farm. The average mortality throughout the PWP was 14.2%: about one death per seven weaned lambs. Total deaths approached the industry target of <4% prior to one year of age (Behrendt, 2003) in only one cohort; in all other cohorts mortality was at least twice the industry target, and

in three cohorts deaths exceeded 20%. As discussed above, although few studies have directly investigated Merino weaner mortality in Australia, the death rates reported here are probably representative of conditions on many farms.

The very strong associations found between bodyweight and mortality identify powerful ways in which the problem of weaner mortality on Australian sheep farms could be addressed. In particular, the results suggest that small increases in post-weaning growth rate could substantially reduce deaths. Furthermore, the greater risk experienced by lightweight weaners shows that the lightest sheep, or 'tail', of a flock should receive particular attention if death rates are to be lowered, as discussed in more detail below.

Although associations between bodyweight and mortality have been previously observed (Lloyd Davies et al., 1988; Hodge, 1990) and form the basis of existing weaner management recommendations, the results of this study quantify these associations more precisely. Small increases in weaning weight had more effect on the survival of lightweight weaners than heavy ones. For example, a weaner weighing 14 kg experienced a 34% (95% CI 30–37%) smaller mortality risk than a 12 kg weaner but a weaner weighing 20 kg only experienced a 22% (20–25%) smaller risk than one weighing 18 kg. The average cohort growth rate at different times during the PWP is a precise and convenient 'summary' measure of the nutrition experienced by a weaner flock, and the average growth rate over summer and autumn (early in the PWP) was, in particular, even more strongly associated with mortality than weaning weight. For example, increasing a weaner's average growth rate from 0.25 to 0.5 kg/month during the five months after weaning was estimated to reduce risk by 74% (72–76%), equivalent to increasing bodyweight at weaning from 12 kg to 19.5 kg.

The study's design meant that causal relationships between weaner bodyweight, growth rate and mortality risk could not be demonstrated. However, the results agree with previous research showing that increasing bodyweight reduces a young sheep's risk of death (Allden, 1968; Hodge, 1990). Heavier or faster growing weaners accumulate body fat that can be catabolized, if required, without threatening the weaner's survival (Doyle & Egan, 1983). The current results are remarkably similar to associations between bodyweight and survival reported in overseas studies of small ruminant production systems (Mandonnet et al., 2003; Nguti et al., 2003). It is therefore likely that they could be broadly applied to sheep production systems in a range of Australian environments.

The substantial associations between growth rate and mortality suggest that modest supplementary feeding could efficiently, and cost-effectively, address the widespread problem of weaner mortality in Australia. This strategy is particularly attractive because the effects on mortality were independent of the cause of death. A general mechanism that reduces death would be an appropriate way of dealing with a syndrome such as weaner ill-thrift, which has no single, easily treatable cause (Gordon, 1981). Although weaning weight must be indirectly, and inefficiently, manipulated via ewe nutrition, growth rates in the PWP can be directly manipulated through supplementary feeding. For example, the 74% decrease in mortality risk described previously, associated with weaners growing at 0.5 kg/month instead of 0.25 kg/month, could be achieved by providing 2.5 kg/head/month of supplementary oats and lupins, costing just $AUD0.40/head/month. Such a strategy would be highly cost-effective, particularly if feeding were directed to the lightweight weaners, which are at greater risk of death. Increasing growth rate is also a mechanism for responding to increased mortality risk that is anticipated because of low weaning weights or has been detected through ongoing monitoring of deaths.

The greater mortality risk amongst lightweight weaners, irrespective of absolute weight, reinforces the importance of drafting off a 'tail' of lightweight sheep for closer attention. Currently, this is often performed some time over summer, but the persistent association between weaning weight and mortality suggests that it should be done at weaning. The attributable fraction for weaners in the lightest weaning weight quintile was 0.71, suggesting that 71% of the deaths amongst the lightest fifth of weaners could be prevented if their bodyweight were increased. Doing so could eliminate 31% of deaths in the entire population; in other words, targeted feeding of just one fifth of the flock could address nearly one third of all mortalities.

There was no difference between mortality risk of shorn sheep after shearing and unshorn sheep when shearing occurred at weaning (in December) or in July (at 10 months of age). However, across all cohorts, shearing weaners in March, May or June (i.e., autumn in the southern hemisphere) increased their risk of death approximately four-fold compared to weaners not shorn at those times. Similar results have been observed in adult sheep (Hutchinson & McRae, 1969). The underlying weaner death rate was still high in March but decreased relatively quickly thereafter with the commencement of the season's opening rains (Fig. 1). Thus, a four-fold increase in risk had the greatest effect on absolute numbers of deaths following March shearing. It was estimated that March shearing caused an additional 62 deaths/1000 weaners/month, compared to an extra 30 deaths/1000 weaners/month following May shearing.

In southern Australia, shearing in autumn coincides with cooling temperatures and comes when weaner sheep have just faced several months of inadequate nutrition on dry summer pastures, leaving them with little or no body fat (Allden, 1982). Sheep respond to cold stress immediately after shearing by mobilising body energy stores and may die from adrenal insufficiency if they cannot do so (Hutchinson & McRae, 1969; Donnelly, 1974). Thus, shearing weaners in autumn increases the risk of death off shears because it coincides with the time when they are least able to metabolically respond to its effects. Avoiding shearing in autumn should therefore be considered as part of a strategy for reducing weaner mortalities.

The risk of death of males was estimated to be 1.6 times greater than females. Similar results have been reported in other survival analyses of weaner sheep and goats (Mandonnet et al., 2003; Nguti et al., 2003). This association may be related to sex differences in susceptibility to gastrointestinal parasitism (Barger, 1993), body composition or management, but more investigation of this widely reported phenomenon is warranted.

Although observation of all cohorts commenced at weaning at about the same time each year, the cohorts were not observed for identical lengths of time. However, the large majority of mortalities occurred early in the PWP, when all cohorts were under observation, and the time of right-censoring of survivors (i.e., the end of a cohort's observation time) was independent of the likelihood of death. The different durations of observation of cohorts was accounted for in the multivariate survival analysis using right-censoring, and the similar results obtained from the univariate and multivariate analyses suggest that bias did not result from the unequal cohort observation periods.

This study has quantified, for the first time in an Australian sheep production system, relationships between mortality and bodyweight, growth rate, shearing time and sex. It has added further weight to the evidence that weaner sheep mortality on Australian farms is often unacceptably high. However it has also identified that manipulating flock growth rates,

especially early in the PWP, may be a highly efficient and cost-effective method of addressing this important problem.

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CAMPYLOBACTER SPP. IN COMMERCIAL BROILER FLOCKS: EPIDEMIOLOGY AND RISK FACTORS

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SUMMARY

The study described here was undertaken to investigate the epidemiology and risk factors for *Campylobacter* spp. in commercial broiler flocks in Northern Ireland. Samples, which consisted of 14 cloacal swabs per flock, were collected prior to the first removal of birds to slaughter on 88 farms over five consecutive production cycles. Overall, *Campylobacter* spp. were isolated from 163 of the 388 flocks sampled (42.0%; 95% CI 35.1% - 48.9%). Random-effects logistic regression modelling was used to investigate the association between farm parameters and the *Campylobacter* status of flocks. Factors identified as being significant included the age of the birds at sampling, the presence of rodents on farms, the season, the number of houses on site, the frequency of footbath disinfectant changes and the tidiness / cleanliness of the broiler house ante-room. In addition, there was weak evidence for non-mains water as a source of infection. There was no significant evidence of direct carry-over of infection from one production cycle to the next, neither was there evidence of other farm species acting as a source of infection.

INTRODUCTION

Over the last two decades, *Campylobacter* has emerged as the most commonly reported cause of bacterial enteritis in humans in the UK and most other developed countries (Anon., 2002; Anon., 2006). In the majority of affected individuals, clinical signs are those of an acute diarrhoeal illness, although more severe complications including Guillain-Barré syndrome, reactive arthritis and a range of extraintestinal infections occur in a small percentage of cases (Skirrow & Blaser, 2000). Although the exact epidemiology of human *Campylobacter* infection remains uncertain, poultry meat is a frequently implicated source (Anon., 2005; Wingstrand et al., 2006) and work to reduce the prevalence of infected poultry flocks is recognised as a priority in relation to reducing the occurrence of foodborne infection.

Vertical transmission has been suggested as a possible route of infection for poultry flocks (Cox et al., 2002), but the majority of studies would indicate this to be unlikely or of minor importance (Shanker et al., 1986; Jacobs-Reitsma, 1995; Sahin et al., 2003). Potential horizontal routes of infection include carry-over from previous crops; entry in feed, litter or water supplies; vermin or insect sources; and the carriage on equipment or personnel entering the poultry house (Newell & Fearnley, 2003). Amongst these, both feed (Berndtson et al., 1996; van de Giessen et

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al., 1996) and fresh litter (Newell & Fearnley, 2003) are regarded as unlikely sources. The normal cleansing and disinfection processes carried out in houses between production cycles, and especially those designed to remove *Salmonella,* are also likely to eliminate carry-over of infection from one flock to the next (Anon., 2005). Much of the focus in *Campylobacter* studies to date has therefore been on the potential carriage of infection into the broiler house on equipment, or on the boots and clothes of staff looking after the birds, although other possibilities include water (Pearson et al., 1993, Kapperud et al., 1993) and the entry of insects, such as house flies (Rosef & Kapperud, 1983, Shane et al., 1985; Hald et al., 2004). Studies have also demonstrated the isolation of *Campylobacter* from transport crates (Slader et al., 2002; Hansson et al., 2005), transport modules and other equipment (McKenna et al., 2001) and the entry of catching teams and equipment during the process of partial depopulation is an obvious potential source of infection for birds remaining in the house.

Although these potential sources of *Campylobacter* infection have been identified, the relative importance of each, the exact routes of transmission, and the appropriate preventative measures are less certain. Factors identified in previous epidemiological and field studies include increasing prevalence of infection with flock age (Evans & Sayers, 2000; Bouwknegt et al., 2004), use of undisinfected water (Kapperud et al., 1993), rats (Kapperud et al., 1993), sites with multiple houses (Refregier-Petton et al., 2001; Bouwknegt et al., 2004) and presence of or working with other farm animals (Kapperud et al., 1993; van de Giessen et al., 1996; Hald et al., 2000; Bouwknegt et al., 2004; Cardinale et al., 2004). Seasonal effects, which generally have been an increased risk in summer and autumn compared to winter and spring, have also been reported (Kapperud et al., 1993; Wedderkopp et al., 2000; Refregier-Petton et al., 2001; Bouwknegt et al., 2004). Most studies have reported associations with a range of hygiene parameters. These have included a reduced risk when staff entering the poultry house follow biosecurity practices such as using disinfectant foot dips, washing hands before tending the flock, and changing boots and/or clothing (Humphrey et al., 1993; van de Giessen et al., 1996; Evans & Sayers 2000). Reduced risks have also been reported when buildings were maintained in a good state of repair and drinking water equipment cleaned and disinfected to a high standard (Evans & Sayers 2000). The process of partial depopulation has been linked to the introduction of infection in some studies (Hald et al., 2000, 2001), although a further study which considered age and season as potential confounders, failed to show any increased risk associated with the process (Russa et al., 2005).

While, as indicated above, quantitative epidemiological studies have been undertaken in other countries, risk factors for *Campylobacter* infection are likely to vary depending on local industry and farming practices as well as for wider geographically and climatic reasons. Few studies have also fully excluded the potential effect of partial depopulation by examining risk factors in the period up to the first removal of birds from a house. The study described here was undertaken as part of a wider project investigating the epidemiology and control of *Campylobacter* in poultry in Northern Ireland and describes the risk factors and sequential patterns of infection in a cohort of broiler farms in the time period to first depopulation.

MATERIALS AND METHODS

Study population

Broiler farms selected for the study were drawn from the population of farms supplying the three major poultry processing companies in Northern Ireland, which together accounted for over 90% of commercial broiler farms in the province. Eighty-eight farms were recruited into the study, based on a stratified cohort of farms that had been selected for a previous study carried out at the beginning of 2001 (Menzies et al., 2003). On farms with multiple broiler houses, one house was randomly selected for inclusion in the study. Farms with non-conventional broiler flocks, such as organic and free-range flocks were excluded.

Sample collection and submission

Samples were collected from flocks on the selected farms over five consecutive production cycles between June 2001 and May 2002. This was undertaken by trained advisory staff from the poultry companies who visited each farm prior to, and as close as practically possible, to the date of the first depopulation (removal) of birds from the house. The sampling protocol used consisted of cloacal swabs collected from fourteen birds chosen at random per flock, which was based on achieving 95% confidence of detecting *Campylobacter* in at least one sample where the flock prevalence was greater than or equal to 20% (Thrusfield, 1995). After collection, swabs were placed into Amies charcoal transport media (Deltalab, Barcelona, Spain) and sent by overnight courier to the laboratory.

Laboratory methods

Each swab was inoculated onto a single plate of modified charcoal-cefoperazone-deoxycholate agar (CCDA) (Oxoid CM739B/SR155H) and incubated at 37^{o}C under microaerobic conditions (10% CO_2, 5% O_2, 85% N_2) in a tri-gas incubator (SANYO Biomedical, Leics.). Plates were examined after 48 to 72 hours incubation and characteristic colonies were subbed onto horse blood agar (Oxoid blood agar base CM271 plus 5% defibrinated horse blood) for further identification. Suspect *Campylobacter* colonies from a maximum of four samples per flock were confirmed and identified to species level by standard phenotypical tests including growth in air at 37^{o}C, growth microaerobically at 25^{o}C and 42^{o}C, hippurate and indoxyl acetate hydrolysis.

Data collection

Data on flock and farm management parameters were collected using standardised questionnaires, which were completed by farm advisory staff from each of the relevant poultry companies. The data collected were based on a combination of poultry company data, the personal observations of advisory staff at the sampling visit and information obtained from the farmer in charge of the flock. In brief, the variables included details of the farm such as the number of houses, presence of other livestock, water source, flock placement and slaughter dates, intercrop procedures, as well as details of the hygiene practices followed by the farmer (Table 1). Following the first two production cycles, more detailed questions on hygiene practices and numbers of other livestock on the farm were added to the questionnaire and this version used for the remaining production cycles.

Table 1. Data collected by questionnaire

Type of Variable	Examples
Farm parameters	Number of houses on site, number of birds in flock and on site, placement and sampling dates
Disinfection procedures	Whether house was disinfected, fumigated, sprayed with insecticide, water tank disinfected
Water	Source, use of chlorination
Personnel	Number of staff; contact with other farm species
Pest control	Evidence of rodents, litter beetle infestation
Litter	Condition, frequency of top-ups
Health of flock	Evidence of clinical disease, use of antibiotic medication
Site cleanliness	Cleanliness of broiler house anteroom, area outside broiler house
Hygiene practices	Use of a hygiene barrier, disinfectant foot-baths, separate protective clothing; washing of hands
Livestock	Type and number of other livestock on the farm

Data handling and statistical analysis

Questionnaire data on farm and management parameters were single entered into a Microsoft Access database (Microsoft Cooperation) and visual checks for accuracy against the original questionnaires made both at the time of data entry and at the end of the survey. This information was combined with the *Campylobacter* isolation data for each flock and the information extracted for analysis as a single data file. After validation checks for data inconsistencies and missing entries, statistical analyses were carried out using STATA version 7.0 (StataCorp, 2001). Confidence intervals for prevalence estimates were calculated using the survey commands in STATA, with stratification and clustering in the dataset adjusted by designating the associated poultry company and the individual poultry farm as separate strata and primary sampling units, respectively. The longitudinal pattern of infection on farms was examined by cross-tabulation of number of production cycles sampled and the number that tested positive.

In the statistical analyses, the unit of observation was taken as the individual flock (production cycle) with a flock classified as positive if *Campylobacter* spp. were isolated from one or more cloacal swabs. Initial analysis showed that responses to the small number of additional variables added to the questionnaire after cycle two, showed a high degree of consistency across the last three production cycles. Rather than limit any multivariable analysis, responses to these additional variables were extrapolated back to earlier cycles and the validity of this approach checked at the univariable stage.

Associations between possible explanatory variables and the *Campylobacter* status of the flock was examined in univariable and multivariable stages, with the likelihood-ratio χ^2-test used to assess statistical significance. In both cases random-effects logistic regression models were used to adjust for the correlated nature of the sampling design, with the cluster variable defined as the individual farm identification. Variables that gave a p-value <0.25 in the univariable screen were considered for inclusion in the multivariable model. This list of eligible variables was further examined for possible collinearity and also to determine the likely relationships between biologically linked variables. For closely related variables, the variable

considered most appropriate on biological or statistical significance was selected for inclusion in the multivariable model or, alternatively, a new combined summary variable produced. As part of the wider project of work, of which this study formed part, data on the *Campylobacter* status at final depopulation, based on the same sampling protocol and laboratory methods as described above, was available for 280 of the flocks and this information was also included in the analysis to assess potential carry-over of infection from one production cycle to the next.

Backwards elimination was the main approach used in the model building process. In this the significance of each variable in the full model was assessed in turn with the least significant variable deleted and process repeated until all remaining variables were deemed significant. Variables for which the association with *Campylobacter* status could be the due to effect of the other variables, such as company or *Campylobacter* status in the previous production cycle, were not included in the main backwards deletion process but evaluated individually by addition to the resultant model. Further checks on the final model were carried out by addition of each eliminated variable in turn and assessing its significance using the likelihood-ratio χ^2- test. Biologically plausible interactions between variables, such as any possible interactions between the number of houses on site and other variables were investigated in the final multivariable model and the reliability of parameter estimates checked using the 'quadchk' command in STATA.

RESULTS

Completeness of sampling

The overall completeness of sampling was high with samples received from 388 flocks on the 88 farms, equivalent to 88.2% of all possible flocks in the study design. The remaining farms were missed due to reasons such as limitations in the availability of company staff to visit farms at the required time. Overall, samples were received from all five production cycles on 50 farms (56.8%), from four cycles on 26 farms (29.5%), from three cycles on 10 farms (11.4%) and from two cycles on two farms (2.3%).

Campylobacter prevalence

In total, *Campylobacter* spp. were isolated from 163 of the 388 flocks (42.0%; 95% CI 35.2% - 48.9%) sampled. In the majority of positive flocks, a single *Campylobacter* spp. was found with *C. jejuni* isolated from 134 flocks (82.2% of positive flocks), *C. coli* from 14 flocks (8.6%), *C. lari* from one (0.6%) and other *Campylobacter* spp. from one (0.6%). In the remaining 13 flocks (8.0%) mixed infections were found with *C. jejuni* isolated in combination with *C. coli* from 11 flocks (6.7%) and in combination with *C. lari* from two flocks (1.2%). The overall prevalence of infection in positive flocks was high with a mean within-flock prevalence of 83.3%, and at least 12 of the 14 swabs taken, culture positive from 74.8% of positive flocks.

Longitudinal pattern of infection

The distribution of farms by the number of flocks (production cycles) sampled and the number of flocks that tested *Campylobacter* positive is shown in Table 2. Of the 76 farms sampled over at least four production cycles, 16 (21.0%) were consistently negative, while 6 (6.8%) were consistently positive. Farms that were positive at either most (3 of 4 cycles or 4 of 5 cycles) or all production cycles accounted for significant percentage of the overall number of

Campylobacter positive flocks, with just over a quarter of farms (20 of 76; 26.3%) responsible for over half of the number of positive flocks in this subset (78 of 151; 51.7%).

Table 2. Distribution of farms by number of flocks sampled and number of flocks *Campylobacter* positive.

No. of flocks sampled per farm	No. of flocks positive (%)						Total
	0	1	2	3	4	5	
2	1	0	1	-	-	-	2
3	5	2	1	2	-	-	10
4	8	4	6	5	3	-	26
5	8	11	11	8	9	3	50
Total	22(25.0)	17(19.3)	19(21.6)	15(34.1)	12(13.6)	3(3.4)	88(100.0)

Analysis of farm and farm management parameters

Key descriptive statistics: Questionnaire data were available for 386 of the 388 flocks (99.5%) sampled. Overall 35.2% (31 of 88) of farms included in the study had a single poultry house, while 26.1% (23) had two houses, 22.7% (20) three houses, and 15.9% (14) four or more houses. The majority of farms (71; 80.7%) had other animal species as well as poultry, with 73.9% having cattle, and 28.4% sheep. None had any other avian species while only one (1.1%) had pigs. The median number of birds per flock (broiler house) was 19,357 (mean 17,663), while the median number on each farm was 39,000 (mean 41,995). In ninety percent of flocks the age of the birds at the first-depopulation sampling was between 33 and 41 days (5 and 95 percentiles respectively) with a median age of 36 days (mean 36.8).

Univariable analyses: In total 20 variables were identified as statistically significant at the $p<0.25$ level in the initial univariable screen. These included differences between poultry companies ($p=0.247$), increased odds of infection with increasing age of the birds at sampling ($p=0.050$), increased odds of infection on larger sites ($p=0.005$), and reduced odds of infection in flocks with a greater number of birds ($p=0.184$). The source of water used for flocks was highly significant with flocks supplied from non-mains sources (mainly boreholes) or a combination of mains and other sources, having 2.30 times the odds of infection compared to those supplied with water from the mains system only ($p=0.017$). Flocks for which the condition of the litter was rated as average or poor showed a higher odds of infection (OR=1.65; $p=0.089$). In general, parameters related to intercrop procedures, such as the time intervals between production cycles, were not significant although there was weak evidence of a lower odds of infection in houses where the water tanks were disinfected between production cycles (OR=0.45; $p=0.206$).

Two variables related to rodent control were highly significant with a higher odds of infection in flocks where the farmer reported have seen rodents during the production cycle (OR=3.60; $p<0.001$) and also in those in which the poultry company advisor observed rodent droppings at the time of sampling (OR=3.72; $p<0.001$). These two variables were strongly associated and were combined into a new single variable, 'presence of rodents', which showed an odds ratio of 2.64 ($p=0.009$) on sites on which the farmer only reported having observed rodents and an odds ratio of 4.78 ($p<0.001$) on sites on which the sampler observed evidence of rodent droppings at the sampling visit. All of the variables on the presence of other animal species on the farm were not statistically significant ($p>0.25$) except for weak evidence of

increased odds of infection on farms with young calves (OR=1.70; p=0.160) and farms with lambs (OR=1.76; p=0.227).

A number of parameters related to biosecurity were statistically significant. These included a reduced odds of infection in flocks where there was a wash-hand basin present in the poultry house ante-room (OR=0.31; p=0.004), when the farmer washed his hands before tending to the birds (OR=0.54; p=0.088), where separate boots and overalls were used when working with the broilers (OR=0.38; p=0.020), presence of a hygiene barrier (OR=0.52; p=0.133) and consistent use of a hygiene barrier (OR=0.35; p=0.019). The tidiness / cleanliness of the ante-room and the tidiness / cleanliness of the outside of the broiler house were also both highly significant (p=0.007 and p=0.016 respectively) with increased odds of infection in flocks with poorer standards. Footbaths were recorded as being present for all but one flock, so it was only possible to assess the effect of differences in the location and number of footbaths rather than the effect of presence or absence of footbaths *per se*. Overall there appear to be little difference between flocks from houses that had a footbath in the ante-room compared to those with a footbath outside (OR=1.07; p=0.908), although flocks from houses with footbaths in both locations did have a significantly lower odds of infection (OR=0.51; p=0.083). The frequency of footbath disinfectant changes was highly significant (p=0.027), with the lowest odds of infection seen in flocks where the disinfectant was changed every 3-5 days and the highest odds of infection when the interval between changes was greater than 7 days (OR=5.14).

Multivariable analyses: The results of the final multivariable model are shown in Table 3. Seven variables were included in the model, five of which were statistically significant at $p<0.05$ and one of which was significant at $0.05<p<0.10$. Data for the variables included in the model were available for a total of 354 flocks (91.2% of those sampled), with the highest number of missing observations relating to the age of the flock at sampling (24 flocks, 6.2%).

The age of the birds at sampling was the most statistically significant variable identified (p=0.004) with an odds ratio for its linear effect of 1.15 for each day of increase in age at sampling, which in the study, ranged from 30 to 57 days. The combined variable on the presence of rodents was also highly significant (p=0.012). Compared to flocks in which no evidence of rodents was reported, an increased odds of infection was seen in flocks where the farmer reported having observed rodents during the production cycle but the sampler had not reported evidence of droppings at the sampling visit (OR=2.01), with the higher odds in flocks in which the sampler reported having observed rodent droppings at the sampling visit (OR=3.08).

Two variables relating to biosecurity i.e. the frequency with which the footbath disinfectant was changed and the tidiness / cleanliness of the broiler house ante-room were both statistically significant in the final model (p=0.048 and p=0.024, respectively). In relation to the frequency with which the footbath disinfectant was changed, the lowest odds of infection occurred in flocks where the disinfectant was changed every 3-5 days, with odds ratios relative to this of 2.33 in flocks where the disinfectant was changed every 7 days and 3.74 when the interval between changes was greater than 7 days. Relative to flocks in which the tidiness / cleanliness of the broiler house ante-room was rated as very good, the odds of infection was 2.02 times greater in flocks where the sampler noted that the tidiness / cleanliness of the ante-room could be improved and 4.52 where the sampled noted that there was a definite need for improvement.

Farms with three or more houses showed a higher odds of infection (OR=2.40; p=0.030) than those with a single house. The difference between farms with two houses compared to those with a single house, however, was not statistically significant (OR=1.27; p=0.580). Farms

that used non-mains sources of water also showed a higher odds of infection (OR=1.56). Although the statistical significance of the variable was relatively weak (p=0.166), the use of non-mains sources of water was closely associated with larger sites, and the variable was retained in the final multivariable model for two reasons. Firstly, exclusion of the variable caused a noticeable increase in the odds ratio in respect of farms with three or more houses (from OR=2.40 to OR=2.86) and secondly, in models which excluded the number of houses on site, the source of water was highly significant (OR=1.93; p=0.033).

Table 3 – Multivariable model: occurrence of *Campylobacter* spp. at first-depopulation.

Variable	Level	No. of flocks	Odds Ratio	95% CI	Wald Test p-value	LRS p-value
Age at sampling [a]	per day	354	1.15	1.04 - 1.27	0.005	0.004
No. of houses on site	1	125	1.00	-	-	0.064
	2	88	1.27	0.55 - 2.91	0.580	-
	≥3	141	2.40	1.09 - 5.30	0.030	-
Water source	Mains only	164	1.00	-	-	0.166
	Borehole +/- mains	190	1.56	0.84 - 2.91	0.163	-
Presence of rodents	Not Reported	233	1.00	-	-	0.012
	Farmer only reported	56	2.01	0.94 - 4.30	0.071	-
	Sampler reported	65	3.08	1.38 - 6.89	0.006	
Tidiness/cleanliness	Very good	188	1.00	-	-	0.024
Of ante-room	Could be improved	150	2.02	1.10 - 3.71	0.023	-
	Needs improved	16	4.52	1.09 - 18.81	0.038	-
Footbath disinfectant	3-5 days	57	1.00	-	-	0.048
changes	7 days	249	2.33	1.00 - 5.45	0.050	-
	>7 days	48	3.74	1.23 - 11.37	0.020	-
Season	Other seasons	274	1.00	-	-	0.035
	Summer	80	1.93	1.04 - 3.58	0.037	-

[a]included in the model as a linear variable

Although not significant in the univariable screen (p>0.25), the effect of season was evaluated in the multivariable models as an *a priori* risk factor. Inclusion of the variable showed some evidence of an overall effect (p=0.192), with a marked increase in the odds of infection for birds sampled during the summer months of June, July and August (OR=1.91) relative to the baseline season of winter. The odds of infection in spring and autumn were very similar to that seen in winter (OR=0.86 and 1.07 respectively). When recategorised as a binary variable (summer versus all other seasons), the effect of variable was markedly significant (p=0.035) with an odds ratio for infection during summer almost twice that in other seasons (OR=1.93).

While univariable analyses showed evidence of an association between the *Campylobacter* status of flocks and the final clearance result in the previous cycle (OR=2.25; p=0.094), addition of variable to the final multivariable model showed no evidence of an effect (OR=1.12, 95% CI 0.40 – 3.17; p=0.826). The *Campylobacter* status of flocks at first-depopulation in the previous cycle was, however, statistically significant (OR=2.46; 95%CI 1.19 – 5.09; p=0.026) but was not retained in the model for the reasons that are outlined in the discussion. Final checks on the

model showed no significant interactions between variables, and parameter estimates were stable when checked in STATA.

DISCUSSION

The overall prevalence of infection found in this study (42.0%; 95% CI 35.2 – 48.9%) should be interpreted with caution, considering that it is based on sampling prior to first depopulation. Nevertheless the prevalence found is similar to that reported elsewhere including studies in France (Refregier-Petton et al., 2001), Denmark (Wedderkopp et al., 2000), and also a longitudinal study in Great Britain that found that more than 40% of flocks were positive by the time the chicks were 4 weeks old (Evans & Sayers, 2000). The high within-flock prevalence (>80%) and the occurrence of *C. jejuni* as the predominant *Campylobacter* species are also consistent with previous reports (Evans & Sayers, 2000).

In total seven variables, five of which were statistically significant at $p<0.05$ and one of which was statistically significant at $0.05<p<0.10$, were included in the final multivariable model. Of these, the age of the birds at sampling was the most statistically significant factor identified. The observed effect of increasing odds of infection with increasing age is consistent with previous reports (Evans & Sayers, 2000; Bouwknegt et al., 2004). The birds sampled in the study here were all over 30 days of age, and the increasing odds of infection observed with increasing age may simply reflect the cumulative risk of introduction of infection over time, from environmental and other sources. A marked seasonal effect, with increased odds of infection in summer versus other seasons was also observed. An increased risk of infection in summer and / or autumn has been reported previously (Kapperud et al., 1993; Wedderkopp et al., 2000; Refregier-Petton et al., 2001; Bouwknegt et al., 2004), although others, including studies in Great Britain (Evans & Sayers, 2000) have not found evidence of such effects. Seasonality in the occurrence of campylobacteriosis in humans is well recognised both in the UK and elsewhere (Tam, 2001; Nylen et al., 2002), with recent work suggesting fly transmission to be a possible explanation (Ekdahl et al., 2005; Nichols, 2005). *Campylobacter* spp. have also been isolated from flies in the ventilation air entering broiler houses, and it has been suggested that this may also be a source of infection for flocks over the summer months (Hald et al., 2004).

The presence of rodents during the production cycle was also statistically significant. Although the measures used to categorise farms were based on reports by farmers and the observations of company staff at sampling visits, both of which are partially subjective, the strength of the associations and the statistical significance suggest the effect to be genuine. *Campylobacter* spp. have been previously isolated from mice and rats on farms with broilers (Annan-Prah & Janc, 1988), and also more recently from approximately 10% of rodents on organic (Meerburg et al., 2006) and dairy farms (Adhikari et al., 2004). In previous epidemiological studies, an increased risk of *Campylobacter* in broilers associated with the presence of rats has been reported (Kapperud et al., 1993), although the number of farms with a rodent problem in this study was small, and the effect of borderline statistical significance. A previous study in Great Britain has also reported an increased risk associated with high rodent populations (Evans, 1997 as cited by Evans & Sayers, 2000), although follow-up studies (Evans & Sayers, 2000) and also epidemiological studies in other countries have not reported an association (Hald et al., 2000; Refregier-Petton et al., 2001; Bouwknegt et al., 2004; Cardinale et al., 2004).

Two parameters relating to biosecurity were significant in the final multivariable model. Flocks in which the footbath disinfectant was changed every 3-5 days had the lowest odds of infection, with the highest odds in those flocks where the disinfectant was changed less than every seven days. The use of disinfectant footdips has been previously reported to delay or prevent flock colonization (Humphrey et al., 1993) and at least weekly changing of the disinfectant found to be statistically significant in epidemiological studies in Great Britain (Evans & Sayers, 2000). The finding is also consistent with results of an intervention trial (Gibbens et al., 2001), which found that twice-weekly replenishment of disinfectant significantly reduced the risk of infection in flocks. Although a number of hygiene parameters such as the use of separate protective boots and overalls, presence and use of a hygiene barrier were significant in the univariable analysis, unexpectedly the only variable that remained significant in the multivariable model was that on the tidiness and cleanliness of the broiler house ante-room. Notably, however, many of these hygiene parameters were strongly associated and it is possible that this particular variable on the tidiness and cleanliness of the broiler house ante-room, may simply have acted as a proxy measure for the combined effect of a number of hygiene parameters.

Other risk factors identified include the number of houses on site, as well as weak evidence of a possible effect of water-source. An increased risk of *Campylobacter* infection on sites with multiple houses has been noted in previous studies, with Refregier-Petton et al. (2001) reporting an increased risk on sites with more than two houses and Bouwknegt et al. (2004) reporting some increase in risk on sites with 3 or 4 houses and a more marked increase on sites with 5 or more houses. Reasons for this association are not clear, but could include the increased risk of entry of infection from the environment into one or more of the houses, with the increased possibility of subsequent transfer between houses on site. Although the overall significance was relatively weak, the study suggests that the use of non-mains sources of water may also have a contributory effect. Field studies have previously indicated a contaminated water supply as the likely source of *Campylobacter* in one field investigation (Pearson et al., 1993) and an increased risk of infection associated with the supply of untreated water has also been reported (Kapperud et al., 1993).

Overall the results of the longitudinal element of the study showed that a significant percentage of farms produced *Campylobacter* negative flocks, at the first depopulation stage, on a regular basis, with 16 of 76 farms (21.0%) consistently negative at this sampling point over at least four production cycles. Interpretation of the association in the *Campylobacter* status at consecutive production cycles is, however, complicated by a number of separate effects. These include the potential association due to the direct carry-over of infection from one production cycle to the next and also the association between cycles that exists because of the continuation in occurrence of the same risk factors at successive cycles. Addition of the final clearance result from the previous production cycle to the multivariable model showed no significant effect (OR=1.12, 95% CI 0.40 – 3.17; p=0.826) and is consistent with previous evidence suggesting an absence of direct carry-over of infection between production cycles including reports of failure to isolate *Campylobacter* from the walls and floors of houses post-disinfection (van de Giessen et al., 1998; Evans and Sayers, 2000). In contrast, the *Campylobacter* status at first depopulation in the previous cycle was statistically significant when added to the final multivariable model (OR=2.46; 95%CI 1.19 – 5.09; p=0.026). Considering the evidence against direct carry-over of infection above, it would seem likely that this association reflects a consistency in *Campylobacter* status caused by the same, or largely similar, on-farm risk factors at successive production cycles, with the variable in effect acting as a partial summary measure of other on-farm risk factors. For this reason, the variable was not retained in the final multivariable model.

Perhaps surprisingly no association was found in the study reported here with the presence of other animals on the farm. *Campylobacter* spp. have been isolated from a wide range of animal species and an increased risk associated with presence of, or working with, other farm animals reported in a number of previous epidemiological studies (Kapperud et al., 1993; van de Giessen et al., 1996; Hald et al., 2000; Bouwknegt et al., 2004; Cardinale et al., 2004). The current study was carried out shortly after the occurrence of Foot and Mouth Disease in Northern Ireland in spring 2001, and it is possible that farmers' awareness of biosecurity issues may have been heightened, though even if this was the case, some increased risk might still have been expected.

CONCLUSION

In conclusion, the study here has identified a number of important risk factors for infection in broiler flocks in the period from placement to first depopulation. While some, such as the increasing occurrence of infection in older birds is well recognized, others, including the increased risk associated with the occurrence of rodents on farms, have been much less frequently reported. The study also reinforces the need for good biosecurity measures on farms with twice weekly changes of footbath disinfectant appearing to a significant protective effect. In addition, the study has shown a significant degree of consistency in the *Campylobacter* status of successive flocks on individual farms, with some farms capable of producing negative flocks, at least at the first depopulation stage, on a regular basis.

ACKNOWLEDGEMENTS

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EXPLORATION OF DIRECT AND INDIRECT CONTACTS BETWEEN CATTLE FARMS

M.L. BRENNAN*, R.M. CHRISTLEY AND R. KEMP

SUMMARY

Little is known regarding the types and frequencies of contact that exist between farms; however, it is likely that farms demonstrate considerable heterogeneity in contact. In this cross-sectional study, we explored the direct and indirect contact types and frequencies that exist between cattle farms within a region. The owners/managers of 56 farms located in a 10km by 10km study area in north-west England were administered an interview-based questionnaire between June and September 2005. Information was obtained relating to contact types and frequencies, including those involving animal movements, equipment sharing between farms and any contractors or companies visiting the farms. Maps of each farm were produced prior to visits (including additional premises used for stock) and contiguous neighbours identified from these.

There was considerable variation in the connectedness of networks arising through different contact types. Some exhibited great connectivity, incorporating approximately 90% of the farms interviewed, whilst other networks appeared more fragmented, with multiple small components (sets of farms not linked with any others). A range of factors influencing contact between farms were identified. For example, contiguous farms were more likely to be linked via other contacts, such as sharing of equipment, direct farm to farm animal movements and use of the same livestock dealers ($p<0.001$, $p=0.02$ and $p=0.1$, respectively).

The frequency of contacts was also investigated; it is likely that the amount of contact a farm receives from a company or contractor will impact on the potential for disease transmission to occur. Similarly, whether or not biosecurity is performed after contact (i.e. personnel, vehicles from that company) is also an important consideration; minimal biosecurity performed infrequently would increase the likelihood of transmission of disease when compared with regular and thorough involvement in biosecurity practices.

These findings lead to greater understanding of inter-farm contact. This may aid development of appropriate biosecurity practices and control procedures, and inform mathematical modelling of infectious diseases.

INTRODUCTION

Infectious disease transmission at the individual, herd and farm level relies on some form of contact, either direct or indirect. It has been shown that as early as the mid-eighteenth century, livestock producers recognised animal movements as an important risk factor for the spread of

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disease (Woolhouse & Donaldson, 2001). Exotic diseases such as foot and mouth disease (FMD) and other notifiable diseases such as bovine tuberculosis are likely to be spread by animal movements (Gibbens et al., 2001; Gilbert et al., 2005; Woolhouse et al., 2005). Other contacts that may result in transmission of infectious agents include sharing of equipment, movement of people and vehicles and contact over/through fences with neighbouring stock; it has also been reported that wildlife and even wind can play a role in transmission between contiguous or proximate premises (Mikkelsen et al., 2003; Woodroffe et al., 2006).

Often there is little knowledge of what contacts (direct and indirect) exist between farms. As was highlighted by the FMD outbreak in the UK in 2001, local risk kernels are often used to model local transmission, as details of contacts between farms are not well known (Woolhouse & Donaldson, 2001; Webb, 2005). Studies during the early phase of the 2001 FMD outbreak found that animal movements were one of the most significant contacts between farms in disease dissemination (Ortiz-Pelaez et al., 2006). Other studies from the Netherlands, California and New Zealand have identified and quantified contacts over time, particularly with regard to the potential spread of FMD. The number of contacts varied greatly when considering characteristics such as type of enterprise, size of farm and number of animals on farm. It was reported in California that there were approximately 11 direct animal contacts and 404 indirect contacts per farm over a 2 week period (Bates et al., 2001), which is substantially more than the 92 direct and indirect contacts per farm seen over the same length of time in the Netherlands (Nielen et al., 1996). In comparison, 50 contacts of people, animals and materials were reported over a 2 week period during a study in New Zealand (Sanson et al., 1993).

Such variability illustrates the structural complexity and heterogeneity of the contacts that exist between farms, some of which can be represented schematically (Fig. 1). This could potentially be described as a 'network' of contacts between farms, which requires further exploration.

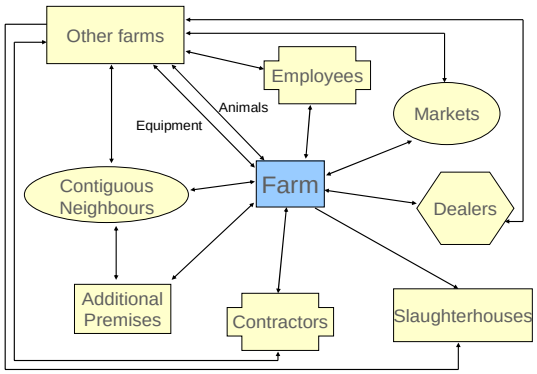

Fig. 1 Schematic representation of potential contact characteristics of cattle farms

Network analysis facilitates investigation of interactions between units of interest ('nodes', e.g. animals or farms) at the population and the individual level and enables identification of key nodes in terms of the connectivity individuals have within a population (Corner et al., 2003). By focusing on the most likely contact types and the most significant individuals within these networks, it is possible to consider how disease may be transmitted through a population (Christley & French, 2003). It has been suggested that heterogeneity is present in all animal movement patterns and to presume homogeneity is likely to be unrepresentative of actual movement patterns (Bigras-Poulin et al., 2006). Furthermore, models that assume random

mixing can overestimate the size of an outbreak and underestimate the initial rate of transmission (Christley et al., 2005). Hence network analysis can play a part in developing understanding of the topology of potential routes of disease transmission and consequently may aid the design of effective surveillance and control programs (Woolhouse et al., 2005).

The aim of this study was to investigate the characteristics of the direct and indirect contact structure of cattle farms in a region and to explore the nature of such contacts using network analysis techniques.

MATERIALS AND METHODS

Study population

A 10 x 10 km area of north-west England was selected and the owners or managers of all known cattle farms were contacted by mail and invited to participate in this cross-sectional observational study. Letters explaining the project were sent to farms in the area; follow up phone calls were made to all farms to ascertain willingness to participate. Of those farms whose phone numbers were not available, visits were assigned to determine farm details and whether participation was possible. Visits to all willing farms were conducted and questionnaires completed.

Questionnaire design

The interview-based questionnaires were administered to owners/farm managers during July – September 2005. A pilot study involving 6 farms outside the study area was completed prior to the main study. The first author conducted all interviews. The questionnaire concentrated on the direct and indirect contacts between farms. This included in-depth questions regarding animal movements on and off the farm and their destinations and departure points. Questions relating to the sharing of equipment between farms, any personnel coming on and off the farm and the types and frequencies of companies/contractors coming onto the farm were included. Social contacts between farmers were also investigated.

Attitudes of the interviewees towards nineteen biosecurity practices were explored; these practices were selected after review of current practices, sourcing information from peer-reviewed papers, current advice from various government bodies and grey literature.

On-farm observations

During visits, maps of each farm were used to gather information regarding farm structure, including additional premises used for stock. Boundaries and fence types bordering the land were noted; boundary fields that were frequented by animals and had fences that allowed potential contact with neighbouring animals (those owned by other farmers) were recorded. A single fence that was reported to not permit nose-nose contact was selected randomly from those on the farm and was examined to ascertain the potential for nose-nose contact with neighbouring stock.

Data management and analysis

The questionnaire was formatted using Verity TeleForm Version 9.1 (Verity Inc) and data managed using Microsoft Office Access 2003 (Microsoft Corporation). Hierarchical cluster

analysis was used to classify farms (or farmers) on the basis of their methods of animal movement (direct to slaughterhouse, farm-farm or through markets and dealers), their use of companies and contractors and their attitudes to 19 biosecurity practices. These groups were compared with regard to the variables used to cluster them and other farm-level variables using chi-squared tests (for categorical data) and the Kruskal-Wallis test (for continuous data). Statistical analysis and cluster analysis was performed using SPSS 12.0.1 for Windows (SPSS Inc.). The Jaccard coefficient was used to assess whether the probability of one contact type between farms was related to the probability of another contact type. The quadratic assignment procedure (QAP) was used to develop standard errors to test for the significance of these associations. This analysis and all other network analyses were performed using Ucinet v6.135 and network diagrams were created using NetDraw v 2.41 (NetDraw and Ucinet; www.analytictech.com/).

RESULTS

Response rate

Questionnaires were completed on 56 out of 81 farms, giving a 68.3% response rate. Of the farms not participating, 7 had ceased trading or did not have cattle and 3 were shortly to cease trading. One farmer could not be contacted despite several visits and phone calls; 13 declined to participate and 1 farmer could not make an appointment in the allotted project time. Therefore, considering only those farms in the area that owned cattle and would continue to do so for the foreseeable future, a 78.8% compliance rate was achieved. Of those farms that were farming cattle at the time of interview, the response rate was 75.7%. The 3 farms that were shortly to cease trading were excluded as we believed that animal movements, visits by companies and contractors and general farm contacts might not be representative of a typical farm in this area. Excluding those farms that did not have cattle/had ceased trading, 15 farms remained that were not interviewed. Of these farms, information solely regarding enterprise was collected on 10 by telephone interview or via external data sources; 6 dairy farms, 2 mixed cattle farms, 1 beef farm and 1 heifer rearing farm declined to participate. All results reported in the following sections are derived used data obtained from the 56 participating farms. The average time spent on farm was 2 hours 8 minutes, ranging from 57 minutes to 3 hours 15 minutes, inclusive of time spent viewing fences.

Types of enterprise and management groups

The majority of interviewed farms in the study area were dairy farms (36 farms), with 19 fat-stock farms, 15 suckler herds, 8 store-animal producers and 3 pedigree breeders. Almost one third of dairy farms had additional cattle enterprises outside of the dairy sector. The median size of each farm was 80.3 hectares (range 6 – 2428; IQR 48 - 137). The majority of cattle were dairy cattle (lactating cows, heifers and dry cows) and the median number of cattle per farm was 170 (IQR 104-320).

Other livestock species and alternative income

Eleven farms had other animal enterprises; 8 farmed sheep, 2 produced turkeys and 1 kept laying hens. Of the 8 farms that owned sheep, 5 farmers stated that they grazed cattle on the same pasture at the same time. Many farms (36 farms) supplemented their cattle enterprise with other ventures (i.e. B & Bs, running kennels); the majority had alternative incomes within the agricultural industry (31 farms).

Types of contact

Animal movements: The most commonly reported mechanism for trading animals was through markets (89% of farms), followed by trading with other farms (73%), dealers (50%) and slaughterhouses (50%). Markets and dealers were used most frequently for the sale, rather than purchase of animals. Most farms trading with dealers used one dealer only. In contrast, most farms purchased animals directly from other farms. The majority of slaughterhouse movements were to a plant outside of the study area.

The combined animal movement network involving interviewed farms and named markets, dealers and slaughterhouses incorporated almost all of the farms in the study area into a single network component (Fig. 2; excludes farm-farm movements). The network exhibited a 'hub and spoke' structure due to the local market within the study area appearing at the centre of the network as the 'hub'. This market plays an important role in connecting the nodes within the network. Although most farms used a single market, one farm bought and sold stock through 5 different markets.

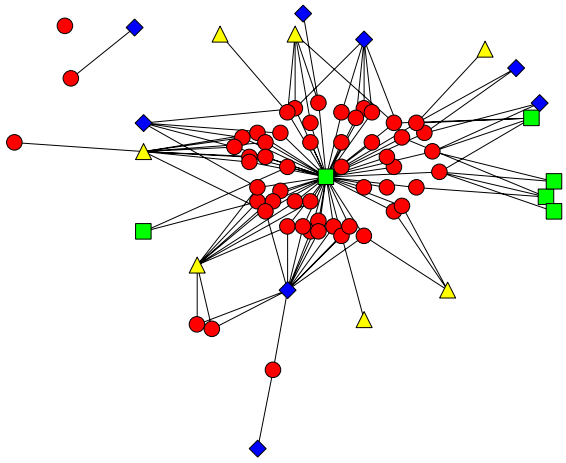

Fig. 2 Network of animal movements between interviewed farms (circles) and markets (squares), dealers (triangles) and slaughterhouses (diamonds) arranged using multi-dimensional scaling

The animal movement network involving farm-farm movements appeared substantially different to the previous network (Fig. 3a). This network was fragmented and involved many movements of animals from farms outside of the study area. Fragmentation of the network increased when only those animal movements between farms in the study area were considered (Fig. 3b).

The patterns of animal movements (M) were explored using cluster analysis. This suggested three main groups: farms in group M1 were solely reliant on markets for sale of animals; all group M2 farms used dealers, the majority used markets and none sold directly to other farms. All group M3 farms sold directly to other farms, most used markets and half used dealers. Farms in all groups purchased directly from other farms. Although an uncommon practice generally, the hiring of animals onto a farm was more common in M2 farms, and was not undertaken by farms in M3. There was no evidence of differences between these groups in terms of acreage, number of animals, types of enterprise (dairy, beef etc.) or in the use of companies or contractors ($p>0.1$ in all cases).

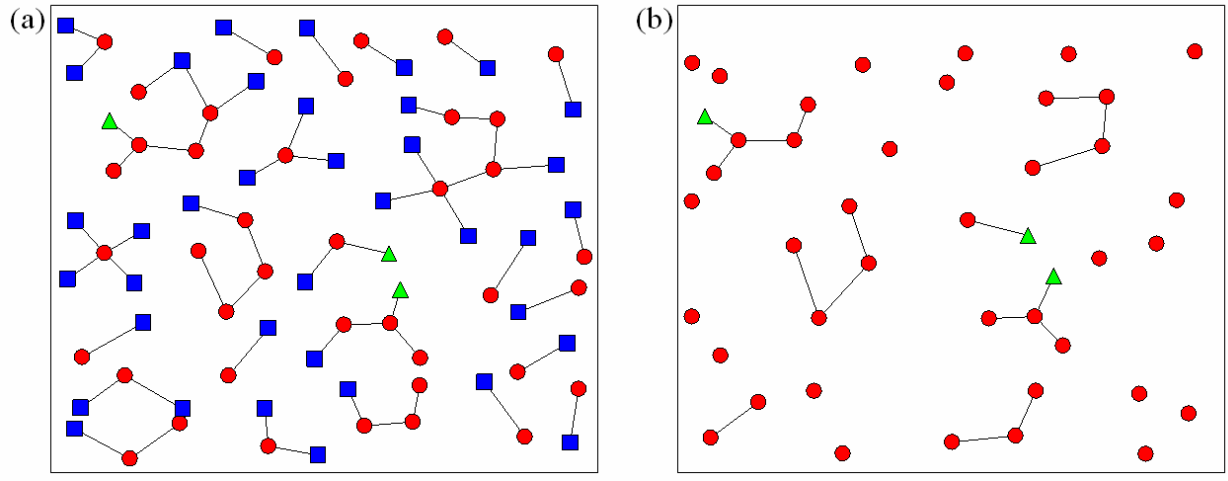

Fig. 3 (a) Network of animal movements between interviewed farms (circles) and other nominated farms (not interviewed) within the study area (triangles) and outside of the study area (squares). (b) Network of animal movements as in Figure 3a excluding nominated farms outside of the study area

Stock on the farm not owned by the farmer: Twenty five percent of interviewed farms responded that they sometimes had other livestock species living on the farm that were not owned by them. Of these 14 farms, 11 had sheep and 4 had cattle from other farms. All of the sheep originated from premises in Wales and all except one group of cattle were from locations within the same county but outside of the study area. The remaining cattle source was located within the study area.

Equipment sharing: Forty three percent of farmers stated they shared equipment with other farms, the majority of farms sharing only one item (63%). Tractors, trailers and wagons were shared most commonly between farms, followed by machinery for harvesting and ploughing, and muck vehicles. Waste handling and feeding were nominated as the 2 most common tasks for which tractors were utilised.

Information regarding the disinfection of borrowed or lent equipment was obtained. Of the 9 responses received from farms lending their own equipment, 4 would clean on return and 5 would clean before lending the item; only one did both. Sixteen farmers reported borrowing equipment from others; 12 cleaned the item prior to returning it and 4 before using it; again, one did both. The network arising through sharing of equipment was sparse and fragmented and involved many farms outside the study area (9 farms) and farms within the study area that were not interviewed (6 farms).

Companies and contractors: There was considerable variation between the number of farms visited by each type of company or contractor and the frequency with which these visits occurred. At the time of interview, each farm had a median of 14 individual contractors visiting their farm per year (IQR 12-16, range 6-22) resulting in a median of approximately 67 visits per month (IQR 36-80, range 4-136).

The networks connecting farms varied greatly between the different companies and contractors. Many exhibited some form of ‘hub and spoke’ topology, such as the AI technician, deadstock collector, farm assurance advisor, government veterinarian, milk company, private veterinarian and Trading Standards networks (e.g. Fig. 4a). Other networks such as the castrator,

animal haulier, hoof trimmer/belly clipper and muck spreader networks were quite fragmented and had components linking 15 or less farms (e.g. Fig. 4b).

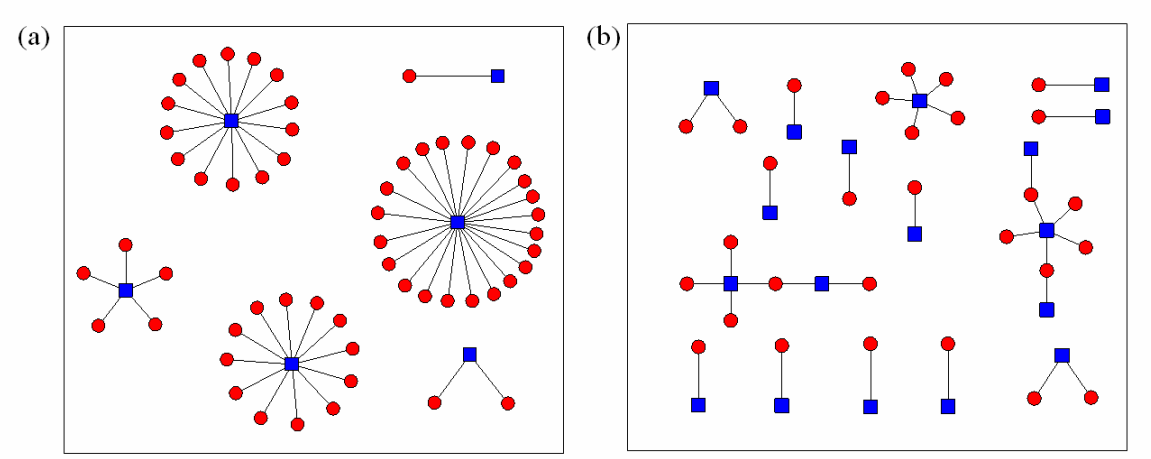

Fig. 4 (a) Network of private veterinarians and (b) animal hauliers and interviewed farms within the study area. In each case, the company or contractor (veterinarians or animal hauliers) are represented by squares and the interviewed farms by circles.

Due to the perceived difference in transmission risk posed by different companies, we divided the contractors and companies into 2 subgroups; those that entered animal areas (defined as areas frequented by stock) and those that did not. Cluster analysis was used to classify farms according to company/contractor usage. There was little evidence of clustering when considering all companies and contractors, whereas three clusters (CC1, CC2, CC3) were evident when considering only those that entered stock areas (Table 1). In all groups farms used muck spreaders, deadstock collectors, milk companies (mostly CC3) and AI technicians (mostly CC3); Trading Standards officers visited only farms in CC1 and CC3. Farms in CC1 were visited by government veterinarians and animal hauliers. In comparison, farms in CC2 were not visited by government veterinarians and only a few were visited by animal hauliers. Farms in CC3 were most likely to be visited by hoof trimmers and farm assurance advisors. CC1 included dairies, beef suckler and store cattle farms; CC2 included dairies and beef fattening farms; CC3 farms were exclusively dairies. The size of the farms (in acres) was significantly different between the groups (Table 1); there was no difference between the groups with regard to types of animal movements (dealers, markets, farm-farm or direct to slaughterhouse, $p>0.2$ in all cases) or herd size ($p=0.2$).

Using cluster analysis, farmers were classified on the basis of their attitudes to 19 biosecurity practices into 3 groups (B1, B2, B3); those with very positive (n=14), positive (n=19) or negative (n=23) attitudes, respectively. Using this classification, the company and contractor groups varied with regard to their attitude to biosecurity ($p=0.07$). There was a significant trend for group CC2 to have more positive attitudes towards biosecurity, compared to group CC1 (Chi-square for trend $p=0.04$). However, no difference was detected between groups CC1 and CC3, or CC2 and CC3.

Table 1. Cluster analyses based on companies and contractors visiting farms using Ward's cluster method

Companies and Contractors	Group CC1 (%; n=19)	Group CC2 (%; n=24)	Group CC3 (%; n=13)	P-value
Milk company	58	50	100	0.008
Government veterinarians	58	4	15	<0.001
Trading Standards	47	0	39	<0.001
AI technician	53	25	77	<0.001
Animal haulier	84	17	69	<0.001
Deadstock collector	95	100	100	0.4
Muck spreaders	63	71	92	0.2
Hoof trimmers	16	8	100	<0.001
Belly clippers	0	0	7.7	0.2
Castrators	11	0	0	0.1
Farm assurance advisors	68	83	100	0.07
Median no. animals per farm (IQR)	151 (92-280)	140 (92-322)	238 (164-367)	0.2
Median acreage of farm (IQR)	146 (75-300)	146 (109-315)	290 (205-707)	0.03

Employees: Eighty two percent of farms employed other workers. Just under half of these farms (44%) had employees that worked on other farms and approximately 26% had employees that ran their own cattle enterprise.

Additional premises: Fifty percent of the farmers had additional farms or other pieces of land separate to their main holding on which cattle were run. Of these 28 farms, 19 had 1 additional premise, 5 had 2 additional premises, 2 had 3 additional premises and 2 had 4 additional premises. Of the 19 farms with one additional premise, 15 reported that they never recorded movements between the premises, 1 reported sometimes recording movements and 3 reported that they always recorded movements. In contrast, 4 farms with more than one additional premise reported that they never recorded movements between the premises, 2 reported sometimes recording movements (or reported recording them for some, but not all additional premises) and 3 reported that they always recorded movements. Farms tended to be more likely to respond that they never recorded animal movements between their premises when they had only 1 additional premise (Fishers exact p=0.07).

Contiguous neighbours and boundary fences: For each perimeter field used for grazing it was determined if the neighbouring field/s were also used for grazing. Boundary fences dividing these fields were classified by the farmers as either allowing nose-nose contact (e.g. wire fences, gapped hedges) or not (e.g. double-fences, thick hedges). A proportion of these boundary fences were randomly selected and examined on 43 farms. The selected fences on 19 farms (44%) were assessed to have no contact possible through them. Of the fences that allowed contact, over 90% permitted contact along only 1-20% of their length. Each farming unit (main holding plus additional premises with stock) had an average of 7.3 neighbouring farms (median 7, range 1-17) and an average of 7.2 grazing fields with potential neighbouring stock contact (median 7, range 0-24). As some neighbouring farms did not use perimeter fields for grazing, the average number of neighbours with potential stock contact was 3.3 (median 3, range 0-10).

Contiguous farms were more likely to be linked via various other types of contact. These included sharing of equipment and social interactions (p<0.001 for both). Contiguous neighbours were also more likely to move animals using the same markets (p=0.01) and dealers (p=0.1) and to have direct farm to farm movements (p=0.02). In addition, equipment sharing

and farm-farm movements ($p=0.05$), equipment sharing and social interactions ($p<0.001$) and farm-farm movements and social interactions ($p<0.001$) were significantly correlated.

Social contacts: Social interactions, which involved visiting other farms, were investigated. Farmers were asked to identify contacts with contiguous neighbours, and with other farms. Forty one farmers (73.2%) responded that they regularly socialised with 1 or more of their contiguous neighbours. Most farmers (18 farms) visited on a weekly basis, and were visited by contiguous farmers on a weekly or monthly basis (16 farms for each category). Only one farmer reported never visiting his contiguous neighbours. Thirty two (57.1%) farmers responded that they regularly socialised with people from other farms that weren't contiguous. More than half of farmers (n=19) visited on a monthly basis, and many were visited by other neighbours on a monthly basis (n=16). Again, a single (different) farmer stated that he never visited neighbours who weren't contiguous.

DISCUSSION

The aim of this study was to investigate the characteristics of direct and indirect contacts arising between cattle farms. Broadly, these contacts arise due to the movement of animals, people, equipment or vehicles, or due to proximity. We have identified considerable variation in these contacts. This variation exists between farms (some contacts are more common on certain farms than others), between contact types (some contacts are more common than others) and in the structure of the networks arising from these contacts.

Patterns of animal movement were investigated between farms and between farms and markets, livestock dealers and slaughterhouses. Most farms in the study area were part of a single component, linked via markets, dealers and slaughterhouses. In this network, the market within the study area acted as a "hub" and hence may facilitate pathogen transmission through this area. Although most farms traded with a single market, one farm traded with 5 markets, potentially increasing the exposure of the network to farms in a wider geographic area.

The trading of animals is a fundamental activity in livestock farming. However, farmers are able to make choices with regard to the mechanisms through which they trade animals. For example, animals may be bought or sold through direct trade with other farms, through markets, or with dealers acting as intermediaries. We used cluster analysis to classify farms according to their animal trading activities, resulting in 3 main groups. These groupings, which could not be explained by simple measures of farm type (acreage, number of animals, enterprise), suggest that other factors contribute to a farmer's decision-making process with regard to the sale and purchase of animals. Given the recent trend in the UK toward increased reliance on markets for movement of animals and a concomitant decrease in farm to farm movements (unpublished data), further investigation of the motivations underlying such decisions is warranted. This trend is concerning as it is well established that trading through markets or dealers leads to an increased risk of disease transmission; this can be due to commingling of animals from various sources, or factors such as transport increasing stress levels potentially exacerbating latent disease conditions (Duncan, 1990; Barrington et al., 2006).

Agistment of stock (i.e. the housing/feeding of animals on pasture for payment) for other farmers was not an uncommon practice. Approximately two-thirds of the agisted stock were sheep, and whilst sheep do not transmit many cattle diseases, pathogens such as *Salmonella dublin* and conditions such as malignant catarrhal fever can potentially be transferred between

these species. Most of the agisted animals originated within the same county or neighbouring areas of Wales. Sending sheep from upland farms to lowland farms to be away-wintered has been a common farming practice over the past 150 years in Scotland and Wales (Jones, 1946); however it is difficult to find any recent studies investigating this practice and the potential risks associated with it. In addition, as part of the initial Countryside Stewardship Scheme (pre-2003) developed for upland farms, producers were financially supplemented to away-winter ewes in order to regenerate/enhance heather moorlands (MAFF, 1999). There is evidence, however, that pathogen transmission occurs between farms due to away-wintering of sheep (DEFRA, 2005). Disease transmission risks associated with practices such as these require further investigation, particularly if it is to be a recommended practice through agricultural schemes.

Almost half the farmers shared equipment with other farms. Equipment sharing was more likely to occur between neighbours, but sharing also occurred with farms outside the study area. The network of farms connected through shared equipment was fragmented, with farms linked in small components only. Importantly, tractors were the most commonly shared item, and farmers reported that tractors were most frequently used for waste handling and feeding. Therefore, application of appropriate biosecurity measures may be an important process in limiting transmission of pathogens via such contacts. Most farmers who borrowed equipment chose to clean and disinfect items only before returning them, suggesting that the cleaning process may have more to do with other factors (such as politeness) than concern over biosecurity. It is documented that contamination of equipment with mucus, faeces and blood can harbour organisms such as *Salmonella* and *Mycobacterium* species; it is recommended that borrowed or hired equipment should be cleaned and disinfected before it is used (Caldow et al., 1998), many producers in our study area did not appear to undertake this practice.

The number and frequency of companies and contractors visiting farms in this area was substantial, suggesting that a median farm would have (on average) more than two visits per day by personnel from an external contractor or company. Similar to the animal movement networks, the networks arising through contact with specific companies and contractors exhibited considerable heterogeneity. Some demonstrated a hub-and-spoke pattern, where a single contractor or company contacted many farms within the study area. Others had a more fragmented pattern, with each company or contractor contacting one or a few farms in the region. These differing patterns are likely to reflect both the geographical range of the companies' and contractors' activities and the differing number of farms they attend.

Cluster analysis suggested 3 farm categories on the basis of company and contractor usage. Broadly, this classification system divided farms according to enterprise (dairy, suckler and so on) and farm size. Farms in CC2 (corresponding to dairy and beef fattening farms) tended to have a more positive attitude to biosecurity, compared to those in CC1 (dairy, beef suckler and store cattle farms). The farmers with the least positive attitudes to biosecurity (CC1) were those most likely to be visited by government veterinarians and Trading Standards officers; whilst those with a more positive attitude tended to be visited by fewer types of external companies and contractors. The cause of these apparent relationships is unknown and the reasons for these associations require further investigation.

Classification of farms is difficult due to the varied enterprises that may be undertaken on a single farm and to the differences in individual management practices. In this study, we have used cluster analysis to classify farms (or farmers) according to three themes; methods of moving animals, use of companies and contractors and attitudes to biosecurity practices. We believe that this approach may provide useful insight into farming in the UK (and elsewhere); it

may help, for example, to inform strategies for interventions to improve biosecurity standards or to develop categories of farm type for refinement of mathematical models of pathogen transmission.

Most farmers in this study area employed people to work on their farms; many of these employees also worked on other farms and/or kept cattle of their own. This finding is in keeping with the current socio-economic trends in the farming community; according to DEFRA there has been a long-term downward trend in the agricultural workforce, the volume of paid labour has dropped by 48% since 1984 with a shift towards part-time work (MAFF, 1998). Although this movement of people for work may aid dissemination of ideas and innovation throughout the farming community, people may act as fomites, particularly when minimal biosecurity is performed. In a previous study, Dutch dairy farms that employed temporary workers who worked on other farms were 3.3 times more likely to be BHV-1 (Bovine Herpes Virus 1) positive (van Schaik et al., 1998).

The use of additional farms or land parcels affects the potential for farms to be in direct contact with other farms, and may increase the geographic range of this contact. In this study, half the farms had additional premises for keeping stock and the majority of these had only one additional premise. Regardless of whether producers possess official documentation (e.g. sole occupancy authority, linked premise status) to permit less restrictive movements between their holdings, all premises are required to keep on-farm records of animal movements between all of their holdings (DEFRA, 2006). It is evident from our findings that many of the producers (particularly those with only one additional premise) are not maintaining on-farm records of these movements. We suggest that this could be due to confusion regarding the recording requirements. Furthermore, in our study several of the farms had additional premises adjacent to their main holding, sometimes only separated by a gate. These were often managed as a single unit, potentially making it more unlikely that movements would be recorded. These findings suggest that effort needs to be made to inform farmers with regard to their obligation to maintain up to date records and that further classification of 'movements' between main and additional premises are required.

The potential for transmission of pathogens across farm boundaries depends on many factors, including the type of perimeter fence existing between farms and stock concentrations on neighbouring farms. Prevention of nose-to-nose contact across farm boundaries has been widely recommended as a means of improving herd biosecurity (Duncan, 1990; SAC, 2002). In the current study, while many boundary fences perceived to prevent contact actually did so, nose-to-nose contact was possible between animals in adjacent farms in more than half. In most cases this contact was possible over a relatively small proportion of the total length of the fence. The effect of these contact points on the potential for disease transmission will depend on the proportion of time animals spend at fence lines and their behaviour during this time which requires further investigation. However, it is likely that such contact points reduce the efficacy of these fences in terms of prevention of transmission of pathogens. This may diminish the potential return for producers after substantial investment in their construction and maintenance.

Whilst contiguous neighbours are clearly linked via common boundaries and general proximity, such farms are also more likely to share other contacts, such as equipment sharing, farm-farm animal movements and social interactions. This suggests that contiguous and local contacts are multi-dimensional. Some of these relationships may be expected; farms that are contiguous are probably more likely to establish social relationships, facilitating sharing of equipment and potentially transmission of infectious agents via vehicles and personnel. In

addition, information regarding sale prices and recommendations of stock from particular sources may be communicated within these social groups. Hence, contiguous farmers may be more likely to use the same markets and dealers as trading partners. Whilst the role of different contact mechanisms in pathogen transmission is pathogen specific, disentangling the components of "local contact" may suggest specific interventions to reduce transmission via this otherwise undefined mechanism.

The large proportion of producers undertaking other enterprises is a reflection of the current trend within the farming community. The percentage of producers with alternative incomes in our study (64%) is similar to that reported in the United Kingdom in 2004 when it was shown that 56% of full-time farmers in England had diversified (DEFRA, 2004). Producers are being encouraged to diversify with financial incentives (e.g. stewardship schemes); approximately 60% of farmers identified extra income as the main reason for diversification in a recent study (Sharpley and Vass, 2006). Producers have also sought out alternative farm enterprises because of the socio-economic decline in rural areas and food scares due to BSE and FMD (Sharpley and Vass, 2006). However, such diversification may complicate application of biosecurity practices due to the increased need to move animals and/or equipment, or the increased access to the farm by people.

In conclusion, contact between farms on a local scale demonstrates considerable heterogeneity; variation exists between farms, between contact types and in the structure of the networks arising through these contacts. Such variation may impact on the farm-level risk of pathogen transmission. Despite this, there have been few investigations addressing these issues. This study involved a relatively small number of farms in a single region; further information is required to extend these results to the wider UK farming community.

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INFECTIOUS DISEASE MODELLING AND CONTROL

ESTIMATION OF PARAMETERS OF A META-POPULATION MODEL OF THE SPREAD OF EQUINE INFLUENZA

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SUMMARY

Within farm, or training yard models for the transmission of equine influenza (EI) have been used to explore optimal vaccination policies and the impact of antigenic drift on spread and control of the infection. However, the deficit of these approaches is that they ignore between-group spread. A meta-population model has been developed that can take into account important factors for transmission, including the demographic and spatial distribution of populations of horses in yards and realistic contact patterns. For this, a vector approach has been used to extend previous SEIR models of EI. This approach allows the scaling up of the models from one yard to a multi-yard scenario, allowing epidemic modelling at a site level. However, these changes lead to an increase of complexity in the model. This paper addresses the problem of parameter estimation from sparse field epidemiological data. The difficulty of this problem arises from two issues, on one hand the increase in the number of parameters to estimate, on the other hand, the increased scale of such models means that the collection of epidemiological data to estimate these parameters is much more difficult to achieve and that these data –when they exist- are inevitably sparse.

This paper uses a set of data, collected by the Animal Health Trust during the 2003 EI outbreak in Newmarket, to fit the parameters of a multi-yard model of the Newmarket site. The fitting of the model allows the derivation of important epidemiological parameters such as the local effective reproductive ratios for the different yards and a comparison of the changes in the propagation of influenza when scaling up from a single yard population to a multi-yard scenario. Using a kernel with two-level of mixing λ_G (global rate; between yards) and λ_L/N_i (local rate; inside yards), the estimation results in values of λ_G=2.46x10^{-3} and λ_L=1.06, which gives a range of values for local effective reproductive ratios between 1.32 and 2.33. This shows that taking into account the structure of the population in groups changes significantly the way a unique R_0 as given by previous models is regarded. This work does not contradict these previous studies, but gives a refined image of what happens during epidemics, splitting the in-yard and the between-yard contributions to the spread of epidemics.

Finally the paper discusses the relevance of the application of the same methods to the study of other datasets and the possibility of using other methods to fit the same type of models. This work is important because it paves the way toward the development of more sophisticated models which could take into account the evolutionary aspects of the virus propagation.

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INTRODUCTION

Equine influenza, like all mammalian influenza viruses is transmitted by close contact, including via aerolisation. Transmission at the population level is therefore determined by contact patterns within and organization of the host population. These vary markedly between species (e.g. pig, horse, man). The work presented here involves development of meta-population models to represent the transmission of equine influenza. Important drivers of transmission in horse populations include the demographic structure and horse movements, due to exercises and competition, as well as sales or purchases. Such models are useful in the development of risk assessments and design of optimal vaccine policies. Current models for equine influenza have been largely restricted to the study of single yards, although dynamics of between-yard transmission are obviously extremely important to understand larger scale influenza outbreaks.

The aim of this paper is to present a meta-population model of the spread of equine influenza based on data from an outbreak that occurred in 2003 in Newmarket, UK. For this, the paper starts by presenting the data and the structure of the model. It then describes some methods to estimate the parameters from the model and present the results for the 2003 outbreak. Finally, there is a discussion of possible developments arising from these results.

MATERIALS AND METHODS

Data

This paper focuses on the 2003 outbreak in Newmarket described by Newton et al. (2005). The available data come from various sources, including trainer questionnaires, Raceform "Horses in Training 2003", and tests and samples taken during the epidemic by veterinary surgeons from the Animal Health Trust in Newmarket. In particular, some serological data giving antibody levels for some of the yards are available with additionally some estimation of the total number of infected horses in some yards at the end of the epidemic. The dates of the first case in each contaminated yard have also been recorded.

Taking all data sources together, there is a reasonably precise description of the situation in Newmarket at the start of the outbreak and at the end; nevertheless, the temporal evolution of the spread of the disease is poorly described.

The model

Description: The previous models of the spread of equine influenza (Glass et al., 2002, Park et al., 2003, 2004) were based on versions of the classical SEIR model. The SEIR model splits the population of horses in four categories, S for susceptible animals, E for exposed, I for infectious and R for recovered or removed. Individuals then move from one category to another with rates depending of the assumptions of the model. The transitions are usually considered as being exponentially distributed, mainly to simplify the mathematical side of the problem, though other distribution are in many aspects often more appropriated especially concerning the distribution of the infectious period (e.g. gamma or empirical distributions (Park et al., 2004)).

Such modelling is justified provided one accepts two main assumptions:

- "Well mixing" of the population, meaning that a horse has the same probability of making an infectious contact with any other horse in the population;

- Homogeneity of the population, meaning that any horse reacts in the same way toward the infection.

In the case of a large outbreak as in 2003 in Newmarket, it is no longer possible to rely on these two assumptions, as the fragmentation of the population, the difference in group sizes and levels of protection induced by vaccination in the different yards clearly played a role in the way the epidemics developed. To tackle this issue, a model was designed in which animals in the epidemic were grouped in "patches", which can be animals from the same yard or animals with the same vaccine history etc. Then, the S, E, I and R populations break down into vectors $S=(S_i)_{0<i\leq n}$, $E=(E_i)_{0<i\leq n}$, $I=(I_i)_{0<i\leq n}$, and $R=(R_i)_{0<i\leq n}$, where the index i defines populations belonging to the same patch.

The way in which an exposed animal becomes infectious or an infectious individual recovers remains the same as in the previous models (see Park et al., 2003), however the way the animals 'mix' now has to be considered i.e. the structure of infectious contacts between animals in different yards. For this the matrix $T=(T_{ij})$ is defined with $0 < i,j \leq n$ which summarizes how the animals mix between each other. The different infectious rates are then given by $\Lambda=(\lambda_i)_{0<i\leq n}= TI$, meaning that the infectious pressure on a yard is a linear combination of the infectious horses all around on the site weighted by the intensities of contact between this yard and each of the other yards. The transition rates of the models are summarized in table 1.

Table 1. Transition rates for the meta-population model.

Transition From	To	Rate
S_i	E_i	$\lambda_i S_i$
E_i	I_i	$a_i E_i$
I_i	R_i	$g_i I_i$

The values of a_i and g_i depend on whether the population is vaccinated or not in the patch i, $1/a_i$ =2.52 days and $1/g_i$=2.48 days for vaccinated populations and $1/a_i$ =1.75 days and $1/g_i$=4.8 days for unvaccinated populations (Park, 2003). In the rest of the study, the population of horses is assumed to be vaccinated and each patch i is considered to correspond to a single yard.

Cases covered by the model: The main advantage of this model is its versatility, indeed, depending of the dimensionality of the model and of the value of the matrix T, the present model can cover a large class of existing models.

- With n=1 and $T=\beta/N$, the model is the classic one-yard SEIR model as used by Glass et al. (2002) and Park et al. (2003). The difference between Glass' model of transmission and Park's model of vaccinated transmission is first in the parameter values, second in the initial conditions of the model. In Glass' model, all horses are assumed to be susceptible to catch the virus, whereas in Park's model, depending on the antibody levels of the horses, a fraction of the population seroconverts when catching the virus, which is translated at the modelling level by a fraction of the horses being considered recovered (removed) horses at the beginning of the simulated epidemics.

- With n=2 and

$$T = \beta \begin{pmatrix} (1-\mu)/N_A + \mu/(N_A+N_B) & \mu/(N_A+N_B) \\ \mu/(N_A+N_B) & (1-\mu)/N_B + \mu/(N_A+N_B) \end{pmatrix}, \quad (1)$$

the model becomes the two-yard model designed by Park et al. (2003) to assess the risk of transmission from one yard to another.

- With any n and

$$T_{ij} = \begin{cases} \lambda_L + \dfrac{\lambda_G}{N} & if \quad i = j \\ \dfrac{\lambda_G}{N} & else, \end{cases} \quad (2)$$

the model is the double mixing household model with latency period. Such a model (in its SIR version i.e. with no latency period) has been extensively studied by Ball et al. (1997).

Methods used to estimate the parameters of the model

The probability that a horse becomes infected when challenged with the virus depends on its pre-exposure antibody level. Park et al. (2003) showed that this can be modelled by considering a proportion of the initial population of horses to be removed. The susceptibility level α_i giving the number $S_i = \alpha_i n_i$ of initially susceptible horses then depends on the distribution of antibody levels in yard i and of the homology or heterology of the challenging strain with the strain in the vaccine (Mumford et al., 1983). Comparing the data of the affected yards with the actual infection, a threshold was derived at which, horses with an antibody level (measured by single radial haemolysis (SRH)) below are considered to be susceptible and horses with an antibody level above are protected.

Local transmission: Local transmission parameters are the diagonal coefficient T_{ii} of the matrix T (see Eq.(5)) in the model. To estimate them, horses inside yards are assumed to make contacts at the same rate, and the difference between the yards concerning the size of epidemics is then assumed to be due to the differences in the level of protection and in purely stochastic effects. It is assumed that sub-epidemics inside yards are mainly driven by internal transmission following a mass action law and are independent. It is then in theory possible to derive, knowing the level of protection of the yard, the probability than an epidemic of a certain size occurs as a function of the transmission rate. This can be done through the recursive resolution of a set of triangular equations (see for example Andersson & Britton, 2000).

Unfortunately, the evaluation of these probabilities –even for relatively small values of n- numerically breaks down because of the appearance of extremely small terms (beyond the precision of standard programming) which can lead to the appearance of negative probabilities. Therefore, the solution chosen was to estimate these probabilities through repeated simulation.

If $P_{N,Z,\alpha}(\lambda_L)$ is the probability that an epidemic of size Z occurs in a yard of size N with a level of susceptibility α and transmission rate λ_L, then the probability that a set of yards with known sizes N_i and known levels of susceptibility α_i endure sub-epidemics of different sizes Z_i is

$$P(\lambda_L) = \prod_{i=1}^{n} P_{N_i,Z_i,\alpha_i}(\lambda_L) \tag{3}$$

and then λ_L^{est} can be estimated as being

$$\lambda_L^{est} = E(\lambda_L) = \frac{\int_0^\infty \lambda_L P(\lambda_L) d\lambda_L}{\int_0^\infty P(\lambda_L) d\lambda_L} \tag{4}$$

Global transmission: To be able to estimate the global transmission (i.e. between training yards), the simplifying assumption is made that global transmission is purely random and does not depend on the two yards involved, and can thus be considered as being a constant λ_G. The contact matrix can then be written as

$$T = \begin{pmatrix} \frac{\lambda_L}{N_1} & \lambda_G & \cdots & \lambda_G \\ \lambda_G & \ddots & \ddots & \vdots \\ \vdots & \ddots & \ddots & \lambda_G \\ \lambda_G & \cdots & \lambda_G & \frac{\lambda_L}{N_n} \end{pmatrix}. \tag{5}$$

Using the actual demographic data of the site, and the actual level of susceptibility (estimated to be on average 71%) the probability $Q(\lambda_G)$ (estimated through repeated simulations) that the final size of the epidemic is between 24 and 25% of the total population is plotted (the estimated number of infected horses at the end of the epidemics is 24.44% of the population) and λ_G^{est} can then be estimated as being

$$\lambda_G^{est} = E(\lambda_G) = \frac{\int_0^\infty \lambda_G P(\lambda_G) d\lambda_G}{\int_0^\infty P(\lambda_G) d\lambda_G} \tag{6}$$

RESULTS

Estimation of the antibody protection

The protection threshold has been set to 200 mm^2 (SRH). At the start of the simulations, any horse below this threshold is considered susceptible and any horses above as removed. It should be noted that two yards (3 and 11, marked in grey in Table 2) contain a number of susceptible

horses that is lower than the final number of infected horses, which by definition should be impossible. The reason for this is that the SRH study measured the level of antibody in the horse blood in the very early stages of the epidemic assuming that the measured antibodies are from vaccine origin. However, for some of the early-infected yards, the blood samples had been taken after the infection had already reached them, giving an artificially high level of protection (low value of α). These have therefore been removed from the data used to estimate λ_L^{est} .

Estimation of the local transmission

$P(\lambda_L)$ is plotted in Fig. 1 as given by Eq. (3) for the nine yards for which an estimation of the final size of the sub-epidemics and of the initial level of protection by pre-exposure antibodies (Table 2) were available at the same time.

Table 2. Size, estimated proportion of infected horses, estimated proportion of initially susceptible horses for 11 yards affected by the 2003 outbreak.

Yard	n	Z/n	α	R_0^i
1	32	0.5	0.5	1.32
2	83	0.469	0.83	2.19
3	103	0.757	0.57	-
4	141	0.528	0.677	1.7
5	70	0.188	0.823	2.17
6	19	0.882	0.822	2.33
7	19	0.824	0.882	2.33
8	190	0.703	0.861	2.27
9	32	0.8	0.882	2.33
10	41	0.414	0.8	2.11
11	25	0.778	0.667	-

Computing Eq. (4) gives an estimated value of the inter-yard transmission λ_L^{est} of 1.065. It is then possible to deduce the local effective reproductive ration $R_0^i = \alpha_i \lambda_i^{est} g_i$ for each yard.

In Fig. 1, $P(\lambda_L)$ can be seen to be very small with maximal values around $5\text{x}10^{-17}$. The reason for this is that the event giving exactly the measured final distribution is an event with extremely small probability among a large space of possible final states. It is not its precise value which is of interest, but rather its distribution given the different values of λ_L.

Estimation of the global transmission

Plotting $Q(\lambda_G)$ in Fig. 2 and computing Eq. (6) gives an estimated λ_G^{est} of $2.46\text{x}10^{-3}$.

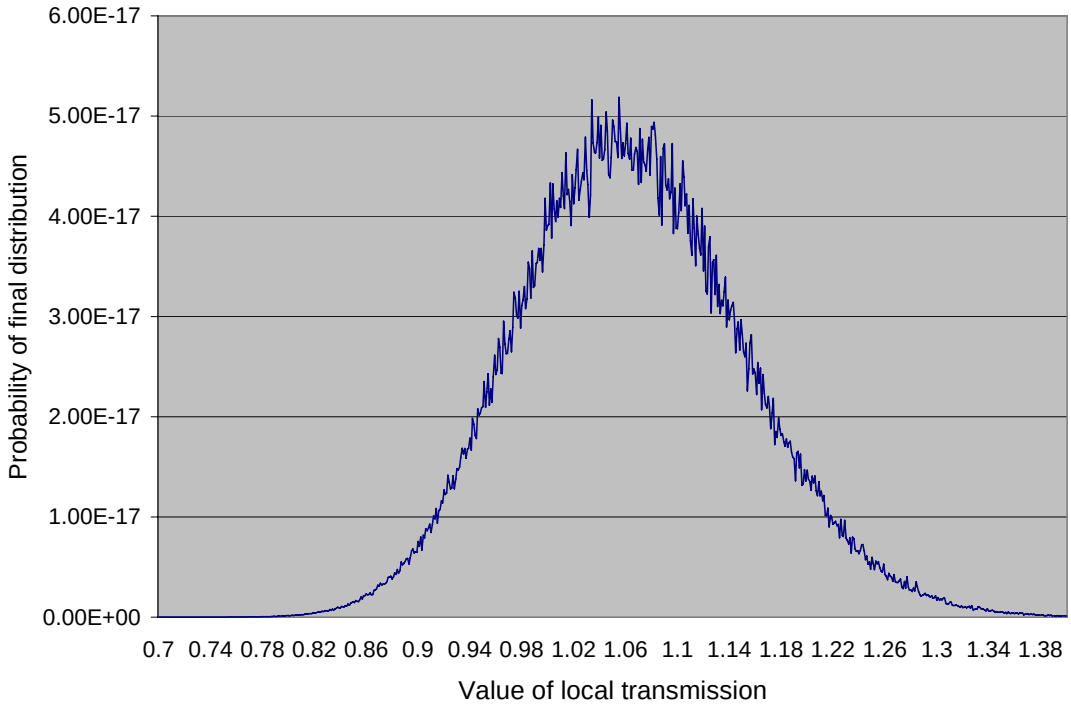

Fig. 1 Probability than sub-epidemics with the same sizes than given by the data occurs as a function of the local (inside-yard) transmission.

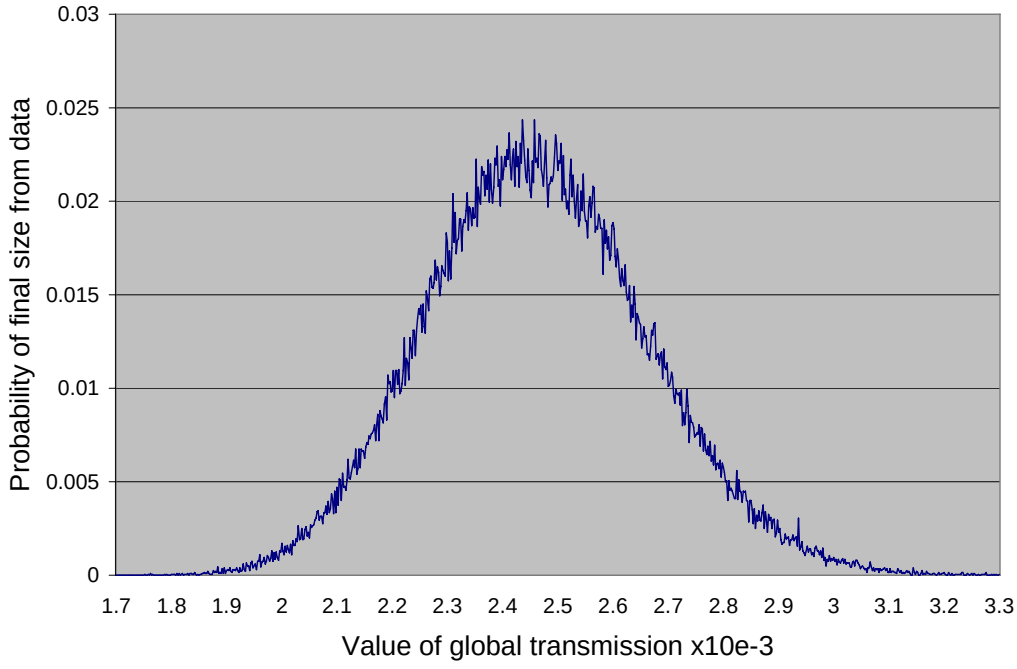

Fig. 2 Probability than an epidemic evolving between 24 and 25% of the horses as a function of the global transmission

DISCUSSION

In the previous sections a model has been developed for the spread of equine of influenza at a site level and some relevant parameters have been estimated from actual data. However, to facilitate the parameterisation of the model two simplifying assumptions were used:

1. the sub-epidemics are independent;

2. the transmission between yards is purely random and does not depend on the two yards involved.

Assumption 1, is justifiable by the fact that the between-yard contact rate is much lower inside yards. Nevertheless, this assumption would tend to slightly over-estimate λ_G^{est} and may not be possible to state for other diseases. One solution would be to use Bayesian inference as done by Demiris & O'Neill (2005) for models of the spread of influenza in human populations. Though promising, this approach, based on representations of epidemics by graphs, would require major modifications. Indeed, the protection yielded by vaccination would need to be included, and, in general this approach would to be less amenable to further developments (e.g. incorporation of geographical data).

Assumption 2 is unlikely to be accurate, but serves to reflect the difficulty in finding clear patterns from the analysis of only one epidemic. A version of the same model is being developed with a contact kernel taking into account the geographical distances between the yards. Though the distance between yards is highly likely to play a role in the probability of a transmission when one yard is infected, there are a large number of other yard-dependent parameters that can be found: sharing of common facilities as gallops, yards being close to the pathways taken by horses to go to these gallops, transmission via visiting veterinary surgeons etc. The contact matrix approach used, would be enhanced by drawing on studies of networks of contacts in the equine world.

One of the interesting results to arise from this model and its confrontation with actual data is the way in which it changes the classical interpretation of the threshold parameter R_0. The basic reproductive ratio R_0 (or any of its variations) is usually viewed as an indication of the possibility that large epidemics take off in population. Generally, if $R_0>1$ large epidemics can take off, if $R_0<1$ they will fail to spread. R_0 is thus the cornerstone of a modelling generated approach to vaccine policy. The vision sketched by this model is slightly different, instead of a unique number, there are set of local numbers depending on the local vaccination policies. The study of the impact of the quality and variability of vaccination on the risks of epidemics in a network of yards should provide valuable results for policy makers.

Finally, the observed pattern (only relatively big sub-epidemics, no "micro-sub-epidemics") is theoretically unlikely. When entering a yard via contamination of a single individual, a viral infection with a transmission rate of the order of the one measured is likely to sometimes fade out without yielding more infections. It is very likely that a lot of micro-sub-epidemics occurred, and were not detected as a consequence of their small sizes. The fact that the infection in vaccinated horses can be sub-clinical does not help to detect such events. The development of models such as this one can hopefully help to identify the relevant data to collect to understand the dynamics of epidemics.

CONCLUSION

This works give an interesting picture of how an epidemic behaves at a site level. In addition to the ability to test different vaccination scenarios and understand the impact of varying quality of this vaccination, this work additionally paves the way toward the development of models that take into account the evolution of the influenza virus (drift). With such models it may be possible to understand the transformation compulsory vaccination has produced and what makes the phylogenicity of the equine and humane lineages different. (There are two sub-lineages co-evolving at the same time for EI, but only one for human influenza).

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FOOT-AND-MOUTH DISEASE MODEL VERIFICATION AND VALIDATION THROUGH A FORMAL MODEL COMPARISON

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SUMMARY

Three foot-and-mouth disease spatial simulation models were compared: *AusSpread* (Australia), *Interspread Plus* (New Zealand) and the North American Animal Disease Spread Model, *NAADSM*. The comparison included: (1) an evaluation of written model descriptions, and (2) 11 scenarios testing spread mechanisms and control measures. The following were compared for all scenarios: descriptive statistics of selected outputs, temporal spread, the size of predicted infected areas and spatial agreement of predictions.

Despite the different approaches used in model building, the three models produced very similar results in most scenarios. All models were improved as a result of this comparison: it identified programming errors and assessed the impact of certain programming decisions. The results show that code verification and validation are critical steps in model development. A comparison of different models should be an important step towards gaining model credibility.

INTRODUCTION

As part of a process designed to improve the ability to deal with animal disease emergencies, the Quadrilateral (QUADS) countries (Australia, Canada, New Zealand, and the United States) held a workshop on foot-and-mouth disease (FMD) modelling and policy development in Canberra, Australia in March 2005. The objectives of the workshop were to present policy makers with disease simulation models developed for contingency planning in the QUADS countries and to review the current status of FMD response strategies. A key outcome of the workshop was the creation of a technical group comprised of epidemiologists from the QUADS countries, Ireland, and the United Kingdom. Following the workshop the technical group developed a work program which included a formal comparison of the three FMD simulation models being used by the QUADS as a priority item.

Models for FMD are developed to support decision-making by being used as contingency planning tools to evaluate various outbreak scenarios and determine optimal control strategies. In order to use outputs from such models, decision makers must have confidence in a model's predictions. The process of model verification and validation seeks to provide such confidence. Verification is defined as the process that ensures that the logic, formulae, and computer code of the model correctly reproduce the logical framework conceived by the model designer (Taylor,

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2003; Sargent, 2005). Validation ensures that the model provides an adequate depiction of the process it is designed to represent (Taylor, 2003; Law, 2005). Simulation models of disease in human and animal populations will always be an approximation of what might (or has) occur(ed) in reality, no matter how much time and money is spent on design and implementation (Law, 2005). Regardless, the validity of a model, when used for the purpose it was designed for, should be assessed (Sargent, 2005).

Unfortunately, a formal approach to infectious disease model validation does not exist. There is no set of specific tests that can easily be applied to determine the "correctness" of a model (Sargent, 2005). Law & Kelton (1982) proposed a three step approach: (1) develop a model with high face validity — a model which, on the surface, seems reasonable to people who are knowledgeable about the system under study; (2) test the assumptions of the model empirically; and (3) determine how representative the simulation output data are. The three modelling groups involved in the QUADS are in the process of or had already taken various steps to verify and validate their models with efforts such as sensitivity analyses, expert reviews of assumptions (Dubé et al., 2004) and comparison of model outputs with real FMD outbreak data (Stevenson et al., 2003). While comparison of model predictions with real outbreak data remains the ideal means for testing model validity, a demonstrated level of agreement between independently developed models using identical data should provide policy makers with reassurance of the consistency of assumptions made by model developers. Conversely, differences in model output provides a means for highlighting differences in assumptions that need to be resolved by modellers and researchers, and provides a better focus for future research.

This paper provides a description of the data used for the QUADS model comparison study arising from the Canberra workshop, the scenarios tested, and a comparison of the results obtained.

MATERIALS AND METHODS

The selected approach was to construct a hypothetical population of farms and then to simulate the spread of FMD among this population following the introduction of virus into a single farm, using each of the FMD simulation models used by the QUADS countries. The models compared were *AusSpread* (Garner & Beckett 2005), *InterSpread Plus* (Sanson, 1993; Stevenson et al., 2006), and *NAADSM* an enhanced version of the Spreadmodel (Schoenbaum & Disney, 2003), which are referred to as the Australian, New Zealand, and North American models, respectively, in the remainder of this paper. A series of 11 outbreak scenarios were developed to evaluate key areas of disease transmission and control. The following sections describe the data, the features of the scenarios simulated, the key features of each of the three models, and the analytical approach that was taken to evaluate model agreement.

Data

A population of 6000 farms was constructed: 3960 of these were defined as cattle and 2040 were defined as swine enterprises. The median number of cattle per farm was 163 (minimum 2, maximum 990); the median number of pigs per farm was 858 (minimum 48, maximum 4892). Enterprises were comprised of single animal types: there were no farms containing a mix of animal species. Farms were distributed in a circular study area of 500 km radius with some clustering (Fig. 1).

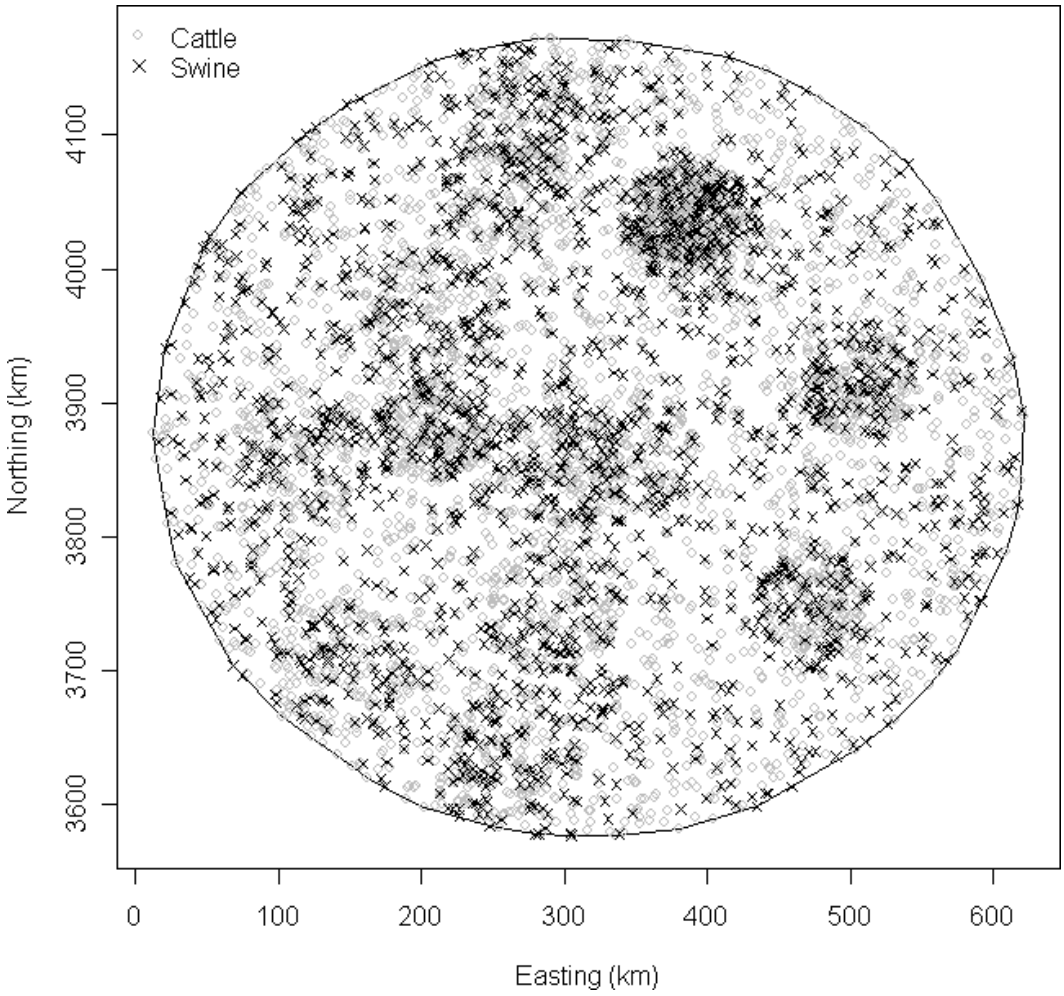

Fig. 1 Map showing the boundary of the study area and the location of the hypothetical population of units

Scenarios

Five categories of scenarios were defined: (1) those where the type of disease transmission among farms was varied, scenarios 1 – 3; (2) those where the nature of direct and indirect contact was varied, scenarios 4 – 6; (3) those where movement controls were applied, scenario 7; (4) those where vaccination was applied, scenarios 8 and 9; and (5) those where depopulation was applied, scenarios 10 and 11. The key features of each of the 11 scenarios are outlined in Table 1. The intent was to devise scenarios of increasing complexity and in many instances (particularly for the first category of scenarios) extreme values were specified for model parameters so that the expected outcome could be easily predicted and easily verified. In the later scenarios more realistic model parameters were specified.

The simulation exercise commenced in May 2005 and concluded in September 2006. At the start of the exercise each modelling group nominated the version of their model which was to be used for each of the outbreak scenarios for comparison. A written description of the models was provided to the Canadian team members so that a common set of parameters for testing could be derived. For each scenario, epidemics were initiated from a single infected farm located in the centre of the study area. Epidemics were simulated in 1-day time steps from incursion for a period of pre-determined number of days and each simulation was repeated 40 times to generate

a distribution of predictions. Scenarios were simulated by each modelling group in sets, according to the categories described above. Outputs from simulations were submitted to the Canadian team members who provided summaries to the rest of the group in terms of descriptive statistics of the predicted numbers of infected farms. Where the results of a simulation for a model varied substantially from the other two models, clarification was sought from the respective modelling team regarding the possibility of any misinterpretation of the specified input parameters for that scenario. When this occurred, the scenario was re-simulated and the outputs from the second simulation submitted for comparison. In most cases, single runs of simulations were carried out by each team.

Table 1. Details of the key features of the 11 outbreak scenarios. All scenarios simulate spread among cattle herds only.

Scenario	Disease [a]	Spread			Control			Duration (days)
		Direct	Indirect	Air	Movement	Vaccination	Depopulation	
1	1	+[b]	-	-	-	-	-	300
2	1	-	-	+[e]	-	-	-	200
3	1	+[b]	-	+[e]	-	-	-	200
4	1	+[c]	+[d]	-	-	-	-	100
5	1	+[c]	+[d]	-	-	-	-	115
6	2	+[c]		-	-	-	-	150
7	2	+[b]	-	-	+	-	-	200
8	2	+[b]	-	-	+	+[f]	-	200
9	2	+[b]	-	-	+	+[g]	-	200
10	2	+[b]	-	+[e]	+	-	+[h]	75
11	2	+[b]	-	+[e]	+	-	+[i]	75

[a] Disease states 1: Latent (triangular 29-30-31 days), infectious (triangular 59-60-61 days), immune (triangular 99-100-101 days). Disease states 2: Latent (triangular 1-3-10 days), infectious (triangular 3-12-35 days), immune (triangular 180-240-360 days).
[b] Shipment rate = 1 per day, distance = triangular 10-20-30 km, probability of infection =1
[c] Shipment rate = 1 per day, distance = triangular 4-5-6 km, probability of infection = 0.5.
[d] Shipment rate = 2 per day, distance = triangular 140-150-160 km, probability of infection =1.
[e] Wind direction = 225º - 315º. Probability of infection at 1km = 0.98, maximum distance of spread = 50km.
[f] Small rings (5 km around identified infected units).
[g] Large rings (35 km around identified infected units).
[h] Stamping-out and pre-emptive depopulation using smaller rings (10 km around identified infected units).
[i] Stamping-out and pre-emptive depopulation using larger rings (30 km around identified infected units).

Model descriptions

Each of the three models differ in the information that defines the unit of interest, that is a herd or flock of animals resident at a single location. All three models record a unit's location - they are all spatial models. In the case of the Australian and New Zealand models, a unit's position in space can be represented by either a point or a polygon (the farm boundaries); in the North American model a unit's location can only be represented by a point. All three models allow a descriptor variable to be applied to each unit, which is typically used to define enterprise type (e.g., "dairy," "beef fattening," or "mixed"). All three models specify a unit size in terms of number of animals. In the Australian and New Zealand models this count may be broken down by species; in the North American model the count is simply a total.

Each of the three models is a state-transition models (Isham, 1993) meaning that units move between the states of susceptible, infected and removed throughout the simulation period. All three models have a susceptible state. The infected state is described as follows by the models: the Australian model has two states, latent and infectious representing whether units are able to spread an agent or not. The New Zealand model also has two states representing the presence or absence of clinical signs (infected or clinical) where the option exists to allow infectiousness to be present in both states. The North American model has three states: latent, infectious-subclinical, and infectious-clinical with the possibility of allowing spread from all states or a subset of states.

The choice of states is tied to other mechanisms in each model. States representing a unit's disease status influence disease spread. States related to the appearance of clinical signs influence detection of disease (and therefore how quickly control measures are implemented). In addition to the disease states, the Australian and New Zealand models use a lookup table to define the level of infectiousness within a unit over time. This gives an ability to simulate within-unit prevalence of disease. The North American model does not currently provide this facility and as a result, the scenarios used in this study specified constant infectivity over time.

The removed state is described as follows by the models: the Australian and New Zealand models have immune and dead (depopulated) states, whereas the North American model has naturally immune, vaccine immune, and destroyed states. In each of the three models the duration of the diseased and immunity states are defined by the user via probability distributions.

The parameters for each scenario developed in this project were based on the common features of all models: a group of animals as a unit in the simulation, a point location, production type and number of animals for each unit, and five possible states: susceptible, two infected states (latent, infected/clinical), immune and dead/destroyed. All three models provide a range of options for simulating disease spread. The Australian model allows disease spread to be based on either a user-specified dissemination rate, a "pathway" model (including direct and indirect contact, local spread, spread through saleyards and airborne spread), or a combination of the two. The New Zealand model allows user-defined movement types to specify direct and indirect contacts in addition to sequential routes that can spread infection (*e.g.*, dairy tanker routes), special locations that can spread disease intermittently (*e.g.*, saleyards, fairs, rodeos), airborne spread, and a type of short-distance spread termed "local spread." The North American model has direct and indirect contact mechanisms resembling those found in the Australian and New Zealand models, plus airborne spread which can also be used to simulate "local spread."

The models share a basic set of disease spread mechanisms: direct contact, indirect contact, and airborne spread. Direct contact (the movement of animals between units) is handled in a similar way by all three models. The number of shipments out of a unit on a particular day is sampled from a (user defined) probability distribution, and the distance over which the shipment travels is sampled from another user-defined distribution. All three models include the notion that the rate and distance can be tied to the characteristics (*e.g.*, production type, presence of animals of a particular species) of source and recipient units.

The Australian model samples a shipment distance from a user-defined probability distribution, then samples a random value for the bearing (direction) of the shipment. It then searches for an eligible recipient in the vicinity of the chosen location and, if none can be found, will re-try (up to a fixed number of times) with a new distance and bearing. The North American model samples a shipment distance from a probability distribution and finds an eligible recipient (in any direction) whose distance from the source best matches the chosen distance. The New Zealand model uses the same approach as the North American model, but if a suitable recipient unit cannot be found at the distance selected it will allow a number of re-tries by sampling a new distance from the distribution.

Airborne spread differs considerably among the three models. The Australian model allows airborne spread exclusively from units containing pigs, and uses historical weather information to determine probabilistically whether conditions at a given location are suitable for airborne spread and the prevailing wind direction, with risk of infection for exposed units varying with type and distance down-wind . The New Zealand model allows the user to define a function for probability of infection versus distance from source, a probability distribution for wind direction on a given day, and infectivity and susceptibility parameters for each animal species. The North American model provides the simplest approach, allowing different infectivity and susceptibility for different production types, using a linear or exponential fall-off function for probability of infection versus distance from source, and using fixed limits on the wind direction throughout the simulation period. The North American model also amplifies a unit's probability of spreading or receiving infection for larger-than-average units, and reduces the probability for smaller-than-average units. For the purposes of this comparison, airborne spread was allowed among cattle units, using a linear falloff and using a fixed wind direction throughout the simulation.

Each model handles detection of infected units differently. In addition to passive surveillance or *ad hoc* reporting based on the presence of clinical signs (a feature found in all models) the Australian and New Zealand models also include the concept of active surveillance (spatially targeted visits around infected units by surveillance teams). Direct and indirect contacts from infected-detected units can be traced in all models. The models differ in how the parameters for detection based on clinical signs are entered. While a probability of detection by day since the appearance of clinical signs within a herd is included in the North American and New Zealand models, the Australian model uses an expected proportion of units, by production type, that will recognize and report clinical signs and a reporting interval in the form of a probability distribution. In addition, the North American model has a second probability distribution that represents the probability of reporting based on the number of days since the first unit was detected. This simulates the awareness of the presence of FMD in the community. For this study, a single probability distribution based on the presence of clinical signs was specified.

Control measures common to the three models are: quarantine, movement restrictions, stamping-out, pre-emptive slaughter of high-risk contacts and/or contiguous culling and ring vaccination. The Australian and New Zealand models also have the capability to include: disease control zones, trace investigations (forward and back) and targeted protective vaccination. The North American model includes only trace forward investigations and disease control zones are currently under testing. The availability of resources is accounted for in the three models.

Analyses

For each of the 11 scenarios the total numbers of infected units were natural-log transformed and a two-sided, one-way analysis of variance (ANOVA) applied to test for differences in the 40 outcomes for each of the three models. The physical boundaries of each outbreak area were determined by constructing a convex hull around those units predicted to become infected at the end of each simulation. The area of each convex hull was calculated and differences among the three models tested using the ANOVA procedure, described above.

Survival analyses were used to describe the temporal onset of infection among the population of farm units. Here, the outcome of interest was the infection date for case units. Units that were depopulated pre-emptively as part of specified control measures were right-censored on the day of depopulation. Units that remained free of infection throughout the simulation were right-censored at the end of the simulation. For each simulation day the median number of infected units (across the entire set of 40 simulations) was determined. Kaplan-Meier survival curves were generated to provide a summary description of the temporal onset of infection for each model. Differences in the distribution of time to event among the three models were tested using the log rank statistic.

To evaluate the level of spatial agreement in predictions a regular grid of cells 30 km × 30 km was applied to the study area (producing a total of 314 cells). Each model was considered in turn and for each simulation of a given scenario, cells were given a score of 1 if at least one unit within its boundaries was predicted to become FMD-positive and 0 otherwise. The mean cell score over 40 iterations was calculated and rounded to the nearest whole number. Fleiss' kappa was used as an index of agreement for the 314 cell scores for each of the three models. A score of > 0.8 is assumed to reflect almost perfect agreement (Dohoo et al., 2003).

RESULTS

This study involved the simulation of 11 outbreak scenarios by the three modelling groups. For brevity, we present descriptive statistics of each of the outcomes under comparison, indicating those outcomes and scenarios that differed significantly at the alpha level of 0.05. Descriptive statistics of the predicted number of FMD-infected units, the size of the predicted outbreak area, and the median number of days to infection for each scenario and model are shown in Tables 2, 3, and 4, respectively. Box and whisker plots showing the distribution of the predicted number of FMD-infected units and the size of the predicted outbreak areas for each scenario and model are shown in Figures 2 and 3. Kaplan-Meier survival curves showing the proportion of units that remained free of infection as a function of time for scenarios 1, 6, 8, and 10 are shown in Figure 4. Table 5 lists the Fleiss' kappa statistics for each of the 11 scenarios.

Table 2. Descriptive statistics of the predicted number of FMD-infected units for the 11 scenarios described in this study. Key: AU Australia; NZ New Zealand; NA North America.

Scenario	Model	Minimum	Q1	Median	Q3	Maximum
1 [a]	AU	1386	1499	1539	1596	1720
	NZ	1377	1461	1492	1560	1697
	NA	1298	1397	1444	1469	1573
2 [a]	AU	728	738	741	746	753
	NZ	733	744	749	753	770
	NA	662	676	681	684	695
3 [a]	AU	1315	1335	1346	1363	1384
	NZ	1305	1344	1354	1370	1422
	NA	1235	1264	1279	1300	1324
4 [a]	AU	75	91	97	102	114
	NZ	70	78	82	89	98
	NA	64	72	76	81	99
5	AU	341	415	460	503	607
	NZ	340	412	468	497	611
	NA	319	416	500	536	689
6 [a]	AU	3956	3958	3958	3959	3959
	NZ	3957	3958	3959	3959	3959
	NA	3957	3959	3959	3959	3959
7	AU	0	151	250	337	521
	NZ	0	135	202	250	398
	NA	0	180	268	308	601
8	AU	0	125	237	298	420
	NZ	0	173	227	320	482
	NA	0	191	230	265	369
9	AU	0	93	132	155	217
	NZ	0	102	129	151	220
	NA	0	98	133	155	205
10 [a]	AU	1395	1595	1714	1795	2052
	NZ	1656	1748	1803	1880	2091
	NA	1598	1781	1846	1902	2279
11	AU	617	724	796	880	1001
	NZ	575	706	793	889	1087
	NA	643	747	795	877	1055

[a]Predicted number of infected units differed significantly among the three models at the alpha = 0.05 level.

Table 3. Descriptive statistics of the size of the predicted outbreak area (× 1000 square km) for the 11 scenarios described in this study. Aus=Australia, NZ=New Zealand, NA=North American models.

Scenario	Model	Minimum	Q1	Median	Q3	Maximum
1 [a]	AU	97	102	103	106	111
	NZ	90	97	99	102	106
	NA	93	95	97	99	101
2 [a]	AU	45	47	48	48	50
	NZ	45	47	48	49	50
	NA	38	40	40	41	41
3 [a]	AU	88	91	92	94	97
	NZ	85	90	91	93	100
	NA	81	82	85	87	88
4 [a]	AU	73	74	74	75	76
	NZ	71	72	72	73	74
	NA	70	72	72	74	112
5 [a]	AU	210	256	259	263	269
	NZ	209	247	255	258	265
	NA	228	253	258	261	265
6	AU	282	282	282	282	282
	NZ	282	282	282	282	282
	NA	282	282	282	282	282
7 [a]	AU	11	26	37	47	65
	NZ	6	18	24	35	65
	NA	4	21	29	35	89
8	AU	9	21	35	47	70
	NZ	7	21	26	42	82
	NA	0	20	25	34	53
9 [a]	AU	10	15	19	26	38
	NZ	6	12	15	18	30
	NA	1	13	15	20	39
10 [a]	AU	27	126	134	141	162
	NZ	132	138	145	149	157
	NA	127	139	145	151	173
11	AU	47	61	70	78	93
	NZ	47	64	72	81	111
	NA	45	61	67	77	96

[a]Predicted number of infected units differed significantly among the three models at the alpha = 0.05 level.

Table 4. Descriptive statistics of the period that infection was active in the population (excluding the unit that initiated each epidemic) for the 11 scenarios described in this study. Aus=Australia, NZ=New Zealand, NA=North American models.

Scenario	Model	Minimum	Q1	Median	Q3	Maximum
1 [a]	AU	32	145	213	268	300
	NZ	31	143	211	267	300
	NA	30	142	208	266	300
2	AU	32	95	127	160	200
	NZ	31	93	125	158	200
	NA	30	92	124	159	200
3	AU	32	96	129	163	200
	NZ	31	94	127	162	200
	NA	30	93	127	163	200
4	AU	52	62	73	86	100
	NZ	51	61	72	85	100
	NA	50	60	71	85	100
5	AU	52	105	110	113	115
	NZ	51	105	110	113	115
	NA	50	104	109	112	115
6 [a]	AU	5	64	87	104	149
	NZ	4	61	80	95	150
	NA	2	57	75	90	142
7	AU	4	28	45	66	200
	NZ	4	26	39	55	199
	NA	3	31	47	72	200
8	AU	4	26	41	58	200
	NZ	4	32	47	75	200
	NA	3	29	41	58	200
9	AU	4	20	29	38	96
	NZ	4	24	33	41	162
	NA	3	23	32	40	87
10	AU	4	17	27	43	75
	NZ	3	20	31	47	75
	NA	2	20	31	48	75
11	AU	4	11	18	25	75
	NZ	3	14	20	29	75
	NA	2	13	20	27	75

[§]In scenarios 1, 2, 3, 4, 5, 8, 10, and 11 the last day of new infections in all models was equal to the length of the simulation period (Table 1).

[a]The median number of days when new infections occurred differed significantly among the three models at the alpha = 0.05 level.

[b]The median number of days when new infections occurred differed significantly among the three models at the alpha = 0.10 level.

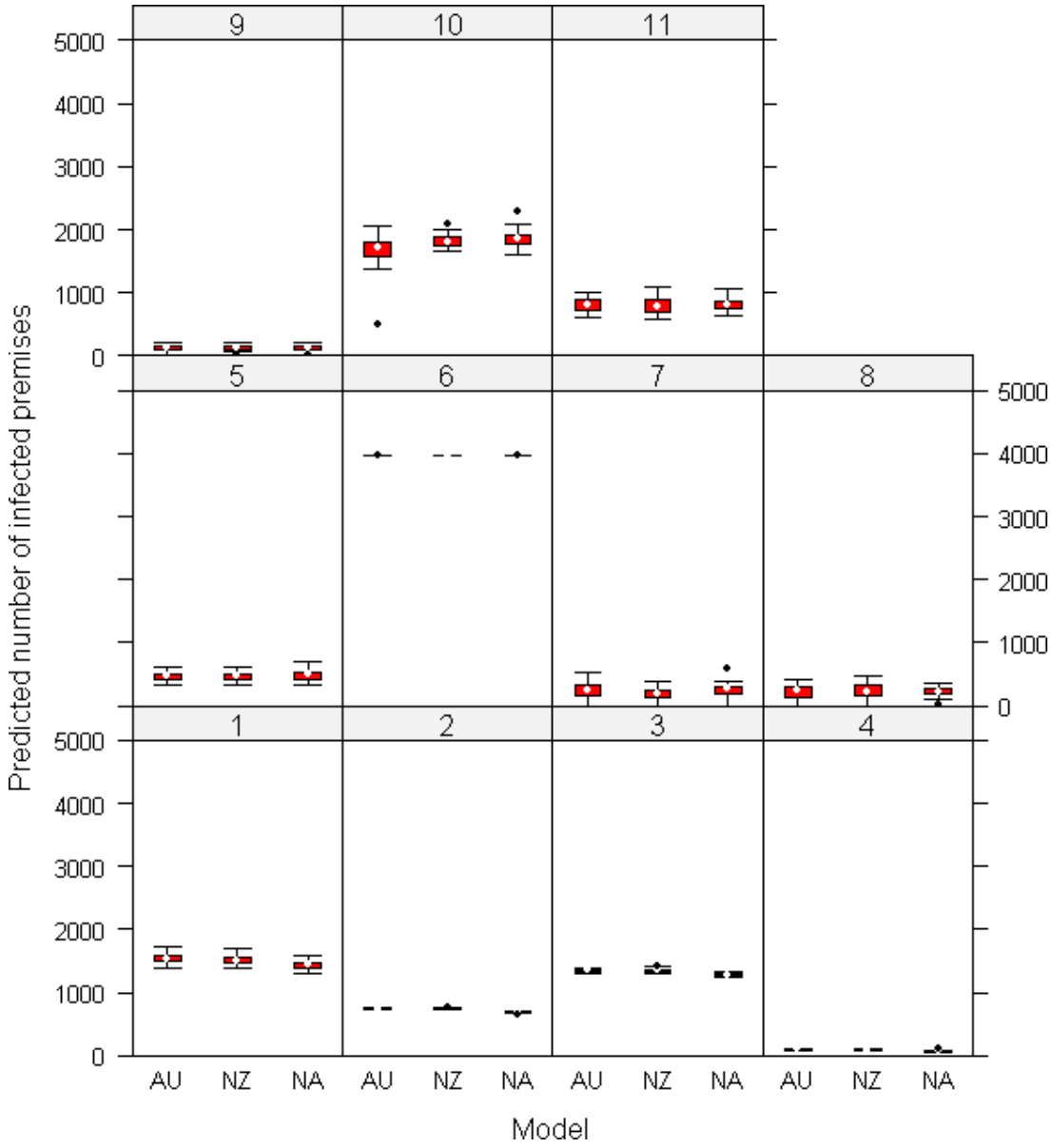

Fig. 2 Box and whisker plots showing the predicted number of FMD-infected units for the three models and 11 scenarios (40 simulations of each scenario). Key: AU Australia; NZ New Zealand; NA North America.

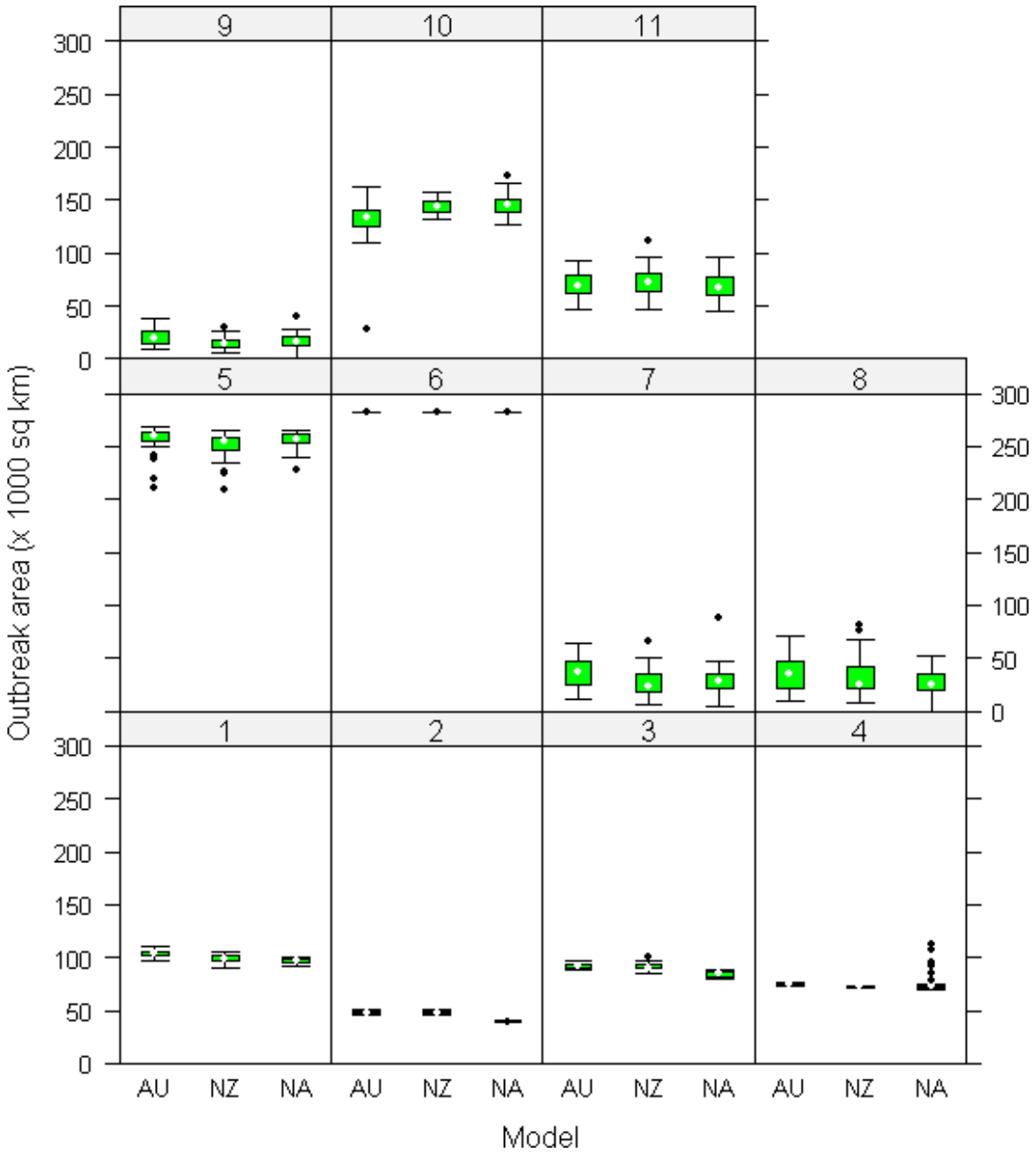

Fig. 3 Box and whisker plots showing the size of the predicted outbreak area (× 1000 square km) for the three models and 11 scenarios (40 simulations of each scenario). Key: AU Australia; NZ New Zealand; NA North America.

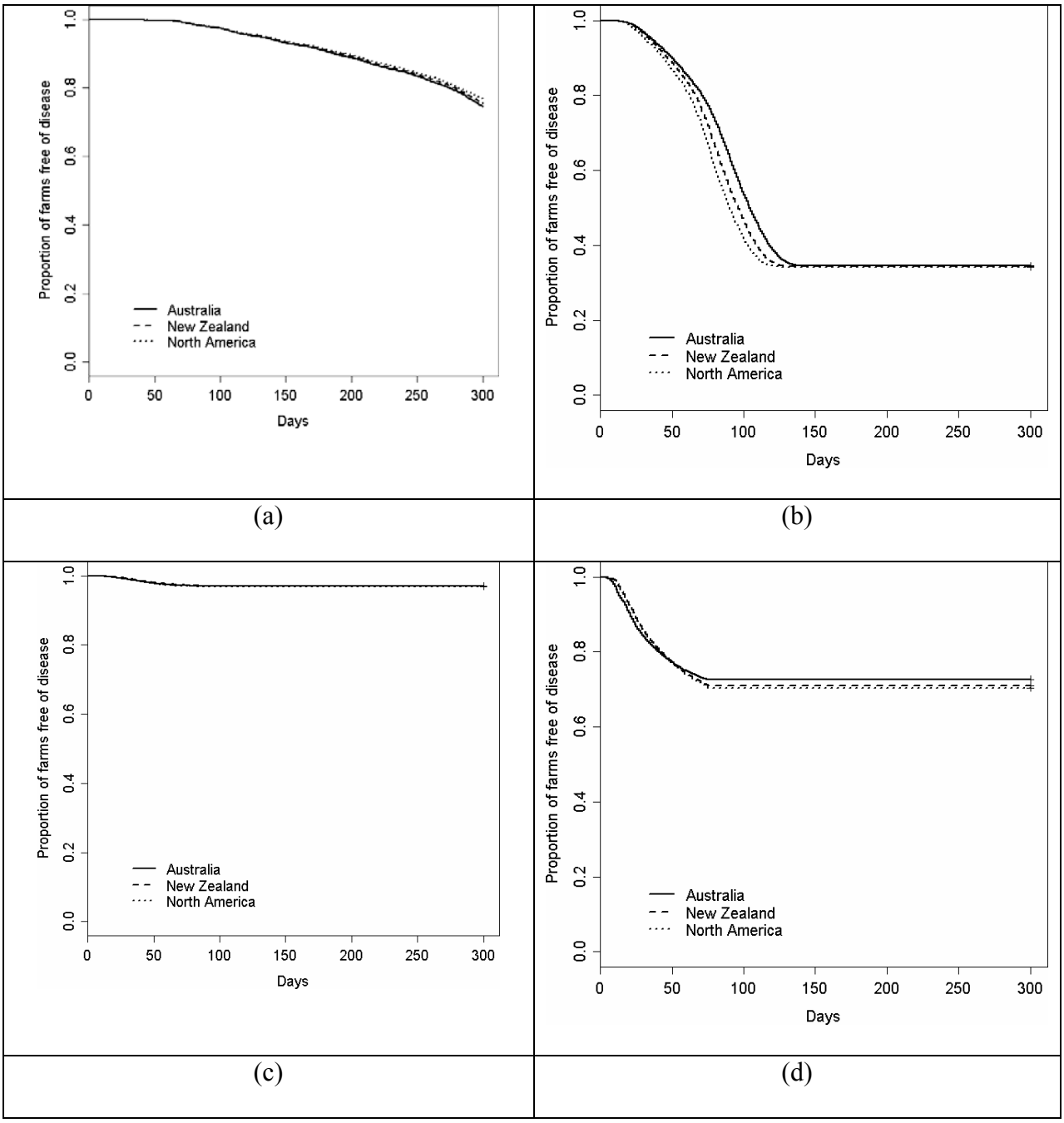

Fig. 4 Kaplan-Meier survival curves showing the proportion of units that remained free of FMD infection as a function of time for: (a) scenario 1 (log rank test statistic 6.8; df 2; P = 0.03); (b) scenario 6 (log rank test statistic 43.8; df 2; P < 0.01); (c) scenario 8 (log rank test statistic 0.4; df 2; P 0.81); and (d) scenario 10 (log rank test statistic 3.7; df 2; P = 0.16).

Although the scenarios specified in this study were unlike those that would be encountered in practice, changes to the scenario specifications produced logical changes in the outcomes of interest. The single effect of airborne spread (scenario 2) produced smaller numbers of infected units and epidemics involving a smaller areas compared with scenarios where direct contact and airborne spread were combined (scenario 3). There were significant differences in the predicted number of infected units (F = 1180; df 2,117; P < 0.01 and F = 149; df 2, 117; P < 0.01) and the

predicted size of the outbreak area ($F = 924$; df 2, 117; $P < 0.01$ and $F = 110$; df 2, 117; $P < 0.01$) among the three models for these scenarios.

Increases in the length of the simulation period (scenarios 4 and 5) resulted in substantial increases in the predicted numbers of infected units and was attributed to a second wave of infection resulting from the transition of previously immune units to the susceptible state. A simulation period of 100 days with no control measures resulted in 64 – 114 infected units (scenario 4, Table 2): increasing the simulation period to 115 days increased the predicted number of infected units to 319 - 689 (scenario 5, Table 2). It also increased the size of the predicted outbreak area from 70000 - 112000 km^2 in scenario 4 to 209000 - 269000 km^2 in scenario 5 (Table 3).

Using more realistic disease parameters and direct contact at shorter scales of distance resulted in all cattle units being infected by each of the models (Scenario 6, Table 2). Vaccination that commenced when 50 units were detected (scenario 8) in combination with spread by direct contact, detection, quarantine, and movement restrictions resulted in smaller epidemics (0 - 482 infected units) than scenario 7 where vaccination was not applied (0 - 601 infected units, Table 2). Increasing the size of the vaccination area (scenario 9) halved the predicted number of infected units (0 – 220, Table 2). Depopulation as a control strategy (scenarios 10 and 11) had a similar effect: larger ring sizes of destruction led to smaller epidemics: lower predicted number of infected units and a smaller outbreak area.

In all scenarios but scenario 5 there was almost perfect agreement in the predicted location of infected units using an inferential grid cell size of 30 km^2 (Table 5). In contrast, significant differences were identified in the size of predicted outbreak areas. Outbreak areas for scenarios 1, 2, 3, 4, 5, 7, 9, and 10 differed significantly among the three models at the alpha level of 0.05. This is expected, given the different mechanisms used by each model to handle direct and indirect contact arising from movement.

DISCUSSION

All three models are based on similar objectives and methodological approaches (all being spatial simulation models) however, given the differences in the way various model components have been implemented, it is not surprising that they provided slightly different results. Although there were statistically significant differences among the number of infected units and the temporal and spatial spread predicted by the three models in some of the scenarios, these differences were generally small and from a practical perspective would have resulted in the same (or similar) management decisions being adopted in each case. Differences in the size of outbreak areas were attributed to variations in the process of recipient units selection among the three models, although in most scenarios those differences were relatively small (Fig. 3).

The model comparison project has had an additional positive outcome in that it has encouraged each of the three teams to take an in-depth look at the way core functions are implemented in their models. For example, whether values selected from distributions were rounded or not had a significant impact on predictions. For Scenario 1, the Australian model was initially run in its default mode, which allows infectivity of a herd to increase with time reflecting within-herd spread of FMD. This resulted in significantly fewer new infections than when infectivity was kept constant (assuming a 100% prevalence from the first day of infection), showing that this is an important issue to explore. Although not included in some of

the mathematical models used in the 2001 UK FMD epidemic (Keeling et al., 2001; Ferguson et al., 2001), there is evidence of within-herd transmission (Hutber & Kitching, 2000). As a result, it is expected that infectiousness will increase over time as the number of animals infected increase within a herd (Taylor, 2003; Honhold et al., 2004; Haydon et al., 2004; Kitching et al., 2006). Future comparisons should assess the impact of within-herd spread in more complex, realistic scenarios.

The impact of delays in state-transitions occurring in the models was observed in scenario 7. By default, the Australian and New Zealand models allowed the transition of a unit from susceptible to latent (following successful exposure) to take place on the day infection occurred while the transition to latent occurred on the following day in the North American model. This meant that the North American model produced significantly smaller outbreaks than the other two models until this issue was identified and accounted for in the Australian and New Zealand models.

The Australian model recorded a lower number of infected units in Scenario 10, in which the infection spread to the west edge of the simulated study area. When infection is active near the edge of the study area, that model can generate contacts that go out of the area, and are recorded as such. The other two models do not simulate out-of-area contacts, and will therefore tend to concentrate new infections along the edge of the study area, leading to an increased number of infected units. Some extra experimentation with this scenario (running for fewer days, so that the infection does not reach the boundaries of the study area, for example) indicated that this explanation was responsible for the differing results. Future comparisons will include further testing of this effect.

Programming decisions relating to the order in which events occurred in a simulation day had important impacts on the results. For example, some models allowed spread to occur before detection in a simulation day whilst another model had detection taking place prior to spread. This produced more spread in the model that had spread occur prior to detection in a day. A conclusion from this study is that models should allow these events to occur in a random order during a simulation day to mimic the fact that in a real outbreak, detection could occur at any time during a day.

For policy makers, it is reassuring that despite the different design and implementation of each of the three models, each produced similar outcomes from the relatively simple outbreak scenarios described in this study. We conclude that despite the differences that were identified, decisions based on the results from any of the three models would not differ. Epidemiological modelling is recognized as a valuable tool that can assist disease managers in identifying and evaluating alternate approaches to disease control (Taylor, 2003; Keeling, 2005; Guitian & Pfeiffer, 2006). They are particularly useful for gaining insights into the conditions under which controversial control measures such as emergency vaccination might become an economically viable option (Burrell, 2002). Suitably designed models can simulate outcomes under different assumptions concerning types of strategy, availability of resources, and reactions of trading partners and therefore help identify conditions under which different approaches to control might or might not be appropriate. These findings need to be kept under review as new technologies ─ such as new diagnostic methods or vaccines ─ and changes to international guidelines and trading protocols might alter the balance.

This comparison study was a valuable exercise in that it provided each modelling group the opportunity to consider all the assumptions made in the model-building process, including the

quantification of the impact of certain programming decisions. In addition, minor bugs were identified in all models and therefore all models have been improved as a result of this exercise. Further discussion on different assumptions and further comparison scenarios will take place among the three groups to test other features of the models with more complex scenarios.

Arising from this project the QUADS technical group will develop a set of guidelines for model testing and validation to ensure that their use for policy development and in some cases, as predictive tools during outbreaks, are computationally sound and based on accepted biological principles. These guidelines will be proposed to the World Organization for Animal Health (OIE) as a template by which other modelling groups may assess their models.

This is the first time an international team of government epidemiologists have formally agreed to collaborate on developing and validating tools for use in animal health emergency preparedness and response. These tools should enhance decision-making by the respective governments if faced with FMD. In addition, the team will provide resources and expertise that can be exchanged during these events.

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EFFECTIVITY OF VACCINATION STRATEGIES TO CONTROL CSF EPIDEMICS

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SUMMARY

To minimize the economic and animal welfare impact of a Classical Swine Fever (CSF) epidemic an effective control strategy is essential. Following the development of marker vaccines, emergency vaccination can be considered an alternative to pre-emptive culling. This paper uses mathematical modelling to assess the efficacy of such a control strategy. The transmission of the virus is described at the individual, farm and national scales. Data obtained from transmission experiments and the outbreak of CSF in the Netherlands in 1997/1998 are used to calibrate the models. In this way, the effect of vaccination of individual animals can be studied on a larger scale. Four control strategies are evaluated. 1 km ring vaccination is found to be less effective than 1 km ring culling, while 2 km and 3 km ring vaccination limit both the duration and size of the outbreak considerably. The multilevel character enables the model to evaluate a large range of control scenarios and thus aid decision-making.

INTRODUCTION

Outbreaks of Classical Swine Fever (CSF) can lead to large economic losses and have a major impact on animal welfare, both of which should be minimised by an effective control strategy. The standstill of all pig transports, as required by the EU, appears to be insufficient to stop CSF spread in pig dense areas. Consequently, additional measures, such as pre-emptive culling, are necessary to halt the epidemic in a minimal time span. The recent development of a marker vaccine allows the consideration of an alternative to pre-emptive culling. Using this vaccine to control the outbreak may take away the necessity of pre-emptive culling of healthy animals, while vaccinated meat can still enter the (local) markets, as serological positivity due to infection can be distinguished from that caused by vaccination with the marker vaccine. Because of these advantages, marker vaccination will possibly be used in the control of future outbreaks of CSF in the Netherlands. As no previous experience exists, mathematical modelling provides a valuable instrument to assess the efficacy of such a control strategy.

As a first step in developing a transmission model, this paper addresses the question of the scale (animals vs. farms) on which the model needs to be formulated in order to describe the transmission process adequately, whilst avoiding excess detail. To study the effect of strategies to control a CSF epidemic in a livestock area, a dynamic model is required that describes the distance-dependent transmission of CSF between herds in that area. In the simplest model of this type, a pig herd is regarded as an unstructured basic unit that is completely susceptible, fully protected by vaccination, infected (and infectious), or removed (i.e. culled). A more realistic approach, however, allows an infected herd, before being detected, to become increasingly more

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infectious as the number of infected animals increases. As the timescale on which transmission within a herd progresses is similar to the timescale on which animals gain protection via vaccination, both processes need to be taken into account explicitly. This requires a model that includes the within-herd dynamics, i.e. that is formulated in terms of individual animals.

To incorporate the effect of (marker) vaccination, results of several (small-scale) transmission experiments are available. From these, estimates are obtained for the parameters of a within-pen model that describes transmission and vaccination simultaneously. Linking up the model description on the different scales of the pen, the herd and the livestock area, generates a model structure that allows the extrapolation of the effects of vaccination on individual animals to the level of an area with many pig farms.

In the next section, each level of the three-level model and its parameterisation is discussed, starting with transmission between animals on the pen level and zooming out to epidemic spread on livestock-area level. This model is used to study the efficacy of different control strategies in a large livestock area with a geographical structure as was present in the Netherlands in 1997. The set-up and results of the calculations are discussed in subsequent sections. In the last section, the main conclusions are set out.

MODEL

Within-pen model

The within-pen transmission of CSFV between animals can be described by a simple SEIR model. The acronym denotes the different stages of the disease: when a susceptible (S) animal is infected, it will be exposed (E) during a latent period, after which it will become infectious (I) until it recovers (R) (or dies). In the model the variables S, E, I and R represent the number of animals in the respective stages. When the residence times in stages E and I are assumed to be exponentially distributed, a deterministic formulation of the evolution of the system in time is given by the following set of differential equations:

$$\begin{aligned}
\frac{dS}{dt} &= -\sigma(t-\tau)\beta(t-\tau)SI \\
\frac{dE}{dt} &= \sigma(t-\tau)\beta(t-\tau)S - \gamma E \\
\frac{dI}{dt} &= \gamma E - \mu(t-\tau)I \\
\frac{dR}{dt} &= \mu(t-\tau)I
\end{aligned} \tag{1}$$

Here N is the total number of animals ($S+E+I+R$), β is a measure of the infectivity of the infectious animals, σ is the relative susceptibility of susceptible animals ($0 \leq \sigma \leq 1$), γ is the rate at which exposed animals become infectious and μ is the rate at which infectious animals recover (or die). The reciprocal value of γ equals the latent period ($T_{lat} = 1/\gamma$) and the reciprocal value of μ equals the infectious period ($T_{inf} = 1/\mu$). We use the fully stochastic equivalent of the deterministic model formulation in Eq. (1). Taking into account stochasticity, i.e. the variation occurring due to chance, is important when the number of infected animals in a herd is small. One such situation is that of an outbreak taking place on a farm that was vaccinated a short time

ago such that vaccine protection is developing whilst the spread of the infection has started. The stochastic model is implemented efficiently using the Sellke construction (Andersson & Britton, 2000).

The parameters of the model need to be estimated from experimental data. As the experiments show, vaccination to some extent reduces the infectivity and infectious period of infected animals, while lowering the susceptibility of non-infected animals. The earlier animals have been vaccinated, the more pronounced these effects are expected to be. Thus the model parameters β, σ and μ do not have constant values, but depend on the time since vaccination $t-\tau$. The detailed parameter estimations and some further model assumptions are now explained.

An animal is assumed to be infectious when it has tested positive for virus in whole blood samples. In transmission experiments animals usually become viraemic 4 to 6 days after challenge (see for instance Dewulf et al., 2001, Dewulf et al., 2004, Dewulf et al., 2005 and Laevens et al., 1998). As contact infections probably take a longer latent time (as the infection dose is smaller), assuming a latent period of 4 days is the most conservative choice. Vaccination is assumed not to influence the latent period of the challenged animals; also it is assumed that the latent period for the contact animals is independent of vaccination.

The infectious period T_{inf} is assumed to be identical to the observed period of viraemia. Transmission experiments without vaccination (Dewulf et al., 2001) give mean viraemic periods of 13,6 (ranging from 2 - 29) and 15,6 (ranging from 5 – 36) days. Laevens et al. (1998) report shorter periods, due to a higher mortality. The average infectious period without vaccination is taken as $T_{inf,0}$ = 15 days. To take into account the observed variation, the infectious period is modelled by a gamma distribution with a shape parameter of 10, i.e. the 95% confidence interval for $T_{inf,0}$ is $\{7-25\}$days.

A series of transmission experiments by Dewulf et al. (2004), following the course of infection for individual vaccinated animals, provides crucial information on the effect of vaccination on the infectious period. Assuming a fixed length of the latent period (4 days), the results of these experiments allow us to relate the length of the infectious period to the time elapsed between vaccination and (contact) infection of the animal. Assuming vaccination has no effect for a time T_{delay} after vaccination, after which the infectious period decreases inversely proportional with time, the function:

$$\begin{aligned} T_{inf}(\tau) &= T_{inf,0} && \text{if } \tau < T_{delay} \\ &= \frac{c_1}{\tau + c_2} + c_3 && \text{if } \tau \geq T_{delay} \end{aligned} \qquad (2)$$

is fitted to the 15 data points for the contact-infected animals, yielding $\{c_1 = 69;\ c_2 = -1{,}8;\ c_3 = 0{,}094;\ T_{delay} = 6{,}4$ days$\}$. A gamma distribution with, again, a shape parameter of 10 is used to model the variance of this reduced infectious period. Figure 1 shows the average infectious period as a function of time since vaccination, as well as its density and the data points

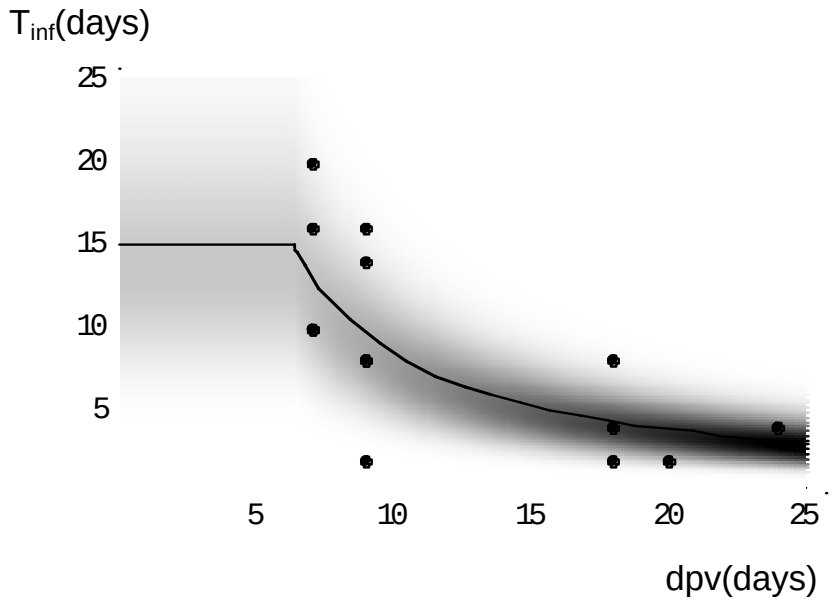

Fig. 1 Infectious period T_{inf} as a function of time since vaccination (dpv) on the day of infection; data points (black dots) from Dewulf et al. (2004), fitted function (black line) and density of gamma distribution (shaded area).

The effect of vaccination on the transmission rate is not as straightforwardly derived as the effect on the infectious period. It is a combination of a reduced infectivity of vaccinated and infected animals and a reduced susceptibility of vaccinated and non-infected animals. To distinguish the two effects, different types of transmission experiments are considered. When unvaccinated seeders infect vaccinated animals, conclusions can be drawn about the effect of vaccination on the susceptibility. On the other hand, when vaccinated seeders infect highly susceptible unvaccinated animals (sentinels) the infectivity of vaccinated animals can be assessed. The infectivity β and susceptibility σ are assumed to be unaffected by vaccination for a time T_{delay} (estimated from infectious period data) after which they decrease exponentially. The susceptibility is assumed to remain constant from three weeks after vaccination onwards, to take into account that a vaccinated animal will never be completely protected against infection (see for instance Dewulf et al. (2005)).

$$\begin{aligned} \beta(\tau) &= \beta_0 && \text{if } \tau < T_{delay} \\ &= \beta_0 \exp\left[-\lambda_{inf}(\tau - T_{delay})\right] && \text{if } \tau \geq T_{delay} \end{aligned} \tag{3}$$

$$\begin{aligned} \sigma(\tau) &= 1 && \text{if } \tau < T_{delay} \\ &= \exp\left[-\lambda_{sus}(\tau - T_{delay})\right] && \text{if } T_{delay} \leq \tau \leq 21 \text{ days} \\ &= \exp\left[-\lambda_{sus}(21 - T_{delay})\right] && \text{if } \tau \geq 21 \text{ days} \end{aligned} \tag{4}$$

The parameters β_0, λ_{inf} and λ_{sus} are estimated from 21 experiments that study three different types of transmission (vaccinated $\rightarrow$ unvaccinated, unvaccinated $\rightarrow$ vaccinated and vaccinated $\rightarrow$ vaccinated) (taken from Bouma et al., 1999, Bouma et al., 2000, Dewulf et al., 2004, Dewulf et al., 2005 and Moormann et al., 2000). Probing the parameter space, the model results are

compared to the experimental results to determine the maximum likelihood estimates for the parameters. All model parameters are summarized in Table 1.

Table 1. Within-pen model parameters.

Parameter	Value	95% CI	Equation
T_{lat} (= $1/\gamma$)	4 days		(1)
$T_{inf,0}$ (= $1/\mu$)	15 days		(2)
T_{delay}	6.4 days		(2)
c1	69 days^2		(2)
c2	-1.8 days		(2)
c3	0.094 days		(2)
β_0	6.7 days^{-1}	5.5 – ∞	(3)
$R_0 = \beta_0 T_{inf,0}$	100	82 – ∞	(3)
λ_{inf}	0.5 days^{-1}	0.31 – 0.63	(3)
λ_{sus}	0.2 days^{-1}	0.16 – 025	(4)

The reproduction number R_0 in the table above gives the number of secondary infections that an unvaccinated infected animal will cause in a fully susceptible and unvaccinated population. The value of 100 agrees well with the reported values of 81.3 (Laevens et al., 1998) and 100 (Klinkenberg et al., 2002). For a vaccinated infected animal the reproduction number decreases with time since vaccination; it crosses the R_0 =1 line after about 14 days.

Within-herd model

An infectious animal is more likely to infect a susceptible animal in its own pen than an animal in another – still fully susceptible – pen, due to the pen barrier. This barrier prevents animals to mix freely, which hampers the transmission between pens, while the direct contact between animals that are in the same pen facilitates the transmission of the virus. Together these two transmission processes – within-pen and between-pen – determine the spread of the virus through a farm. A household model (Andersson & Britton, 2000) combines the two types of transmission. Each pen is considered to be a household of size N_p that has random contacts with other households. When one household member is infected, it exhibits an infectivity β to other household members, but a reduced infectivity $\varepsilon\beta$ to members of other households, due to the different contact rate. If the first infected member can be assumed to infect all other members in its household (i.e. $R_0 >> N_p$), the overall reproduction ratio R_0' is expressed as:

$$R_0' = \varepsilon N_p R_0 \quad (5)$$

This equation relates the information from small-scale experiments to transmission at the farm level as observed during a real-life CSF outbreak on a farm. Such outbreaks occurred on 429 farms during the CSF epidemic in the Netherlands in 1997 and 1998 (Elbers et al., 1999). From serological data that was collected during that period from a large number of infected farms, the reproduction number within the farm was estimated independently by Stegeman et al.(1999a) and Klinkenberg et al. (2002), who found numbers of 2.9 (based on breeding farms) and 2.8 (based on finisher farms) respectively. This agreement is remarkable, considering that sows were then held separately, while finishers were usually held in pens of ten animals on

average. Using the value for finisher farms, Eq. (5) gives a reduction factor of $\varepsilon = 2.8 \cdot 10^{-3}$. Clearly, this value only applies to an unvaccinated population, as was the case during the 97/98 epidemic. However, vaccination status presumably does not have any effect on contact structure or the ratio between within-pen and between-pen transmission. Therefore the reduction factor ε is assumed to be constant under vaccination.

During an outbreak of CSF on a farm, the number of infected animals increases until the disease is diagnosed and confirmed. The time between infection and detection is related to the number of infected animals at that moment, but it also depends on tracing of dangerous contacts and the awareness of the farmer and veterinarian. This is why a large variance is observed in the outbreak data of 97/98. Of 82 farms the infectious period (i.e. the time between infection and detection) is known, ranging from 10 to 57 days. These realistic data, rather than the number of infectious animals, are used to determine the time of detection. The information is fitted to a detection time distribution that was proposed by Klinkenberg (2003), with a minimum detection time of 2 latent periods (i.e. $2\ T_{lat} = 8$ days):

$$\mathrm{pdf}(t_{\mathrm{det}}) = \frac{Cr\exp\left[r(t_{\mathrm{det}} - 2T_{lat})\right]}{\left(C - 1 + \exp\left[r(t_{\mathrm{det}} - 2T_{lat})\right]\right)^2} \tag{6}$$

For finisher farms the fitted parameters are C = 8.5 and r = 0.12 day^{-1}, with a mean detection time of 4 weeks after infection. From this distribution an infectious period, after which the disease is detected, is drawn for each infected farm. As an extra condition it is required that at that time at least N_p animals must be infectious (not necessarily in one pen), to enable clinical detection. If this condition is not fulfilled, the model postpones detection until it is. The extra condition only has a very small effect on the detection times in the non-vaccinated case; it provides a way however to extrapolate the description of the detection process to the case of vaccinated farms. When the outbreak has already ended before the designated detection time, or when the requirement of N_p infectious animals is never fulfilled, the outbreak will go unnoticed. It is assumed that for vaccinated farms the detection time distribution still applies. As outbreaks on (imperfectly) vaccinated farms will generally affect a smaller number of animals than on unvaccinated farms, vaccination increases the chance that outbreaks remain undetected.

Between-herd model

The infection on one farm can spread to other farms, by numerous possible infection routes such as transport of animals and persons visiting different farms. Stegeman et al. (2002) discusses the share of several different infection routes in the part of transmission for which the route was identified in the CSF epidemic in the Netherlands in 97/98. Here a modelling approach that describes the transmission of all routes together as one single entity is used. The infection hazard h_i experienced by farm i depends on all infected farms j at that moment (Keeling et al., 2001):

$$h_i = \sum_j K\left(z_{ij}\right) \tag{7}$$

K is the transmission kernel that describes the probability of transmission per unit of time between an infectious farms and a susceptible farm as a function of the distance z between these farms:

$$K(z) = \frac{\lambda}{1 + \left(\frac{z}{z_0}\right)^{\alpha}} \tag{8}$$

The parameters α and z_0 determine the shape of the kernel curve. When $\alpha > 1$ the chance on infection is smaller for farms that are farther apart and for $\alpha > 2$ the area integral is convergent. The point of inflection is determined by the parameter z_0. The values of α and z_0 are estimated by Maximum-Likelihood along similar lines as in Ferguson et al. (2001), using the outbreak data from the 97/98 epidemic and the location data of all Dutch farms with pigs. Subsequently, the parameter λ, that is an overall multiplicative factor that does not affect the distance-dependence of transmission, was fitted by reproducing the 97/98 epidemic with the three-level model. In 1997, at the time the first herd was detected, 34 herds were already infected. Starting with these herds, we simulated the course of the outbreak 100 times and repeated this for different values of λ. Next, we determined the average effective between-herd reproduction ratio R_h for each set of 100 simulations. In the early stage of the 97/98 epidemic, this number was R_h = 1.3 on average, which agrees with the value reported by Stegeman et al. (1999b). As R_h linearly depends on λ, the value for the latter can be easily determined by interpolation. In summary, the kernel parameters found are $\alpha = 2.2$, $z_0 = 1$ km and $\lambda = 0.0011$ day^{-1}.

COMPUTATIONAL DETAILS

The model described in the previous section comprises three levels of transmission: on individual scale, farm scale and livestock-area scale. This allows us to study the area-level effect of vaccination that is administered at the individual level. We will do this by comparing four different control strategies: ring culling in 1 km around a detected herd and ring vaccination in 1, 2 and 3 km around a detected herd. Calculations are performed for an (inhomogeneous) spatial pattern of farms in the Netherlands in 1997, using the locations of all 21 500 farms present at that time. Each farm is assumed to consist of 900 animals (mean farm size) that all stay in pens of 10 animals (N_p=10). The fixed number of animals is a model simplification justified by the fact that most within-farm outbreaks are detected before saturation effects become noticeable.

In total 1000 stochastic realizations (runs) are computed for each control strategy. Each realization is seeded with between 10 and 20 infected herds. This starting situation corresponds to the situation at the time the first herd is detected. In the first week after the disease is first diagnosed, farms around detected herds are pre-emptively culled one day after in a circle of 1 km radius, to account for the time to obtain permission for emergency vaccination. After the first week, the calculation branches out for the different control strategies. Because every strategy is applied on the same starting situation, they can be properly compared.

When a farm is confirmed to be infected it is culled the day after. When the culling strategy is applied, the farms around the detected farm are pre-emptively culled four days after culling of the detected farm (Elbers et al., 1999). For the vaccination strategies the delay between detection and ring vaccination is assumed to be one day.

RESULTS

Four control strategies were compared: 1 km ring culling that was used in the later stages of the 97/98 epidemic and three vaccination strategies with varying ring sizes of 1 km, 2 km and 3 km radius. One thousand starting situations were generated, on which each strategy was applied. Table 2 summarizes the results by reporting the 5%, 50% and 95% quantile of the duration of the epidemic (i.e. time between first and last detection), the number of detected farms, the number of infected farms that are not detected, the number of pre-emptively culled farms and the number of vaccinated farms.

Table 2. The 5%, 50% and 95% quantiles of the duration of the epidemic and number of detected, not detected, pre-emptively culled and vaccinated farms over 1000 simulations for different control strategies.

Control Strategy	Duration (days)			# Det. Farms			# Not Det. Farms			# Pre. Cul. Farms			# Vacc. Farms		
	5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%
1 km culling	65	317	927	12	99	486	0	0	3	105	1028	4056	0	0	0
1 km vacc.	83	476	1042	14	248	814	1	52	172	6	22	62	105	1936	5408
2 km vacc.	60	187	468	12	44	165	1	12	64	6	22	62	231	1146	3822
3 km vacc.	54	135	335	10	26	81	1	8	33	6	22	62	390	1244	3434

In comparing the efficacy of the strategies, the focus here is on the duration of the outbreak and the number of detected farms. A point to note is that the strategy with the shortest and smallest outbreaks is not necessarily the most effective from an economics point of view. From the two strategies that are applied in 1 km around a detected herd, it is clear that pre-emptive culling is more effective than vaccination. This is as expected, because vaccination takes some time to provide protection (typically two weeks), whereas pre-emptive culling works instantly. For larger vaccination circles, the effectivity drastically increases and surpasses the effectivitiy of the 1 km culling strategy. Examining the distributions of the number of detected farms more closely (Fig. 2), shows that whereas for the 2 km and 3 km vaccination strategies the probability density drops steeply with epidemic size, in contrast the 1 km strategies exhibit a plateau of very large outbreaks with over 200 cases. This suggests that these distributions are in fact a combination of outbreaks where the control strategy suffices and of outbreaks that are out of control.

The vaccination strategies do entail the risk of missing infected herds. When a farm is infected that is partly protected by vaccination, the outbreak will remain small and may go unnoticed. The results in Table 2 suggest that around 20% of the infected farms will not be detected during the epidemic. Note that this percentage does not depend on the vaccination radius. Only part of the undetected farms will be discovered during an end screening, and a small number of seropositive animals is likely to escape detection altogether. Whether this poses an unacceptable risk, depends on policy and export bans.

The vaccination strategies in Table 2 show a small number of pre-emptively culled herds that were culled in the first week of the epidemic. The numbers are identical for all vaccination strategies, as the starting situations were identical. The number of culled farms for the culling strategy might seem high when it is compared to the 97/98 outbreak. During that epidemic 429

farms were detected and 1280 were pre-emptively culled. This high number of detected farms and relatively low number of culled farms are due to the fact that it was only after more than two months that ring culling was applied. When the vaccination radius increases, the total number of vaccinated farms decreases due to improved control of transmission. This suggests that it is worthwhile to vaccinate large numbers of farms early in the epidemic as it repays itself later on in terms of a reduced outbreak size.

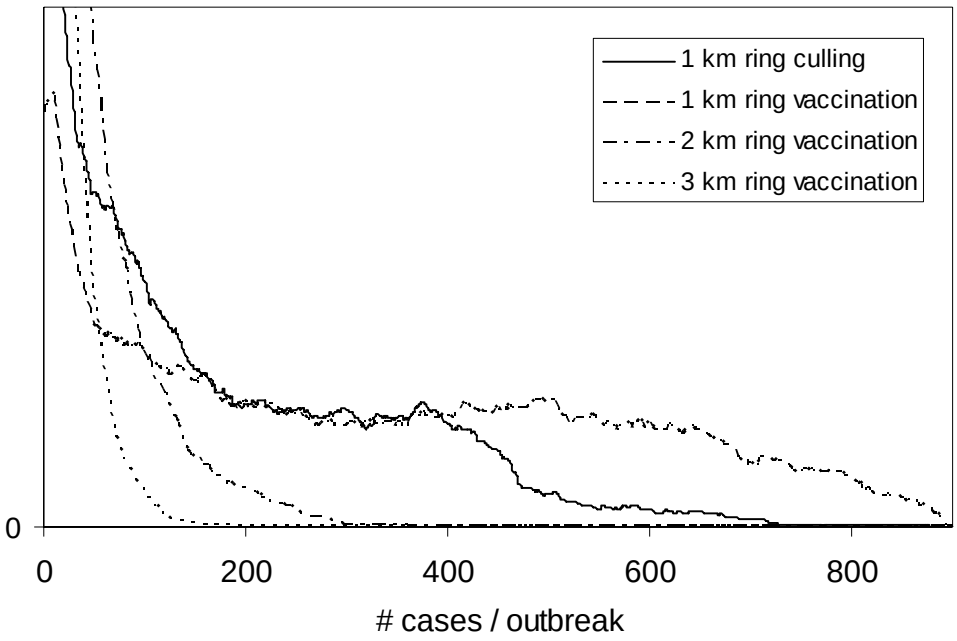

Fig. 2 Distribution of detected herds for 1 km ring culling (—), 1 km ring vaccination (– –), 2 km ring vaccination (· – · – ·) and 3 km ring vaccination (····).

The incidence curves in Fig. 3 show the average number of cases (i.e. detected farms) per day as a function of time for each control strategy. Both 1 km strategy curves have a distinct maximum, whereas the 2 km and 3 km vaccination strategy curves immediately bend down after control measures are taken. This is mirrored by the difference in the shape of the distributions in Fig. 2. Closer examination of the early stages of the incidence curves (see Fig. 3a) shows that the effects of pre-emptive culling take on earlier than the three vaccination strategies.

Finally, we study the effective reproduction number between herds R_h as a function of time. This number expresses the number of secondary infections that an infected herd will cause before it is detected. The number is stored on the day of infection of the source herd. Averaging over all infected herds in all realizations for each strategy, results in Fig. 4. The ineffectiveness of the 1 km strategies is evident as their R_h curves are both above unity for a considerable period. This means each infected herd infects on average more than one other herd, resulting in the growing incidence curves in Fig. 3, until they reach their maximum. The location of these maxima do not coincide with the time the R_h curves cross the R_h=1 line, because the reproduction number is matched to the day of infection of the source herd. It must be noted though that these reproduction numbers are effective numbers and therefore reflect both the effectiveness of the control strategy as well as the depletion of susceptible herds.

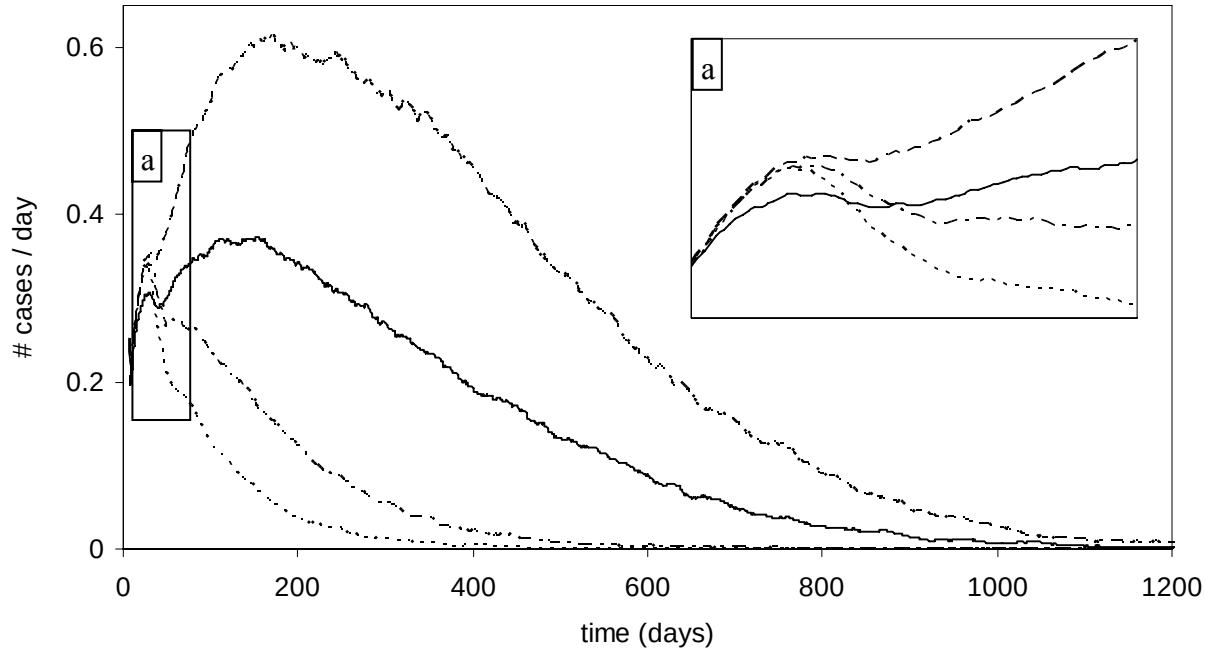

Fig. 3 Average incidence curves for 1 km ring culling (—), 1 km ring vaccination (– –), 2 km ring vaccination (· – · – ·) and 3 km ring vaccination (····).

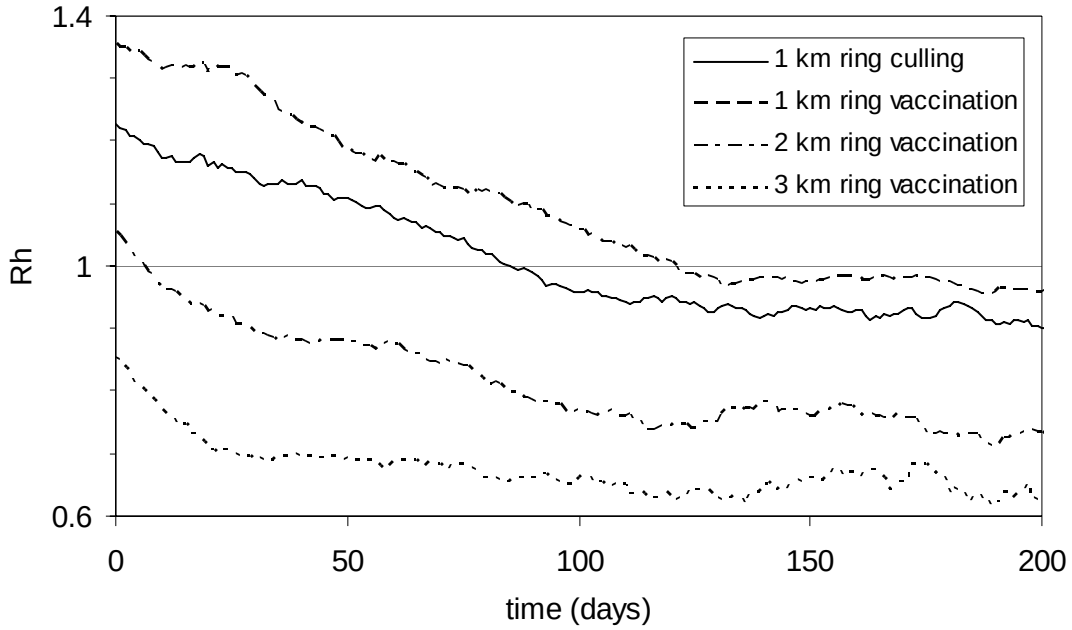

Fig. 4 Average effective between-herd reproduction number as a function of time for 1 km ring culling (—), 1 km ring vaccination (– –), 2 km ring vaccination (· – · – ·) and 3 km ring vaccination (····).

DISCUSSION AND CONCLUSION

This paper has used mathematical modelling to assess the effectivity of CSF control strategies using emergency vaccination with a marker vaccine. The modelling approach is necessarily a multi-level one, that describes transmission of the virus on the levels of individuals, of farms and on a national scale. Experimental data of transmission experiments and data that were collected during the outbreak of CSF in the Netherlands in 1997/1998 have been used to calibrate the models.

The results of four control strategies (i.e. 1 km ring culling and 1 km, 2 km and 3 km ring vaccination) were compared. Although the 1 km strategies are sufficient in some outbreaks, a considerable proportion of the simulated epidemics went out of control. The 1 km vaccination strategy performs worse than the 1 km ring culling, as could be expected. The other vaccination strategies (in 2 km and 3 km rings) are more effective, and limit the size and duration of the outbreaks.

The above results apply to the situation in 1997 in terms of number and spatial distribution of farms. In the past decade the number of pig farms in the Netherlands has declined considerably. In 1997 at the start of the epidemic around 21 500 holdings were present, while in 2005 there were less than 10 000. This decreasing farm density significantly diminishes the infection probability between herds. On the other hand the holdings are larger in size, which might lead to a larger infectivity to the surroundings once such a herd is infected. Moreover, the structure of the holdings has changed drastically. On some farms pens can now contain up to a hundred pigs. In such a large pen it cannot be assumed that the first infected animal will infect all others, so another formulation of the household model (Eq. (5)) is required. Furthermore, due to the closer contact the infection may spread faster through the herd, which would shorten the time to detection as compared to the distribution in Eq. (6) obtained from the 1997/1998 data.

The model analysis might benefit from including some further detail, if only for the purpose of studying the sensitivity of the results to some modelling assumptions made. In particular, inclusion of a distinction between finisher farms and breeding farms could be interesting. Sows are excluded from vaccination to minimize the risk of carrier sows and young piglets that are not protected by vaccination are born during the epidemic. Both aspects diminish the overall protection of a breeding farm. Including the distinction in the model allows the investigation of the effects. Finally, as only data of Dutch pig farms are used in the simulations, the outcomes of the model are of limited use in areas near the frontiers of neighbouring countries Belgium and Germany. Adding cross-border transmission explicitly (by using e.g. approximated farm locations in these countries), would improve the credibility of the results in such areas.

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COMPANION ANIMAL EPIDEMIOLOGY

THE RISK OF ANAESTHETIC-RELATED DEATH IN CATS: RESULTS FROM THE CONFIDENTIAL ENQUIRY INTO PERIOPERATIVE SMALL ANIMAL FATALITIES (CEPSAF)

D.C. BRODBELT*, D.U. PFEIFFER, L.E. YOUNG AND J.L.N. WOOD

SUMMARY

Recent figures describing the frequency or causes of anaesthetic-related death in cats have not been available. The aims of this study were to address these deficits. A nested case-control study was undertaken. All anaesthetics and sedations undertaken and related deaths (i.e. 'cases') within 48 hours were recorded. Details of patient, procedure, and anaesthetic management were recorded for all cases and controls. A multivariable mixed effects logistic regression model of factors associated with anaesthetic and sedation-related death was constructed.

Between June 2002 and June 2004, 79,178 anaesthetics and sedations were recorded and the overall risk of anaesthetic and sedation-related death was 0.24% (95% CI 0.20 – 0.27%). Factors associated with anaesthetic-related death were health status, age, weight, procedural urgency and complexity, endotracheal intubation, fluid therapy, pulse monitoring and pulse oximetry.

The risk of anaesthetic-related death in cats appears to have decreased since the last UK study. The results should aid patient management.

INTRODUCTION

Anaesthetic complications have been infrequently evaluated in veterinary practice (Jones, 2001). The last UK study of veterinary anaesthetic companion animal complications was undertaken in the 1980's and documented the risk of anaesthetic-related death in cats to be approximately 0.29% (Clarke & Hall, 1990). In this study, perioperative deaths in healthy patients (American Society of Anesthesiologists (ASA) physical status 1-2), occurring during or shortly after surgery were considered 'primarily due to anaesthesia' unless an obvious surgical cause was present, whilst in sick patients (ASA physical status 3-5) all deaths were reported and no attempt was made to separate anaesthetic from other causes. Subsequent international work has reported the risk of anaesthetic-related death in cats to be approximately 0.1 – 0.2% (Dodman & Lamb, 1992; Dyson et al., 1998; Joubert, 2000). This is substantially higher than reported in human anaesthesia, where the risk of anaesthetic-related death (death where anaesthesia could not be reasonably excluded as a contributory factor) is approximately 0.02% to 0.05% (Biboulet et al., 2001; Eagle & Davis, 1997; Kawashima et al., 2001).

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Identification of major risk factors for anaesthetic-related death could aid the reduction of complications. Previous studies identified poor health status, administration of the alpha$_2$ agonist xylazine, endotracheal intubation and lack of a technician monitoring the anaesthetic as factors associated with perioperative mortality in cats (Clarke & Hall, 1990; Dyson et al., 1998; Hosgood & Scholl, 2002). Since these studies, new drugs, monitoring and anaesthetic techniques have been introduced into veterinary practice. Based on clinical experience and this previous work we hypothesised that sick patients (ASA physical status 3-5), the use of medetomidine and endotracheal intubation are associated with increased odds whilst the use of acepromazine, propofol, isoflurane, intraoperative fluid therapy and having a separate person monitor anaesthesia are associated with a reduction in the odds of anaesthetic-related death. Hence the aims of this study were to estimate the current risk of death, to test the stated hypotheses and to identify other current risk factors associated with anaesthetic-related death, in order to improve feline anaesthesia in general veterinary practice.

MATERIALS AND METHODS

A case-control study was undertaken between June 2002 and June 2004, nested in a cohort ('nested' case-control study) of all cats anaesthetised and sedated at participating veterinary practices and referral institutions in the UK (Confidential Enquiry into Perioperative Small Animal Fatalities, CEPSAF) during centres' periods of participation. In the cohort study, all anaesthetics and sedations, procedure date and outcome at 48 hours (alive, dead or euthanased) were recorded prospectively by the centres on self-administered questionnaires and were returned to the investigators on a monthly basis. Anaesthesia was defined as chemical restraint, sufficient to allow endotracheal intubation, whilst sedation was defined as chemical restraint insufficient to allow endotracheal intubation. Anaesthetic and sedation-related death was defined as perioperative death (including euthanasia) within 48 hours of termination of the procedure, except where death was due solely to the surgical or pre-existing medical condition, such that anaesthesia or sedation could not be reasonably excluded as a contributory factor. An independent review panel, consisting of 3 Royal College of Veterinary Surgeons (RCVS) Diploma level / European College Diplomate level veterinary anaesthetists and 1 European College of Veterinary Surgeons Diplomate level veterinary surgeon, classified all potential deaths as anaesthetic and sedation-related or not. Deaths for which insufficient information was available for the independent review panel to classify them as cases or not, were included as anaesthetic-related for the estimation of risk of death. Risks of anaesthetic and sedation-related death and ninety-five percent confidence intervals (95% CI) were calculated adjusting for clustering at the clinic level and risks were compared with the normal test for proportions (Kirkwood, 1988; Levy & Lemeshow, 1999).

In the case-control study, cases were compared to anaesthetics or sedations recorded in the cohort that did not die within 48 hours of the procedure (controls). Details of the patient, procedure, anaesthetic and sedative management, and personnel involved were recorded for cases and controls in self-administered questionnaires (Table 1). Intended procedure type and duration were recorded in addition to actual procedure and duration. In cases that died prior to the procedure being performed, intended duration was recorded as the mean duration for controls of the same procedure category. The unmatched controls were randomly and prospectively selected at a 1:4 case:control ratio from the cohort of cats anaesthetised and sedated during the study. The cohort study data were returned to the investigators on a monthly basis and were entered into a relational database (Access, Microsoft), exported to a spreadsheet (Excel, Microsoft) and cumulative frequencies of monthly anaesthetic and sedation events were

calculated. This allowed the identification of individual cat anaesthetic and sedation events by veterinary centre and within that centre by animal number of the month (e.g. 53rd cat of 112 cat anaesthetics and sedations recorded that month at centre 1115). The cat controls were identified by centre number, procedure number of the day, day of the week and week of the month based on the previous month's distribution of anaesthetics and sedations and the control questionnaires were requested shortly after the selected anaesthetic or sedation had taken place.

A pilot study was undertaken between June and October 2002 to refine *a priori* hypotheses, to test the methods and data collection tools, and to check the sample size calculations. Sample size calculations indicated that approximately 150-170 cases would be required to detect risk factors with a prevalence of 5% in the controls and odds ratio of ≥ 2.5 or a prevalence of 10% in the controls and odds ratio of ≥ 2.0 at an 80% power and a 5% level of statistical significance, with a case:control ratio of 1:4. Univariable screening of the association of the risk factors with anaesthetic and sedation-related death was undertaken by standard methods (Schlesselman, 1982; Hosmer & Lemeshow, 2000). Biologically important factors and statistically significant variables ($P<0.2$) were evaluated in a multivariable mixed effects logistic regression model using a manual forward selection approach (Stata 7.0, Statacorp)(Hosmer & Lemeshow, 2000). Continuous variables were evaluated for linearity, for higher order associations and as fractional polynomials (Royston et al., 1999). Clinic identity was treated as a random effect in the mixed model to take account of any clustering of outcome at this level. First order interactions were assessed between the major risk factors, and model fit of the non-clustered model was checked using the Hosmer-Lemeshow test statistic and delta beta and delta deviance influence diagnostic statistics (Hosmer & Lemeshow, 2000). A sample of 20% of the non-responding controls was compared to the controls for major risk factors to assess the likely representitiveness of the controls. Statistical significance was set at the 5% level.

RESULTS

One hundred and seventy five anaesthetic and sedation-related deaths were recorded across 117 participating centres in the UK within 48 hours of anaesthesia and sedation. An additional 14 deaths, for which insufficient information was available to classify them as anaesthetic-related, were included as anaesthetic and sedation-related deaths for the estimations of risk. Sixty one percent of deaths occurred postoperatively, 30% during maintenance, 8% on induction and 1% after premedication. Of the deaths, 177 were general anaesthetics and 12 sedations. During the study, 69,234 cats (87% of patients) were anaesthetised and 9,944 (13%) were sedated. The risks of anaesthetic-related death was significantly greater than the risk of sedation-related death, 0.25% (95% CI 0.22 – 0.29%) and 0.12% (95% CI 0.05 – 0.19%, P=0.01) respectively. The overall risk of anaesthetic and sedation-related death was 0.24% (95% CI 0.20 – 0.27).

In the case-control study, 175 cases (the 14 indeterminate deaths were excluded here) were compared to 555 randomly selected controls. In the multivariable model (Table 2) increasing ASA physical status, procedural urgency, major versus minor intended procedures, increasing age, extremes of weight, endotracheal intubation and the use of fluid therapy were associated with increased odds of anaesthetic and sedation-related death. Pulse and pulse oximetry monitoring were associated with a reduction in odds. ASA physical status represented the major risk factor in this model and increasing ASA physical status by one level in the grouped variable (ASA 1-2 to ASA 3 to ASA 4-5) was associated with a 3-fold increase in odds of death. An increase of one increment in urgency (scheduled to urgent to emergency) was associated with a

1.6 fold increase in odds. Increasing age was associated with increasing odds, with patients 12 years and older twice as likely to die as young adults (0.5 to 5 years). Though the significance level of categorical age was just above the 5% level in the multivariable model (P=0.058), age was retained in the mixed model on biological grounds and because it improved model fit as assessed by the Hosmer-Lemeshow test and the delta beta diagnostic statistic. Extremes of weight were associated with increased odds and cats weighing under 2 kg were nearly 16 times as likely to die as those between 2 and 6 kg. Cats presenting for major procedures were nearly 3 times as likely to die as those presenting for minor procedures, and endotracheal intubation was associated with a 2-fold increase in odds of death. Patients receiving fluid therapy were nearly 4 times as likely to die as those that did not, whilst cats that had pulse or pulse oximetry monitoring were 3 - 4 times less likely to die than those that did not. After adjusting for other variables, particularly ASA physical status and intended procedure, the odds associated with sedation versus general anaesthesia were not significantly different and no drug associations were retained.

The response rate in the cases was 94% and in the controls it was 80%. The non-responding controls were comparable to the responding controls for a number of major risk factors (Table 3). Cases were clustered at the clinic level (P=0.05), and clinic was retained as a random effect. The fit of the logistic regression model (without the random effect) was good as assessed by the Hosmer Lemeshow goodness-of-fit statistic (P=0.90), the delta beta diagnostic statistic (all delta betas < 1.0) and the delta deviance diagnostic statistic (all delta deviances < 9.0, only 7 covariate patterns > 7.0)(Hosmer & Lemeshow, 2000).

DISCUSSION

This study represents the first large scale multi-centre study of anaesthetic-related deaths in small animals in the UK since the mid 1980's and documents a reduction in risk of anaesthetic-related death in cats compared to this previous work (0.25%, 95% CI 0.22 – 0.29% compared to 0.29%, 95% CI 0.22 - 0.37%)(Clarke & Hall, 1990). Veterinary practices involved in the current study were anaesthetising a sicker population of patients than in the previous study (8% compared to 4% ASA physical status 3-5), suggesting the improvements were greater than reflected in this overall risk of death. However the risk of death in feline anaesthesia was substantially greater than the risk of anaesthetic-related death reported in human anaesthesia (0.02 – 0.05%)(Biboulet et al., 2001; Braz et al., 2006; Kawashima et al., 2001). Cats are relatively small, with a large surface area to volume ratio, making them more prone to hypothermia and to the potential for drug overdose than larger species. They have small airways and sensitive larynxes predisposing to upper airway complications (Hall & Taylor, 1994). Nonetheless, in comparison to the risk in human anaesthesia, a major component of this greater risk in cats was likely to reflect different standards of anaesthesia; fluid support was infrequent in the anaesthetised cats, monitoring was often superficial, a specialised anaesthetist was generally not in charge of the anaesthetic, and dedicated postoperative and intensive care facilities were not available for the majority of patients.

The risk of sedation-related death was half the risk of anaesthetic-related death suggesting sedation may be lower risk than general anaesthesia. However, in the case-control study, a tendency to reduced odds of death associated with sedation compared to general anaesthesia was not significant after adjusting for other variables, particularly health status and intended procedure. Though heavy sedation is routinely used in veterinary medicine (patient in lateral recumbency and minimally responsive to moderate stimuli), it is generally reserved for minor

procedures such as diagnostic tests and wound repairs and the reduced risk associated with sedation was likely to reflect this. Further, the sedation data were sparse as the risk reported represented only 12 deaths from 9,944 sedations, compared to 177 anaesthetic-related deaths from 69,234 general anaesthetics. The reduced risk of sedation compared to anaesthesia has not been reported before in veterinary anaesthesia and in human anaesthesia some work recorded a similar risk of death for general anaesthesia and sedation (Arrowsmith et al., 1991). The current study suggests the risks of death associated with sedation and general anaesthesia are broadly comparable after accounting for major risk factors.

Table 1. Risk factors evaluated in the Case-Control study

A. Patients Details	Breed, sex, age, weight. Primary or referred patient. Previous anaesthetics and sedations in the last month.
B. Preoperative Evaluation and Preparation	Patient health status (ASA physical status). Preoperative clinical examination, workup and preparation
C. Procedure	Procedure urgency. Intended and actual procedure Location of procedure and patient recumbency
D. Anaesthetic and sedative drugs administered	General anaesthesia or sedation Premedication, induction, maintenance and other drugs Endotracheal intubation, anaesthetic breathing system used and type of ventilation employed Intravenous catheter placed, perioperative fluid therapy given Anaesthetic machine check performed
E. Monitoring	Person monitoring the patient and other duties of this person Methods of monitoring
F. Recovery	Duration of procedure, times to sternal recumbency and standing Quality and location of recovery Person and frequency of postoperative monitoring Postoperative temperature recorded
G. Personnel Details	Person undertaking procedure, experience and qualifications Person monitoring the anaesthetic, experience and qualifications

The postoperative period was the highest risk period. Many of these deaths occurred at times when patients were not being observed and closer postoperative monitoring could reduce fatalities. Previous work in companion animals documented the majority of deaths occurring during anaesthesia with only approximately 30% occurring postoperatively (Clarke & Hall, 1990) suggesting veterinarians may now be more able to maintain anaesthesia safely but still have problems in recovery. In human anaesthesia, postoperative mortality has also been a concern (Pedersen et al., 1990; Tiret et al., 1986). Early reports from the National CEPOD (1988; 1982) recognised the postoperative period as an important period for complications and

recommended greater support at this time. In subsequent work increased recovery room and intensive care facilities were considered to have contributed to reduced risks (Tikkanen & Hovi-Viander, 1995). The provision of improved recovery facilities and closer patient monitoring particularly in the early postoperative period should be considered to reduce risk further in feline anaesthesia.

Table 2. Mixed effects logistic regression model of the odds of anaesthetic and sedation-related death in Cats

Risk Factor	Categories	β	s.e. β	Odds Ratio (OR)	95% Confidence Interval	P value
Health Status (ASA grade)	ASA 1-2					
	ASA 3					
	ASA 4 – 5					
	Trend[a]	1.16	0.23	3.2	2.0 – 5.0	<0.001
Urgency of Procedure	Scheduled					
	Urgent					
	Emergency					
	Trend*	0.46	0.23	1.6	1.0 – 2.5	0.050
Intended Procedure	Minor procedure			1		
	Major procedure	1.00	0.35	2.7	1.4 – 5.4	0.005
Age	0 - < 0.5 years	-0.97	0.93	0.4	0.1 – 2.4	
	0.5 - < 5 years			1		
	5 - < 12 years	0.51	0.29	1.7	0.9 – 3.0	
	12 years – maximum	0.73	0.32	2.1	1.1 – 3.9	0.058
Weight	0 - < 2 kg	2.75	0.85	15.7	2.9 – 83.6	
	2 - < 6 kg			1		
	6 - < max	1.03	0.50	2.8	1.1 – 7.4	
	Unknown	0.11	0.81	1.1	0.2 – 5.5	0.002
Endotracheal (ET) Intubation	No ET tube			1		
	ET tube	0.66	0.33	1.9	1.0 – 3.7	0.042
Pulse and pulse oximeter used	None			1		
	Pulse assessed only	-1.10	0.34	0.3	0.2 – 0.6	
	Pulse oximeter used only	-1.62	0.43	0.2	0.1 – 0.5	
	Pulse and pulse oximeter	-1.81	0.40	0.2	0.1 – 0.4	<0.001
Perioperative Intravenous (IV) Fluids	No fluids given			1		
	IV catheter used only	-0.34	0.65	0.7	0.2 – 2.5	
	IV fluids given	1.37	0.30	3.9	2.2 – 7.1	<0.001
Intercept		- 5.03	0.58			
Random Effect of Clinic Identity (rho)		0.08 (rho)	0.02 (SE)			0.054

[a]Trend represents the OR for a one-category increase in the risk factor. Number of observations 723 out of 730.

Table 3. Comparison of Controls and Non-Returned Controls

Risk Factor	Proportion of controls	Proportion of non-returned controls	P value	95% CI[a] for the difference in proportions
Sedation	55/555 (9.9%)	2/23 (8.7%)	0.84	-15.5 to 13.1%
ASA 3-5	47/555 (8.5%)	3/23 (13.0%)	0.44	-12.0 to 21.1%
Urgent or Emergency procedure	97/555 (17.5%)	2/23 (8.7%)	0.27	-23.2 to 5.7%
Major Procedure	35/555 (6.3%)	3/23 (13.0%)	0.20	-9.8 to 23.2%
Age[b]	5.3 +/- 5.1 years	4.1 +/- 4.1 years	0.27	-1.0 to 3.6 years

[a]Hauck-Anderson corrected 95% Confidence interval (CI) for the difference in proportion between the controls and non-returned controls.
[b]Mean +/- standard deviation and the 95% CI for the difference between controls and non-returned controls.

A number of risk factors were identified in the case-control study that could greatly aid preoperative patient assessment and identify those patients at greatest risk prior to anaesthesia. Patient health status, as described by ASA physical status was particularly important and this work provides further support for the relevance of the ASA physical status to species other than man. The major association between health status and anaesthetic-related death has been previously documented in veterinary reports (Brodbelt et al., 2006; Clarke & Hall, 1990; Dyson et al., 1998; Hosgood & Scholl, 1998; Johnston et al., 2004) and is consistent with work in the medical literature (Biboulet et al., 2001; Buck et al., 1988; Donati et al., 2004; Morita et al., 2001; Pedersen, 1994; Tikkanen & Hovi-Viander, 1995; Tiret et al., 1986; Wolters et al., 1996). Pre-existing pathology may reduce the therapeutic index of administered anaesthetics, it may predispose to cardiopulmonary depression and it may depress other physiological function significantly. In addition to health status, procedural urgency could be a valuable factor to aid preoperative patient assessment. Increased risk has been associated with increasing urgency in human and equine anaesthesia (Biboulet et al., 2001; Buck et al., 1988; Donati et al., 2004; Johnston et al., 2002; Newland et al., 2002; Pedersen et al., 1990; Tiret et al., 1986) and this was likely to reflect the ability to assess and stabilise patients preoperatively, and due to the tendency for urgent procedures to be presented outside of normal working hours, to reflect staffing levels and personnel fatigue. Greater attention to preoperative assessment of patient health status and procedural urgency and improved stabilisation prior to the procedure could substantially reduce deaths.

Increased odds of anaesthetic-related death with increased patient age has not been reported before in cats, in contrast to work in canine (Hosgood & Scholl, 1998), equine (Johnston et al., 2004; Johnston et al., 2002) and human anaesthesia (Biboulet et al., 2001; Donati et al., 2004; Morita et al., 2001; Pedersen, 1994; Tikkanen & Hovi-Viander, 1995). The lack of an association with age in previous studies in cats, was likely to reflect limits of study power, more than species-specific differences (Clarke & Hall, 1990; Dyson et al., 1998; Hosgood & Scholl, 2002). Fractional polynomial analysis of age suggested best fit with a linear function compared to a categorical representation of age. However, the categorical version was preferred due to it having fewer missing values, as it was possible to classify the age category but not the exact age for twelve cats. Old patients may be more susceptible to the depressant effects of anaesthetics, to

hypothermia via impaired thermoregulatory mechanisms and to prolonged recoveries due to tendencies to reduced metabolic function and hypothermia (Dhupa, 1995; Meyer, 1999) and particular care should be taken when anaesthetising older patients.

The increased odds of death seen with small patients were consistent with findings in dogs (Brodbelt, 2006) and in paediatric anaesthesia (Campling et al., 1990) and were biologically plausible. Smaller patients could be more prone to drug overdose, to hypothermia and to difficulties in perioperative management (e.g. intravenous catheter placement, endotracheal intubation). Increased risk with increasing weight was likely to reflect, at least in part, risks associated with obesity. Obesity could contribute to perioperative complications via increased potential for respiratory compromise, reduced cardiovascular reserves, and slower recoveries after inhalation anaesthesia due to a greater sink for the inhalant agent (Hall et al., 2001). Increasing risk for patients presenting for major compared to minor procedures, was also consistent with the work in canine (Brodbelt, 2006), equine (Johnston et al., 2004; Johnston et al., 2002) and human anaesthesia (Donati et al., 2004; Newland et al., 2002; Tiret et al., 1986). More complex and invasive procedures were likely to impose greater stress on patient physiology and when assessing patient risk prior to anaesthesia, assessment of the planned procedure's complexity should be considered.

Increased risk of major complication with tracheal intubation (compared to no endotracheal intubation) has been previously reported in cats (Clarke & Hall, 1990; Dyson et al., 1998) and in the current study this association remained even after adjusting for major confounders, in particular ASA physical status and intended procedure. In the multivariable model there was a non-significant tendency to an interaction with intended procedure, with endotracheal intubation being associated with increased odds of death in minor intended procedures and reduced odds in major procedures (endotracheal intubation OR=2.3 in minor procedures, OR= 0.6 in major procedures). This was not significant (P=0.08), the model fit was not as good when the interaction was included and thus it was not retained in the final model. Nonetheless, this would suggest that in the more complex procedures the advantage of securing an airway outweighed any risks associated with the process of intubation, whilst in more simple procedures the risks associated with intubation were more important. The cat airway is small and more sensitive to trauma, spasm and oedema than that of the dog or horse (Hall & Taylor, 1994; Mitchell et al., 2000) and as such the process of intubation if poorly performed would be expected to increase complications. More patients that were intubated during the procedure died postoperatively than cases that were not intubated (63% of endotracheal intubated deaths versus 48% non-intubated deaths occurred postoperatively), suggesting laryngeal trauma, spasm or oedema may have been a more common underlying cause than endotracheal tube obstruction. Problems with airway management have also been a major cause of anaesthetic death in human anaesthesia (Biboulet et al., 2001; Braz et al., 2006; Caplan et al., 1990; Gannon, 1991). Topical local anaesthesia (lidocaine / prilocaine mixture) was routinely used in the vast majority of cats in this study to desensitise the larynx prior to anaesthesia whereas neuromuscular blockade to facilitate endotracheal intubation is not currently practiced in feline anaesthesia. Though further work is merited to evaluate this association further, the results suggest endotracheal intubation should be used cautiously particularly in cats undergoing minor procedures whilst for more complex procedures the provision of a patent airway remains more important.

The four-fold increase in odds associated with receiving fluid therapy was surprising. This has not been reported before in animals, was counter-intuitive and may reflect, at least in part, residual confounding particularly by health status, age, procedure type and duration. Though the reported odds were adjusted for health status, age and presenting procedure, heterogeneity

within the categories of each risk factor could have resulted in residual confounding (Dohoo et al., 2003; Royston et al., 2006). Nonetheless, a component of the increased odds may be related to administering fluids and the potential for fluid overload. Cats are a relatively small species, and with few veterinary practices measuring central venous pressure or using fluid pumps to administer intravenous fluids, the potential for volume overload was possible. Careful fluid administration and monitoring should be recommended in cats, though further work is needed to confirm that the observed association is real.

The reduction in odds of anaesthetic-related death with pulse and pulse oximetry monitoring has not been reported in small animals. Theoretical analyses in human anaesthesia support these findings and have suggested pulse oximetry would have detected 40 - 82% of reported perioperative incidents, and when combined with capnography 88 - 93% (Eichhorn et al., 1986; Tinker et al., 1989; Webb et al., 1993). Other human studies involving randomised controlled trials have been unable to demonstrate a significant reduction in complications when pulse oximetry was used (Pedersen et al., 2001) and the current work provides further objective support for the use of pulse oximetry albeit in a species other than man. That monitoring pulse and using a pulse oximeter were associated with reduced odds suggested that some form of assessment of cardiovascular function (pulse quality and rate) and respiratory function (oxygen saturation from pulse oximetry) may be important in minimizing perioperative complications. Pulse oximetry was not routinely available in veterinary practice at the time of the last UK study (Clarke & Hall, 1990), and one could speculate that this now widely adopted monitoring device has contributed to the reduced risk of anaesthetic death reported here. Other methods of monitoring including capnography, blood pressure and electrocardiography were not retained in the model primarily because they were rarely used (<5% of the controls had these methods) and this study would have had limited power to evaluate them.

Sedation and general anaesthesia were both evaluated in the current study. Heavy sedation (patient in sternal or lateral recumbency, unresponsive to minor stimuli) is commonly used in veterinary practice to undertake diagnostic and other minor procedures and the authors were interested in evaluating the role of general anaesthesia versus sedation in perioperative death. Additionally, specific *a priori* hypotheses involved drugs used both for sedation and as part of general anaesthesia in cats (e.g. medetomidine and acepromazine), and their roles in both were of interest. In the univariable analysis in the case-control study there was a trend to reduced odds of death with sedation compared to general anaesthesia (odds ratio= 0.6, 95% CI 0.3 – 1.2) but, as discussed above, after adjusting for major risk factors the odds ratio tended to 1 and this variable was not retained in the multivariable model, indicating that sedation versus general anaesthesia was not a major risk factor for anaesthetic-related death.

Interestingly, no specific drug or class of drugs was associated with death. Though univariable associations were seen, when adjusting for potential confounders principally health status, these associations were no longer significant. There were non-significant tendencies (in the multivariable model) to reduced odds with premedication with acepromazine (adjusted OR=0.6, 95% CI 0.3 – 1.1), benzodiazepines and opioid combinations (adjusted OR=0.5, 95% CI 0.2 – 1.4) and similar odds with medetomidine premedication (adjusted OR=1.1, 95% CI 0.3 – 3.4) compared to no premedication. The tendency to reduced odds with acepromazine is supported by previous work in companion animals (Brodbelt et al., 2006; Clarke & Hall, 1990; Dyson et al., 1998; Johnston et al., 2002), whereas the lack of increased odds with medetomidine contrasts with strong evidence associating xylazine (another alpha$_2$ adrenoceptor agonist) with increased risk in dogs and cats (Clarke & Hall, 1990; Dyson et al., 1998). One of the *a priori* hypotheses based on this latter work was that medetomidine was associated with

increased odds of anaesthetic-related death and given the power of the study to detect major associations there was no evidence to support this. There were no clear associations with induction or maintenance agents and anaesthetic-related death, suggesting in general drug-related effects were less important than patient, procedure and management factors.

The validity of this study rested on a clear and consistently applied definition of anaesthetic and sedation-related death. The inclusive definition, such that all deaths were considered cases unless it was reasonable to exclude them, attempted to provide a clear and objective cut-off. The appointment of an independent review panel to assess all potential cases was undertaken to increase objectivity and consistency of classification. The high response rate for cases indicated that the cases were representative of the anaesthetic-related deaths in the study population. The random selection of the controls from the cohort of anaesthetised and sedated cats was undertaken to reduce the potential for selection bias. The response rate for controls was reasonably good (Dohoo et al., 2003) and comparisons between responders and non-responders suggested the controls were largely representative of the population under study. Misclassification of exposure history was minimised by checking patients against their anaesthetic record when available and assessing the plausibility of data. Recall bias was minimised by requesting the controls soon after the procedures were undertaken, such that both case and control questionnaires were completed in the immediate period after the event and control selection could not influence exposure status (Dohoo et al., 2003).

In summary, this is the first large scale prospective multicentre study of perioperative mortality in cats undertaken in the UK for nearly 20 years. The risk of anaesthetic-related death has reduced over this time period but further improvements are required if the risk is to approach that reported in human anaesthesia. The postoperative period was particularly high risk and greater care at this time could substantially reduce deaths. Risk factors have been identified that could improve preoperative assessment of cats; in particular health status, age, weight, and procedure urgency and type. These factors should aid the identification of patients likely to be at greatest risk of death. Pulse and pulse oximetry monitoring could reduce complications and should be routinely used. Endotracheal intubation may increase the risk of anaesthetic-related death, greater care should be exercised during and after the intubation of cats and for minor procedures the provision of oxygen without an endotracheal tube may be more appropriate. Current use of perioperative fluid therapy may be adversely affecting cats and greater attention to monitoring and management of fluid therapy should be considered.

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FACTORS AFFECTING THE SUCCESS OF REHOMING DOGS

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SUMMARY

Every year there are many unwanted dogs rehomed by welfare organisations throughout the UK. Successfully rehoming these dogs is a difficult process with some dogs being returned to rehoming centres. There have been very few studies examining these processes in the UK. This study was conducted to determine factors that affect rehoming success.

A prospective cohort study was conducted using 5,750 dogs rehomed by Dogs Trust between January 2005 and January 2006. Dogs were followed for a period of 6 months after adoption to determine if they were still in their placement home. Data were analysed using multivariate logistic regression. The results of this study suggest that there were many factors involved in a successful adoption, including: behavioural problems, owner expectations and attendance at training classes. New owners should be informed of what to expect when rehoming a dog and should be prepared to work at any behavioural problems that they might face if successful rehoming is to be achieved.

INTRODUCTION

Every year thousands of dogs are cared for and rehomed by animal shelters or welfare centres throughout the UK. These dogs are either strays or are relinquished to the centres because their owners can no longer look after them. The rehoming of these dogs to new owners is not always successful, resulting in these dogs being returned to the rehoming centres, which is stressful for both the dog and the owner. Successful adoption is dependent upon the dog, the previous owner, the potential owner and the staff at the welfare centre (Posage et al., 1998).

Studies have found that the proportion of dogs that are unsuccessfully rehomed varies depending on the country or area in which the study was done and on the welfare centre policies. In the USA, studies have found that over 10% of adoptions were returned (Posage et al., 1998) and Patronek et al. (1995) found that 18.8% of the adoptions in his study were returned. Of all the dogs adopted in a one year study of Australian centres, 7.2% were returned (Marston et al., 2004). A study carried out through the Blue Cross in Oxfordshire, found that 81.4% of dogs that had been adopted during the year prior to the study interview were still in their original placement home, 8.2% were euthanased for medical reasons, died of old age or medical conditions or were returned for non-behavioural reasons and 10.4% were returned, euthanased or rehomed by the owner for behavioural reasons (Bailey et al., 1998). A recent study in Italy

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found that 58% of the adopted dogs were returned due to behavioural problems (Mondelli et al., 2004).

Previous studies have shown conflicting results for factors that affect the success of rehoming and have highlighted differences between the countries in which they were conducted. The objective of the current study was to determine the factors affecting the success of rehoming or which were associated with the return of dogs post-adoption in the UK.

MATERIALS AND METHODS

A prospective cohort study was conducted using dogs which were rehomed from Dogs Trust kennels throughout the UK between January 2005 and January 2006. Dogs Trust is the largest dog welfare charity in the UK and at the start of the study had 15 rehoming centres. A sample of dogs was selected by the staff at the centres each month when they were adopted using stratified systematic sampling, stratified by rehoming centre and whether the dog was relinquished or stray. The target sample size was 5000 dogs, aiming to detect an odds ratio of 1.5 with 95% confidence and 80% power, assuming a 14% return to kennel proportion. Every dog that was relinquished was included in the study and every second stray dog that was adopted was included in the study. For the purposes of this research "relinquished" refers to dogs for which we have a completed relinquishment questionnaire from the previous owner and "stray" refers to any dog without this information. The Dogs Trust's rehoming centre in Leeds was excluded from this study as it was not using computerised records at the start of the study.

When the dog was adopted the new owners were informed about the study and asked if they would complete the questionnaire that would be sent to them. Once the dog was selected by the staff at the centres, copies of its veterinary record, behavioural assessment and handover form (if the dog was relinquished) were made and sent to Dogs Trust head office. This information along with further information collected from the Dogs Trust database was then entered into a relational database (Access 2003, Microsoft). This database used data entry forms with data entry checks and validation rules to minimise data entry errors. The data were automatically coded as they were entered to minimise errors that could occur during recoding of the data.

The veterinary record and behavioural assessments used in this study were those used routinely by the Dogs Trust centres. The handover forms and postal questionnaires were designed at the start of the study. They consisted of a combination of questions taken from the previous handover form used by Dogs Trust and additional questions aimed at obtaining more detailed information about the home environment. It was decided to use closed questions to simplify categorising the data with an open question at the end to allow the respondents to add more information if they felt it was needed (O'Cathain & Thomas, 2004). The questionnaires were pre-tested using owners relinquishing their dogs during December 2004 at 3 Dogs Trust centres.

Approximately 6 to 8 weeks after the dog was adopted the new owners were sent a postal questionnaire with a pre-paid reply envelope. The questionnaires were sent at this time in order to reduce recall bias with regards to the questions about the dog's health immediately after adoption, to allow time for the dog to adjust to its new surroundings and thus to allow the owners to get a more accurate picture of the dog's behaviour. At 6 months after adoption a follow-up telephone call was made to a random sample of new owners to determine if they still had their dog. The sample size of 700 phone calls was based on an expected prevalence of 2% of

people no longer having the dog at a 95% confidence level and an accepted error of +/-1%. Dogs Trust requests that if a new owner is no longer able to care for the dog, that they should return the dog to one of the Dogs Trust centres and therefore the number of dogs rehomed privately was expected to be very small. Dogs Trust bases all their estimates of a successful adoption on a 6-month period; that is they consider that if a dog is returned at any point after 6 months after it was adopted, this would be considered to be a successful adoption. The postcode information, which was available for all previous owners and all new owners, was used to classify all the owners into consumer classes based on a program called Mosaic (Experian Micromarketing). It classifies all postcodes into 61 types aggregated into 11 groups in terms of their socio-demographics, lifestyle, culture and behaviour. The program uses 400 variables from demographics, socio-economics and consumption, financial measures, property characteristics, property value and location to enable the classification.

All analysis was carried out using Stata version 9.0 (Stata Corp.). Descriptive and univariate analyses were conducted using cross-tabulations, chi ^{2} tests for association and the calculation of odds ratios using univariate logistic regression. Those variables that had a p-value less than 0.2 were put forward for evaluation in the multivariable model. The multivariable model was developed using a forward fitting logistic regression model, assessing the addition of each variable using likelihood ratio tests ($p \leq 0.05$). The data were correlated as some dogs were rehomed, returned and rehomed again, in some cases several times, and therefore it was checked whether there was any need to account for this in the model by comparing a model of the data with the dog-id as a random effect and a model without this. The fit of the model was assessed using a ROC curve and Hosmer-Lemeshow goodness-of-fit test. Estimates of population attributable risk (PAR) were calculated using odds ratios from the logistic regression model output (Cox, 2006).

RESULTS

A total sample of 5,750 dogs was recruited into the study of which 2,185 were relinquished dogs. There was a 78% response rate to the follow-up postal questionnaire sent to new owners shortly after adoption giving results on 4,500 dogs. There were 700 follow-up phone calls made to new owners 6 months after adoption and only 2 owners had rehomed the dogs themselves (0.3%). There was a 14.7 % failed adoption rate during the 6-month follow-up period. Of those dogs that were returned within the 6-month follow-up period, 39.1% were returned within 2 weeks of adoption. Figure 1 shows the distribution of time from adoption to return during the follow-up period, with a median time of 27 days (95% CI 23 – 32 days).

On univariate analysis, 55 variables relating to the dog, new owner, previous owner and rehoming centre had a p-value of less than 0.2 and were put forward for evaluation in the multivariable model.

Figure 2 shows the frequency with which different reasons were given for returning a dog within 6 months of adoption and it can be seen that the most common reason given was behavioural problems.

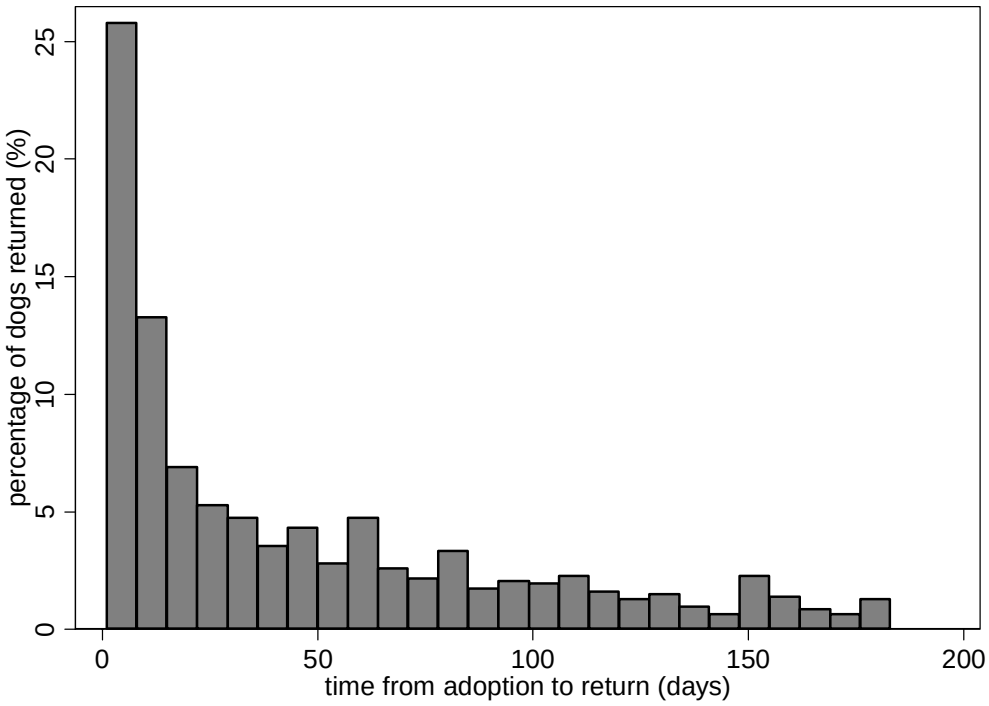

Fig.1 Percentage of dogs returned (each bar indicates 7 days)

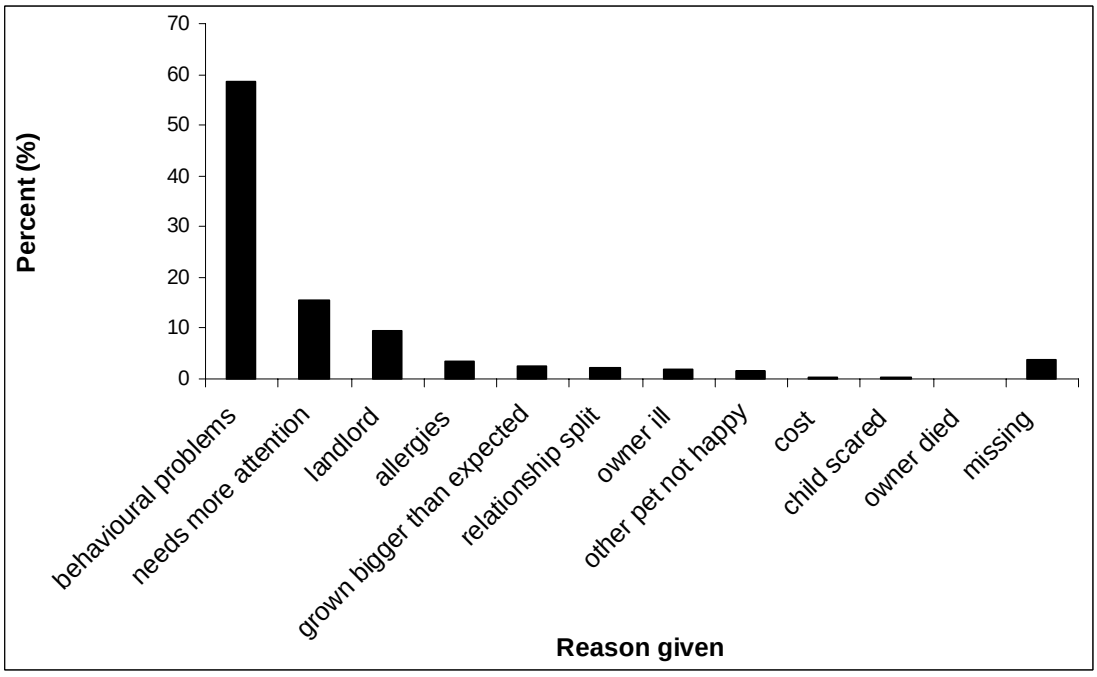

Fig. 2 Reasons given for return of dog post-adoption

The results of the multivariable analysis are shown in Table 1, suggesting that behavioural problems, attending training classes and the expectations of the new owners were particularly associated with success of adoption.

Table 1. Results of multivariable analysis of factors associated with unsuccessful adoption.

Variable	Variable category	Odds ratio	p-value	95% confidence interval	Likelihood ratio test p-value
Size	Small (<10Kg)	1.00			0.003
	Medium (10-25Kg)	1.78	0.002	1.23 – 2.59	
	Large (>25Kg)	2.08	0.002	1.30 - 3.33	
Centre	A	1.00			0.001
	B	0.18	0.006	0.05 – 0.62	
	C	0.64	0.419	0.21 – 1.91	
	D	0.60	0.419	0.18 – 2.06	
	E	0.49	0.003	0.30 – 0.79	
	F	1.27	0.265	0.83 – 1.95	
	G	0.42	0.251	0.09 – 1.86	
	H	0.35	0.008	0.16 – 0.76	
	I	1.00	0.995	0.52 – 1.94	
	J	0.75	0.248	0.46 – 1.22	
	K	0.75	0.405	0.37 – 1.49	
	L	0.71	0.250	0.40 – 1.27	
	M	0.84	0.452	0.53 – 1.33	
	N	0.59	0.085	0.33 – 1.08	
New owner reported behavioural problem	No behavioural problem	1.00			<0.001
	Aggression toward people with advice	5.61	<0.001	3.37 – 9.35	
	Aggression towards people, didn't get advice	11.13	<0.001	6.61 – 18.76	
	Destructive and got advice	2.14	0.003	1.30 – 3.52	
	Destructive and didn't get advice	2.07	0.002	1.32 – 3.24	
	Other problems with advice	1.73	0.021	1.09 – 2.76	
	Other problems without advice	1.85	0.002	1.26 – 2.73	
The level or work and effort the new owner expected	Less	1.00			<0.001
	Same	1.79	0.194	0.74 – 4.28	
	More	9.99	<0.001	4.05 – 24.64	

Variable	Variable category	Odds ratio	p-value	95% confidence interval	Likelihood ratio test p-value
Was the dog sick soon after adoption	No	1.00			<0.001
	Yes	3.20	0.079	0.87 – 11.73	
New owner adults age	>50 years	1.00			<0.001
	35-50 years	1.28	0.227	0.86 – 1.92	
	25-35 years	1.91	0.002	1.26 – 2.87	
	<25 years	2.91	<0.001	1.68 – 5.02	
New owner children	None	1.00			0.002
	<13 years	1.79	<0.001	1.30 – 2.47	
	>13 years	1.03	0.895	0.71 – 1.49	
Where does the dog sleep	In house	1.00			0.002
	On family members bed	0.56	<0.001	0.41 – 0.76	
	Outside (kennel / garage)	0.34	0.154	0.08 – 1.50	
Did the new owner attend training classes	No	1.00			<0.001
	Yes	0.26	<0.001	0.16 – 0.42	

It was found that the area under the ROC curve was 0.858 (95% CI 0.833 – 0.876). Population attributable risk (PAR) estimates for attendance at training classes was -2.3% with the current attendance rate being 18.1% of the population. However, if the attendance was increased to 50% of the population a PAR of -8.6% could be expected. For the expectations of the new owner the PAR for the category of work and effort being more than expected was 8.9%. PAR estimates for a dog that had shown aggression towards people and the owners had or had not sought advice were 2.9% and 4.8% of dogs returned respectively.

DISCUSSION

The results of the analysis suggest that there are many factors involved in the successful adoption of a dog. The results agree with previous studies in showing that factors relating to the dog, the new owner and the welfare centre are important (Posage et al., 1998). There was only little evidence that the previous owner also influenced how successful an adoption would be.

However, factors relating to the previous owner are likely to play an important part in the behaviour of the dog and therefore could be masked by the variables relating to behaviour.
In this study it was found that 14.7% of all dogs that were rehomed were returned to the rehoming centre within 6 months of adoption. The most common reason for the return of dogs was behavioural problems with almost 60% of the dogs returned for this reason. The second most common reason was that owners felt they were unable to give the dog the attention that it needed. In a study at Blue Cross in Oxford, 81.4% of dogs were in their original placement home 1 year after adoption, 8.2% were euthanased for medical reasons, died of old age or medical conditions or returned for non-behavioural reasons and 10.4% were returned, euthanased or rehomed by the owner for behavioural reasons (Bailey et al., 1998). A study in Sao Paulo found that of the dogs that were adopted from the city shelter between 4 years to 3 months before the study, only 40.9% were still with the owner, much less than the current study, 34.9% had died, 15% had been given to other people, 4.3% had run away and 3.2% had been returned. Of the 741 dogs that were adopted only 279 dogs were able to be traced and followed-up (Soto et al., 2005). However, in Sao Paulo no effort was made to match dogs to owners and no behavioural testing was performed, in contrast to procedures at Dogs Trust. A study in Melbourne, Australia found that of 4,405 dogs that were adopted only 7.2% were returned of which 26.4% were returned due to owner problems, 22.3% for dog related factors (size, health) and 22% for behavioural problems. Forty percent of the dogs returned were euthanased. Most dogs with problems with an existing pet were returned within 1 week of adoption while those returned for owner factors or behavioural problems were returned within 1 month (Marston et al., 2004).

In Milan, Italy, a study carried out looking at returns of adopted dogs looked at a 6-year period during which 2,830 dogs were adopted and 15.2% were returned. Out of those returned only 71.2% completed a questionnaire. More males were returned than females, 7% of dogs were returned more than once, returns were due to: 39% misbehaviour (barking, chewing, inappropriate elimination), 15% aggression and 40% management problems (no time, small house, personal problems). Length of adoption ranged from a few hours to 9 months, but 40% returned the dog within 1 week, mostly due to behavioural problems. Again, no attempt was made at these shelters to match dog and owner and no behavioural assessment was carried out (Mondelli et al., 2004). In the current study it was found that 39% of the dogs that were classified as being adopted unsuccessfully were returned within 2 weeks of adoption. Whereas, in a study in USA, Shore (2005) found that 54% of returns were within the first 2 weeks of adoption. When asked about the problem that resulted in them returning the pet, 50.6% of owners said the problem developed within the first 24 hours of obtaining the dog and a further 16.9% within the first week. The most common reasons for return were: didn't get along with other pets and not good with children. Shore (2005) said that returning a dog which was only recently adopted from a welfare centre may be very different from the relinquishment of a pet which has been owned for many years as with a recently adopted animal the length of ownership is short and therefore the bond between the dog and owner may not be fully developed. It was also discussed that returning a recently adopted dog may result in the owner feeling "disappointment and a sense of failure".

From these studies, it can be seen that the return rates vary greatly between countries and welfare centres. However, it should also be noted that the policies of welfare centres are likely to have a large impact on the return rates. Dogs Trust carries out behavioural assessments and attempt to match the ideal owner with each dog and this should result in fewer returns. However, Dogs Trust also has a non-euthanasia policy which means that they tend to work with and try to rehome more difficult dogs which many other welfare centres would euthanase, this is likely to

result in a higher return rate at Dogs Trust. In addition to this they also encourage owners who can no longer care for their dog to return the dog to one of their rehoming centres rather than try to rehome the dog themselves privately.

The multivariable model suggests that the size of the dog is a significant variable with large sized dogs being more likely to be returned to the rehoming centres after adoption when compared to small sized dogs, a result also found in previous studies (Marston *et al.*, 2004; Posage *et al.*, 1998). This is most likely because larger dogs require more exercise, they are more expensive to keep and can cause more damage than smaller dogs and what appears a small problem in a smaller dog can be quite a big problem in a larger dog and more difficult to control. As expected, behavioural problems were an important factor in the success of adoption with those dogs showing aggression towards people having 11.2 times the odds of being returned to the rehoming centre when compared with those dogs without behavioural problems. It was shown that if the dog had shown aggression towards people and the owners had called the rehoming centre for advice they then only had 5.6 times the odds of returning the dog. Assuming a causal association, the population attributable risk was 4.8% for those dogs where the owner had not sought advice when the dog had shown aggression, where as for those dogs where the owners had sought advice it was 2.9%. This indicates that owners should be encouraged to seek advice with regards to aggression as soon as the problem is noticed as this should result in fewer dogs being returned. Those dogs that showed destructive tendencies had 2.2 times the odds of being returned. However, if they sought advice from the rehoming centres, it seemed to have no effect on the likelihood that the dog would be returned. Other studies have shown that behavioural problems are often the most common reason for a dog to be returned. A previous study found that 17.4% of dogs were returned, with boisterousness (16.9%), aggression towards other dogs (7.7%) and aggression towards people (19.2%) being the most common reasons (Ledger & Baxter, 1997). Eighty-nine percent of the people who returned a dog to the shelter in Northern Ireland did so because of behavioural problems. Studies have shown that more male dogs and strays showed undesirable behaviours as compared to females and relinquished dogs respectively. Puppies were less likely to have behavioural problems than juveniles or adults (Wells & Hepper, 2000).

The model suggests that the expectations of the new owner played an important role in how likely a dog is to be retuned, with those who found the work and effort in looking after their dog to be more than they expected 9.9 times more likely to return their dog. The proportion of dogs returned to kennels in the study population that could have been prevented and successfully adopted by educating owners and providing more advice to ensure that they have realistic expectations was estimated to be 8.9%, assuming a causal association. Kidd et al. (1992b) showed that veterinary clients rejected significantly fewer pets as compared to owners who adopted pets from welfare centres. They thought this was due to the fact that vet clients would have fewer unreasonable expectations and access to more advice before obtaining a pet. However, the vet clients were older than the shelter adopters which may have affected the results (Kidd et al., 1992b). A study in the USA found that work expectations had an effect with those owners with more work than they expected being 4.3 times more likely to return the dog (Patronek et al., 1996). Stafford et al. (2003) noted that the second dog a person owns often does not meet the owners expectations, this has been especially demonstrated with police dog handlers and with guide dogs, and this can often result in a dog being returned. An Italian study showed that people who had previously owned a dog were less tolerant of behavioural problems than new owners and were therefore more likely to return a dog (Mondelli et al., 2004). However, Kidd et al. (1992a) showed that those people who had previously owned a pet were more likely to have successfully adopted a dog. Shore (2005) showed that 85% of the people

who were returning a dog had previously owned a pet. In the current study it was found that the variable of whether the owner had previously owned a dog appeared important on univariate analysis but was dropped from the multivariable model suggesting it might be confounded by other variables.

It was also found that family structure was important. Young adults were much more likely to return a dog post-adoption as compared to those older than fifty. Families with children less than 13 years were also more likely to return the dog compared to those families without children. These results are similar to those found by Kidd et al. (1992a), who showed that parents rejected pets more than non-parents. A study in the USA found that families with children were more likely to relinquish a pet. They suggested that this may be because behavioural problems were more common in homes with children (Miller et al., 1996). However, it is also likely that families with children have less time to spend working with a dog with behavioural problems or the impact of behavioural problems could be greater in households with children.

Where the dog slept also influenced how likely it was that a dog would be returned. Those owners who allowed the dog to sleep on a family member's bed were less likely to return the dog. This is possibly an indication of the bond or attachment between the owners and the dog, and may also indicate a certain level of tolerance to behavioural problems. A similar result was found where a study showed that puppies which slept on or near the owner's bed were more likely to be adopted successfully (Duxbury et al., 2003). It was also interesting to note that those who kept their dog outside, in kennels or the garage were also slightly more likely, although this was not a significant difference, to keep the dog as compared to those who kept the dog in the house but not in the bedroom. This could be due to the fact that behavioural problems of the dog would have a lower impact on the owner.

Attending training classes significantly reduced the likelihood that a dog would be returned to kennels due to the owner having better control and understanding of his dog, possibly resulting in a closer bond with the dog and it behaving better. Other studies have shown similar results. A study in the USA examining the adoption of puppies showed that there was a higher retention of dogs in the home if the puppy had attended socialization classes, wore a head-collar as a puppy and was handled frequently as a puppy (Duxbury et al., 2003). A study examining factors associated with relinquishment of a pet found that those attending training classes shortly after acquiring the dog were 5 times more likely to keep the dog (Patronek et al., 1996). It was estimated that amongst the study population, attending training classes reduced the number of dogs returned to kennels by 2.3%. This is quite a small effect considering the strength of association. This is due to the small proportion of people who attend training classes, only 18.1% of the new owners said that they attended training classes. By increasing the number of new owners who attend training classes to 50% you would expect the reduction in dogs returned to kennels to increase to 8.6%. This is likely to be economically beneficial as the cost of running training classes would be relatively small in comparison to the costs of housing the dogs that are returned and it would also be less stressful for the dogs and owners.

The rehoming centre from which the dog was rehomed also played an important role in how likely a dog was to be returned. This could be due to the staff at the centre and how much effort they put into trying to place the right dog with the right owner, and in the pre-adoption talks to try and prepare the owner or the level of post-adoption care, for example the advice given if the owner phoned with any problems after adoption. It may also be due to differences in surrounding populations, however, socio-demographic classification was dropped from the

model as it was not significant. Shore (2005) found that the most common suggestion given by failed adopters was that people should put lots of thought into the adoption and secondly that adopting a pet from a shelter was an uncertain process. Lloyd (2000) using focus group discussions about the matching process between guide dogs and their user, found that owners felt that more emphasis should be made on lifestyle changes, family dynamics and other pets.

CONCLUSION

The study shows that there are many factors that affect the success of rehoming. However, it was shown that providing training classes and the opportunity for owners to ask for advice will reduce the number of dogs being returned to rehoming centres after adoption. The results highlight that rehoming centres should be aware of these factors so that they can properly advise potential new owners on what to expect. Attending training classes significantly reduced the likelihood that a dog would be returned to the rehoming centre and therefore it may be possible for rehoming centres to change their policy and ensure that all people who adopt a dog attend a minimum number of training classes. Training classes also provide a relaxed environment in which owners may feel more comfortable to ask for behavioural advice. It also shows that further work needs to be done in this area to assess whether there are ways of better matching potential owners to dogs thereby reducing the number of dogs which are unsuccessfully adopted.

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USING A CASE-CONTROL APPROACH TO ANALYSE THE SPATIAL DISTRIBUTION OF ATOPIC DERMATITIS AMONG INSURED SWEDISH DOGS

A. NØDTVEDT*, A. EGENVALL, U. EMANUELSON AND D.U. PFEIFFER

SUMMARY

The purpose of the study was to further investigate whether living in densely populated areas is a risk factor for canine atopic dermatitis (CAD). A case-control approach was utilized, where cases of bone tumours from the same insured Swedish population that gave rise to the CAD cases, served as controls. Prior to analysing the case-control data, the assumption of spatial randomness for the controls was checked by mapping the incidence rate (IR) of bone tumours by postal code area and performing Moran's *I* and LISA tests. A logistic regression model with a spatial random error term was developed to determine the relationship between case status and human population density. In conclusion, the study supports the hypothesis that living in densely populated areas is a risk factor for CAD but some weaknesses of the design and choice of control population are discussed.

INTRODUCTION

Canine atopic dermatitis (CAD) has been defined as "a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features (…) associated most commonly with IgE antibodies to environmental allergens" (Olivry et al., 2001). No diagnostic test for the disease is available and therefore the diagnosis is based on a typical clinical presentation (pruritus of the face, ears, paws, extremities, axillae and/or ventrum) and exclusion of differential diagnoses such as pyoderma, Malasezzia dermatitis, flea allergy dermatitis, ectoparasites and adverse food reactions (DeBoer & Hillier, 2001).

CAD can serve as a model for its human counterpart as the mechanisms and manifestations of the disease are similar between the two species. In the human population, the prevalence of atopic dermatitis has increased substantially over the past decades and it is seen mainly as a disease of western, industrialized countries. Risk factors described in the literature include higher socio-economic status and smaller family sizes, and it seems reasonable to assume that the development of clinical manifestations in humans is due to interactions between genes and the environment (Williams, 2000). However, less is known about the epidemiology of CAD and hence this has been the main focus of an ongoing research project in Sweden.

In a previously published study, a large animal insurance database in Sweden was used to investigate the epidemiology of CAD, estimating the incidence rate (IR) of the disease as well as

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evaluating various risk factors (Nødtvedt et al., 2006a). It was also assessed whether spatial patterns exist for this diagnosis in a similar way as for allergic diseases in humans. A geographical analysis showed evidence of spatial heterogeneity regarding the IR of CAD among insured Swedish dogs, with clustering of cases in cities (Nødtvedt et al., 2006b). Furthermore, an increasing IR of CAD was found to be associated with increasing annual rainfall and human population density using a spatial Poisson regression model for CAD data aggregated by three-digit postal code areas (PCA). The model also revealed that there was a significant increase in CAD incidence if a veterinarian with special interest in dermatology was present in the county. A diagnostic bias was considered to be present as a result of veterinary dermatologists being more likely to work in cities than in the rural parts of the country. The ability to determine if a patient suffers from CAD will strongly depend on the skills of the veterinarian because it is a subjective diagnosis. The main question as to whether dogs in cities are more likely to develop CAD could therefore not be answered satisfactorily, and further investigation into this issue was warranted. Furthermore, the regression analysis was performed on aggregated data and individual dog level factors could not be included. There is a strong breed-predisposition for development of CAD and this was "missed" in the spatial Poisson model as well as any age or gender effects.

When performing case-control studies based on registry data, it is important to select controls from diagnostic categories that are not related to the exposure of interest (Dohoo et al., 2003). For the current study, dogs with malignant bone tumours were chosen as controls. Osteosarcoma is the most common primary malignant bone tumour in the dog, contributing more than 85% of all cases (Dernell et al., 2001). Therefore, the majority of the bone tumour cases recorded in the insurance database used were assumed to be osteosarcomas. The rationale behind using bone tumour cases as the control population in the analysis of CAD distribution was that it is also a diagnosis that will require some degree of specialization by the diagnosing veterinarian (such as possibilities for diagnostic imaging) but the two diagnoses are likely to be made by different practitioners. Furthermore, the aetiology of the two diseases is different as environmental risk factors are not reported for osteosarcoma and hence their distribution is likely to represent a spatially random sample of dogs diagnosed at specialized small animal hospitals.

The objective of the current analysis was to assess whether dogs living in urban areas are at increased risk of developing CAD by utilizing a case-control approach where bone tumour cases were used as controls. A partial aim was also to investigate the spatial distribution of bone tumour cases to assess whether the assumption of spatial randomness was valid, and to compare this distribution to that of CAD.

MATERIALS AND METHODS

Study population

Claims records from the Agria insurance company between 1995 and 2002 were accessed, and all dogs included in the analysis were covered by veterinary care and life insurance at some point during this time. For further details regarding the insurance process and the insured population see Egenvall et al. (2000). Cases were defined as dogs coded with the diagnosis CAD, while controls were all dogs with primary malignant bone tumours (Egenvall et al., unpublished observations). Time at risk was defined as years from first enrolment in the insurance plan until a diagnosis of either CAD or bone tumour, or until the dog died or left the

insurance plan. The IR by PCA was calculated as the number of cases over dog years at risk (DYAR) for CAD and bone tumours, respectively. Because CAD is a disease of young individuals, only dogs born from 1994 and later contributed time at risk to the denominator of IR for this disease. The overall IR of CAD was 1.70 cases per 1000 DYAR (95% confidence interval 1.67 – 1.77) (Nødtvedt et al., 2006a), and the IR of bone tumours 0.55 cases per 1000 DYAR (95% CI 0.51 – 0.59) (Egenvall et al., unpublished observations).

Descriptive spatial statistics

Each case and control was geo-referenced to the PCA of the owner's address when insurance was first purchased. The exact location of each dog was not known and individual observations were therefore aggregated at the centroids of each PCA. The spatial distribution of cases and controls was visualized separately as crude IR per PCA and presented as choropleth maps. Spatial empirical Bayes (EB) smoothed IRs were calculated based on a first-order, common-border contiguity matrix to account for the small sample size in many PCAs (Bailey & Gatrell, 1995). Global spatial autocorrelation of the IR of CAD and bone tumours was assessed using Morans's *I*. A local indicator of spatial association (LISA) test statistic was used to identify spatial clusters in the IR of the two diagnoses (Anselin, 1995). These analyses were performed in order to confirm the spatial randomness of the bone tumour cases. Maps were generated and manipulated using the geographic information system software ArcView version 9.1 (ESRI Inc., Redlands, California, USA), while the public domain software GeoDA version 0.9.5-i was used to generate the EB smoothed IR and to calculate Moran's *I* and the LISA test statistic (https://geoda.uiuc.edu/).

Logistic regression model

A logistic regression model for the case-control data was developed where the individual dog was the unit of analysis. The independent variables evaluated in the model were: age at diagnosis, average annual rainfall, average July temperature, average January temperature, the natural logarithm of human population density (ln(hpd)) and presence of a veterinary dermatologist in the county. For details on how these variables were obtained and included in the dataset, see Nødtvedt et al. (2006b). The assumption of linearity for continuous variables was tested by categorizing each variable into ten groups and plotting the group mean against the proportion of cases per category. To account for the lack of independence between observations that were in close spatial proximity to each other, a (Gaussian) spatial covariance matrix based on the distance between PCA centroids was included as a random error term in the model. The PROC GLIMMIX procedure in the statistical software package SAS version 9.1.3 was used for the spatial logistic regression model.

RESULTS

Study population

The current analysis included 1235 cases of CAD while 755 cases of primary malignant bone tumours were included as controls. The included individuals originated from 456 different PCAs.

Descriptive spatial statistics

Maps of the crude IRs showed that a majority of CAD cases appeared to be located in the south while no obvious pattern was observed for the IR of bone tumours (data not shown). The spatially smoothed EB rates of CAD (Fig. 1) and bone tumours (Fig. 2) show a similar pattern. The Moran's *I*, adjusted for population at risk, showed less evidence of spatial clustering of bone tumour cases than for cases of CAD (I=0.05, p=0.02 vs. I=0.37, p=0.001). The LISA statistic revealed high incidence clusters of CAD in the major Swedish cities, while no pattern of statistical significance was detected for the IR of bone tumours. Overall, these results support the notion that the spatial distribution of canine bone tumour cases appears randomly throughout Sweden.

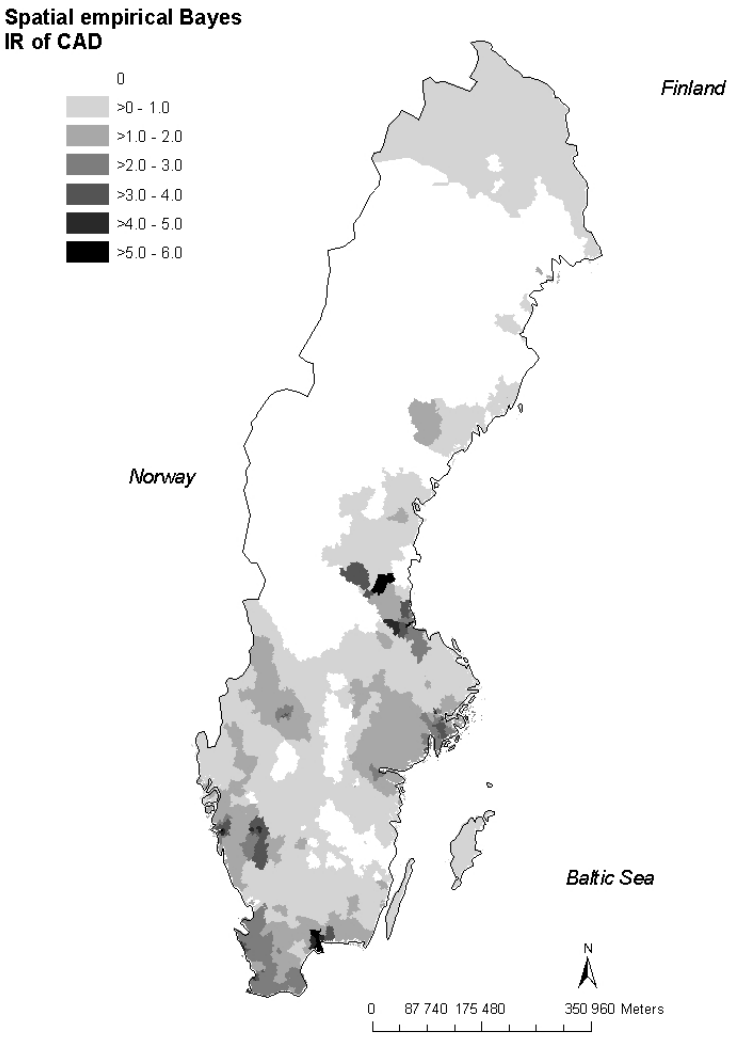

Fig. 1 The spatially smoothed empirical Bayes incidence rate (IR) of canine atopic dermatitis (n=1235) per 1000 dog years at risk by postal code area among insured dogs in Sweden 1995 – 2002. © Lantmäteriverket Gävle 2006. Permission number I 2006/1011.

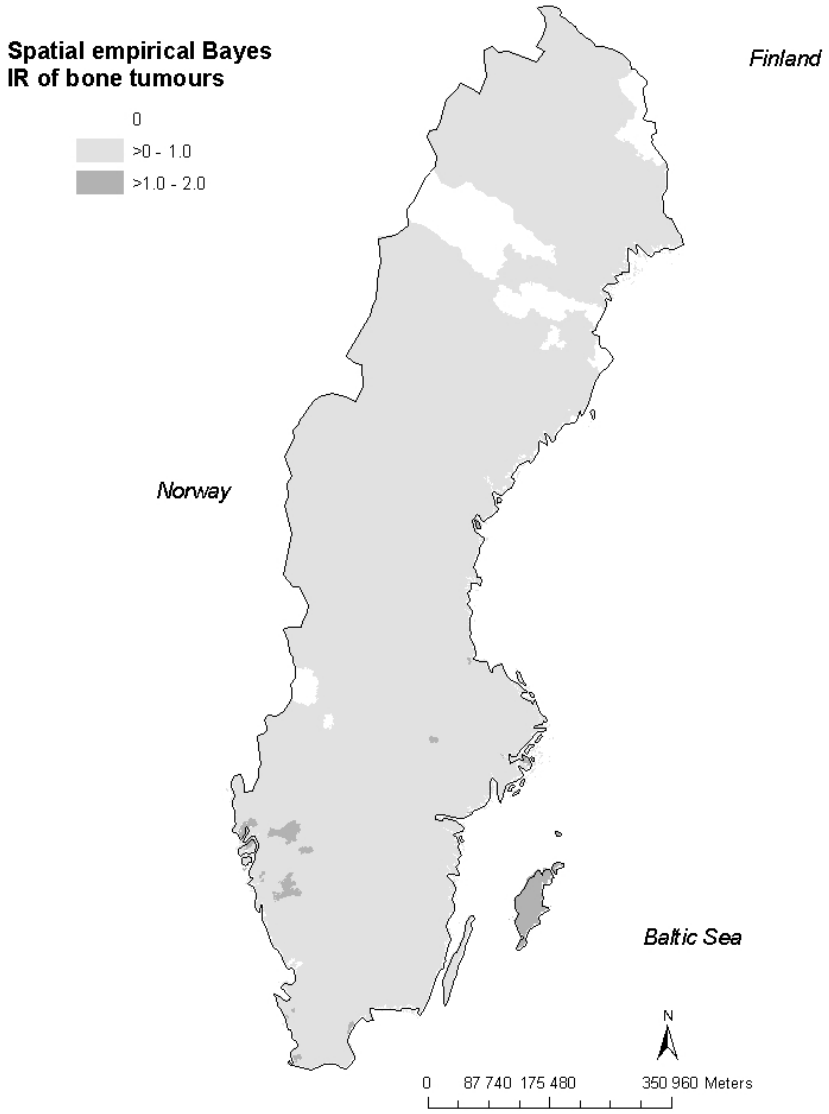

Fig. 2 The spatially smoothed empirical Bayes incidence rate (IR) of bone tumours (n=755) per 1000 dog years at risk by postal code area among insured dogs in Sweden 1995 – 2002. © Lantmäteriverket Gävle 2006. Permission number I 2006/1011.

Logistic regression model

Human population density remained a significant risk factor for CAD after controlling for age at diagnosis and long-term average January temperature as seen in Table 1. The variance of the spatial random error term was 0.26 with a standard error (SE) of 0.15, and excluding the term only marginally altered the coefficients in the model. By transforming the coefficient for ln(hpd) to an odds ratio it can be estimated that a one unit increase in the predictor (i.e. from 1 (=2.71 inhabitants per km^2) to 2 (=7.39 inhabitants per km^2))leads to a 13% increase in odds of being a case.

Table 1. Results from a spatial logistic regression model of risk factors for canine atopic dermatitis among insured Swedish dogs between 1995 and 2002, using cases of bone tumours in the same population as controls. Ln(hpd)= natural logarithm of human population density.

Variable	Coefficient	S.E.	p
Age at diagnosis	-0.82	0.03	<0.001
January temp. (C)	0.08	0.03	0.012
Ln (hpd)	0.12	0.04	0.004
Constant	4.19	0.30	<0.001

DISCUSSION

The results support the hypothesis that living in a densely populated area is a risk factor for CAD, in the same way as a higher prevalence of human atopic dermatitis has been observed in urban areas. Among the possible explanations for this phenomenon are increased levels of pollution, lifestyle factors or different climate in cities.

The purpose of using bone tumour cases as the control population was the notion of a separate mechanism of disease and the assumption of their spatial randomness. The investigations described under "Descriptive spatial statistics" support this assumption. Due to the different manifestations of the two diseases with CAD mainly occurring in young and tumours in older dogs, the time at risk for the two diseases is different. The difference in time at risk becomes apparent when comparing maps of IR for the two diseases on the same scale, as the range of values for bone tumours is more limited than can be explained purely by the smaller number of cases. The maps of spatially smoothed empirical Bayes IR make overall trends more obvious than in maps of raw data, because the effect of small sample sizes are taken into account by smoothing the IR for each PCA towards a neighbourhood mean. The effect of different age of onset was controlled for analytically in the logistic regression model.

The analysis was performed with dog as the unit of analysis but the breeds at risk of the two included diagnoses are not the same, thus impeding the possibility to separate the breed effect and the case definition. The "Top five" breeds at risk of developing CAD in this insured population were Bull terrier, Welsh terrier, Boxer, Westie and Staffordshire bull terrier (Nødtvedt et al., 2006a), while Irish wolfhound, St Bernard, Leonberger, Great Dane and Rottweiler had the highest risk of being diagnosed with bone tumours (Egenvall et al., unpublished observations). An attempt was made to include breed as a random effect in the logistic regression model but this was unsuccessful due to the lack of overlap between the case and control populations (data not shown). Therefore, results might still be biased because there is a strong breed pre-disposition for CAD and the distribution of breeds throughout the country is likely to be heterogeneous.

Due to the lack of information on the exact location for each individual, PCA was used as the spatial reference and hence data were clustered at this level. Even though the significance of the spatial error term appears dubious, a model which ignored the clustering provided signs of severe lack of fit (data not shown) and is intuitively wrong for this kind of data.

The case-control approach was aimed at evaluating the spatial distribution of CAD in Sweden while controlling for the diagnostic bias introduced by the location of veterinary

dermatologists in previous analyses. A case-cohort study could be an alternative design. A random sample of insured dogs with a recorded claim for any disease would then serve as the control group for the series of CAD cases, and the problem of non-overlapping breeds at risk would not be as prominent. However, the bias caused by many "specialists" being located in cities would then re-emerge.

In conclusion, the study lends further support to the hypothesis that urban living is a risk factor for canine atopic dermatitis. However, the effect of the geographical differences in breed-distribution on the result could not be assessed through the current analysis.

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ANIMAL HEALTH ECONOMICS

PERSUADING FARMERS TO INVEST IN ANIMAL HEALTH

A.W. STOTT* AND G.J. GUNN

SUMMARY

Using the example of bovine viral diarrhoea (BVD) in Scottish 50-cow suckler (cow-calf) beef herds, this paper illustrates a method to establish the maximum average net benefit of disease control under specific epidemiological and farm business circumstances. The method puts a relative value on alternative control/prevention options and can establish the financial benefit of health related information such as freedom from a specific disease. Results suggest that such information might affect the best control strategy to adopt and its cost effectiveness. The method can also allow for constraints on farm resources used to control animal disease and competition for their use from other investment opportunities. These attributes are missing from most previous cost-benefit studies of endemic farm animal disease. It is argued that these attributes are important to persuade farmers to invest in animal health.

INTRODUCTION

The Animal Health and Welfare Strategy for Great Britain (AHWS)

The objectives of the AHWS (Defra et al., 2004) are ambitious (e.g. disease status to be amongst the best in the world within 10 years) and will therefore require a radical shift in approach to farm animal disease prevention and control if they are to be achieved. Part of this shift in approach is to place greater responsibility for animal health and welfare on animal keepers. However, despite the Foot and Mouth Disease crisis, few cattle and sheep farmers in Great Britain have shown much interest in making radical changes in their approach to animal health (Paterson et al., 2003). The solution to this problem given in the AHWS is to establish cost benefit of alternative disease control measures so that best practice can be understood, accepted and adopted. If this solution is to be successful, cost benefits must appropriately reflect farmers' decision choices and be communicated to them in ways that best meet their needs, i.e. they identify most appropriate actions under specific circumstances.

Now that farm subsidies in the EU have been de-coupled from production, it is believed farmers (especially extensive livestock producers) will need to make considerable reductions in their costs of production and/or receive much greater market prices if their farming enterprises are to remain viable (Oglethorpe, 2005). It is therefore now particularly important to identify cost-minimising approaches to animal health in extensive farming systems.

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Limitations of existing cost benefit studies

A loss-expenditure frontier (LEF) method identifying endemic disease control strategies that minimise total costs (eliminate avoidable losses) has been clearly set out and justified by McInerney and colleagues (McInerney et al., 1992; McInerney, 1996). It is only by persuading farmers to adopt such strategies that improvements in farm animal health and welfare will be achieved. Simply publicising the average total cost of a specific disease has little or no value (McInerney, 1996). Such action may even be counter-productive as the figures imply greater benefits for animal health than are likely in practice, possibly leading to loss of credibility with farmers and other stakeholders (Stott, 2003).

Unfortunately, very few studies have been conducted using the methods of McInerney et al. (1992) (for exceptions, see Yalcin et al. 1999 and Chi et al., 2002). Lack of data is often cited as the main constraint (Bennett, 2003). However, this constraint may be overcome by simulation modelling (e.g. Stott et al., 2003) or by using decision analysis methods that are not necessarily dependent on detailed epidemiological information (e.g. Stott et al., 2005).

Relating cost-benefit results to the farm

The total cost minimising disease control strategy established for a sample of farms using the LEF method (see Yalcin et al., 1999) may not necessarily identify the best strategy at the individual farm level. Special epidemiological or economic circumstances may apply that require a different response. For individual farms, an epidemiological model of the disease in question may, in theory, allow a farm-specific LEF to be established. However, even with the necessary farm-specific data and a model capable of accurately reflecting all-important aspects, it is unrealistic to assume that the efficiency frontier implicit in the LEF model applies in practice to an individual farm. In other words, the individual farm will not obtain the maximum possible reduction in loss from disease (benefit) for every possible level of expenditure on control. Maximum possible reduction in loss can only be established from a sufficiently large sample of farms operating a wide range of alternative control strategies on and behind the frontier. Tisdell (1995) describes an alternative to the LEF method based on a benefit function (BF). The BF can be established using data representative of the decision-maker's farm (or of a homogeneous group of farms). These data may need to be obtained from an appropriately configured epidemiological model, as they are very unlikely to be available otherwise. However, the BF is not based on the concept of an efficiency frontier.

Another problem with applying cost-benefit studies of disease at the farm level is that farm decision-making is not confined to disease control. Animal disease control must compete for limited resources with other investment opportunities both on and off the farm. The optimal disease control strategy may therefore not be implemented because of better investment prospects elsewhere. Also animal disease influences, and is influenced by, decisions not directly concerned with animal health. For example, a farmer may decide to reduce the scale or intensity of an enterprise with a disease problem rather than tackle the disease head on. Stott et al. (2003) took a whole-farm approach to animal health economics in order to address these issues. The BF method can also be modified to incorporate some of them (Tisdell, 1995).

The objective of this paper is to demonstrate how economic analysis of an endemic farm animal disease using a BF can address specific decisions facing farmers and hence help persuade them to invest in animal health. Although the analysis is confined here to bovine viral diarrhoea

(BVD) in typical Scottish hill suckler-beef (cow-calf) herds, the general approach is likely to be relevant to many other diseases and farming systems.

MATERIALS AND METHODS

This example was based on a herd of 50 suckler beef cows. This was the approximate weighted average number of beef cows per holding across all regions and size groups in Scotland in the Agricultural Census of June 2004 as reported by the Scottish Executive (2005). In that year, cattle and calves constituted the biggest sector of Scottish agriculture at over 28% of gross output.

BVD model

A description of the epidemiology and economic importance of BVD is given by Houe (1999). The epidemiological model of Gunn et al. (2004) was used to estimate the annualised total cost per cow (output loss plus control expenditure) of BVD over a 10-year horizon. A variety of control options and disease scenarios were used (see below) to build the BF required for the decisions explored. As the model is stochastic, each disease cost used was the mean of 100 Monte Carlo simulations.

The model is based on a Markov-chain (state transition) methodology (Agrawal & Heady, 1972) with annual time steps. Transitions between states (animal type (calves, heifers, cows), disease status (susceptible, transiently infected, immune or persistently infected (PI)) and herd exit (replacement or death)) were tracked using a matrix of transition probabilities derived as explained in Gunn et al. (2004). A key transition probability is that of susceptible individuals becoming transiently infected in any one year (P), which was based on the Reed-Frost relationship (Abbey, 1952):

$$P = (1-Q^{Ct}) \quad (1)$$

where Ct is the number of PIs in the herd at time t (years) and Q the probability of avoiding effective contact, set to 0.4 throughout (Gunn et al., 2004). All calves born were assumed to be susceptible unless born to a PI cow (all PI) or a transient cow (susceptible, immune or PI according to stage of pregnancy at time of infection based on Houe et al. (1993). Sufficient female calves were retained to ensure a closed herd operated. Replacement rate was adjusted to maintain constant herd size throughout the course of each simulated epidemic.

All individuals in the herd were assumed susceptible in year 0 or the herd was of unknown BVD status. If the latter, animals were allocated at random to disease states at the start of each simulation to reflect assumed national prevalence of 0.95 antibody-positive herds and 0.5 antigen-positive as described by Stott et al. (2003).

The BF

In the model of McInerney et al. (1992) the LEF can be represented as:

$$LEF = A - f(E) \quad (2)$$

(Tisdell, 1995), where A is the losses from a disease when expenditure on control (E) is zero. Tisdell (1995) defines the BF as:

$$BF = f(E) \quad (3)$$

In this example, E was either expenditure on biosecurity and/or the cost of maintaining a vaccination programme on the farm (Santarossa et al., 2005). Following Stott et al. (2003), biosecurity was defined as the probability (P(Avoid)) of avoiding introduction/reintroduction of BVD virus on to the farm in any one year. If biosecurity was not maintained, then a fixed proportion of susceptible cows or heifers (0.75) would become transiently infected due to a source of virus from outside the farm. The chances of such an event occurring can be reduced by taking a variety of precautions to isolate cattle on the farm from cattle on other farms (van Schaik et al., 2002). Using the precautions required under the Premium Cattle Health Scheme (SAC, 2001) Stott et al. (2003) devised a production function (Debertin, 1986) to represent the relationship between biosecurity and expenditure on such precautions of the form:

$$P(Avoid) = U - (U - L)\, e^{\,b(m-x)} \quad (4)$$

where x is the expenditure on biosecurity precautions (£/year), $x \geq m$ and U, L, m and b are constants. The constants U and L represent the upper and lower limits of biosecurity that can be achieved in practice. The BVD model assumed that the herd rears all its own replacements, thus providing a base level of biosecurity (P(Avoid)=0.3) in return for an opportunity cost of £100/year (based on van Schaik et al., 2001) and represented in the function as m. Perfect biosecurity (P(Avoid)=1.0) was assumed unachievable under current Scottish conditions where prevalence is known to be high (Bennett & Ijelpaar, 2003) regardless of the level of expenditure, and so the curve tends towards an asymptote of P(Avoid)=0.95. The constant b governed the shape of the curve and was set to 0.005 to reflect diminishing marginal returns (to P(Avoid)) up to an expenditure of £600/year with little further improvement beyond this figure. The function provided for an expenditure of £612/year at P(Avoid)=0.90 and of £1386/year at P(Avoid)=0.949 which was taken as the uppermost level of expenditure and biosecurity in the range of options available. These figures are broadly comparable with a similar exercise conducted for a Dutch dairy farm by van Schaik et al. (2001) and a case study of biosecurity costs in Scottish cow-calf herds by Henderson (2001) as reported by Stott et al. (2003).

To find the cost of biosecurity (£/herd/year) (x), for a set of biosecurity levels (P(Avoid)), equation 4 was rearranged to give:

$$-1/b[\ln[(P(Avoid)-U)/(L-U)]] + m \quad (5)$$

Vaccination was represented in the BVD model by increasing the proportion of BVDV immune cows and heifers in the initial and subsequent state vectors of the Markov-chain. The proportion remaining susceptible was set to 0.2 (good vaccination, denoted V80) or 0.6 (poor vaccination, denoted V40) (following Santarossa et al., 2005) to reflect the risk that some animals (for a variety of reasons) may fail to be effectively immunised. It should be noted that these rates are not known to reflect the efficacy of vaccines currently on sale to farmers as the authors had no access to such information. They were chosen to further the objectives of the paper i.e. assist in demonstrating the utility of the BF approach. Results should be interpreted accordingly. The cost of vaccination was set at £4.46/cow, covering vaccine at £2.50/dose (Bennett and Ijelpaar, 2003) and opportunity cost of farm labour required to vaccinate based on Stanger (2006) (2 people vaccinating 25 cows per hour). The vaccine cost was the annual equivalent of the net present value of a 10-year programme starting with two doses to all adult animals with single doses to cows and two doses to heifers each year thereafter. In line with the model of Gunn et al.

(2004), farm labour for vaccination was charged at just £1/hour, reflecting the low opportunity cost of family labour in extensive Scottish hill farms.

Simulation for BF

The average annual benefit (output losses saved) per cow was established using the BVD model over the range of expenditures on biosecurity given by equation 5. An appropriate polynomial curve was fitted to the data in order to find function (3) using the 'add trendline' facility in an excel spreadsheet (Microsoft Corporation, 1997). The differential of this function was set to 1 and solved to find the point where the marginal benefit was equal to the marginal cost, i.e. the level of biosecurity that maximised net benefit (the last £1 spent on control yields £1 of benefit) (Tisdell, 1995). The Monte-Carlo simulation was then re-run using this level of P(Avoid) to obtain a mean maximum net benefit and its associated standard error. This process was carried out for a BVD-free herd and for a herd of unknown BVD status in order to compare the level of biosecurity justified in each case and the extra benefits derived from known BVD-free status. Results from these two analyses were then combined to establish the best allocation of a fixed biosecurity subsidy of £10/cow between BVD-free herds and herds of unknown BVD status.

The above process was repeated for herds using vaccination and biosecurity. This established the level of biosecurity expenditure justified when using vaccine. Finally, the net benefit of vaccination without additional biosecurity expenditure was estimated. By comparing these results, the hypothesis that vaccination replaces the need for investment in biosecurity could be tested.

Financial assumptions

The financial assumptions in the model of Gunn et al. (2004) were updated for this analysis using SAC (2006) to provide output typical of contemporary spring-calving hill suckler cow units in Scotland. The revised estimates are given in Table 1. All other assumptions are as reported in Gunn et al. (2004).

Table 1. Approximate values for the main output losses due to BVD in Scottish cow-calf herds used as inputs in the BVD model.

Source of Loss	Value
Immunosuppression of calves	£3 per calf at risk
Immune calf (congenital defects, growth retardation etc.)	£28 per calf
PI calf	£183 per calf
Transient cow aborting (replace or foster a calf)	£246 per cow
Transient cow no abortion (delayed rebreeding or replace)	£61 per cow
PI cow (mortality and replacement costs)	£501 per cow

RESULTS

Figure 1 shows the BF for an unvaccinated BVD-free herd. The cost function is simply a 45-degree slope representing the locus of alternative expenditures on biosecurity. At the point of greatest distance between the benefit and cost functions, net benefit is maximised. The R^2 of the

trend line was 0.99. The R^2 values for all other scenarios were 0.9 or higher. The mean maximum net benefits for this and all other control strategies investigated in BVD-free herds are shown in Table 2 together with their standard errors (SEM). The equivalent results for a herd of unknown BVD status is shown in Table 3.

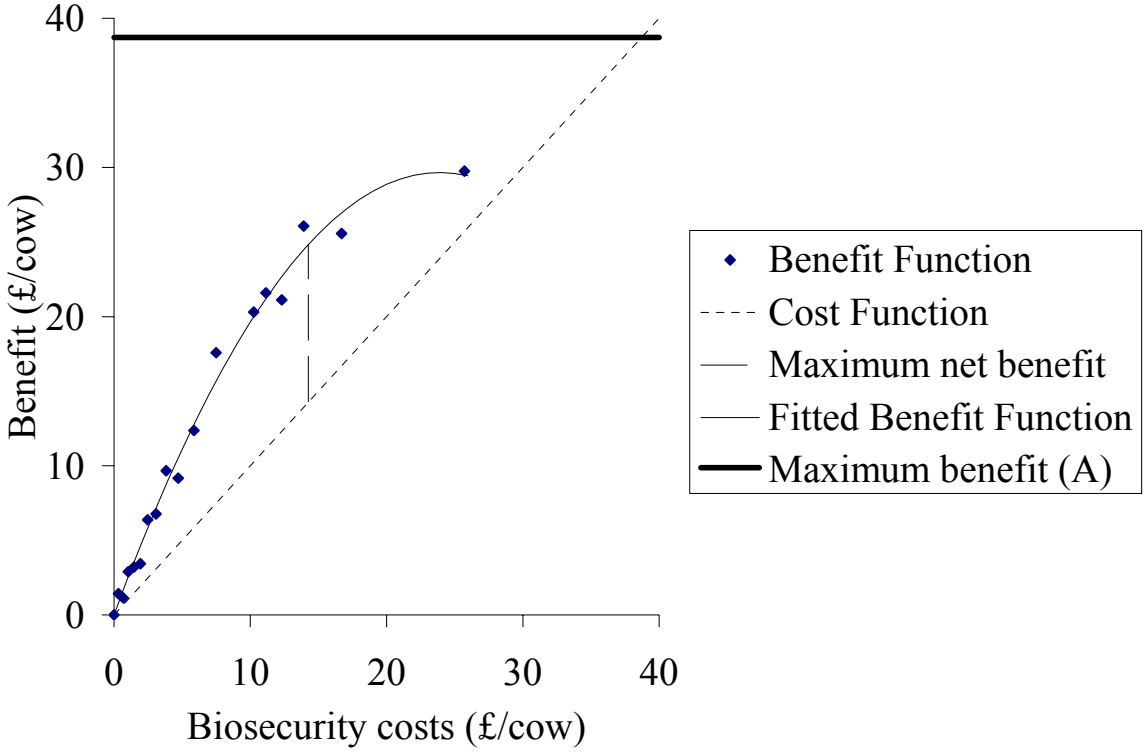

Fig. 1 Benefit function for a BVD-free 50-cow beef suckler herd showing the point of maximum net benefit from investment in biosecurity.

Table 2. Mean maximum net benefits for control of BVD and associated biosecurity level (P(Avoid)) in initially BVD-free herds.
(B=Biosecurity, V=Vaccination, all net benefits in £/cow/year)

Rank	Control Method	P(Avoid) %	Net Benefit	SEM
1	V80B	53	25.85	0.49
2	V80	30	25.67	0.43
3	V40B	87	16.85	0.93
4	B	93	11.99	1.44
5	V40	30	11.18	0.47

Table 3. Mean maximum net benefits for control of BVD and associated biosecurity level (P(Avoid)) in herds of unknown BVD status.
(B=Biosecurity, V=Vaccination, all net benefits in £/cow/year)

Rank	Control Method	P(Avoid) %	Net Benefit	SEM
1	V80	30	14.24	0.47
2	V80B	68	13.08	0.63
3	V40B	67	5.87	0.76
4	V40	30	5.73	0.62
5	B	76	2.18	1.02

The maximum potential benefit, i.e. BVD losses when E=0 (A in equation 2) for BVD-free herds (Table 2) was £38.71/cow/year. The gap between this figure and the maximum net benefit (£12.86/cow/year) represents the minimum total costs of BVD (losses plus control costs). Of this figure, £6.21 is control costs (£4.46/cow/year on vaccine plus £1.75/cow/year on added biosecurity). This leaves just £6.65/cow output losses (12.86-6.21) i.e. this best strategy cuts out 83% of BVD losses. The value of A for herds of unknown BVD status was £28.22, i.e. the minimum total cost was £13.98/cow/year. In this case, the best strategy is V80 vaccination only (£4.46/cow/year), leaving £9.52/cow/year output losses. For unknown status herds therefore, the best strategy cuts out only 66% of BVD losses.

BVD-free herds enjoyed greater rewards from BVD control than herds of unknown BVD status. The difference between the best strategies in each case was £11.61/cow/year (approximately 82% more benefit). Vaccination (V80) was the best control method in each herd type; adding biosecurity did improve net benefit in BVD-free herds but the difference was not significant. Adding biosecurity to V80 in herds of unknown BVD status could be done at no significant detriment to net benefit. For reasons discussed later, this suggests that added biosecurity is justified even with V80. In BVD-free herds, biosecurity added significant benefit to V40. For herds of unknown BVD status, V40 offered significant benefit over biosecurity alone.

Figure 2 shows the trade-off for different allocations of £10/cow/year spend on biosecurity between herds of differing BVD status. The maximum iso-benefit line (£19.79/cow/year) shows that the best allocation was £9/cow/year invested in BVD-free herds and £1/cow/year in herds of unknown BVD status.

DISCUSSION

The extent to which the results reported here reflect specific experiences in Scottish cow-calf herds is of course dependent upon the validity of the assumptions used and on the capacity of the model to reflect accurately the dynamics of BVD epidemics within commercial herds. These issues have been addressed in previous publications related to the model used here (Stott et al., 2003, Gunn et al., 2004, Humphry et al., 2005 and Santarossa et al., 2005). Many gaps remain in our understanding of the main relationships involved and the value of key parameters is uncertain. However, these difficulties are implicit in all cost-benefit studies of animal disease. Unlike many other studies that simply quote an average cost of BVD, the method reported here provides some insights into the relative importance of factors that contribute to costs and

benefits and hence provides a link to control decisions. These decisions must be taken regardless of the level of information available to assist. If the insights provided by this approach lead to better decisions than would otherwise be taken, then the approach is worthwhile.

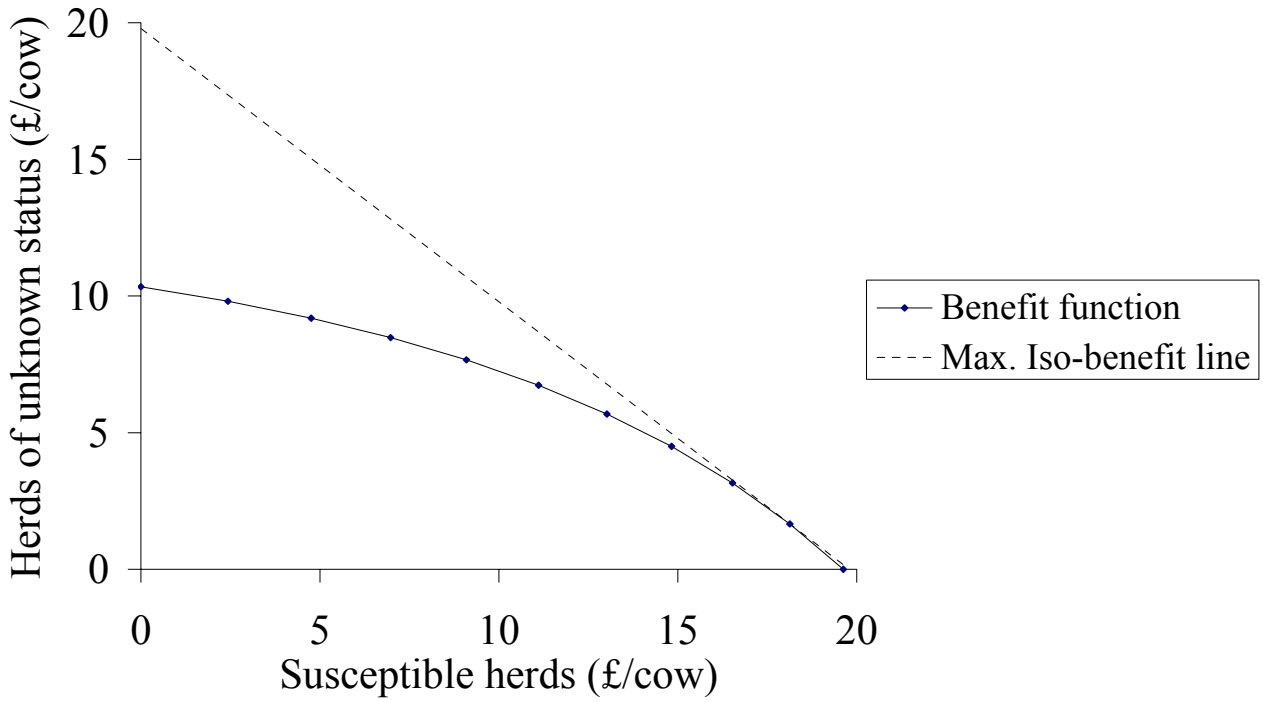

Fig. 2 Predicted maximum net benefit from alternative allocations of £10/cow/year on biosecurity between herds susceptible to BVD in year 0 of a simulated epidemic and those of unknown BVD status.

The most important factor contributing to cost-benefit in this study is the BVD status of the herd at start of treatment. Herds known to be free of BVD gain much more from investment in BVD prevention. This suggests that if farmers can be encouraged to 'test and cull' via a health scheme (Thrusfield, 1995), then a more persuasive argument can be made for further investment in animal health. Such encouragement is likely to bring benefits beyond the farm gate i.e. deliver public good (Holden, 1999). For example, this study shows that BVD-free herds operating for maximum economic benefit have much lower losses from BVD and hence pose less challenge to animal welfare than herds of unknown BVD status. Furthermore, BVD-free herds present no BVD risk to neighbouring herds, thus reducing risk of biosecurity breakdown at regional level and hence increasing the collective benefit of investment in biosecurity. Encouraging farmers to 'test and cull' may therefore be an attractive way for the farming industry to address its increased responsibility for animal health under the AHWS. Figure 2 demonstrates how limited incentives to further this aim might be allocated in favour of BVD-free herds, thus discouraging 'free-riders' (farmers who may seek to benefit from collective action against BVD without investing themselves (Holden, 1999)).

The method used in Fig. 2 is an application of a generic extension to the BF method described by Tisdell (1995) designed to deal with the problem of optimal control of more than one disease as well as the issue of limited investment funds. Tisdell (1995) points out that

interdependence may be important when dealing with two or more diseases. For example, investment in biosecurity against BVD is likely to increase biosecurity against other diseases too. In that case, these results will underestimate the relative benefits of biosecurity. However, adequately modelling these interactions between diseases is likely to prove difficult at current levels of knowledge (Stott et al., 2003). If such information does become available then the method illustrated here can be used to exploit it for the benefit of animal health and farm business viability.

The results presented here suggest that vaccination alone may not be a sufficient response to BVD. There is a considerable opportunity cost of vaccinating in ignorance of BVD status. Also, in BVD-free herds there remains benefit in added investment in biosecurity, especially if vaccine efficacy is low (even if not believed to be low, then at least efficacy is likely to be uncertain). These results may help vets, for example, to persuade farmers to take advice before instigating a vaccination programme and counter any tendency there may be for farmers to assume that vaccination alone is a complete solution to an animal health concern.

Biosecurity results observed in practice will depend on the cost and efficacy of the biosecurity actions taken and on the risk of biosecurity breakdown. These factors will be highly variable and dependent upon individual circumstances. Results reported here should therefore not be taken as generally indicative but as a demonstration of what decision support may be given using the methods described if more specific information can be had. For example, larger herds may gain some economies of scale and therefore be able to apply biosecurity strategies more efficiently than implied here. Vaccination, where the main cost is the dose given per animal may exhibit less economy of scale. At present, only general observations can be made as the data required precisely to characterise the relationship between investment in disease control and consequent benefits under specific farm circumstances are lacking. Research is currently underway to provide such information alongside the analysis methods demonstrated here. This will ensure that as further information becomes available, it can be quickly put to use for decision support

The higher variability (risk) associated with the biosecurity outcomes is shown in the higher SEM values for biosecurity only options in Tables 2 and 3. It is therefore particularly important to consider risk as well as expected maximum net benefit when making decisions about biosecurity investments. How risk may be dealt with in this context is dealt with in detail by Stott et al. (2003) and by Santarossa et al. (2005).

CONCLUSIONS

While specific estimates of financial benefits from investment in animal health will never be accurate, the method demonstrated here does give pointers towards priorities for action that in the absence of more accurate information are likely to lead to better decision making. The BF method can put a value on information such as farm health status. This can then be compared with the cost of obtaining such information. It can also rank disease control strategies under specific business and epidemiological circumstances, taking account of competition for limited resources with other investment opportunities. Most of these attributes have not been part of past cost-benefit analysis of endemic farm animal diseases but are likely to be important if such studies are to help stakeholders persuade farmers to invest more effectively in animal health.

ACKNOWLEDGEMENTS

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ECONOMIC IMPLICATIONS OF POTENTIAL CLASSICAL SWINE FEVER OUTBREAKS FOR THE FINNISH PIG PRODUCTION SECTOR

J. K. NIEMI*, H. LEHTONEN, K. PIETOLA, T. LYYTIKÄINEN AND S. RAULO

SUMMARY

Rapid structural change and regional concentration of pig production has raised concerns about whether the risk of large-scale disease losses has increased in Finland. This paper examines the pig industry's losses due to classical swine fever (CSF) epidemics. The analysis was carried out with a sector model, which simulated the recovery of pig production, starting from the recognition of the disease in the country, and took into account producers' profit-maximising behaviour and the export shock.

Epidemiological evidence suggests that the present structure of Finnish pig farming and related industries provides inadequate circumstances for severe disease outbreaks. Even in large epidemics (5-33 infected farms) with major demand shock, the average loss was simulated only at €20.7 million (mainly market losses). While it incurs higher production costs than the most intensive structures in Europe, the current farming structure does not seem to incur catastrophic losses providing incentives for tightening CSF control policy.

INTRODUCTION

European livestock production has undergone rapid structural change during the past decades. The number of pig farms in Finland, for instance, has decreased by 49% between years 1995 and 2005, whereas pig meat production has increased by 21% (by about 35 million kg) (Niemi & Ahlstedt, 2006) and the average size of production unit has doubled. Investment in larger and more efficient production units than in the past has allowed producers to specialise and to gain economies of scale and scope. Pig production in the less favourable agricultural areas of the country has gone down and production volume is now increasingly concentrated in regions where major meat processors are located (Appendix 1 in Lehtonen & Pyykkönen, 2005).

Rapid structural change and regional concentration of pig production has raised concerns as to whether the risk of large-scale disease losses has increased. If catastrophic losses are strongly related to geographical concentration and the size of production units, then bio-security, as a part of the multifunctionality of agriculture, is one argument for sustaining small production units spread out across the country. Studies suggest that geographically concentrated and contact intensive farming systems are more susceptible to large-scale disease epidemics than systems lacking these features (cf. Mangen et al., 2002; Stegeman et al., 2002; Mintiens et al., 2003; Raulo & Lyytikäinen, 2005). One of the potential problems in recently established vertically

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integrated production systems (e.g. sow pools) is that the economising of production increases the frequency of live animal transports and thus can speed up the spread of animal diseases.

The introduction of a highly contagious animal disease, such as classical swine fever (CSF), into a country has several financial consequences. Increased production costs and lost business opportunities can severely reduce the profitability of pig meat production, particularly if the outbreak is large. Disease eradication, screening and surveillance measures also tend to be costly in large outbreaks. Hence, risk management policy should be chosen accordingly. Large epidemics typically permit authorities to apply disease control measures more vigorously than small epidemics (cf. Mahul & Gohin, 1999; Mangen et al., 2002).

Disease outbreaks can reduce the economic viability of livestock production in Finland even if the number of infected farms is expected to be low compared to the most intensive pig production areas in Europe. For instance, the livestock sector can be hit by market reactions, such as the closure of export markets which, in turn, can have large price and income effects on agricultural producers (e.g. Mangen & Burrell, 2003; OECD, 2006). The potential for trade distortions has gained importance over the past decade in Finland when pig meat exports have grown fivefold, to about 20% of output (Niemi & Ahlstedt, 2006).

When designing animal disease control policies and estimating their economic implications it is critical to understand that economic agents (i.e. producers and consumers) respond to economic incentives. An essential part of economic analysis is therefore to take into account that the disease can affect the behaviour of economic agents in multiple ways, and even if the risk never materialises. If the epidemic is large, an important question is whether producers can minimise income losses due to trade distortions and loss of animal stock by adjusting the recovery of the pig population from the disease outbreak.

The goal of this study was to estimate the maximum size of financial losses that a CSF epidemic could cause to Finnish pig producers, meat processing industry and government. Disease losses were simulated with a sector model presented by Niemi et al. (2006). In addition, direct loss due to disease eradication and losses to the meat processing sector were calculated and losses to farms were minimised. The sector model allowed the adjustment of pig production over time. The model was based on dynamic programming (Bellman, 1957). It took into account that producers can minimise disease losses by adjusting production decisions according to observable trade shocks and the size of the disease outbreak. One advantage of the model is that it allowed markets to be in disequilibrium while simulating the recovery of markets from the disease shock. Epidemiological data were derived by simulation (Raulo & Lyytikäinen, 2005). Combinations of two disease and two trade scenarios (‘what if…’) were examined.

MATERIALS AND METHODS

The analysis has two main components. First, there is a model for the pig sector which simulates the market implications of a trade shock and animals removed from the stock. At each moment, the sector model takes into account the production capacity on farms currently unaffected by any official disease eradication or preventive measure. Secondly, there is epidemiological data based on Monte Carlo simulations which determine how large an impact the disease initially has on pig supply, and the extent of the applied measures. Epidemiological data relate only to farms affected by any official disease eradication or preventive measure,

counting farms infected and uninfected by the virus. The data characterise the mean and variance of production capacity lost due to the disease outbreak (Fig. 1).

The starting point of the current modelling approach is that the flows of pigs and pig meat in pig markets can be characterised with demand and supply equations. Demand and supply are connected to the prices of pigs and pig meat, as they characterise the behaviour of economic agents. Furthermore, biological constraints affect supply of pigs to slaughter. In the short term, the supply of pigs depends on the number of pregnant sows, pigs and piglets currently on farms. The import and export of live animals can also have an important role (e.g. Mangen & Burrell, 2003). As the foreign trade of live pigs is negligible in Finland, it is ignored in this model.

The logic of the sector model formulates the way in which profit-maximising pig producers on farms unaffected directly by disease make production decisions. The model assumes that producers know how demand and prices respond to changes in the volume of pig meat supply and that they have an option for inter-temporal adjustments of animal stock. Optimising producers refer to how pig producers as a group behave on markets. This adjustment is implemented with dynamic programming, as an option to optimise the number of pigs produced. It is conditional on the observed characteristics of disease outbreak. The sector model assumes market clearing, i.e. the price settles down to a level at which the demand for pig meat meets the supply. The model takes into account that market prices and quantities adjust along the demand and supply equations if the disease affects the volume of pig meat production. If the outbreak closes down export markets, the export demand for pig meat shifts exogenously and less meat is traded at price P_t than before the shift. Further details of the sector model are given in Niemi et al. (2006).

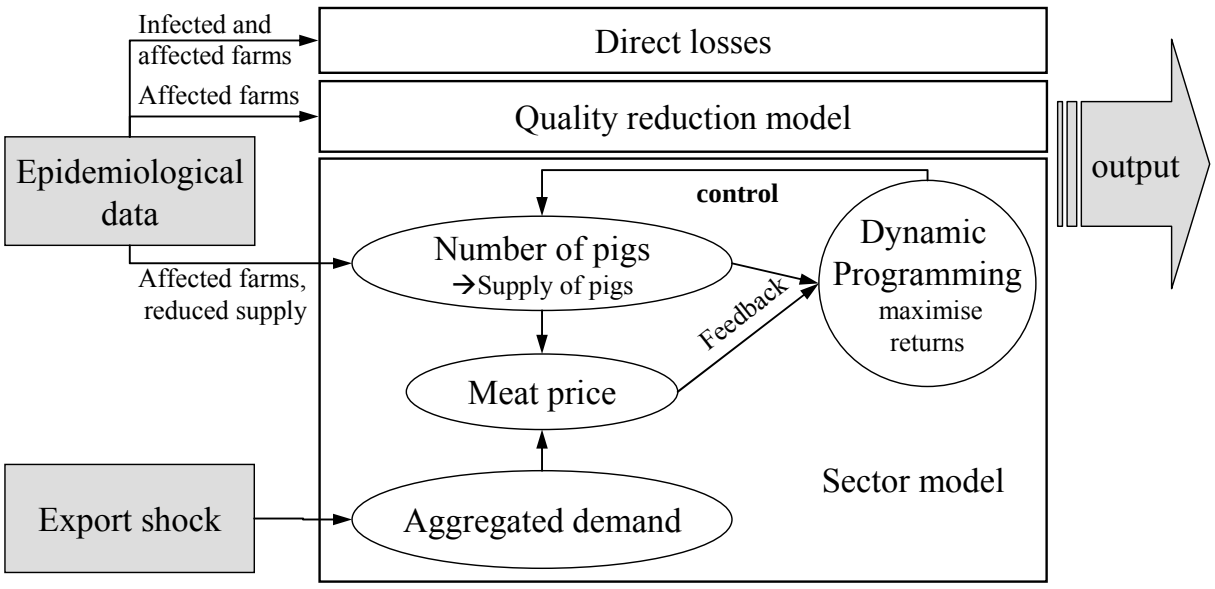

Fig. 1 Linkages between epidemiological data, the trade shock and models used in the analysis, and the impact of infected and uninfected farms affected by official measures on models

Demand for pig meat

The aggregate demand for meat was stratified into three market regimes and demand equations. They were 1) domestic demand for Finnish pig meat, 2) domestic demand for imported (non-Finnish) pig meat, and 3) export demand for Finnish pig meat. At each period t, the demand for pig meat was characterised with the following equation:

$$D_t^i = f^i(P_t, X_t^i) \quad (1)$$

where D_t^i is the demand i for pig meat, superscript i indicates demand type, i={domestic demand, export demand, import demand}, P_t is the pig meat producer price at time t in Finland, and f^i indicates that demand is a function of pig meat price in Finland and other relevant variables $\mathbf{X}_t^i$. Other variables, i.e. $\mathbf{X}_t^i$, include information on factors which affect the demand for pig meat, viz. exogenous demand shifters such as livestock epidemics in other countries, the prices of meat products competing with pig meat and pig meat prices in representative import sources and export destinations. The price indicator for the representative import source was pig meat price in Denmark. Prices in major export destinations were presented by pig meat prices in Russia, Sweden and Japan. Time index t is measured in months. When a disease outbreak occurred, export demand was scaled down by the amount of the trade shock.

In the market clearing, the sum of export and domestic demand was conditioned to meet domestic supply of pig meat. This implied that imported and Finnish pig meat were imperfect substitutes. Empirical evidence supports such an asymmetric treatment of exports and imports.

Supply of pigs and pig meat

Demand equations were estimated econometrically in the reduced form, whereas the supply of pigs and pig meat was obtained by solving the profit-maximisation problem of pig producers on farms unaffected by official measures, with dynamic programming. Dynamic programming is well suited for optimising sequential processes, such as pig production, and for cases where uncertainty is involved in decision-making. The model took into account biological time lags and short term adjustment costs. Time lags can be quite important in determining disease losses, because the supply of pigs and pig meat is predetermined in the short term. For instance, if a sow is inseminated today, piglets born from this insemination would be finished for slaughter 10 months from now. On the other hand, if some of the forthcoming piglets were allocated for re-production, they would be ready to be inseminated for the first time 12 months from now. An unexpected reduction in the number of sows can therefore result in production capacity becoming idle in the short term.

The problem for a representative farrowing-to-finish unit was to maximise the value of production capacity (V_t) by adjusting the supply of pig meat over time. This implicitly included the minimisation of disease costs. The value of pig production was the net present value of instantaneous returns gained from the production activities over the planning horizon t=1,..,T. At each period, instantaneous returns were market revenues of pig production minus production costs and the possible cost of keeping production capacity idle.

The profit maximisation problem can be recast in the Bellman equation:

$$V_t(Z_t, Z_{t-4}) = \max_{u_{t+2}} \left\{ Y_t \; P_t \; - C_t(Z_t, Z_{t-4}, u_t, u_{t+2}) + \beta E(V_{t+1}(Z_{t+1}, Z_{t-3})) \right\}, \; t=0,1,\ldots,T \qquad (2)$$

$$\text{subject to: } Z_{t+6} = Z_t(1-r) + u_t - \eta_t^{sow}$$

$$Y_t \; = y^{\text{meat}}(Z_{t-4} y^{\text{piglet}} - u_{t+2}) - \eta_t^{\text{pig}} + Z_{t+2} r y^{\text{sow}}$$

$$P_t \; = f_t^{\text{P}}(D_t^{\text{domestic demand}}, D_t^{\text{export demand}}, Y_t \;),$$

where V_t is the net present value of pig meat production capacity at time t; subscript t is the time index measured in months; Z_t is the state variable which measures the number of sows which have farrowed and are ready for re-insemination at time t or to be removed from the stock; Y_t is the amount of pig meat produced in period t (kg); P_t is the pig meat price; u_t is the decision variable referring to the number of pigs inseminated for the first time at time t; $C_t(.)$ is the production cost incurred when producing Y_t and inseminating u_t new gilts and a number of old sows; β is the discount factor; $E(.)$ is the expectation operator applied on the next-period value function (V_{t+1}); r is the percentage of farrowed sows removed from the stock; the output figure y^{piglet} is piglet yield per sow at each parity; y^{meat} is the yield of marketable meat upon slaughter (kg/pig); y^{sow} is the meat yield from a removed sow (weighted by meat value); and η_t^{sow} and η_t^{pig} indicate how the disease shock affects the number of sows and fattener pigs kept on farms. The last line denotes that the price of pig meat depends on the price at which supply meets demand.

Market losses, e.g. losses to pig producers due to abrupt price reductions, were obtained as the difference in the market value of pig meat between simulating markets with and without a disease shock. The aggregate change in the sow stock (and meat supply) over time depended on the percentage (r) of removed sows, the percentage of piglets allocated for reproduction and how disease affected the number of pigs on farms. The reduction of pig supply due to disease eradication and preventive measures was included in η_t^{sow} and η_t^{pig}. The reduction of supply due to eradication measures affected pig numbers, whereas lost value of animals compensated by government was included in direct losses. Reduction of supply due to preventive measures included lost production potential of uninfected farms, as official restrictive measures distorted pig farming in the protection and surveillance zones. Reduction in the quality-adjusted value of pig meat due to delayed slaughter of pigs on farms affected by official measures was simulated with the model of Niemi et al. (2004). Profit losses, such as a reduction in farm income after optimal production adjustments, take into account the size and type of the herd kept on farms, and the duration of restrictive measures.

Epidemiological data

A disease outbreak was considered as an exogenous and unexpected shock on the primary pig production in Finland. Since no empirical data were available on actual disease outbreaks in Finland, potential disease outbreaks were based on output of the Monte Carlo simulation model (Raulo & Lyytikäinen, 2005). The data included 4,455 iterations of epidemic outbreaks. The model simulated the spread of CSF from farm to farm starting from the point when the first farm in Finland became infected with the virus and stopping at the point where all infected farms were detected, their pigs culled and premises cleaned. The virus could spread between farms via four types of different contact mediators. In the course of simulation, uninfected pig farms

affected by restrictions down to the protection and surveillance zones, or down to a potentially infective traced contact, were summarised. Modelled official control measures derive from the European Union (EU) directive 2001/89/EC (European Commission, 2001). Both the mean and the variance of the simulated time in days, the number of infected farms and the number of uninfected farms affected by restrictive measures were gained from the output.

Epidemiological data were further classified according to the simulated number of infected farms and disease outbreak scenarios were created accordingly. Moreover, epidemiological data allowed identification of farms revealing their size, herd type, and location at the point when the official restrictive or disease eradication measures were enforced on the farms.

Direct losses

Direct losses arising from the disease outbreak were computed based on the epidemiological output data and farm-specific information. Direct losses included the lost value of rendered animals, the cost of rendering potentially infected material and disinfecting premises, the costs due to screening and surveillance of farms, and the administrative costs associated with eradication and preventive measures. Slaughter due to animal welfare reasons was excluded from the analysis because the number of infected farms was generally small.

Direct losses could vary between simulated outbreaks due to heterogeneity in the number and type of infected and affected farms. The main types of cost were: 1) the fixed charge for eradication (€ per infected farm), 2) the variable cost for eradication depending on the number and type of animals kept on the infected farm (e.g. the compensation paid for culled animals), 3) the cost based on the location of the infected farm (e.g. the cost of transporting carcasses to rendering plant from a remote area), 4) the fixed charge for screening (€ per affected farm) 5) variable cost for screening depending on the number and type of animals kept on each affected farm. Per unit costs were calculated based on information obtained from various sources (see Acknowledgements), statistics and terms of reference. The average total direct losses for farms with different statuses are presented in Table 1. In addition, each infected farm incurred administrative costs, which were, on the average, €43 174 per farm. There was a variation in administrative costs. Direct costs per affected farm were higher than in some of the previous studies (e.g. Saatkamp et al., 1997; Meuwissen et al. 1999).

Table 1. Direct loss (€ per farm on the average) for disease eradication and preventive measures according to herd type and size for farms infected by the disease and for uninfected farms affected by official measures.

Type of Measures	Herd Type \ Size	0-100 Pigs	101-300 Pigs	301-600 Pigs	601- Pigs
Disease eradication measures on infected farm [a]	Sows	9 337	19 928	33 607	87 889
	Fatteners	4 873	9 556	16 277	36 179
Screening measures on uninfected farm [b]	Sows	1 294	2 040	2 589	3 834
	Fatteners	621	675	909	1703

[a]Average loss related to sampling and laboratory analysis, stamping-out, i.e. disinfection measures, rendering contaminated material, and repopulation of farm. Figures exclude the value of pigs.

[b]Average loss related to inspection procedures, sampling and laboratory analysis, related by: 100% for a farm located within the protection zone, by approximately 67% for a sow herd and 62% for a fattener pig herd farm located within is the surveillance zone, and by 22-58% for a farm traced to received potentially infective contact.

Losses of the processing industry

The loss of profit margin in the meat processing and feed industry was computed in addition to other losses based on simulated meat quantities. The lost profit margin was €0.67 per kilogramme of meat, which included value added plus additional fixed costs incurred minus saved variable costs. The figure was calculated based on standard industrial classification (SIC) statistics in 1997-2003. The data were supplied by Statistics Finland.

Market data

Data on slaughterhouse demand and domestic meat prices (pig meat, beef, chicken) for April 1995 to September 2003 were obtained from the Information Centre of the Ministry of Agriculture and Forestry (TIKE). The import and export of fresh pig meat were based on TIKE statistics and Finnish customs. Danish and Swedish pig meat prices were obtained from Eurostat. Pig meat prices in Russia and Japan were from Russian and Japanese statistics (Table 2). Other variables in $\mathbf{X}_t^i$ included lagged demand for pig meat t-6, the population of Finland or linear trend, and demand shifters (dummy variables) including seasonal effects, major CSF, foot and mouth disease and BSE epidemics in the EU and the first BSE case in Finland.

Production costs of pig meat and piglets, as well as productivity and production cycles of pigs, were based on the data from the Finnish farm advisory organisation Proagria.

Table 2. Mean values and descriptive figures of selected market and production data

VARIABLE	UNIT	MEAN
Monthly domestic demand for Finnish pig meat	1 000 t	13.13
Monthly exports of pig meat from Finland	1 000 t	1.45
Pig meat price in Finland	€/kg	1.41
Own-price elasticity of domestic demand	-	-0.58
Own-price elasticity of export demand	-	-3.54
Weaned piglets per insemination (normalised)	pieces	9.5
Variable production cost of a piglet	€/piglet	59
Price of a newly inseminated gilt	€/gilt	553
Production cost (excl. piglet cost) of a fattened pig	€/pig	61
Slaughter premium	€/carcass	23
Interest rate	% p.a.	6
Production cost of pig meat	€/kg	1.36 [a]

[a]Normalised figure for the case where the number of sows remains constant.

Estimation method

The effects of markets shocks were estimated in three steps. First, three demand equations for pig meat were estimated from Finnish meat market data with three-stage least squares (Zellner & Theil, 1962; LeSage, 2001). Domestic meat prices were allowed to be endogenous. Exogenous and lagged endogenous variables were used as instruments.

Second, the supply of pig meat (Eq. 2) was solved numerically with dynamic programming. The state and control spaces were discretised and interpolation was applied between the nodes. The model was calibrated ex-post by adjusting the capacity cost in order to obtain the best fit to

the markets in 1997-2003, as explained in Niemi et al. (2006). Adjustment costs incurred when production decreased and production factors became idle. In the numerical solution, T was set large enough for the value function to converge.

Thirdly, the effects of disease outbreaks were calculated by simulating markets with different disease and demand shocks and then comparing the results between scenarios. During the trade shock, the export demand equation was scaled down by the percentage of closed markets. The accumulation of losses was examined with avoidable losses. Avoidable losses were caused by farms which may become infected in the future. They were computed by first subtracting the estimated average loss resultant from the first infected farm of the epidemic (as corresponding to a sporadic case) from the total loss in each iteration. Secondly, the result was multiplied with the share of farms which became infected after the confirmation of the first CSF case. These steps were to examine losses caused only by farms infected after the first case confirmation.

Scenarios

Scenarios comprising two different demand shocks (the difference in the magnitude and duration of the shock) and two different epidemics (the influences in piglet and the meat supply) were analysed. The most favourable scenario in this study represented a medium-sized CSF epidemic comprising 76% of epidemic simulations, accompanied with minor export demand shock. The least favourable scenario represented a large CSF epidemic comprising 24% of epidemic simulations, accompanied by a full closure of export markets for Finnish pig meat. Besides these, two other combinations of the previous trade and disease shocks were analysed. Table 3 summarises the scenarios.

According to the epidemiological data included in the study, the scenario assumes that most farms became infected prior to the first confirmation of CSF infection (comprising 90.7% and 72.7% of simulated infections in medium-sized and large epidemics, respectively). Export market responses stood for a minor and a major demand shock. In the minor demand shock, export demand for pig meat decreased by 10%. In the major demand shock, export markets were fully closed. The duration of an epidemic and the duration of the trade shock were not equivalent, but they were linked to each other.

Business on infected farms was assumed to be interrupted and their pigs culled. For uninfected farms affected by any restrictive measures, transport of pigs and inseminations of sows were temporarily prohibited. This affected the quality and the size of the pig stock. Detailed information on epidemic impacts, duration and size of business interruptions, used as an input to the ensuing models (Fig. 1) is given in Table 4.

RESULTS

The relative forecast error (Pindyck & Rubinfeld, 1998, pp. 210-211) of the calibrated model in the absence of disease was 6% for domestic demand, 7% for supply and pig meat price, and 29% for export demand. Forecast errors were mainly due to two semi-annual periods in 1997 and 2003.

Table 3. Summary of trade and epidemic shocks in four scenarios examined in this paper, described by the number of animals removed from the markets (supply shock), percentage reduction in export demand (demand shock), and typical duration of the demand shock.

Type of Epidemic and Export Demand Shock	Medium-sized Minor	Medium-sized Major	Large Minor	Large Major
Immediate change in meat supply [a]	-0.7%	-0.7%	-1.9%	-1.9%
Immediate change in the sow stock[a]	-0.7%	-0.7%	-1.7%	-1.7%
Reduction in the export demand	10%	100%	10%	100%
Demand shock duration (months)	6-8	7-9	6-8	7-9

[a]Instantaneous change of the total production capacity of unaffected farms. Figures include animals on affected farms, which cause only temporal shifts in production. See Table 4.

Table 4. Impact of epidemics, average duration and size of business interruptions, used as an input to each of the ensuing economic models (direct losses, quality reduction losses, sector model), given as the average number and 95% range (Min-Max) of variation around mean.

	Medium-sized Epidemic			Large Epidemic		
	Min	Mean	Max	Min	Mean	Max
DIRECT LOSSES						
Infected farms	2	2.6	4	5	7.3	16
Culled sows	0	123	452	59	387	1 446
Culled fatteners	59	841	2 728	538	2 731	7 449
Screened farms on protection zones	0	11	33	5	31	77
Screened farms on surveillance zones	3	51	139	18	138	345
Screened farms traced as contact farms	3	69	267	45	228	636
QUALITY REDUCTION LOSSES						
Fatteners under restrictions ≤ 7 days	838	19 073	72 229	12 036	62 656	178 681
Fatteners under restrictions > 7 days	82	5833	27 382	238	14 862	49 242
Business interruption days on farm	44	47	54	45	47	54
SECTOR MODEL						
Sows removed from the stock	0	123	452	59	387	1 446
Fatteners removed from the stock	59	841	2 728	538	2 731	7 449
Sows under restrictions for ≤ 7 days	173	3 945	14 939	2 486	12 959	36 955
Sows under restrictions > 7 days	0	1 138	5 634	67	2 892	9 916
Fatteners under restrictions > 7 days	82	5 833	27 382	238	14 862	49 242
Business interruption days on farms	44	47	54	45	47	54

Impact of disease outbreak on markets

When markets obtained information from the shock, export demand for pig meat fell by 10% or 100%. Changes in pig meat price in minor demand shock were at most ±2% due to medium-sized epidemic, and from -3% to 4% due to the large epidemic. In the major demand

shock, pig meat prices decreased 15-20% below disease-free outcome, as excess supply sold to markets still open was larger than in the minor demand shock. The price depression continued until the trade shock was removed. This was likely to last one month longer in the large epidemic than in the medium-sized epidemic.

After trade restrictions were removed, pig meat price recovered to greater than the price in the absence of disease shock, particularly if it was a major demand shock. The increase was affected by the lag structure of supply and demand equations. The demand shock caused only small changes in the amount of pig meat traded in the markets in the short term.

Disease losses

The model estimated overall losses likely to be below €40 million. In a medium-sized epidemic with a minor demand shock, the model simulated the pig industry's overall loss at €1.9 million. In a large epidemic with major demand shock, the average loss was €20.5 million. In the latter case, losses were mainly market losses, whereas in the first case direct losses had a more prominent role. These average figures correspond to less than 1% and about a 9% reduction in the gross return of pig production at market prices in 2003. The figures take into account the dynamic profit maximization behaviour of pig producers. The figures exclude welfare effects to consumers, because the focus was on simulating direct losses and income losses to pig producers, the meat industry and the government.

As Table 5 indicates, depending on the strength of the trade shock, a large epidemic resulted €4.7-€6.8 million on average in greater losses than in a medium-sized epidemic. On the other hand, a major demand shock resulted €11.9-€13.9 million in greater losses than in the minor demand shock. Simulated losses were mainly consequential losses which were not compensated by government. Government expenditure was included in figures reported in Table 5. Government expenditure was simulated, on the average, at €0.6 and €1.9 million in medium-sized and large epidemics, respectively. Expenditure was assumed to depend on the number and characteristics of infected farms and farms uninfected but affected by restrictive measures.

There was considerable variation in simulated losses, even if the number of infected farms and the strength of demand shock were set as constant. For instance, losses due to a large epidemic and a major demand shock were between €12.3 and €37.8 million in 95% of simulations. In some cases, a major demand shock or a large epidemic already sufficed to incur more than €19 million in losses. In all scenarios, the maximum loss was at least 51% greater than the average loss. In a minor demand shock, it was possible for the loss to be estimated as negative.

Variation in losses was due to a large number of factors. One of the most important factors was that the number of uninfected farms affected by the restrictive measures varied. This variation resulted in delayed slaughter, temporal shifts in meat supply and costly surveillance activities. All new reproduction activities were assumed to be forbidden in the restriction zones. Indirect and direct losses, therefore, varied even if the number of infected farms remained unchanged.

Simulations in which a large number of uninfected farms were included in restriction zones typically caused the largest losses. In a few simulations, a large slaughterhouse in the protection zone also incurred severe consequential losses. The outbreak caused interference in the delivery of pigs to slaughter and hence, losses due to carcase quality and maintenance feeding.

Consequential and direct losses both increased when the number of infected farms increased. Even if the strength of the demand shock was independent of the number of infected farms, the duration of the demand shock partly depended on it.

Table 5. The average overall and direct loss (€ million) per epidemic in four disease and market scenarios, and upper and lower boundary 95% percentile range (min-max) of simulated losses.

	Medium-sized Epidemic			Large Epidemic		
	Min	Mean	Max	Min	Mean	Max
Overall loss, minor demand shock	-1.5	1.9	6.9	0.4	6.6	19.4
Overall loss, major demand shock	10.7	13.7	20.7	12.3	20.5	37.8
Direct loss	0.2	0.6	1.4	0.7	1.9	4.7

Policy aspects

The profitability of control policies was not explicitly evaluated in this study. Epidemiological data already showed that most infected farms became infected prior to the confirmation of the first CSF case. Even for large epidemics where successive farms became infected after the first confirmation of CSF infection, the incursion of additional losses per day was generally lower than would have been required for losses to justify drastic control measures. For instance, the expected rate of losses incurred per day was generally lower than the cost of ceasing the delivery of pigs to slaughter for one day in the entire country.

Since stakeholders do not need to put effort in preventing infections which have already occurred, most losses were unavoidable by the time information on the presence of CSF in Finland was obtained. For instance, in the minor demand shock, the first detected farm already incurred approximately €0.7 million in losses. In the major demand shock, the figure was €11.4 million. In the minor demand shock, the average avoidable loss was approximately €0.1 million in the medium-sized epidemic and €1.8 million in the large epidemic. In the major demand shock, avoidable loss was €0.3 million in medium-sized epidemic and €3.0 million in the large epidemic. Avoidable losses exceeded €10 million in less than 1% of iterations. Their contribution to the average was small due to the low probability of occurrence.

The option for producers to be able to optimise the recovery of production could reduce disease losses if epidemic were large or if there were a lengthy demand shock. In some instances the reduction could be almost 50%. Nevertheless, chances to optimise supply appeared to be limited, on average, due to the small number of infected farms and short epidemic durations.

Simulated losses responded to changes in model parameters. An increase in the own-price elasticity of domestic pig meat demand decreased losses. It reduced the impact of excess supply as a more elastic demand required smaller price change for the markets to clear. An increase in the cost of pig production or in the capacity cost increased losses.

DISCUSSION

This paper described simulated losses which potential CSF epidemics could cause to Finnish pig producers, to the meat processing industry and the government. The analysis took into account profit-maximising behaviour of pig producers. In the two extreme scenarios, average losses were simulated at €1.9 and €20.5 million. Losses simulated in this study were generally low compared to those estimated for densely populated livestock areas of Europe (cf. Saatkamp et al., 2000; Mangen & Burrell, 2003).

The results suggest that a small epidemic can already be expensive, because non-epidemiological factors can amplify losses. One of the reasons for variation of losses around the average is that there is considerable heterogeneity among the type and number of farms and regions which face restrictions or become infected. The number of uninfected farms under restrictions can range from zero to hundreds and the duration of restrictions can vary. These farms may have to delay marketing and the insemination of pigs. A business interruption in a large meat processing company could amplify losses even more. Losses can also be large if the trade of disease-affected products is distorted; if major demand shock took place, losses were mainly market losses. This result is in accordance with previous studies (e.g. Garner et al., 2001; Mangen & Burrell, 2003; OECD, 2006). Trade can be distorted even if the number of infected detected farms is limited to only one farm, but large epidemics could further motivate trade partners to impose trade bans. Occasionally, a combination of favourable prices and high production quantities during and after the epidemic could also create added value and market income sufficient to outweigh other losses.

The results suggest that although the current scattered farming structure and small farm size incur higher production costs in Finland than the most intensive production structures in Europe, it does not yet seem to promote spread of the disease into severe outbreaks and thereby reduces the industry's potential losses. In fact, epidemiological risk assessments have shown that the probabilities of CSF incursion into Finland (Rosengren et al., 2002) and spread within the country are low (Raulo & Lyytikäinen, 2005). The results therefore suggest that in most cases mandatory measures suffocate the disease effectively enough and CSF alone provides little incentive for tightening control policy from the current level.

When used jointly with an epidemiological simulation model or appropriate empirical data, the approach taken in this study can be utilised to investigate 1) economically viable policies to control disease outbreaks, 2) implications of rapid structural change of pig production on disease control, and 3) costs of increased disease risk due to structural development towards more efficient low-cost pig production. When investigating the effects of structural development, the emphasis is on changes in productivity and disease spread. Structural and technical change tends to improve the efficiency of production and reduce per unit production costs. In competitive markets, this reduces per unit value of lost output. Structural change may also speed up the spread of the disease. Hence the question: what is the trade-off between increasing the efficiency of pig production and increased potential for disease losses?

If organised properly, the ongoing rapid growth in the size of production units and regional concentration of production may not necessarily pose significant increases in the probability of severe epidemics and extra costs to the Finnish pig industry in the next few years. A very important and relevant theme for future studies, therefore, is to provide more detailed information on cost-efficient options to manage changes in production structures without

affecting disease losses. One research approach is to analyse the competitiveness of production structures to project aforementioned trade-offs, i.e. to simulate how a change in farming structures affects production costs, and to simulate first how changes in contact frequency and vicinity of production units affect the transmission of disease between farms and then how this could reflect on the number of farms involved. However, further studies are first needed to investigate in more detail the relationship between different production structures and disease transmission.

In conclusion, the results suggest that a small classical swine fever epidemic can already be expensive, because non-epidemiological factors, especially a negative export demand shock, can greatly amplify losses. If exports completely cease as a result of a CSF epidemic, the direct losses related to disease eradication measures are only a small fraction of the total loss. Despite rapid increases in the size of production units and regional concentration of pig production, Finland can still be considered as a region where a CSF outbreak would cause relatively small losses compared to the total value of production, and where incentives for tightening control policy are low.

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MEASURING AND COMPARING CONSTRAINTS TO IMPROVED BIOSECURITY AMONGST UK FARMERS AND VETERINARIANS

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SUMMARY

Constraints to the introduction of enhanced biosecurity systems are rarely considered in sufficient detail when population medicine specialists initiate new control schemes. The main objective of our research was to investigate and compare the different attitudes constraining improvement in biosecurity for cattle and sheep farmers and practising veterinary surgeons in Great Britain (GB). This study was carried out through focus groups for the farmers and a questionnaire survey of veterinary practitioners. It appears that farmers and veterinarians have their own relatively clear definition for biosecurity in relation to some major diseases threatening GB agriculture. Overall, farmers believe that other stakeholders, such as the government, should make a greater contribution towards GB biosecurity. Veterinary practitioners saw their clients' ability or willingness to invest in biosecurity measures as a major constraint. Veterinary practitioners also felt that there was need for additional proof of efficacy and potential economic benefits of proposed farm biosecurity practices.

INTRODUCTION

Biosecurity as yet has no Oxford dictionary definition; however, it literally means 'safe life' from the Greek 'bios' meaning life. Interested parties have created and adapted different definitions to suit their own particular needs or ends. The National State Association of State Departments of Agriculture (NASDA, 2001) in USA provided this definition: "*Biosecurity itself is more than just a buzzword; it is the vital work of strategy, efforts and planning to protect human, animal and environmental health against biological threats. The primary goal of biosecurity is to protect against the risk posed by disease and organisms; the primary tools of biosecurity are exclusion, eradication and control, supported by expert system management, practical protocols, and the rapid and efficient securing and sharing of vital information. Biosecurity is therefore the sum of risk management practices in defence against biological threats.*" Our current literature review reveals this to be most comprehensive description available.

The majority of appropriate alternative definitions are directed towards farmers in the form of technical, advisory or commercial documents. The UK Department for Environment, Food and Rural Affairs (Defra, livestock knowledge transfer, 2001) livestock knowledge transfer initiative advisory leaflet 311 stated that biosecurity means: "*protecting farm units from the introduction of new diseases and minimising the spread of disease within the herd*." The Scottish Executive Environment Rural Affairs Department (SEERAD, 2006) had a slightly

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different slant along a similar theme, stating that: "*Biosecurity is a set of management practices which, when followed, collectively reduce the potential for the introduction or spread of animal disease causing organisms onto and between farms.*" Whatever the selected final definition, biosecurity is increasingly becoming noteworthy at international, national, regional and farm levels. Governments; veterinarians; the farming industry; the media and the general public are becoming increasingly more involved in issues of food security and safety and the associated areas of zoonotic diseases and animal welfare.

Biosecurity now has become the 'buzzword' in the post foot and mouth disease (FMD) agricultural climate of the United Kingdom (UK). The outbreak of February 2001 demonstrated the vulnerability of the UK livestock industry to disease threats. The wide spread of the disease was predominantly due to multiple stock movements and lack of preventive measures before the outbreak (Gibbens et al., 2001). Despite this, uptake of and implementation of biosecurity measures on UK cattle and sheep farms remains poor (Holliman, 2003; Bennett & Cooke 2005). Burrell (2002) posed the question: "*What can producers do to protect themselves regarding disease epidemics?*", stating that the industry clearly had a major role to play. In recent years, there has been a growth of independent biosecurity initiatives amongst motivated sheep and cattle farmers and between the farmers and their veterinary advisors in several regions of the UK; and also elsewhere within the European Union (EU). Historically, the roles and responsibilities of government, industry and animal owners regarding disease control have been based on a set of assumptions that have not always resulted in the major stakeholders working together.

Defra commissioned this study to evaluate the constraints to improved farm level biosecurity within GB in advance of The Animal Health and Welfare Strategy for Great Britain (AHWS) (Defra, 2004). The objectives of the AHWS are ambitious (e.g. disease status to be amongst the best in the world within 10 years) and depend upon a radical shift in approach to farm animal disease prevention and control if they are to be achieved. Part of such a shift in approach will be to place greater responsibility for animal health and welfare on animal keepers. Our perception was that constraints to the introduction of enhanced biosecurity systems are rarely explored in sufficient detail when population medicine specialists initiate new livestock disease control schemes. The main objective of our research was therefore to investigate the different attitudes constraining biosecurity for GB cattle farmers, sheep farmers and practising veterinary surgeons.

MATERIALS AND METHODS

Farmer Focus Groups

The farmer focus groups were carried out from November 2002 to March 2003. One pilot group (Wiltshire) and eight data gathering focus groups located at Inverness, Perth, Lanark, Borders, Cheshire, Brecon, Glastonbury, and Truro were recruited. Four of the groups represented mainly beef and sheep farmers, two groups represented mixed species farming systems and two groups comprised of only dairy farmers. An analytical framework, based on The Theory of Reasoned Action (TORA), was used to design a discussion guide for the focus groups (Ajzen, & Fishbein, 1980). All focus group discussions were recorded and transcribed and analysis and coding of the text was carried out using N-Vivo™ software (SAGE, Ltd.). The codes were analysed using the TORA as a framework illustrated in Fig. 1.

Veterinary Postal Survey

Cattle and sheep veterinary practitioners in the UK were surveyed with a postal questionnaire. The questionnaires were piloted twice, using six different veterinarians each time, and lists within the questionnaires were randomly organised into six different order-sequences to avoid bias by order. Contacts for the cattle practitioners were supplied by the Royal College of Veterinary Surgeons (RCVS) lists of practitioners with cattle clients (n = 1,121) and the British Cattle Veterinary Association (BCVA) membership list (n = 807). The list of sheep practitioners was supplied by the RCVS lists of practitioners with sheep clients (n = 1,294). Additional comments by veterinary practitioners were gathered under broad headings for both sheep and cattle practitioners and are presented here as a proportion of the total number of comments.

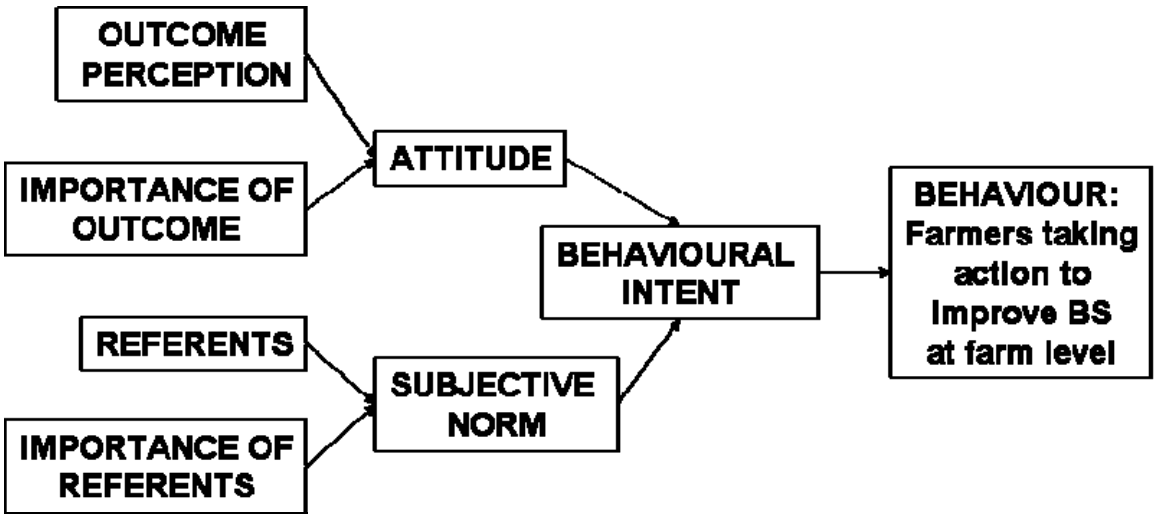

Fig. 1 The TORA framework

RESULTS

Farmer Focus Groups

The mean age of the participants in the eight farmer data gathering groups was 49 years (range: 28-72 for individuals, 38-54 for group means). The majority of the participants were male: (51 from 58). Five of the groups had at least one female participant. The farmer focus groups presented two clearly defined positive definitions of biosecurity: disease prevention and good husbandry; and two negative definitions; disinfecting and bureaucracy & rules. This is illustrated in Table 1.

The positive categories were related to the things farmers perceived themselves to be doing on their farms in order to maintain good biosecurity. The negative definitions were related to things that farmers considered to be imposed on them by others in order to maintain or introduce biosecurity that may or may not be efficacious. The most important biosecurity issues within a whole biosecurity framework identified by the focus group participants are shown in Table 2.

The important biosecurity measures that focus group participants implemented included operating a closed herd/flock (6/8 groups), disinfection of trailers and footbaths (6/8 groups),

Table 1. Categorisation of biosecurity from farmer focus groups

Category	Region	Proportion of Groups	Group Farm type
Positive			
Disease Prevention	Scotland & N. England	4/4	3 sheep /beef & 1 mixed
	S. England & Wales	3/4	2 dairy & 1 mixed
Good Husbandry	Scotland & N. England	1/4	1 sheep/beef
	S. England & Wales	2/4	1 sheep/beef & 1 dairy
Negative			
Disinfection	Scotland & N. England	2/4	2 sheep/beef
	S. England & Wales	2/4	1 sheep/beef & 1 dairy
Bureaucracy	Scotland & N. England	1/4	1 sheep/beef
	S. England & Wales	2/4	1 sheep/beef & 1 dairy

Table 2. The most important biosecurity issues by the focus group participants in 2002-2003

Biosecurity Issue	Groups where issue raised	Region	Proportion of groups	Group farm type
Border controls	6/8	Scotland & N. England	3/4	2 sheep /beef & 1 mixed
		S. England & Wales	3/4	1 dairy & 1 sheep/beef & 1 mixed
Farmers' personal action	6/8	Scotland & N. England	3/4	2 sheep /beef & 1 mixed
		S. England & Wales	3/4	2 dairy & 1 sheep /beef
Disease eradication /surveillance	4/8	Scotland & N. England	3/4	3 sheep/beef
		S. England & Wales	1/4	1 dairy
Public access to farmland	4/8	Scotland & N. England	1/4	1 mixed
		S. England & Wales	3/4	1 dairy & 1 sheep/beef & 1 mixed
20-Day standstill rule	3/8	Scotland & N. England	1/4	1 sheep/beef
		S. England & Wales	2/4	1 sheep/beef & 1 mixed
Control of bovine TB	3/8	Scotland & N. England	0	
		S. England & Wales	3/4	2 dairy & 1 mixed

vaccination (6/8 groups), and general cleanliness and tidiness (4/8 groups). Particular to the Scotland and Northern England focus groups, additional issues raised included quarantine and testing (3/4 groups), health schemes (3/4 groups) and using a reputable feed supplier. Two further issues were raised by the English and Welsh groups: fencing off badgers (2/4 groups) and segregating stock from pick up/drop off (2/4 groups).

The TORA-analysis of the focus group discussions reveals that farmers have both negative and positive outcome perceptions with regard to improving biosecurity on the farm. Inserting the results into the TORA framework outlined in Fig. 1, the results are diagrammatically presented in Fig 2.

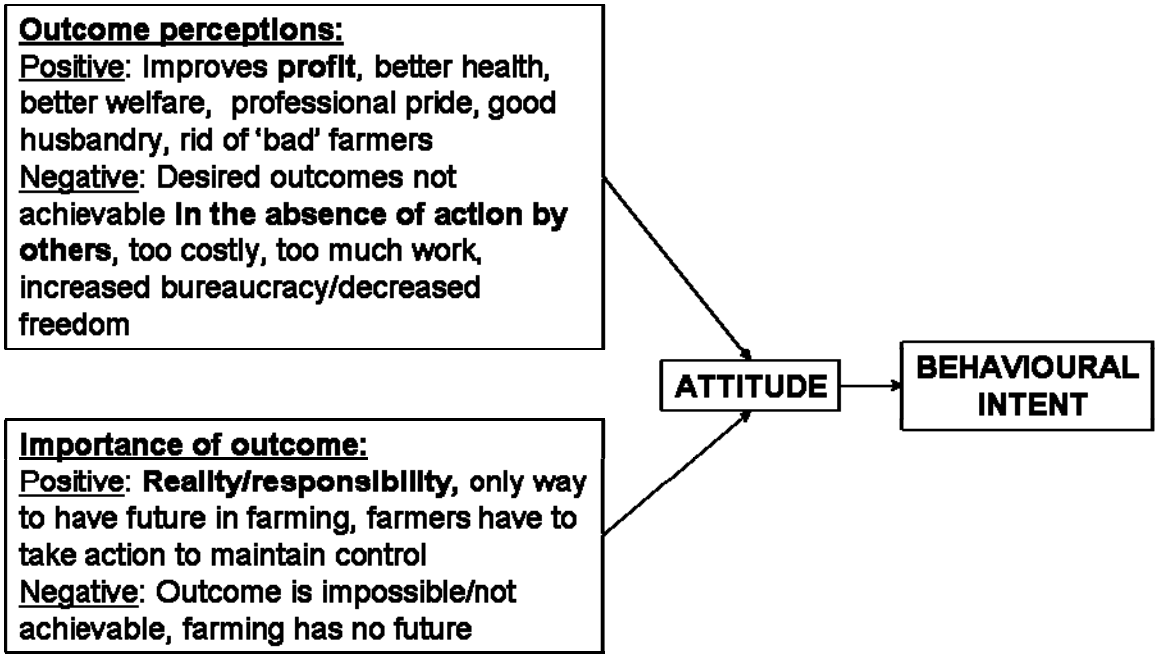

Fig. 2 TORA focus groups results for positive and negative biosecurity outcomes

Among the positive outcome perceptions, improved profitability through better health and welfare on the farm was an issue that was expressed most readily by the farmers. Issues relating to professional pride, such as good husbandry, to be seen to be clean, reputation of farmers etc., were also offered voluntarily as potential outcomes of improved farm biosecurity. The negative outcome perceptions were dominated by the 'Why us?' issue that was not prompted at any point of the discussion by the facilitator, whereas issues, such as cost and hassle, were less readily expressed, in spite of being used as prompts for this part of the discussion. The farmers appeared to have little faith in the efficacy of farm level biosecurity measures in the absence of action by others, such as the public using the footpaths and the government in securing the borders from illegal high risk imports. It is also notable that farmers expected to lose some of their autonomy and/or freedom as a result of improved biosecurity.

The TORA analysis also investigated the importance of the farmers' referents, that is, their main sources of information regarding biosecurity issues, outlined in Fig. 3. It can be seen that the main referents are the veterinary practitioners, followed by the government and other farmers; however, governments featured a negative response for the importance of the referents.

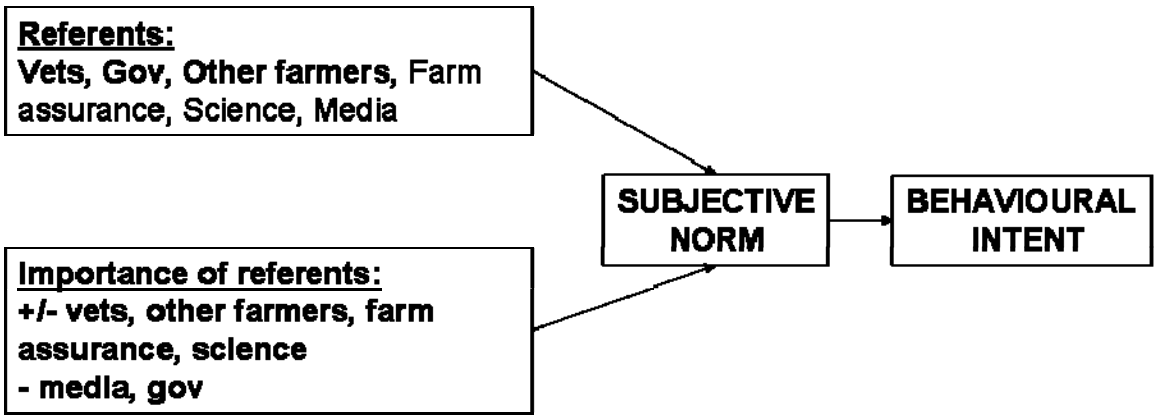

Fig. 3. TORA focus group results for farmer's referents (sources of advice).

Veterinary Postal Survey

The survey of veterinary practitioners elicited response rates of 17% for cattle vets (n = 319) and 16% for sheep vets (n = 208). There was a good geographic spread of the responses. A total of 94% of sheep veterinarians and 92% of the cattle veterinarians reported that they were in practice and 85% of all veterinarians who responded to the cattle questionnaire were members of the BCVA. Of the cattle practitioners, 64% had participated in the BCVA Herd Health Planning courses at level one, and 14% had undertaken the level two courses, while only nine percent had participated in BCVA biosecurity training. In excess of 50% of the cattle veterinarians used the BCVA Herd Health Plan with their clients, with the number of plans made by the veterinarian ranging from four to 71. Both cattle and sheep veterinarians reported being involved with a number of farm assurance schemes, ranging from National Dairy Farm Assurance Scheme (NASFAS, 50% of the cattle veterinary respondents) to the Maedi-Visna Accreditation Scheme (84% of the sheep veterinary respondents). A clearly defined biosecurity policy for farm visits was in place for 30% of cattle practices and 23% of sheep practices. However, the majority of veterinarians did take specific measures when visiting farms as outlined in Table 3.

Table 3. The percentage of large animal practitioners who indicated that they took particular biosecurity measures between visiting farms

Biosecurity Measure	Proportion of cattle vets (%)	Proportion of sheep vets (%)
Clean and disinfect hands[a]	n.a.	92
Wash boots	96	90
Clean and disinfect equipment[a]	n.a.	85
Disinfect boots	81	82
Use waterproof and disinfect	70	75
Leave car always from livestock areas	69	71
Change to clean overalls/coat	36	32
Wear gloves when handling stock	36	23

[a]this option was not offered in the cattle vet question

The general constraints to adequate uptake of on-farm biosecurity measures outlined by the veterinary practitioners are illustrated in Fig. 4.

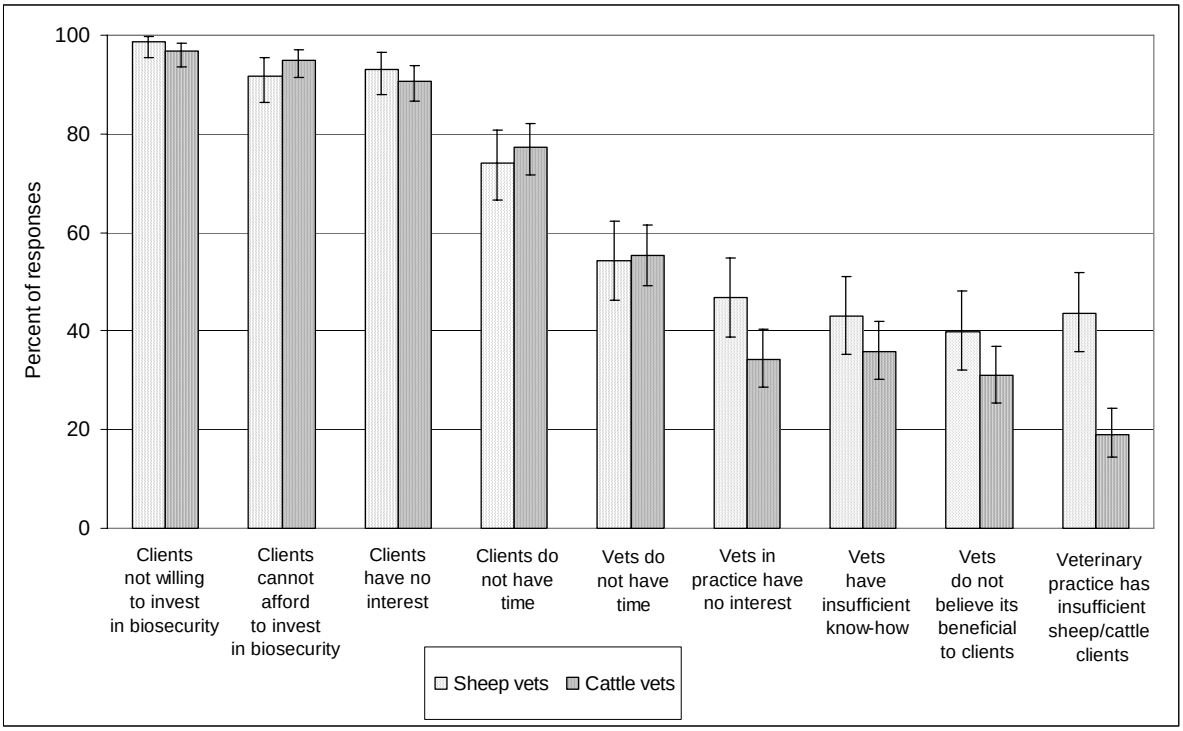

Fig. 4 Constraints towards promoting and improving biosecurity amongst clients (showing 95% confidence interval as error bars)

The majority of practitioners felt that their clients were not willing to, or could not afford to invest in biosecurity measures coupled with a similar majority believing that clients had no interest in instigating these measures (Fig. 4). Over 70% of cattle and sheep specialists felt that clients did not have time to instigate biosecurity measures. In respect of their own profession, over 30% of cattle practitioners and 40% of sheep practitioners thought that practising vets had no interest in biosecurity with similar proportions indicating partners did not believe in biosecurity and that vets lack the know-how regarding on-farm biosecurity.

The veterinary practitioners also categorised farm level biosecurity measures into ‘easy’ and ‘difficult’ measures. Difficult measures included reducing contact with wildlife and vermin; operating a closed herd on beef units; avoiding contaminated surface water and avoiding contact with neighbouring stock on hefted upland sheep farms.

Large numbers of additional comments both positive and negative were received from veterinary practitioners. A total 162 cattle and 78 sheep veterinary respondents included additional free text comments within their returns. When these responses were coded, a total of 385 cattle and 154 sheep related comments were identified. These are detailed in Table 4.

DISCUSSION

In GB, biosecurity and its implementation are complex and potentially value-laden issues for both farmers and their closest partners for biosecurity issues, the local veterinarians. Whole farm biosecurity is a complex combination of both ‘difficult’ and ‘easy’ measures that

complement and, sometimes, contradict each other with farmers and veterinary practices appearing to be fully aware of this complexity. On the basis of the limited data gathered within this survey, it appears that farmers and veterinarians have a relatively good understanding of biosecurity measures in relation to some diseases threatening GB agriculture. Interestingly, neither farmers nor veterinarians raised zoonotic diseases as an important driver or factor in decisions taken with regard to biosecurity implementation at farm level.

Table 4. A summary of additional 385 and 154 comments offered by 162 and 78 cattle and sheep veterinarians respectively

Comment Relating to Biosecurity and Identifying:	Cattle Vets N (%)	Sheep Vets N (%)
Questionable or unproven efficacy	85 (22)	36 (23)
Lack of or inadequacy of public policy	81 (21)	17 (11)
Suggestions on how to use public policy to enhance	71 (18)	12 (8)
Lack of veterinary influence or interest in promotion	55 (14)	44 (29)
Negative farmer attitudes	49 (13)	32 (21)
Financial constraints	44 (11)	14 (9)
Total	**385**	**154**

There is a clear difference in opinion between the vets and farmers regarding the classification of biosecurity measures. Farmers felt that biosecurity is too bureaucratic and that government were doing little to protect the country from disease threats. Overall, farmers regard biosecurity measures as expensive and not fully their responsibility. The vets did not see themselves as prime providers of biosecurity information to farmers, although many responders suggested that formal health planning would help to promote biosecurity. The main constraints were seen to be that veterinary clients were not willing to invest in, or couldn't afford to invest in, biosecurity measures. The veterinary surgeons are aware of their clients' attitudes and financial constraints. Interestingly, the TORA analysis revealed the Veterinary Surgeon to be the most trusted source of advice regarding biosecurity issues. Paradoxically, the vets do not regard themselves the primary biosecurity advice providers.

There is a need to address the perceived barrier of 'Why us?' (i.e. a farmer perception that only farmers are expected to do something about biosecurity and/or that farmer efforts will be useless due to lack of action or wrong action by others) among farmers. The evidence presented by the farmer focus groups suggests that there is a need for more information regarding a number of issues. Specifically, the action taken/not taken by the government in protecting the UK borders needs to be addressed by providing transparent information about the real level of threat and measures in place. Furthermore, clear information on the actual risks posed by public access to farmland is required to clarify the issues for the farmers and provide a clear biosecurity message to pressure groups promoting access to the countryside. This research also suggests that farmers require more information about biosecurity issues associated with farm visitors that includes the potential risks posed by veterinary surgeons. These latter two points indicate a need for more research. The focus groups also revealed a potential for collaboration between farmers and other stakeholders to improve biosecurity.

While demonstrating good understanding of biosecurity and its role in livestock industry, many veterinary respondents appeared to have substantial negative perceptions about disease control by farm level biosecurity and about their own role in promoting biosecurity. A considerable number of veterinary respondents suggested that one of the constraints to local veterinary involvement in farm level biosecurity was lack of interest among vets. Furthermore, few veterinary practices appeared to operate an explicit and planned biosecurity code as part of their working procedure. The majority of the additional comments expressed by the veterinary respondents were negative. Due to their perceived, important role as referents by the farmers, it would be important to address the veterinary attitudes on biosecurity as well as the farmer attitudes.

Finally, if the biosecurity message is to be adequately addressed by both farmers and practising veterinarians, there needs to be more research carried out into the efficacy and potential economic benefits of proposed farm biosecurity practices. This would be particularly true for research into the synergistic costs and benefits of undertaking biosecurity measures for a number of endemic diseases rather than just considering each disease threat individually.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Department for Environment Food and Rural Affairs for project funding. Special thanks must go to The Royal College of Veterinary Surgeons, The Sheep Veterinary Society and The British Cattle Veterinary Association for their help in sourcing and encouraging participants. The authors would like to acknowledge the input from Dr Gemma Harper, Dr Anni McLeod, Mr Nick Taylor and Dr Alistair Stott. Finally, our extended thanks to all the farmers and veterinary practitioners who gave up time in their busy schedules to attend focus groups and fill in postal questionnaires.

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EMERGING DISEASES

BLUETONGUE VIRUS SEROTYPE 8 EPIDEMIC IN NORTH-WESTERN EUROPE IN 2006: PRELIMINARY FINDINGS

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SUMMARY

In the middle of August 2006, North-western Europe was shaken by the start of an extensively propagating Bluetongue (BT) epidemic. BT is an arthropod-borne viral non-contagious disease of domestic and wild ruminants, particularly affecting sheep with severe clinical disease and mortality. To date, 24 different BT-serotypes have been identified and the disease is transmitted by biting midges (*Culicoides*). Soon after the start of the epidemic it was determined that BT virus serotype 8 was involved. BTV8 is new to this European region. The gene sequence is closely related to an isolate from sub-Saharan Africa (Nigeria) from the beginning of the 1980s. BTV-8 has also been found in Pakistan/India, Africa, and the Caribbean. This paper describes the specific aspects of the BTV8 epidemic: onset and course of the epidemic, clinical findings, spatial spread, vector surveillance, the effect of wind on the spread of BTV8, control measures taken and the possibilities for vaccination.

INTRODUCTION

Bluetongue (BT) is an arthropod-borne viral non-contagious disease of domestic and wild ruminants, particularly affecting sheep with severe clinical disease including mortality. At present 24 different BT-serotypes have been identified and the disease is transmitted by biting midges (*Culicoides*). BT has a worldwide distribution between approximate latitudes 35°S and 40°N, although in parts of western North America, China and Kazakhstan BTV may extend up to almost 50°N. This part of the world contains the habitat of *C. imicola*, the most important BT-vector of the *Culicoides spp*. BT is endemic in southern member-states of the European Union (EU), and several new incursions have been seen in Italy, Greece, Turkey, the French island of Corsica, the Spanish islands of Menorca and Mallorca and Portugal. Up to now, BT-serotypes 2, 4, 9 and 16 have been involved in epidemics in the EU member-states.

Starting in August 2006 a major epidemic of BT was diagnosed in the North-Western part of Europe, affecting the Netherlands, Belgium, Germany and the Northern France. This epidemic is unique for several reasons. First, this part of Europe is at latitude 51-52°N, a latitude at which *C. imicola* is unknown. Moreover, the causative virus was of serotype 8, a serotype previously unknown in the EU. This virus also caused considerable clinical disease in cattle, whereas clinical disease is usually restricted to small ruminants. This paper provides a preliminary

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overview of the BTV-8 epidemic in North-Western Europe in 2006 and discusses control measures to contain the epidemic.

ONSET OF THE EPIDEMIC AND SEROPTYPE OF BT VIRUS

On the 14th August 2006, a private veterinary practitioner in the southern province of Limburg in the Netherlands notified the veterinary authorities of BT-suspect cases in four different holdings with sheep. Blood samples were taken and submitted to the National Reference Laboratory. Two days later, on the 16th August, BTV infection was confirmed by PCR-test in one of the four sheep flocks.

As a result of the official announcement to the OIE and neighbouring countries of a BTV-infection in the Netherlands, these countries were put on full alert. The first laboratory confirmed BTV case in Belgium was announced on the 19thAugust, in Germany on the 21st August and in France on the 30th August. This was the first observation of a major BTV-epidemic that affected cattle and sheep holdings in the Netherlands, Belgium, Germany, France and recently also Luxemburg. The affected area lies between latitudes 49°N and 52°N.

Since BT was previously absent in this part of Europe, farmer's and veterinarian's awareness of its clinical signs was likely to be low in the initial stage of epidemic, resulting in an unknown level of underreporting bias. In the first 10 BT outbreaks in sheep in the Netherlands, almost all farmers indicated that the first clinical signs started 12 – 18 days before a suspicion was reported to the veterinary authorities, the earliest observed clinical signs dating back to the 31st July – 1st August. Investigations in Belgium suggest that the first clinical signs of BT appeared in mid June in cattle herds. These data indicate that in the first BT outbreaks, there was a period of at least two to four weeks between observation of the first clinical signs by the farmer and reporting of suspicion by the farmer or veterinary practitioner. Although BT was identified as a possible cause for the observed clinical problems soon after notification to the veterinary authorities in the Netherlands (Van Wuijckhuise et al., 2006), the time taken between observation of the first clinical signs by the farmer and reporting was too long. Since the clinical signs of BT fall within the differential diagnosis of for instance Foot-and-Mouth Disease (FMD), it was lucky indeed that it was not FMD this time. A long time period between occurrence of clinical signs and reporting of suspicion can lead, in the case of infectious diseases such as Classical Swine Fever and Avian Influenza, to a dramatic spread of the disease (Elbers et al., 1999; 2004). Once again in this instance, experience with late reporting of an animal disease emphasizes the strong need to facilitate the process of excluding the possibility of a notifiable disease as a cause of clinical problems (Elbers et al., 2006).

On the 28th August 2006, the Community Reference Laboratory (CRL) in Pirbright announced that BT-serotype 8 was causing the outbreaks (ProMED-mail 1, 2006). BTV-8 is new to the European region. The gene sequence data is closely related to an earlier isolate from sub-Saharan Africa (Nigeria) at the beginning of the 1980's. However, no regular isolations have been made from this region in the last 20-25 years, and therefore the source of the BTV-8 introduction into North-Western Europe remains unclear. BTV-8 has also been found in India (Prasad et al., 1992), Africa (Gerdes, 2004), and the Dominican Republic in the Caribbean (Mo et al, 1994).

COURSE OF THE EPIDEMIC

From the start of the epidemic until the 3rd December, a total of 1988 outbreaks occurred: 1014 in cattle, 966 in sheep and eight in wild ruminants. The overall trend of the epidemic curve (Fig. 1) indicates an increase in the total number of confirmed cases, with a peak in mid-October (week 42). From week 43, the number of new cases per week has decreased, except for another peak in week 45. In week 43, there was an episode of several days with four to 10 °C higher daily maximum temperatures than normal for that time of year, which might be associated with the peak in week 45. Both in Belgium and the Netherlands relatively more sheep flocks have become infected compared to cattle herds, while in Germany the opposite is true.

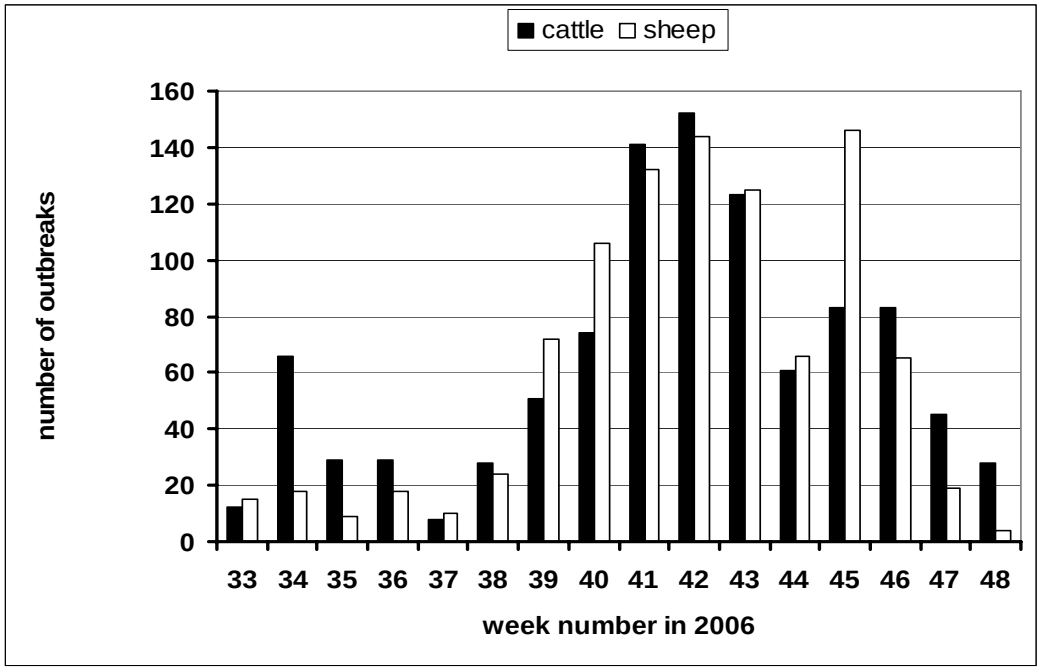

Fig. 1 Distribution of the number of confirmed BTV-8 outbreaks by week in sheep and cattle holdings for the Netherlands, Belgium, Germany, France and Luxembourg together.

CLINICAL SIGNS IN BTV-8 INFECTED CATTLE AND SHEEP

There have been earlier reports of BTV-8 outbreaks in India, Africa, and the Caribbean, but there have not been detailed accounts of the clinical signs associated with BTV-8. Although BTV may infect many different species of ruminants, clinical disease signs are generally associated with sheep and consequently most descriptions of the disease apply to sheep (Erasmus, 1990). Although experienced farmers in BTV endemic areas of South Africa believed that from time to time they had observed clinical BT in their cattle, researchers believed that BT did not produce more than transient and mild, if any, clinical signs in cattle (Hourrigan and Klingsporn, 1975).

Morbidity

The median flock size of 575 BTV-8 infected sheep flocks in the Netherlands and Germany was 12 sheep (range: 1 – 1248). Ninety-four percent of the sheep holdings in the Netherlands

(N=263) were described as hobby type, and 4% as grazing herds. The majority of flocks had one to four clinically affected sheep at the time of clinical investigation (Fig. 2). Morbidity at the flock level was expressed as the number of clinically (BT associated) affected animals in a flock, at the time of clinical investigation, divided by the number of animals in the flock at risk of becoming BT-affected, at the time of clinical investigation. Mean flock level morbidity was 15% (range: 0 – 100%).

The median herd size of 729 BTV-8 infected cattle herds in the Netherlands and Germany was 66 cattle (range: 1 – 540). Sixty-six percent of the cattle herds in the Netherlands (N=178) could be described as dairy type, 15% as hobby, 14% as beef type, and 3% as breeding type. The majority of herds had one to two clinically affected cattle at the time of clinical investigation (Fig. 3). Mean herd level morbidity was 7% (range: 0 – 100).

Mortality

Seventy-three percent of the infected sheep flocks showed no mortality at the time of clinical investigation (Fig. 4). Mortality at the flock level was expressed as the number of BT-associated dead animals, at the time of clinical investigation, divided by the number of animals in the flock at risk of dying, at the time of clinical investigation. Mean mortality in sheep flocks was 3% (range: 0 – 100).

Ninety-four percent of the infected cattle herds showed no mortality at the time of clinical investigation. Mean mortality in cattle herds was 0.1% (range: 0 – 20).

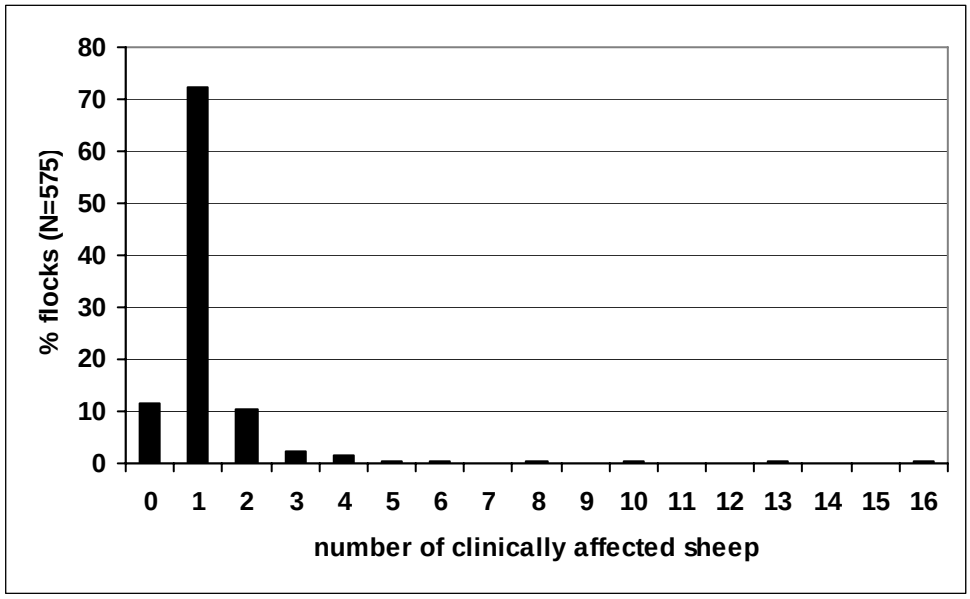

Fig. 2 Distribution of clinically affected sheep in 575 confirmed BTV-8 infected sheep flocks in the Netherlands and Germany

Case fatality

Case fatality at the herd level was expressed as the proportion of diseased animals that had died at the time of clinical investigation. Seventy-two percent of BTV-8 infected sheep flocks had a case fatality of 0% (Figure 5). Mean case fatality in sheep flocks was 18% (min-max: 0 –

100%). Ninety-three percent of BTV-8 infected cattle herds had a case fatality of 0%. Mean case fatality in infected cattle herds was 3.9% (min-max: 0 – 100%)

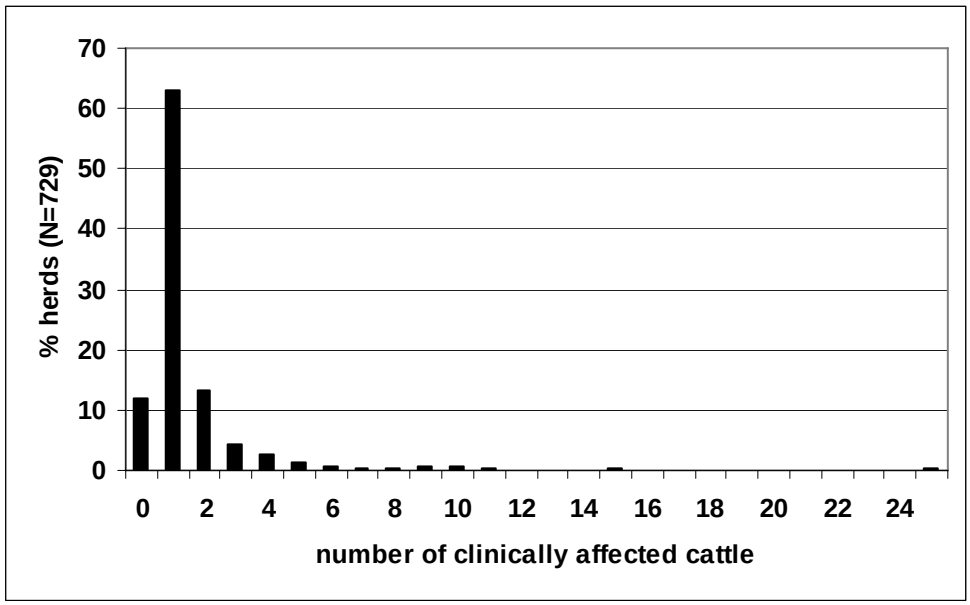

Fig. 3 Distribution of clinically affected cattle in 729 confirmed BTV-8 infected cattle herds in The Netherlands and Germany.

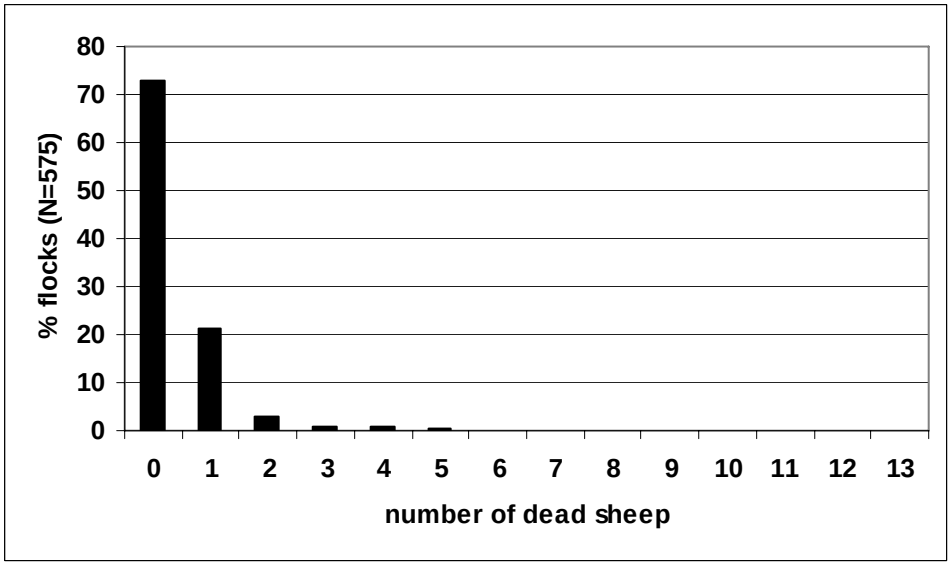

Fig. 4 Distribution of number of dead sheep in 575 confirmed BTV-8 infected sheep flocks in The Netherlands and Germany.

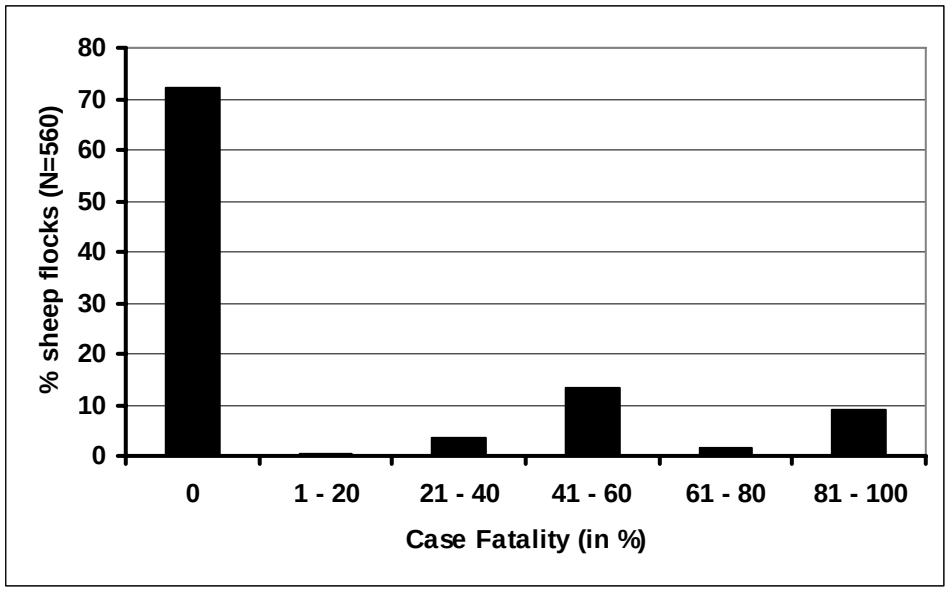

Figure 5. Distribution of case fatality in 560 confirmed BTV-8 infected sheep flocks in The Netherlands and Germany.

Distribution of clinical signs in sheep and cattle

The most prominent clinical signs in affected animals from BTV-8 infected sheep flocks were erosions of the oral cavity, fever, salivation, facial oedema, depression, oedema of the lips, congestion, erythema and redness of the oral mucous membranes, dysphagia and lameness (Table 1.). The most prominent clinical signs in affected animals from BTV-8 infected cattle herds were erythema and redness of nasal mucous membrane, erosions of the oral cavity and lips, salivation, fever, , conjunctivitis and coronitis (Table 1.).

Table 1. Distribution of clinical signs in BTV-8 infected sheep and cattle herds in the Netherlands

Clinical Sign	% Cattle Herds (n=178)	% Sheep Flocks (n=263)
Fever	19.9	43.3
Salivation	24.3	40.3
Grinding of teeth	2.8	2.2
Oedema lips	3.3	30.7
Facial oedema	7.2	37.7
Congestion, erythema, redness of oral mucous membrane	10.5	29.6
Erosions of oral cavity	24.3	48.1
Erosions of lips/ crusts around nostrils	25.4	23.6
Lesions of nasal mucous membrane	18.8	3.0
Congestion, erythema, redness of nasal mucous membrane	47.0	14.3
Conjunctivitis	19.3	2.6
Coronitis	19.3	17.5
Lameness	11.6	28.8
Dysphagia	13.3	28.8
Hyperaemic/purple coloration/lesions of teats	13.3	0.4
Decrease in milk production	5.6	0
Depression/apathy	12.7	34.5
Recovered at clinical inspection after raising suspicion	1.7	7.9

SPATIAL DISTRIBUTION AND CLUSTER ANALYSIS OF THE EPIDEMIC

Mapping of the outbreaks demonstrated that the infection went Eastward, Westward (range 470km) and Southward (range 360 km) but that the spread to the North of the Netherlands was very limited. The infected area in the Netherlands did not change during the period whereas spread was obvious in Germany and Belgium. The six outbreaks identified in France seem to belong to the fringe of the epidemic. The identification of a core and some peripheral outbreaks is visually obvious but could be related to a heterogeneous distribution of the susceptible animals (Fig. 6).

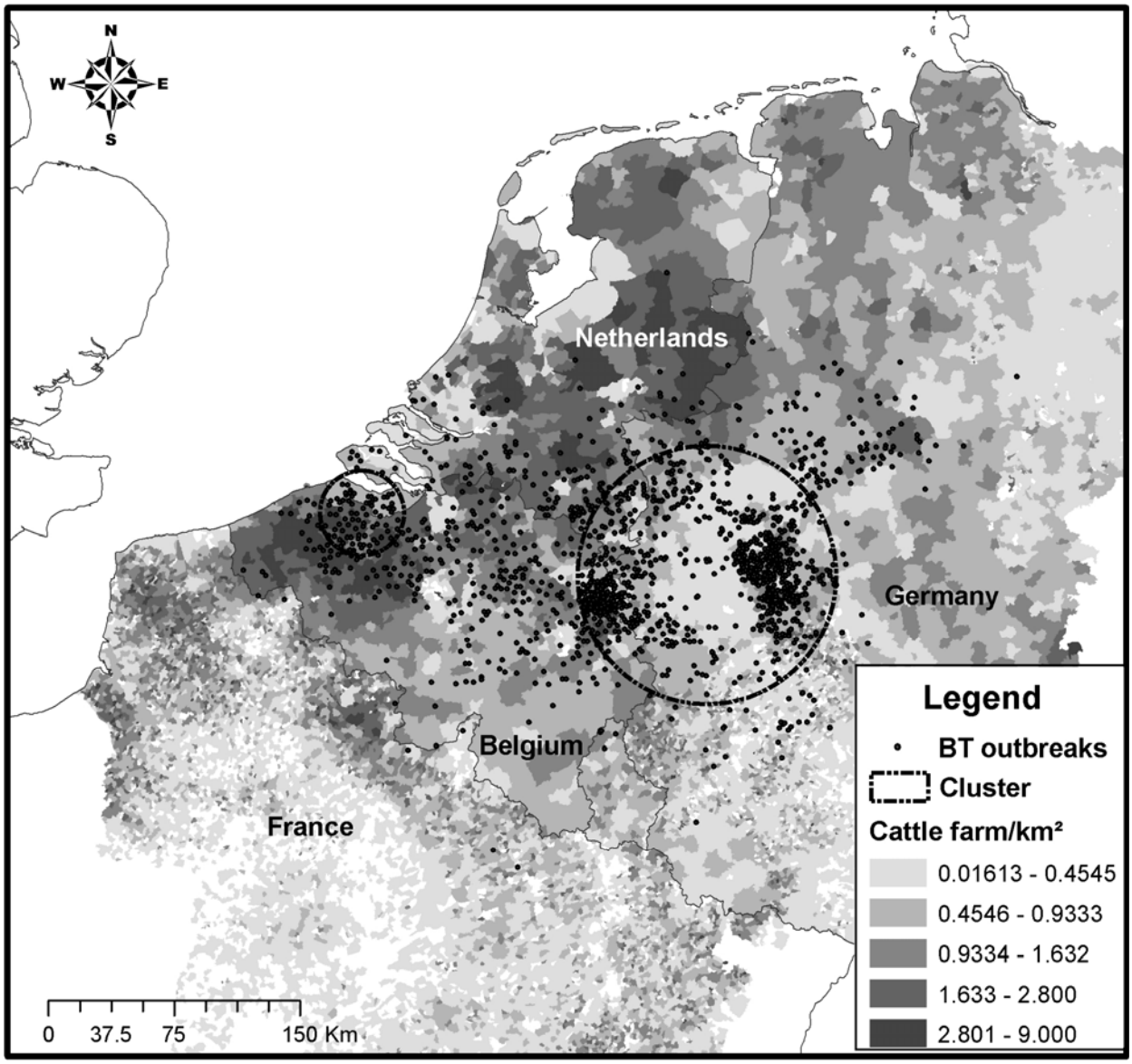

Fig. 6 Spatial distribution of the BTV-epidemic in North-western Europe in 2006.

Collection of the number of cattle farms at the municipality level in the 4 countries allowed the identification of clustering during the epidemic. Because of a lack of consistency of data between countries the same approach was not performed on small ruminants. Using Scan statistic method (Kulldorff, 1997), two statistically significant clusters were identified. The biggest one included the two Eastern visual clusters. The gap (relative absence of outbreaks) that can be identified within this cluster represents a low density of farms in this area. The identification of clusters is important for further studies, as analysing the whole epidemic is difficult to justify given the spatial and temporal structure of the epidemic. This will allow the characterisation of local spread and the identification of differences between different infected areas. BT can be transmitted from one place to another through infected animals or products or through infected midges. In more than one month, the disease spread over around 150 km. Knowing that the active flight of *Culicoides* is approximately 2 km/day (Lillie, 1981), the observed propagation is consistent with a diffusion of the virus in the insect population.

VECTOR SURVEILLANCE

A 'snapshot' was developed to rapidly assess over a wide area the *Culicoides* vector population levels in the Netherlands. The country was divided into 110 20km x 20km grid cells; within each cell a farm was selected to be sampled for *Culicoides* using an Onderstepoort-type blacklight trap. Prior to deployment of the light traps nine veterinarians were trained in their usage and in the preservation of captured *Culicoides.* Each veterinarian was given a light trap and all the necessary collecting equipment, armed with a wayfinder (TomTom) and a map of designated grid cells. *Culicoides spp.* were collected for one night only on five separate farms (one farm in each of five grid cells). Each morning collections were dispatched by courier to a field laboratory where the *Culicoides* were counted and identified. The 'snapshot' commenced on the 12th of September 2006 and was completed on the 28th of September during an intensive period of BT activity in the Netherlands.

The most widely distributed taxon in the Netherlands was the Obsoletus Complex and was found in 93.3% of the grids sampled. The second-most prevalent taxon was the Pulicaris Complex (75% of grids), followed by *C. dewulfi* (70.2%), *C. chiopterus* (68.3%) and the Nubeculosus Complex (27.9%).

The relative abundances of the *Culicoides* in all 106 collections were as follows: The Pulicaris Complex dominated the collections comprising at least six species, the most abundant being *C. punctatus*. The remaining taxa included *C. pulicaris, C. newsteadi* (*sensu* Delécolle 1985), *C. halophilus, C. lupicaris* and *C. impunctatus*. The second-most dominant taxon was the Obsoletus Complex comprising at least two species (*C. obsoletus* and *C. scoticus*). The relative abundances of the two species remains unknown as the respective females cannot be identified morphologically with certainty.

Preliminary mapping of the 'snapshot' results showed that each of the six or more species of the Pulicaris Complex are not widely distributed across the Netherlands. Instead, they are mostly restricted to certain regions because of their preference for specific breeding habitats e.g. saline coastal areas for *C. halophilus*. The Obsoletus Complex is the most widespread taxon in the Netherlands and may be linked to the fact that they are known to breed in forest leaf litter, which enables them to penetrate also into urban environments. Because the Obsoletus Complex is a known vector of BT in southern Europe its widespread occurrence (and heightened abundances) place a significant portion of the Netherlands at risk. *C. dewulfi* and *C. chiopterus* also occur widely across the Netherlands; this is because they breed exclusively in the dung of cattle. During the more intensive investigations in the region of Gulpen, Limburg province, *C. dewulfi* was implicated as a vector of bluetongue.

WIND AFFECTING THE SPREAD OF BTV-8

The flight ranges of *Culicoides* are generally short but can be vastly extended by prevailing winds (Sellers, 1992; Braverman & Chechik, 1996). Sellers (1992) differentiated between two types of flight. In the absence of wind or at low speeds *Culicoides* may fly short distances in any direction, both up- and downwind. When the wind speed exceeds the unaided flight speed of *Culicoides*, the midges can be carried by wind and flight is possible up to 700 km. The start of a flight is either due to active insect movement or passively due to warm updrafts. Landing may

also either be active or passive due to by wind shifts (Sellers, 1992) or terrain topography (Bishop et al., 2000; Bishop et al., 2005).

The establishment of the vector upon arrival and its ability to transmit the virus would then be affected by weather conditions at the destination, the presence or absence of the virus at the source or at destination and the presence or absence of livestock at the destination. Bishop and Alba and their teams were amongst the first to use a more quantitative approach to support these hypothesis. Bishop et al. (2000) tracked *Culicoides brevitarsis* over a long distance starting from the coast. They found that the dispersal of the midges was related to wind speed and direction, as well as topography. Alba et al. (2004) investigated the possibility of introduction of infected midges on the Balearic Islands from Sardinia during the 2000 epidemic. They calculated backward wind trajectories for a number of days when the active spread of the midges was considered to be highly probable given the climatic conditions (humidity, temperature and wind). Using this approach, the authors demonstrated that infected *Culicoides* could have been transported by wind from Sardinia to the Balearic Islands, thereby causing the recorded bluetongue outbreaks.

More recently Ducheyne et al. (submitted) tested the hypothesis that *Culicoides* species can be propagated by wind over long distances both over sea and land, and that this wind-borne spread can cause outbreaks in remote areas. Horizontal and vertical wind components were derived from European ReAnalysis-40 dataset from the European Centre for Medium-Range Weather Forecast (ECMWF) and the hypothesis was tested using available historical bluetongue outbreak data between 1999 and2001 for Greece, Bulgaria and Turkey. Depending on available data forward (i.e. where did wind-vectors point to) or backward (i.e. where did wind-vectors originate from) trajectories were calculated from eight days prior to the first outbreak date until one week after that date. Results showed a consistent relationship between wind patterns and downwind and upwind outbreak sites. It was concluded that the proposed methodology was a good basis for quantifying the risk of spread of infected bluetongue midges by wind and if further developed and validated it could be used in a regional early warning system.

In this study, the methodology developed by Ducheyne et al. (submitted) was taken one step further to analyze the impact of wind on the spread of the current BTV8 epidemic. Six-hourly isentropic forward wind trajectories originating from infected premises were calculated at five pressure levels (700hPa, 850hPa, 925hPa and 1000hPa, respectively approximately 3000m, 1450m, 760m and 100m under stable weather conditions). For these pressure levels pan-European ECMWF wind data are routinely available. Near-surface wind data (10m) are also included. The date of the first identified symptoms was used as the start date.

The obtained trajectories were filtered for suitability and aggregated to produce cumulative weekly wind density raster layers. These raster layers represent the number of ‘passing’ wind events originating from infected premises to each pixel. The weekly data were further analysed using spatial statistics and statistically compared to the observed spread of the epidemic in The Netherlands, Belgium, Germany, France and Luxemburg.

Confounding factors such as animal movement records, herd distributions, terrain topography and climatic conditions were taken into account. In future analyses data from an area-wide serological survey will be included.

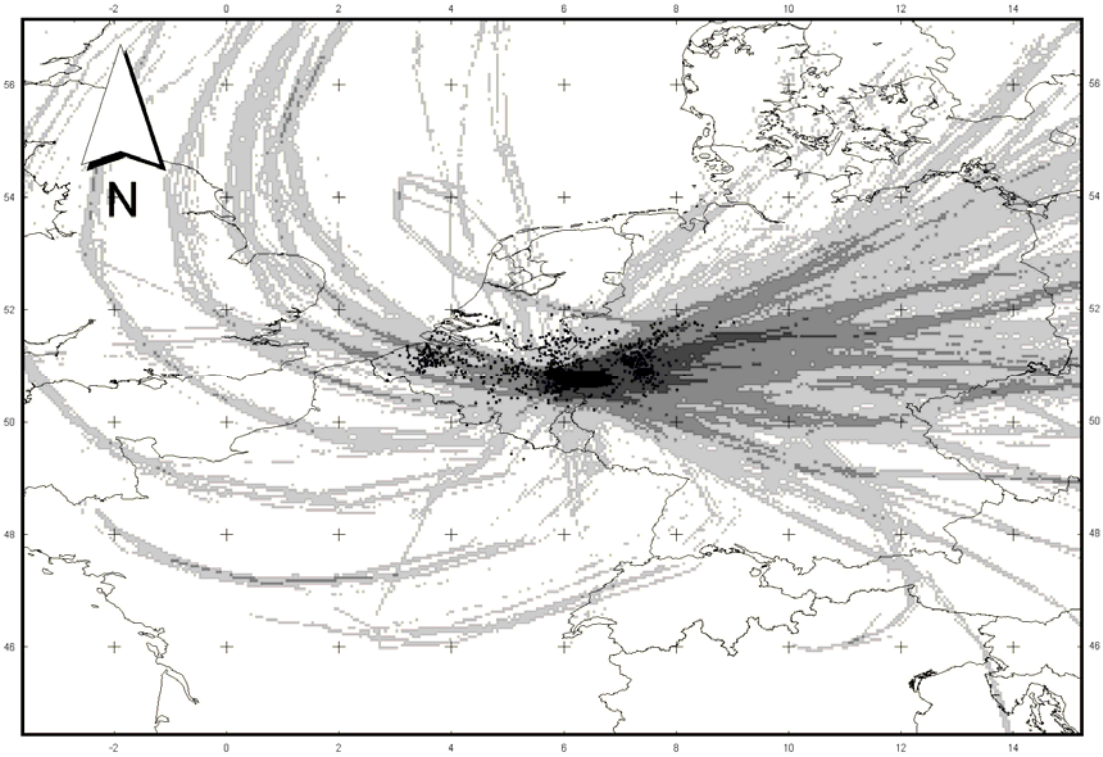

Fig. 7 Wind density map showing cumulative density of wind events originating from positive premises. Period covered: June – August 2006. Categories from light to dark: 0-5, 6-21, 22-57, 58-151 and 152-530. Points show the maximum extent of the epidemic.

Preliminary results based on wind model outputs at 850hPa from June until September 2006 suggest that wind played an important role in the long range dissemination of the epidemic (Fig. 7). The density of wind events appeared to be linked to spread pattern of the epidemic, at least in its early stage. This may contribute to explaining the predominantly east-west spread pattern of the epidemic. An important part of the long range spread may have occurred prior to official detection of the first outbreak further emphasising the importance of efficient monitoring systems. The UKwas at risk of introduction of BT, during the first part of the epidemic, andwith increasing numbers of outbreaks in East- and West-Flanders after September 2006, this risk may have increased.

CONTROL MEASURES

BTV is not contagious and spreads primarily via vector species of *Culicoides* midges or incidentally via transfer of blood, semen, egg cells or embryos. According to Council Directive 82/894/EEC (CEC, 1982) BT is a notifiable disease in Europe and affected Member States should implement control measures according to Council Directive 2000/75/EEC (CEC, 2000). Control measures include the control of vectors (use of insecticides in the animal premises and in the areas where these insects live and insect repellents onto animals), restrictions of live ruminants moving from affected areas to non-infected regions where the vector is present and the use of vaccines. A key element is the demarcation of a 20-kilometre zone (minimum 20-km radius), a protection zone (minimum 100-km radius) and a surveillance zone (minimuml 150-km radius) around an infected herd. The conditions for movement of live ruminants following an

official confirmation of the presence of bluetongue are stated in Council Directive 2005/393/EEC (CEC, 2005).

After the confirmation of the first BTV-8 case on the 16th August, the different restriction zones were demarcated. The 20-km zone covered parts of the Netherlands, Germany and Belgium, the protection zone additionally covered a part of Luxembourg and the surveillance zone additionally covered parts of France. Throughout the course of the epidemic, the different zones further extended together with the further dispersion of confirmed BTV-8 cases over the affected countries. The control measures that were implemented were discussed between the competent authorities of the affected countries in order to reach a uniform control strategy. The measures, described below, apply to Belgium but are generally in line with those applied in the other affected Member States.

When a suspicion of BTV-8 infection on a farm was notified, the farm was placed under surveillance resulting in all ruminants being registered and prevented from leaving the farm. When the suspicion was confirmed by laboratory analysis, the farm was registered as a case and the above mentioned control zones were demarcated around the farm. No additional measures were taken for animals that were confirmed to be infected with BTV-8.

Control measures in the 20-km zones

At the start of the epidemic in August 2006, the following control measures were implemented in 20-km zones. All ruminants that were kept in this zone, including newborn ruminants, were to be registered by the animal keeper. The transport of ruminants within the 20-km zone was prohibited. All cattle were to be housed indoors from one hour before sunset until one hour after sunrise (this measure did not apply to sheep and goats). All ruminants and animal shelters were to be treated with registered insecticides. The export of ruminants, semen, egg cells and embryos from ruminants produced after 1 May 2006, out of the 20-km zone, was prohibited

The initial control measures were modified throughout the course of the epidemic due to their economic consequences and to allow trade of uninfected animals under certain conditions. In a first modification transportation of ruminants, originating from outside the 20-km zonedirectly to a slaughterhouse, within the zone was allowed. This modification was later extended to ruminants originating from any affected Member States but outside a 20-km zone. A further modification permitted the movement, under certain conditions, of ruminants from non-infected farms, within the 20-km zone and the movement of ruminants or veal calves to slaughterhouses or veal calf feed lots, respectively, within the protection zone. A subsequent modification enabled, under certain conditions, the movement of ruminants from non-infected farms within a 20-km zone to any destination (slaughterhouse, feed lot, breeding farm, etc) in a protection zone in any affected Member State.

The conditions for movement of animals within or outside a 20-km zone included the treatment of animals, vehicles and premises with insecticides, clinical inspection of the animals, and for certain movements the establishment of the infection status of the animals by application of serological or PCR tests.

Control measures in the protection zones

The control measures that were implemented in the protection zone at the onset of the epidemic were that all ruminants kept in this zone as well as newborn ruminants were to be registered by the animal keepers and that the export of ruminants, or semen, egg cells and embryos from ruminants outside this zone was prohibited. Treatment of ruminants and premises with insecticides was advised but not compulsory.

In further stages of the epidemic the measures in the protection zone were also adjusted, first to allow movement of non-infected ruminants to slaughterhouses in any control zone in any affected Member State and later to allow movement of ruminants to any destination (slaughterhouse, feed lots, breeding farms, etc.) in a 20-km or protection zone in any of the affected Member States. The conditions for moving ruminants were the same as those applied in the 20km-zone.

Control measures in the surveillance zones

The initial control measures that were implemented in the surveillance zone at the onset of the epidemic were the same as those in the protection zone with the only exception that movement of ruminants to the protection zone was still allowed. The modifications that were made to the movement restrictions were the same as those in the protection zone which resulted in the control measures in the protection and surveillance zones becoming equivalent. Therefore it was decided to lift the surveillance zone and to extend the protection zone to a radius of 150 kilometres.

VACCINATION

Vaccination is implemented to control BT in many regions where the virus is endemic. Vaccination can be either restricted to sheep to minimise clinical BT, or include all susceptible ruminants if the aim is to interrupt virus circulation. For both purposes successes have been claimed in the literature. Breard et al. (2004) reported a successful vaccination campaign against BTV2 infections in sheep on Corsica, during the winter and spring of 2001/2002. No cases of BT were observed during 2002. Moreover, Dungu et al. (2004) reported successful vaccination against clinical BT in Africa. During the last decade Italy has suffered incursions of BTV2, BTV4 and BTV9. In Italy all domestic ruminants have been vaccinated in affected areas in order to stop the infection cycle. On the basis of a quantitative risk analysis it was decided that at least 80% of the susceptible population in a region has to be vaccinated to reach that goal (Giovannini et al., 2004). Caporale et al. (2004) observed a strong negative correlation between the level of vaccination in a region and the number of outbreaks. Clinical disease disappeared completely and infections were reduced to low levels as documented from the results of serological surveillance of sentinel animals. However, it still has not been proven that BTV can be eliminated from an area by vaccination. Moreover, to the authors are not aware ofreports that demonstrate that vaccination can reduce the transmission of BTV8.

Vaccines against BT can be either inactivated, attenuated or derived by genetic modification. Since the first category generally lacks efficacy due to the high concentration of antigens needed and the last category lacks acceptability in many countries, the attenuated vaccines are the preferred choice at present. However, vaccine virus can be transmitted to other animals and even to other herds (Ferrari et al., 2005). This poses the question whether reversion to virulence or

reassortment with other BTV strains can occur. Moreover, vaccines have been shown to have a foeto-toxic effect, which makes them unsuitable for use during gestation. Despite these disadvantages, because it is a disease of a region and not of a herd, vaccination seems to be the only realistic option for control in regions endemically infected. However, according to current theory it is unlikely that the virus will survive during winter in North-western Europe, meaning that vaccination should not be considered at present.

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QUANTIFICATION OF WITHIN-FLOCK TRANSMISSION OF H5N1 AVIAN INFLUENZA VIRUS IN CHICKEN FLOCKS IN THAILAND

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SUMMARY

Transmission dynamics of H5N1 avian influenza virus within a poultry population have not been determined during the recent epidemics in Asia. The aim of this study was to quantify the within-flock transmission of H5N1 virus in infected chicken flocks during the outbreaks in Thailand. Mortality data collected at the flock level during the epidemic were used to estimate the transmission rate parameter (β) and the basic reproduction ratio (R_0). Several models with varying infectious periods and chicken types were parameterized using a Generalized Linear Model (GLM). Based on the SIR assumption, β was estimated with 95% confidence intervals (CI) from 2.26 per day (95% CI, 2.01-2.55) to 0.66 per day (95% CI, 0.05-0.87) for 1-day and 4-day infectious periods, respectively. The accompanying R_0 ranged from 2.26 (95% CI, 2.01-2.55) to 2.64 (95% CI, 2.02-3.47). Although a lower R_0 in backyard chickens and fighting cocks compared to broilers and layer hens was found, this difference was not statistically significant. Moreover, the results indicated a critical vaccination fraction of 75-80% of the susceptible population was necessary to prevent a major outbreak within a flock.

INTRODUCTION

Highly pathogenic avian influenza A virus (HPAI) of the subtype H5N1 caused widespread outbreaks among domestic poultry and wild birds in Asia, Africa, Europe, and the Middle East resulting in enormous losses in poultry production, wildlife conservation, and socioeconomics (OIE, 2006). Sporadic transmission from birds to humans and other mammals was reported (Tiensin et al., 2006; WHO, 2006). Several measures to stop the spread of HPAI, e.g. stamping-out, movement restriction, and hygienic measures were implemented (Capua et al., 2003; Stegeman et al., 2004; Tiensin et al., 2005). However, with the exception of Europe and Japan, the measures have not yet resulted in the elimination of HPAI (Stegeman et al., 2004; Nishiguchi et al., 2005; Tiensin et al., 2006).

Quantitative knowledge of the within-flock transmission could be useful 1) to help in the design of programs to rapidly detect infected flocks, 2) to estimate the period when avian influenza virus was most likely introduced into infected flocks, 3) to determine the fraction needed to vaccinate in order to stop the spread of the virus in poultry flocks, and 4) to assess intervention measures during the epidemic (van Nes et al., 1998; Stegeman et al., 1999;

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Stegeman et al., 2004; Le Menach et al., 2006; Savill et al., 2006). However, until now transmission of H5N1 avian influenza has not yet been quantified from natural outbreaks in Asia. The reason for this may be that the control strategy in most affected countries included depopulation of infected flocks as soon as possible in order to control spread of the disease. As a consequence, the stamping-out strategy also hampered measuring within-flock transmission. However, during an epidemic, mortality data recorded in field situations could be very valuable sources for epidemiologic analyses for HPAI which has a close to 100% mortality rate. It has been suggested that a better understanding of the transmission dynamics of HPAI within flocks can help improve the effectiveness of the control measures (Stegeman et al., 1999; Stegeman et al., 2004).

In this study, a method to quantify the transmission of H5N1 infections from mortality data registered during the outbreaks in Thailand is described. Mortality data were back-calculated to obtain the number of susceptible (S) and infectious (I) animals during the days before the flock was stamped out. Next, the transmission rate parameter (β) was obtained from a generalized linear model (GLM), selecting the model that fitted the data best. Subsequently, the basic reproduction ratio (R_0) was estimated. Differences of transmission among different types of chicken flocks, i.e. backyard chickens, broilers, fighting cocks, and layer hens were investigated.

MATERIALS AND METHODS

The within-flock transmission of HPAI H5N1 virus was quantified by means of the transmission rate parameter *(β)* and the basic reproduction ratio *(R_0)*. R_0 is defined as the average number of secondary infections caused by a primary infectious case per total infectious period (Anderson & May, 1992). If $R_0>1$, a disease can spread, but if $R_0<1$, chains of transmission will inevitably fade out (Anderson & May, 1992; Diekmann & Heesterbeek, 2000). The transmission rate parameter β determines the rate per time unit at which susceptible animals become infected and hence the rate of spread within a flock.

Data

The study included 139 flocks that were diagnosed as H5N1 avian influenza virus infected from July to November 2004 in Thailand. The data were collected as part of a national active surveillance program known as “the X-ray survey” (Tiensin et al., 2006). For each of the 139 flocks, outbreak investigations were done by local veterinarians using a standardized investigation form. For commercial chicken flocks, logbooks containing a history of ongoing activities in those infected farms were also obtained. These flocks were selected because they had daily mortality data, recorded for at least 2 days prior to culling, considered suitable for analyses. Because the goal of this study was to describe within-flock transmission from mortality data, the number of dead birds per day in a flock was essential information.

Assumptions for model development

This study was conducted under the following assumptions 1) no individual chicken had immunity against H5N1 virus at the time of virus introduction, 2) all susceptible chickens were equally susceptible, 3) all infected chickens were equally infectious and shed the virus throughout the flock, and 4) all infected chickens eventually died of H5N1 virus (Tian et al., 2005; Webster et al., 2006).

Dataset construction for statistical model

It was assumed that transmission in a flock could be described by an SIR or SEIR model. Thus, the transmission datasets were constructed based on back-calculation using SIR and SEIR assumptions (de Jong, 1995; Stegeman et al., 1999; van der Goot et al., 2005). In these models, S is the number of susceptible animals, E represents the number of latently infected animals, I is the number of infectious animals, and R represents the number of removed animal from the population, in this case through death (de Jong, 1995; Diekmann & Heesterbeek, 2000). Estimation of a transmission rate parameter (β) requires knowledge of the number of susceptible chickens (S) and the number of infectious chickens (I) per day (Stegeman et al., 1999; Stegeman et al., 2004). Because the disease is highly lethal (Capua and Alexander, 2004; Li et al., 2004), it was assumed that the number of dead chickens per day in a flock represented the number of newly infected chickens at an earlier time (cases, C) when the virus was introduced into a flock. For the analysis, the mortality data of a flock were back-calculated into the format C(t), S(t), E(t), I(t), and R(t) for numbers of birds in each category at time t (day 0, 1, 2, 3, etc.). The total number of chickens in a flock at a specific time t was also designated as N(t) = S(t) + I(t) for the SIR assumption and as N(t) = S(t) + E(t) + I(t) for the SEIR assumption. On the basis of experimental infection studies of H5N1 virus (Lee et al., 2005; Tian et al., 2005; Swayne et al., 2006; Webster et al., 2006), datasets were constructed assuming that the chickens died after an infectious period of 1, 2, 3, or 4 days, represented in Table 1 for SIR. The number of dead chickens in each flock was back-calculated, based on a default 1-day incubation period and a varying 1-, 2-, or 3-day infectious period, for the SEIR model.

Statistical model

The analyses of the transmission based on SIR and SEIR assumptions were tested. Throughout, the analyses were aimed at estimation of β and R_0. In all analyses, the statistical program R (version 2.4.0) was used (R Development Core Team, 2006). Infection of susceptible chickens was assumed to occur with rate

$$\frac{\beta SI}{N} \tag{1}$$

and removal of infectious chickens with the death rate αI. In this study, we only estimated β. According to the SIR and SEIR models, the number of newly infected chickens (C) arising in each time period (Δt) is Poisson distributed:

$$C \sim Poisson(\frac{\beta SI}{N}\Delta t) \tag{2}$$

and has an expected value (McCullagh & Nelder, 1989).

The number of newly infected cases depends on β, S, I, and N in the given time period. Based on the Eq. (2), ln(β) could be estimated with a Generalized Linear Model (GLM) with a log-link function (McCullagh & Nelder, 1989; Dohoo et al., 2003). In the analysis, C was a response variable and $ln(S(t)I(t)/N(t))$ was included as an offset variable. In addition, flock types e.g. broilers, layer hens, fighting cocks, and backyard chickens were added as a categorical fixed effect and flock as a random effect. The latter term was needed because more observations from the same flock were included in the dataset (Dohoo et al., 2003). As a consequence, the negative

binomial distribution was used to correct for overdispersion of the outcome of the model. The Akaike's Information Criterion (AIC) was used to select the best fitting models. The model with the lowest AIC value receives the most support from the data (Akaike, 1973; Motulsky & Christopoulos, 2004).

Table 1. Example of dataset construction of a layer flock based on SIR assumption at hypothetically different infectious periods of 1, 2, or 3 days

Back-calculation based on SIR assumption						
Infectious period	Day	C†	S	I	R	N
1 day	0	55	4100	0	0	4100
	1	115‡	4045	55	0	4100
	2	203‡	3930	115	55	4045
	3	415‡	3727	203	115	3930
	4	837‡	3312	415	203	3727
	5		2475	837	415	3312
	6		2475		837	2475
	7					2475
2 days	0	55	4100	0	0	4100
	1	115	4045	55	0	4100
	2	203‡	3930	170	0	4100
	3	415‡	3727	318	55	4045
	4	837‡	3312	618	115	3930
	5		2475	1252	203	3727
	6		2475	837	415	3312
	7				837	2475
	8					2475
3 days	0	55	4100	0	0	4100
	1	115	4045	55	0	4100
	2	203	3930	170	0	4100
	3	415‡	3727	373	0	4100
	4	837‡	3312	733	55	4045
	5		2475	1455	115	3930
	6		2475	1252	203	3727
	7			837	415	3312
	8				837	2475
	9					2475

*Raw data of daily mortality records at specific date

†Assumed that the number of dead animals represents the number of newly infected animals at an earlier time after virus introduction into a flock (C)

‡After back-calculation of raw data, records containing data in S, I, N, and C columns were used to estimate the transmission parameter (β)

The standard errors (S.E.$_\beta$) of all estimated β's were calculated separately for each flock type and different infectious periods. The standard error (S.E.) for ln(β) was used to calculate the 95% confidence interval (CI) assuming a normally distributed variable:

$$\beta \pm 1.96 * \text{S.E.}_\beta \quad (3)$$

Subsequently, R_0 was calculated as Eq. (4), the products of the infectious period T (days) and the transmission rate parameter β (individuals per day) (Anderson & May, 1992; Stegeman et al., 2004), with a 95% CI based on the limits of the 95% CI of ß in Eq. (3).

$$R_0 = e^{ln(\beta)} * T \quad (4)$$

RESULTS

Characteristics of the H5N1 infected flocks

Table 2 describes the characteristics of the 139 H5N1 infected chicken flocks used for analyses in this study. Most flocks (66%) were backyard flocks with relatively small flock sizes. In contrast, broiler and layer flocks were generally much larger. Cumulative mortality of the 139 infected flocks was 2-100%, whereas average daily mortality was 1-33%. In this study, repeated measures of the infection process within a flock were present and needed to be accounted for. Of the 139 flocks, 21% had 5-6 measurements of daily mortality records and 22% had 4, 38% had 3 and 19% had 2 repeated observations.

Model building

Table 3. shows the results of various models based on the SIR assumption with a default scenario of a 2-day infectious period. In this study, we used Eq. (2) to estimate the expected number of new cases, *E(C)* arising in each time period (per day). Use of a Poisson distribution resulted in overdispersion of the model. As a consequence, the negative binomial distribution was used to correct for overdispersion of the outcome of the model. The AIC values of various models based on the SIR assumption are shown in Table 3. SIR models produced better fits of the data compared to SEIR models, for which the results are not shown.

Estimation of transmission parameters

Table 4. presents the outcomes of the statistical model using different lengths of infectious periods and different types of chicken flocks, and the calculated R_0, based on the SIR assumption. As expected, the estimated β differed for different infectious periods (1, 2, 3, and 4 days). Depending on the assumed infectious period with all flock types included in the best fitting model, the point estimates of β varied from 2.26 day^{-1} (95% CI, 2.01-2.55) for a 1-day infectious period to 0.66 day^{-1} (95% CI, 0.50-0.87) for a 4-day infectious period, whereas the values of R_0 remain more or less unchanged, as theoretically expected (Table 4A.). β and R_0 for the different types of chicken flocks at the specified infectious periods are also shown in Table 4B. The point estimates of β of backyard chickens and fighting cocks combined are lower than those of layers and broilers combined, but this difference was not accompanied by a different AIC value from the model without flock type added (Table 3.).

Table 2. Characteristics of the 139 H5N1 HPAI infected chicken flocks during the 2004 Thai epidemic used in this study

Characteristics	Number of flocks				
	Backyard chicken	Broiler	Fighting cock	Layer	Total
Type of poultry	92	20	9	18	139
No. of chickens in the flock (birds)					
< 100	76	-	9	-	85
100-1,000	16	3	-	7	26
1,000-5,000	-	6	-	10	16
5,000-10,000	-	7	-	1	8
> 10,000	-	4	-	-	4
Cumulative mortality (%)					
1-10	-	5	-	3	8
10-30	13	6	1	11	31
30-50	25	5	1	3	34
50-100	54	4	7	1	66
Average daily mortality (% of chickens died a day)					
1-5	4	9	-	10	23
5-10	12	6	1	4	23
10-20	44	5	4	4	57
20-36	32	-	4	-	36
No. of records of daily mortality prior to culling (days)					
2	17	4	4	1	26
3	38	4	2	9	53
4	19	6	2	4	31
5	14	6	1	3	24
6	4	-	-	1	5

Table 3. Results of various models based on S-I-R model showing a default scenario of a 2-day infectious period with different types of chickens

Models based on SIR assumption	Coefficients* (ln β)	Std. Error	AIC
Model 1: Poisson			
intercept	0.36	0.005	12,284
Model 2: Negative binomial			
intercept	0.28	0.04	1,714
Model 3: model 2 + random flock effect			
intercept	0.21	0.05	405
Model 4: model 3 + poultry type (4 types)			
layer	0.25	0.13	405
fighting cock	0.28	0.23	
broiler	0.46	0.12	
backyard chicken	0.13	0.06	
Model 5: model 3 + poultry type (3 types)			
layer	0.25	0.13	403
backyard chicken + fighting cock	0.14	0.06	
broiler	0.46	0.12	
Model 6: model 3 + poultry type (2 types)			
layer + broiler	0.36	0.09	406
backyard chicken + fighting cock	0.14	0.06	

*coefficients (ln β) of model 4, 5, and 6 are shown individually for each poultry type in which flock types were added in the models.

Table 4. A) Estimates of the transmission rate parameter (β) and the basic reproduction ratio (R_0) with 95% confidence intervals, based on the SIR assumption of 139 infected chicken flocks in Thailand with flock as a random effect, B) Results from the SIR assumption with two groups of flock types added

Model	Transmission rate parameter β (chicken^{-1} day^{-1})				Reproduction ratio R_0			
	1 day n = 346*	2 days n = 208	3 days n = 94	4 days n = 35	1 day n = 346*	2 days n = 208	3 days n = 94	4 days n = 35
A) All type	2.26 (2.01–2.55)	1.23 (1.11–1.36)	0.87 (0.75–1.02)	0.66 (0.50–0.87)	2.26 (2.01–2.55)	2.46 (2.23–2.72)	2.62 (2.25–3.07)	2.64 (2.02–3.47)
B) layer + broiler	2.30 (1.92–2.76)	1.43 (1.20–1.71)	1.16 (0.90–1.50)	0.79 (0.50–1.25)	2.30 (1.92–2.76)	2.86 (2.41–3.41)	3.49 (2.70–4.50)	3.17 (2.01–5.00)
backyard + fighting cock	2.18 (1.94–2.46)	1.15 (1.02–1.30)	0.75 (0.63–0.91)	0.60 (0.43–0.84)	2.18 (1.94–2.46)	2.31 (2.05–2.60)	2.26 (1.88–2.72)	2.40 (1.71–3.36)

*n – number of back-calculated records used in the model

DISCUSSION

Estimates of transmission parameter (β) and the basic reproduction ratio (R_0)

In this study, the within-flock transmission of H5N1 HPAI virus in infected chicken flocks during the epidemic in Thailand for different types of chickens was quantified. The values of β resulting from this study are between 0.6 and 2.3 day^{-1} (Table 4.), which are much lower than the point estimate of 33 day^{-1} for a 6-day infectious period of H7N7 virus given by van der Goot et al. (2005). However, their estimate was only based on observations in two contact-infected chickens resulting in a confidence interval of 1.3–∞ for R_0, which includes the estimates of R_0 with a range of 2-5 in this present study. In addition, the value of R_0 in this study is much lower than the one used by Savill et al. (2006) for H5N1 virus. However, comparison is difficult because their R_0 estimate was modelled using data from experiments on individually challenged birds, not data originating from H5N1 infected flocks under field conditions as in this study. Although the values of β may seem low in the current study, they are comparable with that of *Campylobacter* spp. in broiler flocks (van Gerwe et al., 2005) moreover, the accompanying value of R_0 is still higher than the assumed R_0 of the rapidly spreading influenza in humans (Ferguson et al., 2005).

Differences in H5N1 HPAI transmission between chickens of various types were considered likely because of differences in contact structure among chickens, age, flock size, breed, and management. Moreover, differences in the density at which the chickens are housed are likely to have an impact on transmission (Stegeman et al., 1999; Klinkenberg et al., 2002; Keeling et al., 2003; Stegeman et al., 2004). In this study, no significant difference of within-flock transmission among different chicken types was found. Nevertheless, the resulting point estimates support the intuitive suggestion that the within-flock transmission of backyard chickens and fighting cocks is lower than that of broilers and layer hens.

In this study, within-flock transmission from mortality data is described, which is a different approach from previous studies that have used the serological or virological results of a flock or a herd for quantifying transmission (van Nes et al., 1996; Stegeman et al., 1999; van Gerwe et al., 2005). The previous studies used 1-day point per flock or herd, whereas in this study repeated mortality data was used. In addition, the above studies had some data on known introduction dates, but in this study a date of a single contact when the virus had been introduced could not be traced. Therefore, it was not possible to estimate the number of days that the virus was present in the populations before the appearance of clinical signs.

Why negative binomial distribution?

Most previous studies to estimate transmission parameter β were based on a Poisson Regression Model or Binomial distribution (Stegeman et al., 1999; Keeling et al., 2003; Stegeman et al., 2004; van der Goot et al., 2005). In this study, initial analysis was based on a Poisson model. However, large overdispersion or extra-Poisson variation was present in the dataset. Such overdispersion often arises when the data are clustered, often caused by animals within herd or flock (Dohoo et al., 2003). Overdispersion can be dealt with by using a negative binomial distribution which is as a Poisson distribution with extra or overdispersion corresponding to a random effect model (McCullagh & Nelder, 1989; Dohoo et al., 2003). For the data used in the current study, a negative binomial model with additionally flock as a random effect showed the best (lowest) AIC value (Table 3.) (Motulsky & Christopoulos, 2004).

Bias, validity, and limitations

Records of dead birds might not have been kept with the same accuracy by all poultry keepers. Moreover, there might have been a difference in this respect between the different flock types. The number of chicken houses on large commercial poultry units was not known, which may have caused an underestimation of the transmission parameter if the disease occurred in only 1 house on a commercial unit with several houses. Therefore, data such as total number of animals and number of deaths per day should be recorded per house to facilitate epidemiologic analysis. In the assumptions made in this study, all chickens within a flock were equally infectious for a designated length of the infectious period. Likewise, this may differ from biological conditions, leading to overly precise results in the current study. Moreover, based on the lethality of H5N1 HPAI, we assumed that all infected chickens eventually died.

For other species (e.g. quails, geese, and domestic ducks), not enough data were available to estimate transmission. In addition, the mortality-based approach cannot be used for domestic ducks as they show very little or late mortality. Transmission studies in domestic ducks should be based on longitudinal serological results.

Practical implications

In this study, the upper limit of the R_0 estimate was 5.0 (Table 4B.) based on the model with broiler and layer combined as a categorical variable (in a worst case scenario). Therefore, if vaccination is applied, approximately 80% of susceptible animals will need to be vaccinated, 100% effectively, to prevent major outbreaks within a flock, based on the fraction of $1–1/R_0$ (Anderson & May, 1992). It seems feasible in commercial flocks to apply and maintain vaccination coverage at greater than 80% of a total flock. However, vaccination coverage of 80% might be more difficult in backyard chickens. Another problem is that vaccine efficacy is seldom 100%. Hence the vaccination coverage will need to be higher than the value of estimated $1-1/R_0$ (>80%) but not as high as suggested by others who assumed a much higher R_0 (van der Goot et al., 2005; Savill et al., 2006).

The point estimates of the transmission rate parameter (β) and the reproduction ratio (R_0) of H5N1 HPAI in this study were estimated by using mortality data collected at flock level from natural outbreaks. This quantitative information can be applied when planning a future control program of HPAI.

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EPIDEMIOLOGICAL INVESTIGATION OF INFECTIOUS SALMON ANAEMIA (ISA) OUTBREAKS IN NORWAY 2003-2005

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SUMMARY

Epidemiological information was summarized from 32 outbreaks of infectious salmon anaemia (ISA) in Norway 2003-2005. Virus isolates from the outbreaks were genotyped, and postulated associations between outbreaks and risk factors were assessed. The ISA outbreaks were distributed along most of the Norwegian coast and showed a variable clinical picture. The virus genotypes clustered into three genogroups, and tended to scatter in time and along the coast. Pairs of ISA outbreaks matched for the risk factors proximity or contact; all shared genogroups, which was a significantly higher number of successes than expected. For the smolt supplier risk factor, corresponding genogroups appeared in seven out of 12 matched pairs, which was not significant. In conclusion, genotyping of virus isolates from ISA outbreaks supports associations between adjacent outbreaks. This is consistent with horizontal transmission. The present study failed to find patterns of genogroups related to smolt suppliers or brood fish companies.

INTRODUCTION

Infectious salmon anaemia (ISA) is a viral disease of Atlantic salmon (*Salmo salar* L.) which was first diagnosed in Norway in 1984 (Thorud & Djupvik, 1988). Since then, the disease has been described in Atlantic salmon in Scotland, Canada, United Kingdom, and the Faroe Islands. The ISA virus (ISAV) has also been recorded from in Coho salmon (*Oncorhynchus kisutch*) in Chile (Cipriano & Miller, 2003). ISA was made notifiable as a List B disease in Norway in 1988. Within the EU, ISA is classified as a List 1 disease. A total of 438 outbreaks have been reported in Norway between 1984 and 2005. The yearly number of outbreaks peaked in 1990 with a total of 80 cases. In the late 1980's and the early 1990's, the Norwegian veterinary authorities implemented several measures such as a ban on the use of non-disinfected seawater in hatcheries and the movement of fish from one seawater site to another. Furthermore, compulsory health certificates for aquaculture farms, regulations on disinfection of waste water from slaughterhouses and processing plants were implemented (Thorud & Håstein, 2003). Since 1993, the annual incidence of ISA outbreaks has varied between one and 20 (Fig. 1).

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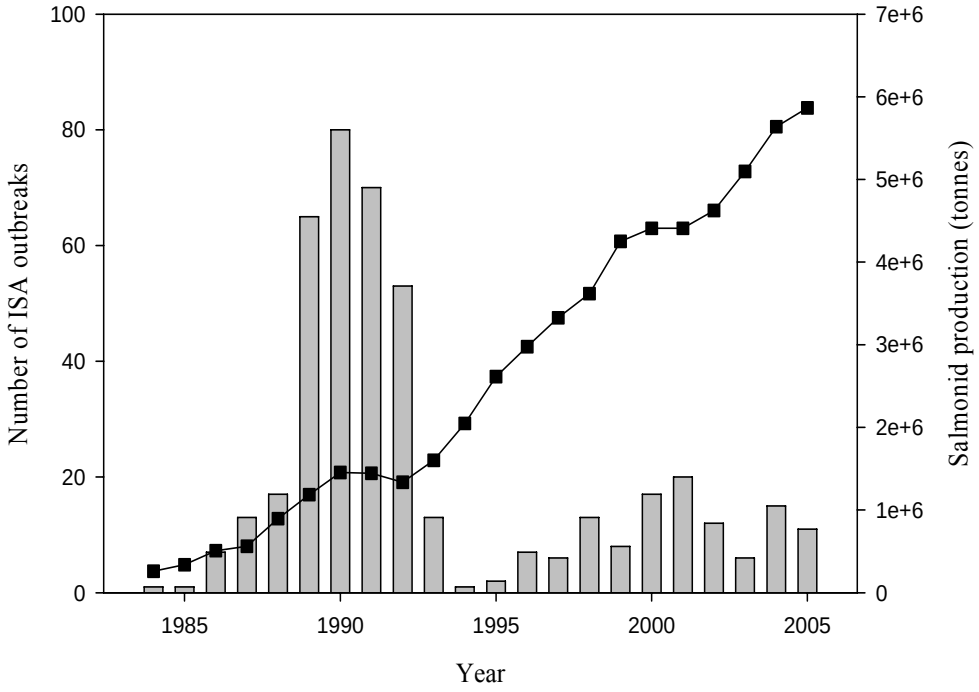


Fig. 1 Verified ISA outbreaks and the salmonid production in Norway from 1984 to 2005 (Source: Directorate of Fisheries and the National Veterinary Institute, T. Håstein).

ISAV has a segmented genome with eight different segments. The haemagglutinin-esterase (HE) gene is located on segment 6 and codes for the main surface glycoprotein in ISAV. It contains three different domains: an N-terminal portion with some sequence variation, which constitutes the surface-exposed region, a transmembrane domain, and a more conserved C-terminal part. A short hyper variable region (HPR), characterized by variable amino acid deletion patterns, is located right upstream of the transmembrane region. The database includes a large number of sequences for the HE gene from different isolates. Even though the sequence variation is not substantial for the European isolates, a sub classification of the European isolates into three groups (G1, G2 and G3) has been proposed, based on the most varied part of the HE gene (the 5'-flanking region). This classification may form a basis for the assessment of kinship between ISAV isolates (Nylund et al., 2003). The HPR-region varies significantly between related isolates due to deletions, and is not suited as an indicator of kinship. A grouping of ISAV isolates into HPR groups has however, been made, with the full-length HPR (without deletions) as the HPR0 (Nylund et al., 2003; Nylund et al., 2007). HPR0 appears to be associated with low virulence, while every other type of HPR has been isolated in association with disease outbreaks (Mjaaland et al., 2002; Cunningham et al., 2002; Nylund et al., 2007). The number of different HPRs being detected is increasing, as there seems to be a potential for a great variety of HPR0-deletions.

Epidemiological studies in the 1990's indicated that ISA disease was most often transmitted by movement of infected live salmon, animal waste, or effluents (blood or somatic cells or organic particles infected with the ISA agent). The risk of an ISA outbreak was associated with the site's proximity to infectious sites and slaughterhouses (Vågsholm et al., 1994; Jarp & Karlsen, 1997). Similar conclusions were reached in a study from New Brunswick (McClure et al., 2005). A recently developed stochastic model quantified the relative importance of seaway

distance and contact network as risk factors for horizontal transmission between sites (Scheel et al., submitted). An association between the number of vessel visits moving fish between sites and site contamination has also been demonstrated (Murray et al., 2002). It is commonly believed that the virus is not transmitted vertically (Cipriano & Miller, 2003). However, Nylund et al. (2007) suggest that some sort of vertical or transgenerational transmission may occur. These authors used genotyping of the haemagglutinin-esterase gene of the ISAV in conjunction with data on the origin of smolt, eggs and broodfish in an attempt to trace the origin of the virus from different outbreaks of ISA in Norway.

The aim of the present study was to summarize ISA outbreak case reports in Norway 2003-2005, present results from genotyping of virus isolates from the outbreaks, and to test if the genotyping supports associations between ISA outbreaks and risk factors for transmission.

MATERIALS AND METHODS

Case definition

An ISA outbreak is defined in accordance with The Contingency Plan for Control of ISA in Norway (Anon., 2004). A positive diagnosis is based on clinical signs, post mortem findings, and laboratory investigations.

Data collation

The case reports from the individual ISA outbreaks in 2003-2005 were collated by the Local District Offices of the Norwegian Food Safety Authority in accordance with EU Directive 93/53/EEC Art. 8.1. The reports provided information on diagnostic data, fish health, stock and management. The recording was not standardized until 2005 when a questionnaire was designed for the data collection. The level of detail therefore differs between years in the study.

Data on broodstock origin was compiled from Nylund et al. (2007) (Table 3.).

Genotyping

RNA was isolated from head kidney of two individuals from each outbreak with Qiagen RNEasy mini kit or on an automated NucliSens® easyMAG™ from BioMerieux following the protocol for off-board lysis. RT-PCR with the primers klon1EGFPF1 & klon1EGFPR1 (Mjaaland et al., 2002), and the alternative primer set: ILAHA1F 5'-GCAAAGATGGCACGATTCATA-3' & ILAHA1R 5'-AGCAACAGACAGGCTCGAT-3' and Superscript III/Qiagen HotStar enzyme was performed in order to transform RNA into cDNA and propagate the ISAV HE gene. The PCR products were purified using a Qiagen PCR purification kit or Qiagen Gel Extraction kit, depending on the quality of the PCR product. A sequencing-PCR using a BigDye Terminator Sequencing kit (Applied Biosystems) and the primers listed above and also internal primers Seg6U 5'-GGAATCTACAAGGTCTGCATTG-3' and ILAHA2R 5'-TAGGAACAGAGCAATCCCAAA and 4 internal primers described previously (Devold et al., 2001) was then performed . The resulting products were run through a 3100Avant Genetic Analyzer (ABI) or Megabace 1000 (AME BioScience). The sequences were then analysed using the Sequencher 4.1.4 software from GeneCodes, and the amino acid configuration of the hyper variable region (HPR) was examined. BioEdit (©T. Hall, Dep. Microbiol., North Carolina State University) was used to create an alignment of the 5' flanking region of HE genes (c. nucleotide 50-950) from the various outbreaks, as well as representative

isolates which had previously been sequenced from genogroups G1, G2 and G3 (Nylund et al., 2003). Phylogenetic analysis was carried out using the PHYLIP Package, version 3.65 (Joe Felsenstein, Department of Genome Sciences, University of Washington, Seattle, Washington, USA, http://evolution.gs.washington.edu/phylip.html), and the phylogenetic tree was visualised using TreeView (Win32) version 1.6.6 (Roderick D. M. Page, Division of Environmental and Evolutionary Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK, http://taxonomy.zoology.gla.ac.uk/rod/treeview.html).

Associations between genogroups and risk factors

An exploratory retrospective analysis was conducted to test if the geno-grouping supported associations between ISA outbreaks and the risk factors; 1) proximity between outbreaks, 2) possible contact between outbreaks, or 3) the sharing of smolt suppliers. Starting with the last occurring outbreak in 2005 we sequentially moved backwards in time through the list of outbreaks, and matched pairs if they conformed to a set of rules that complied with a possible association to a given risk factor. A common rule for this procedure was that a matched pair of outbreaks did not have their date of outbreak verification more than 12 months apart. If this criterion was met, outbreaks were matched for "proximity" as a risk factor if they were located within 10 km seaway distance of each other, and outbreaks were matched for "contact" as a risk factor if they were registered under the same concession identification. Geographical coordinates and concession identifications were compiled from the aquaculture licence register of The Directorate for Fisheries (www.fiskeridir.no) in September 2004. Finally outbreaks were matched for "smolt supplier" as a risk factor if they shared one or more suppliers. Thus, a sequence of seven, five and 12 matched pairs of outbreaks were obtained for the three risk factors, respectively. For each pair the genogroups of the two outbreaks in matched pairs were compared and assigned a success (S) if they were alike or a failure (F) if they were different (Table 4.).

A binomial test was used to test if there was a significantly higher number of S's (one-sided) than expected by chance for each of the three risk factors, unconditionally. The probability of an S was set to 0.40, which corresponds to the number of Ss (n=213) divided by the total number of possible outcomes (N = 528) when two genogroups are randomly picked from the distribution of genogroups given in Table 3. Note that the probability of an S outcome has been adjusted with regard to the two outbreaks from where two different genogroups have been demonstrated.

RESULTS

Case characteristics

In the period 2003-2005 the Norwegian Food Safety Authority reported a total of 32 outbreaks of ISA. The Norwegian Food Safety Authority as primary outbreaks and nine as secondary classified twenty-three of the outbreaks. Case reports from all primary outbreaks and four secondary outbreaks were received in the present study. The ISA outbreak sites were distributed along most of the Norwegian coast (Fig. 2).

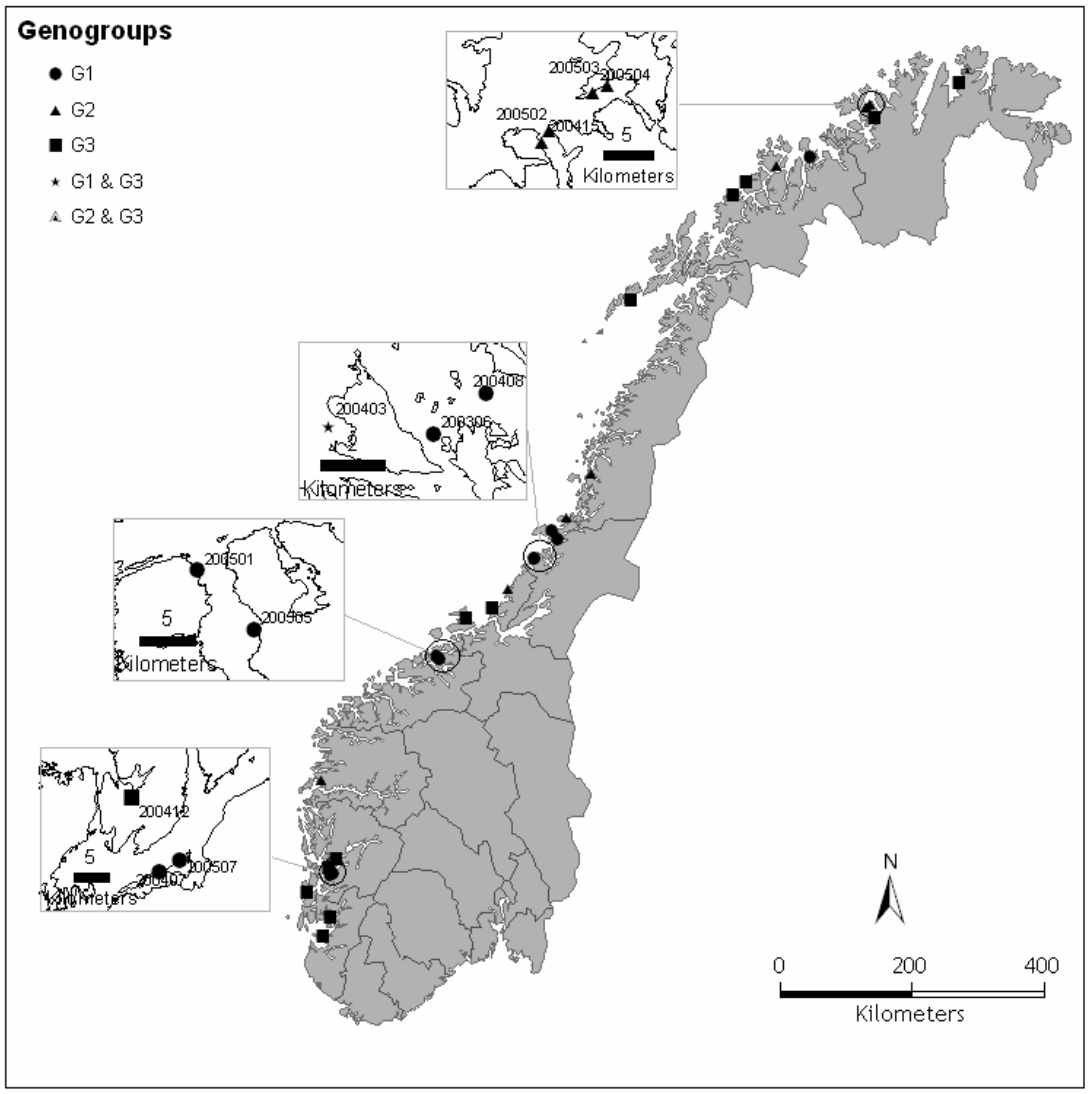


Fig. 2 Verified ISA outbreaks in Norway in 2003-2005 denoted according to genogroups. Four areas are enlarged (in frames) due to proximity between outbreak sites.

All outbreaks in 2003-2005 were on seawater sites rearing Atlantic salmon. Two of the outbreak sites reared rainbow trout in addition, but no clinical signs of ISA were recorded in this species. The ISA outbreaks occurred in all seasons with a peak during April - June (Table 1.).

Table 1. Seasonal distribution of ISA outbreaks in Norway throughout the years 2003-2005.

Season	Number of outbreaks
January - March	5
April – June	12
July - September	7
October - December	8

For 2005, the mean weight of the fish at time of outbreak was 3.6 kg (min 0.8 kg - max 6.4 kg). On average, the fish were reared at sea sites for 14 months before ISA was verified (Fig. 3). For 2005, maximum cage mortality at an ISA outbreak site varied from 0.4 % to 23 % in the last

month prior to the outbreak. Increased mortality was most often registered in only one or two cages on a site.

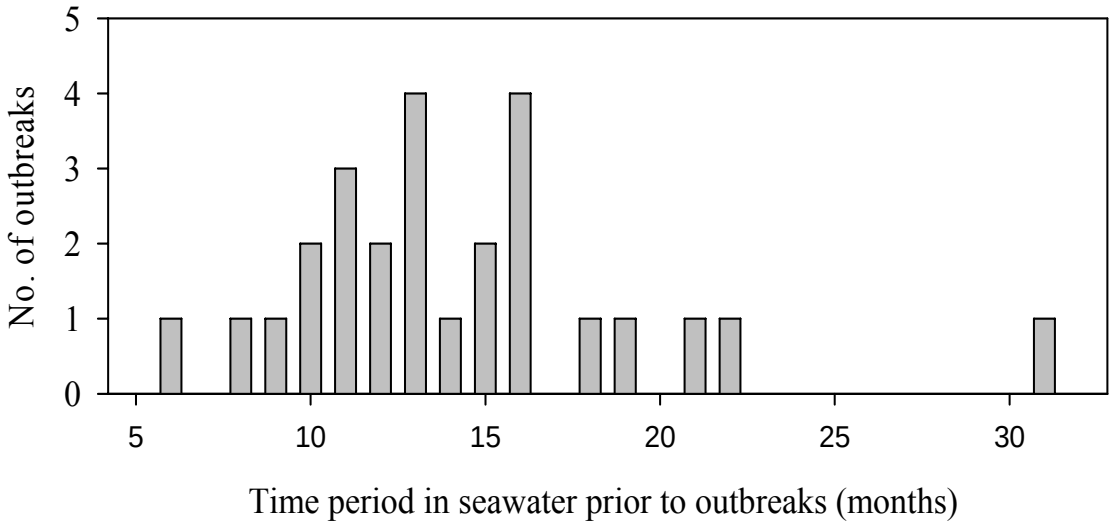


Fig. 3 Frequency distribution of the time period that Atlantic salmon had been reared in seawater sites prior to outbreak verification

Nineteen of the 32 outbreak sites reported clinical signs consistent with ISA. Three outbreaks in 2005 reported contact with other ISA outbreak sites as a suspected reason for ISA. Five of the outbreaks in 2005 occurred on sites located within ISA control zones. None of the ISA outbreak sites in 2005 had an earlier record of ISA. Infectious pancreatic necrosis was reported from 14 of the 32 outbreak sites before ISA was diagnosed. Parvicapsula was reported from five sites, winter ulcer from five sites, Cardiomyopathy syndrome and Heart and skeletal muscle inflammation from four sites each. Pancreas disease was reported twice. In 2005 ISA was frequently diagnosed in the same fish groups that had a history of other diseases. Management activities that may have induced stress situations three month prior to outbreak were reported in all but three sites in 2005.

A total of 46 different smolt suppliers supplied smolt to the 32 sites experiencing ISA outbreaks. Thirty-six suppliers delivered to one outbreak, and one supplier delivered to a maximum of six ISA outbreaks (Table 3.). The mean number of smolts transferred to the different outbreak sites was 586,408 (min 228,900 – max 892,276).

All sites received smolts by well boat transport, and up to 12 visits were reported prior to an outbreak. Smolts on outbreak sites had been transported from 10 to 1800 km along the coast before sea transfer (Table 3.). Sites with ISA outbreaks were on average less than 5 km from the main coastal transportation route, but no transport was reported to have gone through an ISA control zone.

Genotyping

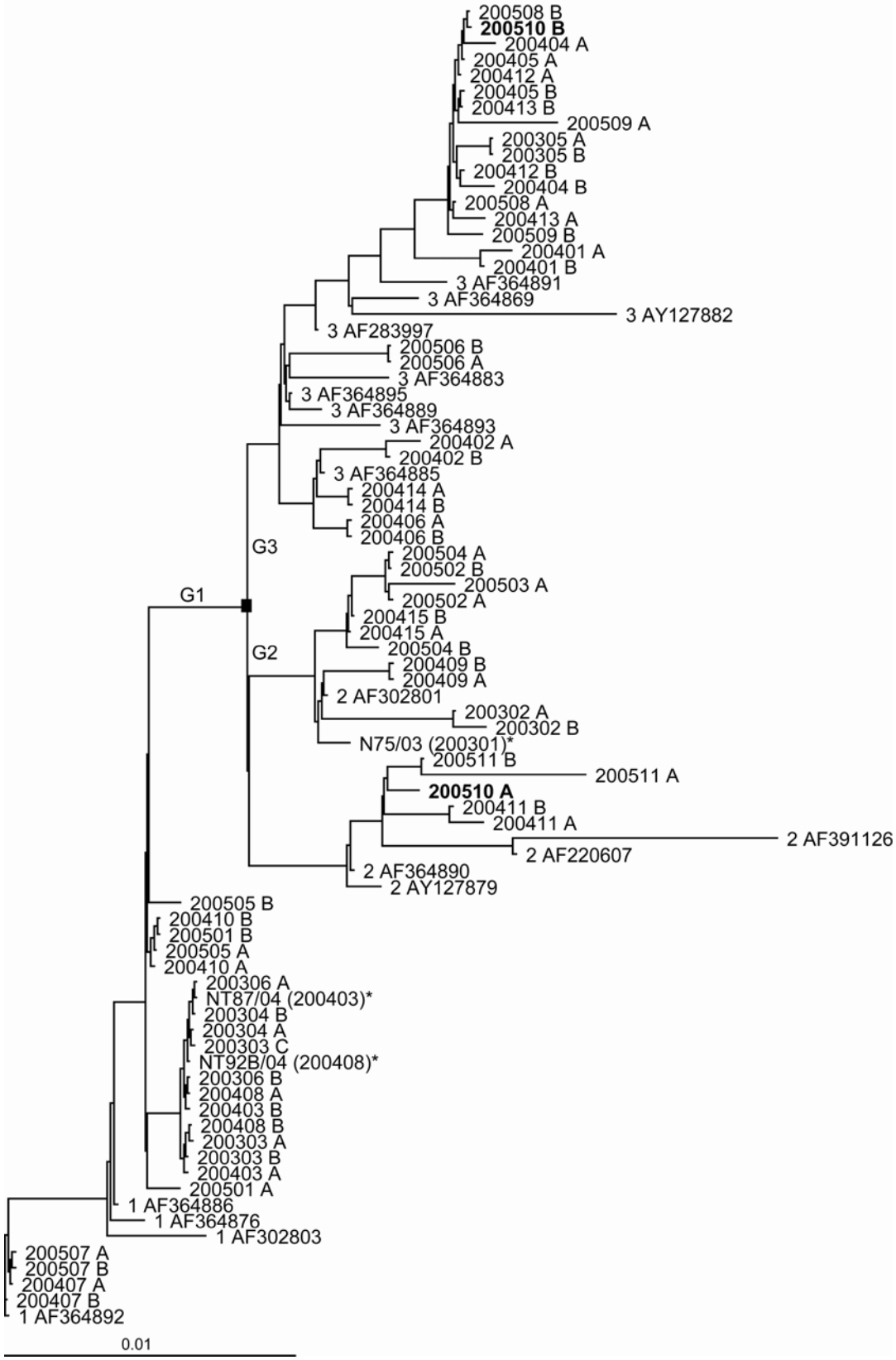
Thirty-one of the ISA virus isolates from the outbreaks in 2003-2005 were genotyped at the National Veterinary Institute. The genogroup from an ISA virus isolate from outbreak number 200301 was identified by Nylund et al. (2007). From outbreak number 200408 Nylund et al.

isolated both G1 and G3, while the National Veterinary Institute only isolated G1. Results from genotyping show variations in the HPR, whereas analyses of the more conserved 5` region support the clustering of European ISA virus isolates into three genogroups, G1-G3. These genogroups tended to scatter randomly along the coast and were represented in outbreaks from each of the three years in study (Table 3., Fig. 4).

The ISAV HE gene has an open reading frame, which varies in length from 1,161-1,233 nucleotides, depending on the length of the hyper variable region (HPR), which varies between 11 and 35 amino acids. The amino acid configurations in the HPR region were determined for two isolates from each outbreak. There was considerable variation between the different outbreaks with respect to their amino acid configuration in this region, and in total 15 different HPR configurations were found. In one case, outbreak number 200404, variation in the HPR region sequence was even found between two specimens from the same outbreak, even though the remainder of the sequence used for genotyping indicated close kinship between these two isolates (Table 2.). On the other hand, in one case (200510) the isolates A and B belonged to two different genogroups (G2 and G3, respectively), but showed identical sequences in HPR (Table 2., Fig. 4). No isolate from the outbreaks showed the HPR0 sequence (European consensus sequence).

Table 2. Amino acid configuration in the HPR-region from selected ISAV-isolates from outbreaks in 2003-2005

HPR 0	T	D	V	K	I	R	V	D	A	I	P	P	Q	L	N	Q	T	F	N	T	N	Q	V	E	Q	P	A	T	S	V	L	S	N	I	F	I	S	M	G	V	A	Geno-group
Isolate	Aa			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35					
200404 A	T	D	V	K	I	R	V	D	A												N	Q	V	E	Q	P	A	T	S	V	L	S	N	I	F	I	S	M	G	V	A	G3
200404 B	T	D	V																							P	A	T	S	V	L	S	N	I	F	I	S	M	G	V	A	G3
200405 A	T	D	V	K	I	R	V	D	A												N	Q	V	E	Q	P	A	T	S	V	L	S	N	I	F	I	S	M	G	V	A	G3
200405 B	T	D	V	K	I	R	V	D	A												N	Q	V	E	Q	P	A	T	S	V	L	S	N	I	F	I	S	M	G	V	A	G3
200412 A	T	D	V	K																								T	S	V	L	S	N	T	F	I	S	M	G	V	A	G3
200412 B	T	D	V	K																								T	S	V	L	S	N	T	F	I	S	M	G	V	A	G3
200509 A	T	D	V	K	I	R	V																			P	A	T	S	V	L	S	N	I	F	I	S	M	G	V	A	G3
200509 B	T	D	V	K	I	R	V																			P	A	T	S	V	L	S	N	I	F	I	S	M	G	V	A	G3
200413 A	T	D	V	K																								T	S	V	L	S	N	I	F	I	S	M	G	V	A	G3
200413 B	T	D	V	K																								T	S	V	L	S	N	I	F	I	S	M	G	V	A	G3
200305 A	T	D	V	K	I	R	V	D	A												N	Q	V	E	Q	P	A	T	S	V	L	S	N	I	F	I	S	M	G	V	A	G3
200305 B	T	D	V	K	I	R	V	D	A												N	Q	V	E	Q	P	A	T	S	V	L	S	N	I	F	I	S	M	G	V	A	G3
200508 A	T	D	V	K	I	R	V	D	A	I	P	P	Q								N	Q	V	E	Q	P	A	T	S	V	L	S	N	I	F	I	S	M	G	V	A	G3
200508 B	T	D	V	K	I	R	V	D	A	I	P	P	Q								N	Q	V	E	Q	P	A	T	S	V	L	S	N	I	F	I	S	M	G	V	A	G3
200510 A	T	D	V	K	I	R	V	D	A	I	P	P	Q	L	N	Q	T																			F	I	S	M	G	V	A	G2
200510 B	T	D	V	K	I	R	V	D	A	I	P	P	Q	L	N	Q	T																			F	I	S	M	G	V	A	G3
200511 A	T	D	V	K	I	R	V	D	A	I	P	P	Q	L	N	Q	T																			F	I	S	M	G	V	A	G2
200511 B	T	D	V	K	I	R	V	D	A	I	P	P	Q	L	N	Q	T																			F	I	S	M	G	V	A	G2
200411 A	T	D	V	K	I	R	V	D	A	I	P	P	Q																				H	I	F	I	S	M	G	V	A	G2
200411 B	T	D	V	K	I	R	V	D	A	I	P	P	Q																				H	I	F	I	S	M	G	V	A	G2



* Sequence published by Nylund et al (2007)

Fig. 4 Phylogenetic tree of the ISAV-isolates from outbreaks in 2003-2005 constructed using the maximum likelihood method

Table 3. ISA outbreaks in Norway (2003-2005) ordered chronologically (Broodstock and Smolt suppliers refer to different egg and smolt producing companies; Maximum transport given as approximate sea way distance to sea site)

Outbreak no.	County	Geno-group	Broodstock[a, b]	Smolt suppliers[b]	Maximum transport (km)
200301	Nordland	G2[a]	C, D	aj, h, i	200
200302	Sør-Trøndelag	G2	-	ac, ar, d, u, aa	300
200303	Troms	G1	-	a, at	-
200304	Nord-Trøndelag	G1	A, B	-	-
200305	Troms	G3	-	a, m	1,100
200306	Nord-Trøndelag	G1	A, B	ac, d, w, aa	200
200401	Hordaland	G3	-	r	10
200402	Sør-Trøndelag	G3	-	ah, z	50
200403	Nord-Trøndelag	G1	-	-	-
200404	Finnmark	G3	-	a, g	>1,800
200405	Nordland	G3	B	a, aq	>1,100
200406	Troms	G3	A, B, D	k, v	>1,000
200407	Hordaland	G1	A, B	ap, l	40
200408	Nord-Trøndelag	G1&G3[a]	-	-	-
200409	Troms	G2	A, B	e, f, m, t	>1,300
200410	Nord-Trøndelag	G1	A, B	an, oe, v	450
200411	Sogn og Fjordane	G2		ag, o	75
200412	Hordaland	G3		a	20
200413	Hordaland	G3		am, n	36
200414	Finnmark	G3	A	af, ak, j	600
200415	Finnmark	G2	A, E	ab, af, al	1,500
200501	Møre og Romsdal	G1	A, B	ad, b	56
200502	Finnmark	G2	A, E	ab, af, al	>1400
200503	Finnmark	G2	A	ab, y	>1100
200504	Finnmark	G2	E	af	>1400
200505	Møre og Romsdal	G1	A, B	-	-
200506	Sør-Trøndelag	G3	A	ae, x	14
200507	Hordaland	G1	-	ao, q	570
200508	Rogaland	G3	-	c, s	130
200509	Rogaland	G3	B	a, as, p	21
200510	Finnmark	G2&G3	-	-	-
200511	Nord-Trøndelag	G2	-	ai	86

[a]Nylund et al (2007)
[b]Individual brood stock companies and smolt suppliers are denoted by letter codes separated by comma.

For the purpose of genotyping, the 5'-flanking end of the haemagglutinin-esterase gene was used. A phylogenetic analysis was performed on sequences from two specimens from each of the ISA outbreaks in 2003 to 2005, as well as on previously sequenced isolates obtained from the GeneBank database. The resulting phylogenetic tree is shown in Fig. 4.

All the three postulated genogroups were represented in the ISA outbreaks in 2003-2005 with 10 outbreaks belonging to G1, 10 outbreaks to G2, and 14 outbreaks to G3. For the outbreak 200510, the isolates belonged to two different genogroups (G2&G3). This was also the case for outbreak 200408 (G1&G3), although only one of these genogroups (200408 A, B & NT92b:G1) is included in our map. The sequence for NT92a available in GeneBank was too short to be included in our phylogenetic analysis, but is previously reported to belong to the G3 genogroup (Nylund et al., 2007).

Associations between genogroups and risk factors

Outbreak pairs that were matched for the risk factors "proximity" and "contact" all shared genogroups, which was a significantly higher number of successes than expected by chance for both risk factors. All pairs matched for the "contact" risk factor were also matched for "proximity" (Table 4.). For the "smolt supplier" risk factor corresponding genogroups appeared in seven out of 12 pairs that matched, which was not a significantly higher number of successes than expected by chance (Table 4.).

DISCUSSION

In the present study genotyping of the haemagglutinin-esterase gene was used in conjunction with postulated associations between outbreak sites due to risk factors. Specifically ISA outbreak sites were postulated to be associated if they were infected within a reasonably short time span, and if they were located in proximity, if they were registered within the same concession, or if they shared one or more smolt suppliers. Outbreak sites thus associated were assessed with regard to kinship based on the genotyping.

All the ISAV isolates from the outbreaks in 2003-2005 grouped within three genogroups, G1,G2 and G3, which agrees with the geno-grouping first proposed by Nylund et al. (2003). There was no apparent geographic trend, nor any apparent time trend, with respect to the incidence of the different genogroups. In the retrospective analysis of the ISA outbreaks, pairs of outbreaks that were matched due to being located in proximity or that were registered within the same aquaculture concession, invariably belonged to the same genogroup (Table 4.). The probability for this sequence of events occurring by chance is very low. Hence, we conclude that the observed genogroups support a common origin of the virus isolates from outbreaks that are located in proximity. This suggests that ISA is spread locally and is consistent with a process involving horizontal transmission of the disease (Vågsholm et al., 1994; Jarp & Karlsen, 1997). Whether this is due to transmission through contact between outbreak sites, passive transmission via seawater, or some other local factor, is not discernable with the present data. Since all the pairs of outbreaks that were matched for the contact risk factor also were matched for the proximity risk factor, it is not possible to assign associations singularly to either of the two risk factors. However, two pairs of outbreaks were matched on proximity but not on contact, implying that the association between outbreaks due to proximity was most strongly supported by common genogroups.

Pairs of outbreaks that were matched due to sharing smolt suppliers did not share genogroups significantly more often than expected by chance, although the observed number of successes was higher than expected. This means that we cannot conclude that there is significant evidence for a common origin of the virus isolates from outbreaks that share smolt suppliers. Nor can we rule out infected smolt being a risk factor for ISA outbreaks. However, it is

noteworthy that for the most northerly cluster of 4 outbreaks (see Fig. 2), which were all in close proximity and registered under the same company, there was no common smolt supplier that covered all of the outbreak sites. Hence, the small-scale epidemics in this area cannot solely be attributed to one batch of infected smolt distributed to all the four sites.

Table 4. Matched pairs of ISA outbreaks for the risk factors proximity (seaway distance < 10 km), contact (registration within the same concession) and smolt supplier (sharing one or more smolt suppliers). Common genogroups between pairs are denoted S, different genogroup are denoted F. Months apart refers to months between verification of outbreaks for the pairs.

Matched Outbreaks	Months apart	Geno-group	Proximity	Contact	Smolt Supplier
200509 - 200412	9	G3, G3			S
200507 - 200407	11	G1, G1	S		
200505 - 200501	1	G1, G1	S	S	
200504 - 200503	<1	G2, G2	S	S	
200504 - 200502	1	G2, G2			S
200503 - 200502	1	G2, G2	S	S	S
200502 - 200415	3	G2, G2	S	S	S
200415 - 200414	<1	G2, G3			F
200412 - 200405	3	G3, G3			S
200410 - 200406	3	G1, G3			F
200409 - 200305	9	G2, G3			F
200408 - 200403	8	G1, G1	S		
200405 - 200404	1	G3, G3			S
200404 - 200305	7	G3, G3			S
200403 - 200306	4	G1&G3, G1	S	S	
200306 - 200302	5	G1, G2			F
200305 – 200303	3	G3, G1			F
Summary statistics binomial test					
N			7	5	12
Observed S			7	5	7
Expected S			2.8	2.0	4.8
P			0.002	0.011	0.16

The basic assumption underlying the use of genotyping in tracing pathogen sources is that genetic similarity reflects kinship. Since there is little genetic variation in the ISAV genome, including the HE gene, and since the evolution of the virus probably does not conform to a molecular clock (Nylund et al., 2007), direct links between outbreak strains can not easily be drawn. The fact that viruses identical in the HE gene were identified on two different locations years apart shows that an identical sequence does not imply direct recent transmission events (Devold et al., 2006). However, relatively large genetic differences, such as those between the genogroups used in the present study, indicate that findings may not be compatible with direct transmission (Hungnes et al., 2000). Hence, this study presents a strong case for not rejecting the hypothesis that ISAV spreads locally and horizontally, even though there were few outbreak cases that were matched for risk factors and compared for genogroups. The study also supports

the conclusions from Scheel et al. (submitted) who attributed a significant proportion of ISA outbreaks to horizontal transmission from adjacent sites or sites within contact networks.

The HPR-region varies significantly between related isolates probably due to serial deletions. However, in this study in one occasion two isolates from the same outbreak belonging to G2 (200510A) and G3 (200510B) shared the same HPR (Fig 4, Table 2.). This HPR type has mostly been found in G2 isolates, and strongly differs from the HPR sequences from the G3 isolates otherwise most related with 200510 B, suggesting a possible recombination event between 200510 A and B in the HPR. This, in addition to the fact that HPR appears to vary in the course of a single outbreak, confirms that HPR is not a good indicator of kinship between isolates.

The present study failed to find any significant patterns of genogroups related to smolt suppliers or broodfish companies. This may be due to the data being too fragmented and the number of outbreak sites too few. Furthermore, the design of the retrospective analysis regarding infected smolt or broodfish companies as risk factors may be flawed due to the complex infrastructure of the industry. These finding are in contrast to Nylund et al. (2007), who concluded that sources of eggs, i.e. the broodfish companies, best explained the origin of ISAV. These authors further suggested that some sort of transgenerational transmission may occur. No coherent analysis, however, was presented to support this hypothesis. However, it is noteworthy that no outbreaks of ISA were reported in the freshwater phase of the salmon production cycle during 2003-2005, and only three outbreaks have been recorded in freshwater in Norway since 1984 (Knut Falk, personal communication). To study the importance of smolt or broodfish as risk factors there is a need for prospective studies with genotyping of ISAV in eggs and juvenile fish, followed by studies of outbreaks in sea sites coupled to ISAV positive eggs or juvenile fish.

The ISA outbreaks were distributed along most of the Norwegian coast and show a variable clinical picture. ISA seems to develop slowly which indicates that a site may be infectious for many months before a correct diagnosis is established. The present findings are thus in accordance with previous descriptions of ISA outbreaks in Norway (Thorud & Djupvik, 1988; Thorud & Håstein, 2003).

Numerous well boat visits and long transport distances (up to 1800 km) were characteristic for the ISA outbreaks. As we do not have information from non-diseased sites, the importance of such risk factors cannot be evaluated. However, from Scotland it has been shown that numerous well boat visits were associated with ISA outbreaks (Murray et al., 2002). Since well boats are involved in many different operations and ISA may stay undetected for prolonged periods, the potential risk associated with well boats requires clarification.

ISA is controlled through different biosecurity measures at the production and transport levels. These measures focused initially on horizontal transmission. Our findings indicate that different forms of horizontal transmission may still be important for disease mitigation and underlines the necessity to maintain a strict biosecurity regime.

In conclusion, genotyping of virus isolates from ISA outbreaks supports associations between adjacent outbreaks. This is consistent with a process involving horizontal transmission of ISAV. The present study failed to find any significant patterns of genogroups related to smolt suppliers or brood fish companies.

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EPIDEMIOLOGICAL METHODS

DATA SPARSITY AND SEPARATION IN MULTIDIMENSIONAL COVARIATE SPACE:

APPROACHES TO A COMMON EPIDEMIOLOGICAL PROBLEM

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SUMMARY

Sparse data in epidemiological data sets can lead to biases and inaccurate inferences, and so must be considered in every analysis. The extreme of sparse data is data separation, which is either complete or quasi-complete. In exploring the risks associated with the presence of *E. coli* O157 on 222 cattle hides during the harvest process, five different approaches to addressing this issue were examined. The methods included reclassification or downweighting of selected outcomes; exact methods using median unbiased estimates (MUE); penalized maximum likelihood methods; and application of a profile likelihood approach. In general, the methods adopted did not alter the biological inferences made using the original data and ordinary logistic regression. However, the key issue is that the confidence one has in the conclusions drawn from a model should be based on an understanding of the relative merits and assumptions associated with the statistical methods. Failure to do so may lead to inaccurate and biased results.

INTRODUCTION

Sparse data is a problem frequently encountered in epidemiological studies that incorporate multivariable analyses, and can lead to inaccurate parameter estimates and inferences, as well as misleading conclusions on confounding and effect modification (Greenland et al., 2000). Separation of data can be qualified as either complete or quasi-complete separation. Complete separation occurs if there is a vector of coefficients that correctly allocates all observations to their group; that is, when a predictor variable or a combination of predictor variables perfectly predicts the outcome variable. Quasi-complete separation is the most common form of separation, and occurs when a predictor variable or combination of predictor variables can perfectly predict the outcome except at a very few values of the predictor variable (Albert & Anderson, 1984). In a 2x2 contingency table with a dichotomous outcome and a dichotomous predictor variable, complete separation will appear as two zero counts in either pair of diagonal cells, and quasi-complete separation will appear as one zero count in any of the four cells. The result of either form of data separation is that the maximum likelihood estimate of at least one parameter is undefined; that is, it is either negative infinity ($-\infty$) or positive infinity ($+\infty$) on the logistic scale, corresponding to associated odds ratio estimates of 0 or ∞, respectively.

By reviewing and applying several of the different methods available for dealing with sparse data, the aim of this paper is to provide a practical overview, using a veterinary

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epidemiological data set as an example. By examining the methods and results described here, and the advantages and disadvantages associated with each method, the aim is that others can choose and apply a method that is most appropriate for their own data.

MATERIALS AND METHODS

The data set and the issue of quasi-complete separation

The data relate to a study examining risk factors for cattle hide contamination with *Escherichia coli* O157 during the harvest process (Mather et al., 2007). Briefly, of 222 cattle submitted to 10 slaughterhouses, risk factors concerning the following were considered: various animal characteristics (e.g., age, breed); on-farm *E. coli* O157 shedding; previous occurrence of *E. coli* O157 on the farm; number of animals in the group sent to slaughter; type of transport to the slaughterhouse and mixing with cattle from other farms *en route*; and slaughterhouse, lairage and killing line features. Logistic regression analysis was used to determine the potential risk factors for whether or not a hide was contaminated with *E. coli* O157. A 'Feed in lairage' variable recorded whether or not feed was supplied to the cattle waiting in lairage, and if so, what type of feed was provided. There were three levels to this variable: no feed provided, hay only provided and straw only provided. Quasi-complete separation of the data was observed: all animals provided with hay did not have contaminated hides, and all animals provided with straw did have contaminated hides (Table 1). The final model contained five variables, one of which was 'Feed in lairage'.

Table 1. Contingency table of *E. coli* O157 hide contamination status of 222 Scottish cattle and feed in lairage, demonstrating quasi-complete separation

	Feed in Lairage		
Contaminated Hide	None	Hay	Straw
0 (No)	81	19	0
1 (Yes)	102	0	20

Methods used to address quasi-complete separation

Re-classifying select outcomes: The first method used was to re-classify the outcome of one record in each level of *Hay* and *Straw* so that there was a count of one in the previously empty cells. This solution has been suggested by Greenland et al. (2000) and has also been mentioned by Agresti and Hartzel (2000). The data were analysed using maximum likelihood estimation (MLE), using the R statistical software (R Core Development Team, 2005).

Down-weighting select outcomes: The second method of resolving the issue involved downweighting one record in each level of *Hay* and *Straw* by a constant value. One record in *Hay* was downweighted to 0.05, and one record in *Straw* was downweighted to 0.95. This alteration was sufficient to obtain model convergence and finite parameter estimates using MLE. This method is a modification of that suggested by Platt et al. (1999), Agresti (2002) and Sweeting et al. (2004), among others, which involves adding a small positive constant to every cell. To determine how sensitive the results were to the choice of downweighting amount and so to determine the stability and consequently the validity of the results, a sensitivity analysis was performed using five different downweighting values and the results were compared. These

values were 0.5, 0.1, 0.05, 0.01 and 0.001. The adjusted contingency table for the 0.05 case is shown in Table 2.

Table 2. Adjusted contingency table of *E. coli* O157 hide contamination status and feed in lairage, after downweighting one outcome each in the *Hay* and *Straw* levels by 0.05.

	Feed in Lairage		
Contaminated Hide	None	Hay	Straw
0 (No)	81	18	0
0.05 (No)	0	1	0
0.95 (Yes)	0	0	1
1 (Yes)	102	0	19

Exact methods: The software LogXact 4 (Cytel Software Corporation, 1999) was used to implement this approach. This version of the software calculates the median unbiased estimates (MUE) in situations where empty cells are encountered, using the distribution of the sufficient statistics of the model parameters. Details on the computation of MUEs are found in Hirji et al. (1989). Median unbiased estimates are the average of the endpoints of a 50% confidence interval estimator (Hosmer & Lemeshow, 2000).

Penalized maximum likelihood: Penalized maximum likelihood was implemented using the R statistical software with the logistic package (R Core Development Team, 2005). A notable advantage of this method is that it produces solutions that exist in both situations of data separation, complete and quasi-complete. Developed by Firth (1993) and applied by Heinze and Schemper (2002) to the problem of data separation, this method introduces a small bias, or penalty, into the score function whose zeros then provide the (penalized) maximum likelihood estimates.

Profile likelihood: Profile likelihood can be used to compute confidence intervals for logistic regression parameters, and can also be used to obtain point estimates for parameter values (Venzon & Moolgavkar, 1988; Heinze & Schemper, 2002; Stryhn & Christensen, 2003; Bull et al., 2006). A concise summary of how profile likelihood is used is provided in Stryhn and Christensen (2003), but the overall method is an iterative one, reducing the log likelihood of a parameter vector to a function of a single parameter of interest (in the case, *Hay* or *Straw*) by treating the remaining parameters as nuisance parameters and maximizing over them (Venzon & Moolgavkar, 1988). The maximum likelihood estimate of this parameter is that value which maximizes the profile likelihood. The profile-likelihood confidence interval contains those parameter values whose corresponding profile log-likelihood values are close to the maximal value. For a 95% confidence interval, this criterion is $0.5 \times \chi^2_{.95,1} = 1.92$ where $\chi^2_{.95,1} = 3.84$ is the 0.95 quantile for a chi-square distribution with 1 degree of freedom. Alternatively, this confidence interval can be thought of as merely the set of parameter values not rejected by a likelihood ratio test. For the two parameters in this study, the 95% joint confidence region is provided by the contour of constant profile log-likelihood within $0.5 \times \chi^2_{.95,2}$ of the maximal value. The advantage of this approach is that these contours can be constructed even when the maximum likelihood estimates do not exist. Even if a point estimate is not obtained, profile likelihood confidence intervals can be used as an indication of the direction and significance (or lack thereof) for a particular factor.

RESULTS

The results of the *ad hoc* and more formal methods for *Hay* and *Straw* are presented in Table 3 (results for the 0.5, 0.1, 0.01 and 0.001 downweighting methods are not shown). Although the point estimates vary for the different sparse data methods, all results for *Hay* were in agreement with respect to the direction and significance of effect, and the majority of results for *Straw* were in agreement with respect to the significance of effect. For *Hay*, all results were statistically significant and indicated a protective effect of providing hay in lairage on hide contamination with *E. coli* O157. Even if a lower limit was undefined, the lower limit of an odds ratio confidence interval is bounded by zero. With the exception of the 0.001/0.999 downweighting method, the results for *Straw* were not statistically significant.

Table 3. Comparison of the different sparse data methods on the *E. coli* O157 data set for *Hay* and *Straw*

Variable	Method	Coeff.	S.E.	OR	95% CI*
Hay	Unaltered data; MLE	-18.3	1410.6	$1.16e^{-8}$	(0.00, $1.4e^{+33}$)
	Switching; MLE	-2.7	1.1	0.07	(0.004, 0.39)
	Downweighting 0.05/0.95; MLE	-5.8	4.5	0.003	(NA, 0.16)
	Exact (MUE)	-3.0	NA	0.05	(NA, 0.34)
	Firth's PMLE	-3.5	1.5	0.03	(0.0002, 0.27)
Straw	Unaltered data; MLE	15.3	1433.8	$4.43e^{+6}$	($1.0e^{-40}$, NA)
	Switching; MLE	-0.09	1.2	0.91	(0.11, 20.2)
	Downweighting 0.05/0.95; MLE	2.8	4.5	15.98	(0.22, NA)
	Exact (MUE)	-0.13	NA	0.87	(0.09, NA)
	Firth's PMLE	0.60	1.6	1.82	(0.15, 260.0)

*Lower limits that are NA are bounded by zero; upper limits that are NA are not bounded (i.e. go to infinity)

Similar results were obtained using profile likelihood. The 95% and 99% confidence intervals for the parameter estimates (in the logit scale) for *Hay* were less than and did not include 0, indicating that receiving hay in lairage was a statistically significant protective effect. The confidence intervals in the logit scale for *Straw* do encompass 0, indicating that the effect was not statistically significant.

DISCUSSION

The *ad hoc* methods described above, i.e., the switching and downweighting of outcomes, in general produced similar results to the more advanced techniques. The *ad hoc* methods are attractive by virtue of their simplicity and ease of implementation, and do produce finite estimates. Disadvantages associated with these methods include the fact that the parameter estimate depends strongly on the value of constant chosen to add to or downweight the zero cells (Agresti & Hartzel, 2000), and the very nature of these methods creates artificial data.

The results obtained by computing the median unbiased estimates were similar in pattern to the other methods, i.e., that hay was protective and significant, and straw was not significant. However, due to the nature of the estimates (the midpoint of a 50% confidence interval), there are no associated standard errors. Therefore, inferences about the parameter exhibiting separation should be based on the confidence interval associated with the MUE, as opposed to

the MUE itself (Collett, 2003). The advantages of calculating MUEs in situations of data separation are that finite estimates are produced, and as the algorithms are incorporated into software packages, they are relatively easy to compute. A particular drawback is that exact logistic regression is computationally intense, and so is suited only for small data sets. In addition, exact methods are more suitable for categorical variables, as it is more difficult to obtain the exact distribution of the sufficient statistics, which are used in the estimation of parameter effects, for continuous variables (Collett, 2003). In data sets exhibiting complete separation, exact logistic regression does not result in estimates for all predictor variables, only that variable exhibiting the separation (Allison, 2004).

The penalized maximum likelihood estimates (PMLEs) obtained using Firth's method were also consistent with the results found using the other methods. PMLEs have an advantage over exact methods (MUEs), as they can be used when there are continuous covariates (Bull et al., 2006). In small to moderate sample sizes with separated and unseparated data, PMLEs were found to be less biased and more efficient than unconditional maximum likelihood estimates (Bull et al., 2002). An additional feature of penalized likelihood estimation is that it can be used in data sets exhibiting complete separation (Allison, 2004).

The profile likelihood results were similar to those obtained using the majority of the other methods. As with Firth's penalized method and the exact methods, no artificial data are produced. The use of profile likelihood confidence intervals is more appropriate in situations of data separation, especially in conjunction with penalized maximum likelihood estimation. Although profile confidence intervals based on the unconditional MLEs can also be calculated, Wald confidence intervals are typically computed for unconditional maximum likelihood estimates, which are based on large-sample standard errors. However, in situations with small sample sizes, the log-likelihood is not quadratic (which is what the estimate of the variance is based on), and so these symmetrical Wald confidence intervals are not appropriate. Instead, non-symmetric profile-likelihood confidence intervals should be calculated, which for PMLEs have two finite endpoints (Bull et al., 2006; Heinze & Schemper, 2002).

In the current analysis and on the data set in question, most methods dealing with sparse data used in the analyses produced comparable results. As the non-*ad hoc* methods all returned results demonstrating that the effect of hay in lairage was protective and significant, and that of straw was not statistically significant, this conclusion (as originally stated) appears to be a valid one. However, although this was the case for the data set used here, it may not necessarily occur with all data sets. Firth's method of penalized maximum likelihood is attractive in that finite estimates are always obtainable, and can be used in instances where either complete or quasi-complete separation is observed. Profile likelihood is another appealing option, as no modifications to the data are required and it is also applicable in both situations of separation. Approximate confidence intervals can always be obtained using this method even when point estimates do not exist. Although the downweighting methods produced similar results to those obtained using Firth's method and profile likelihood, the *ad hoc* nature of these downweighting methods means that it is not possible to comment on the reproducibility of these results using other data sets.

In summary, the selection of a technique to deal with data separation should include an examination and comparison of how each method works, and the relative merits associated with each technique. Although the significance of the parameter estimates obtained for this data set was not dependent on the method used to deal with the data separation, the overall conclusion of these analyses is that biological inference, and the confidence associated with such inference,

should be based on an understanding of the methods used and the associated assumptions. Failure to do so, with the data presented here, could have led to false precision and poorly understood biases.

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AGE DEPENDENT WINDOWS FOR COHORT CULLING BOVINE SPONGIFORM ENCEPHALOPATHY (BSE) HERDS

A. STOCKMARR*

SUMMARY

Prior to 2005 the practice in Denmark was to entirely cull any herd with a bovine spongiform encephalopathy (BSE) case to avoid the risk of further cases. However, a growing dissatisfaction with this has led to a desire to be able to cull a fraction of a BSE herd only, while still removing the majority of the risk. One proposed method has been cohort culling in which all animals whose age is within a given number of years of the age of the infected cow are culled, assuming that the BSE case and all other potential cases were infected at roughly the same time. This method works well for BSE cases aged six years or less, but for older BSE cases the method is inadequate, making age-dependent windows a necessity. A case study with a nine-year-old infected animal is studied.

INTRODUCTION

Bovine spongiform encephalopathy (BSE) in domestic cattle was first diagnosed in 1986 (Wells et al., 1987). The origin of the disease remains unknown. Evidence has pointed towards meat and bone meal (MBM) contaminated with infectious material from sheep (Wilesmith et al., 1988, Wilesmith et al., 1991), but recent research concludes that the problem remains apparent (Burke, 2006). BSE has been linked to the fatal human brain disorder variant Creutzfeldt-Jacob Disease (vCJD) (Hill et al., 1997), and indeed data from the Great Britain BSE epidemic strongly suggests this link (Fig. 1). The Great Britain BSE epidemic contains more than 95% of the BSE cases reported worldwide. BSE is therefore a public health issue as well as an economic issue.

The importance of preventing infected cattle from entering the human food chain is therefore clear and many risk analyses have been carried out to assess the problem (reviewed by Paisley et al. (2007). To combat the disease, a series of bans on the use of MBM in feed were imposed. In 2001 the European Union introduced harmonised control measures, after which feeding of processed animal proteins to animals was banned (EC Regulation no. 999/2001). However, none of the bans have completely eliminated the disease, as BSE cases born after the latest ban in 2001 have been confirmed (Burke, 2006). The EC regulation no. 999/2001 also contained new minimum rules for culling cows in herds where BSE cases had been confirmed. These were based on the so-called *cohort cull* method in which member states since 2001 have been required to cull the one-year birth cohort connected with any BSE case in a herd. That is cull the cattle in the same herd born up to a year before and after the birth of the BSE case, as

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these cattle are the most likely to have shared the contaminated food that infected the BSE case. However, this is a minimum rule and more strict national rules may apply.

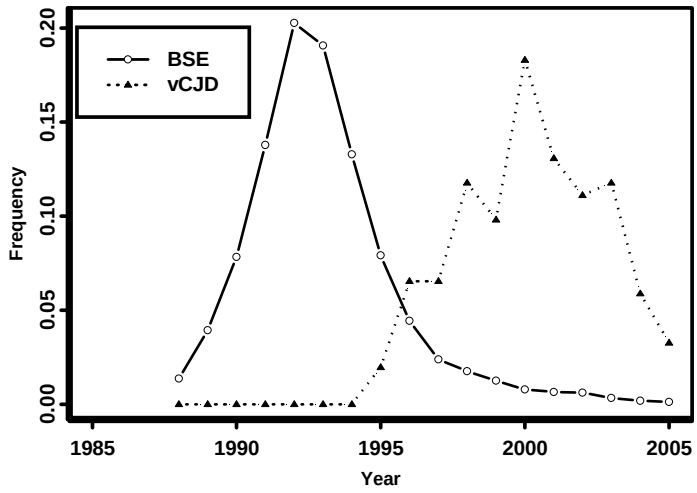

Fig. 1 The Great Britain BSE and vCJD epidemics, normalized so that yearly entries sum to 1. The difference in years between the curves are estimated to be nine years. Based on public data from Office International des Epizooties and The National Creutzfeldt-Jacob Disease Surveillance Unit, UK

This paper focuses on the situation in Denmark, although results and methods may be amended and applied to other settings. Although the EC feed ban came in the middle of the Danish BSE 'epidemic', control measures had already been implemented. Drawing up the Danish BSE cases by year of birth reveals that at present no Danish BSE cases have been born after the EC feed ban came into force (Fig. 2).

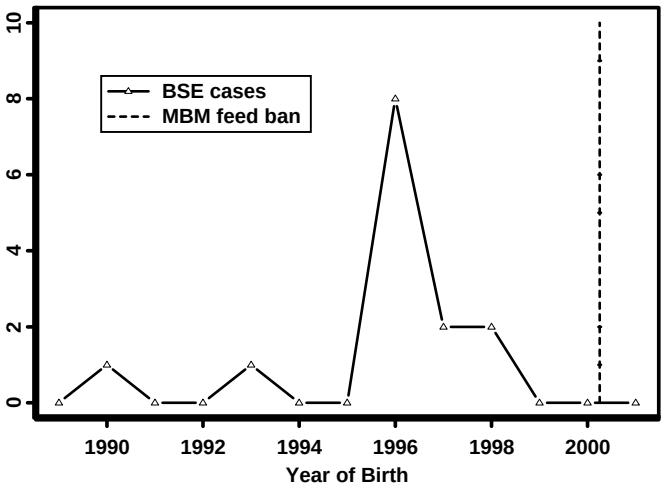

Fig. 2 Number of Danish BSE cases plotted against year of birth

The policy in Denmark had up to 2005 been to perform an entire herd cull if a BSE case was discovered, but with the main Great Britain BSE epidemic essentially over, focus was shifted to farmer losses when culling herds. This included loss of genetic material for breeding, and the direct economic value of the culled herd. The average herd size for a Danish cattle chosen uniformly at random is 214 animals. The work reported here was initiated to investigate the level of risk that the EC *cohort cull* method would impose upon Denmark. Denmark has very few registered BSE cases and the focus is on the risk that *cohort cull* will allow a BSE case to remain in a single herd, where a BSE case has been found.

METHODS

In this study the EC cohort culling method is referred to as *cohort culling with a one-year window*, to indicate that the age interval (window) around the age of the BSE case is up to one year either side of the age of the BSE case. Cohort culling with different windows is considered by calculating the probability that no cattle will result in a BSE case after cohort culling the herd (with any window). Probability modelling is used, assuming that a BSE case has been detected in a herd. The following further assumptions are made:

- Exposure occurs only once, at the time when the BSE case was infected.
- All animals alive when exposure occurs are exposed.
- Animals are infected at random.
- Infected animals develop BSE at random.

While exposure is possible at other times than when the BSE case was infected, this is considered no different from herds that have no detected BSE case. This reflects the excess risk that a herd with a BSE case has, which is the risk of infection from the feed batch, which has infected the identified case. This approach assigns distributions to the above 'random' statements based on studies of the phenomena and use these to derive the probabilities.

Primary and secondary cases

The concept of *cases* plays a central role for stochastic models. A *case* is an animal infected with BSE, which will develop the disease before it dies if not culled. Whether animals from a herd are cases or not will not be observable if culling applies, except for the first animal that is detected, which is the reason that the herd may be subject to cohort culling. This animal is referred to as the *primary case* (PC). All other cases will be alive at the time of detection, and they will be referred to as *secondary cases* (SC). Secondary cases are those who must be culled if additional BSE cases are to be avoided. The efficiency of cohort culling with any window is measured by the probability of culling all SC. In practice, the size of the herd will be observed, so the number of *cases* will be random and binomially distributed, with the size of the herd as the number parameter.

The probability of efficient cohort culling

The probability of efficient cohort culling with a one-year window is the probability that all SC are present within the one-year cull window. This is formally described by the equation:

$$\sum_{n=1}^{k}\sum_{r=0}^{n-1} P(\text{Herdsize}=n)P_n(N=r)\int_0^{\infty}\left[\int_{b-1}^{b+1} sc(a\,|\,b)\,da\right]^r pc_r(b)\,db \qquad (1)$$

where P_n is $P(\cdot\,|\text{Herdsize}=n)$, the conditional probability given the herd size equal to n, k is the maximum herd size, N is the number of SC, sc(· |b) is the probability density function (pdf) for the conditional distribution of any SC given that the PC is of age b at the onset of disease, which shall equal the time of detection, and pc_r is the pdf for the age of the PC at the onset of disease, the *age at onset*, given $N=r$. In order to compute this probability, a number of concepts for the animals and BSE need to be considered. These include the age distribution of the animals, their mortality, their susceptibility to infection with BSE, the incubation period of BSE, the actual infection probability, and the herd size. These are considered below.

The Danish cattle population

The *age* distribution of the Danish cattle population was obtained by smoothing data from the Danish CHR register of 8th December 2005 twice with a constant kernel with a length (age-time) of one year. No significant seasonal effect was detected. As most Danish cattle are dairy cows and farmers are penalized for over-production, replacement of cattle is governed by the rate that cows are removed from milk production. From this distribution, the mortality pdf was derived by simple differentiation of minus the pdf for the age distribution and subsequent normalization, using the argument that the absence of seasonal variation yields does not affect the age distribution. Age and mortality is depicted in Fig. 3.

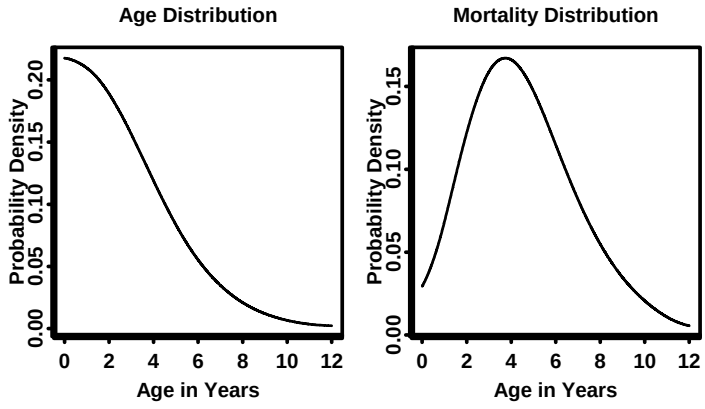

Fig. 3 Age and mortality distributions for Danish cattle

BSE related characteristics

There is agreement that susceptibility to BSE is age dependent (Ferguson et al., 1997, Arnold & Wilesmith, 2004). Ferguson et al. (1997) fitted a number of models to a set of data and scored them with a goodness of fit statistic simultaneously with models for the incubation time. The model with the best fit is used here (model 7C in Table 4 from Ferguson et al. (1997)). According to this, the susceptibility, as a function of age a, has the following pdf:

$$i(a) = \gamma_2(\gamma_1 a)^{\gamma_2 - 1} exp\left[-(\gamma_1 a)^{\gamma_2}\right](1 - exp\left[-(\gamma_3 a)^{\gamma_3 + \gamma_4}\right])$$

$$+(\gamma_2 + \gamma_4)(\gamma_3 a)^{\gamma_2 + \gamma_4 - 1} exp\left[-(\gamma_3 a)^{\gamma_2 + \gamma_4}\right](1 - exp\left[-(\gamma_1 a)^{\gamma_2}\right]) \quad (2)$$

The values used for the parameters are γ_1=1.45, γ_2=0.645, γ_3=0.800 and γ_4=3.77.

The distribution of the age-dependent susceptibility is purely descriptive, while the corresponding model for the incubation time in model 7C from Table 4 in Ferguson et al. (1997) is based on exponential increase of prions over time, with a Gamma-distributed initial dose. As a function of time *u*, it has the following pdf *s*:

$$s(u) = c^{-1}(\alpha_2 e^{-u/\alpha_1}/\alpha_3)^{\alpha_2^2/\alpha 3} exp\left[-\alpha_2 e^{-u/\alpha_1}/\alpha_3\right] \quad (3)$$

where *c* is a normalising constant. The values used for the parameters are *c*=1.135, α_1=1.146, α_2=0.0241, α_3=5.71e-4.

Distributions derived from both population-related and BSE-related characteristics

The probability of an animal being infected with BSE given exposure through contaminated feed is probably the most difficult quantity within this framework to assign. The notion of susceptibility suggests that the immediate risk of infection given exposure at age *a* in the age interval Δa is of the form $\beta s(a)\Delta a$ with *s* the susceptibility function. It has been argued for taking β=1, this being the smallest value of β which will ensure certain infection given exposure, assuming infinite life of the animal (L Paisley, personal communication). This is the approach used here, and the exposure time is taken to be one week, which is deemed a conservative estimate. Thus, the age-dependent infection probability *p* is given as

$$p(a) = P(Infection \mid Age = a) = \int_a^{a+7} s(b)\, db \quad (4)$$

where the susceptibility function *s* is taken in days. Averaging over the age distribution, this results in an average probability of infection of $4.27e^{-3}$. The level of this probability is comparable to results in Arnold & Wilesmith (2004).

The probability of infection is used to compute the *age at infection*, which is the age distribution of the infected cows at the (unknown) time when the infection occurred. The pdf for this distribution is constructed by simply multiplying the probability of infection with the age distribution, and subsequently normalising. This results in the density depicted in Fig. 4.

The *age at infection* describes the age of the infected cows at the time of infection, and will be used to calculate the probability that an infected animal results in a case. Formally, this happens when *death*, as outcome of the mortality distribution from Fig. 3, occurs at a later stage than the sum of the *age at infection* and the incubation period, and computing this results in a probability of 0.28. Combining this figure with the probability of infection, the probability that an exposed animal results in a case is found to be $1.20e^{-3}$. From this, the distribution of *N* in Eq. (1) may be computed.

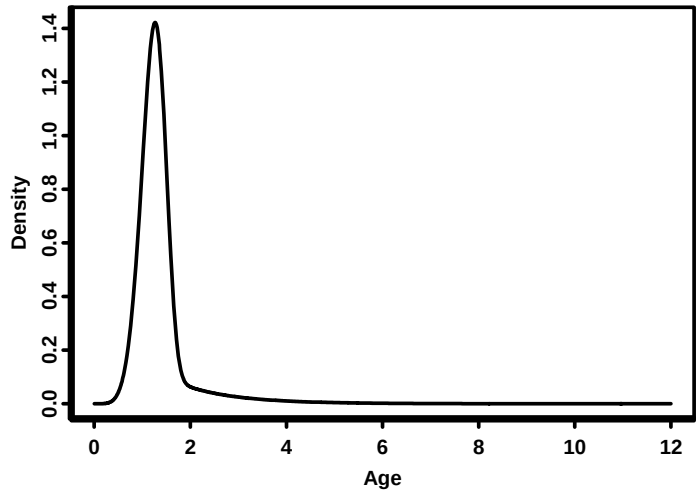

Fig. 4 Age at infection among Danish cattle

Now, the *age at onset* for the PC can be found by adding the *age at infection* and the incubation time for the PC, assuming that the animal is alive at this time, i.e. *death* as the outcome of the mortality distribution has not yet occurred and similar assumptions are made for the SC. However, all of these quantities are stochastic, but it is still possible to derive a pdf for the *age at onset*, by conditioning on the number of cases.

Assuming that $N=r$ for given values of r, i.e. that there are r SC in the herd, the incubation time for the PC, which is the first of the $r+1$ cases in the herd to be detected, is then the minimum of $r+1$ stochastically independent incubation time, all defined through the pdf in Eq. (2). The pdf i_r can be found by differentiating the cumulative density function $P(I_r \leq u)$, with I_r the stochastic minimum of $r+1$ independent incubation times.

Using this, the pdf for the *age at onset* may be computed as

$$pc_r(a) \sim \int_0^a i_r(a-b)\,p(b)age(b)\int_a^\infty m(u)\,du/(\int_b^\infty m(u)\,du)db \qquad (5)$$

where *age* and *m* are the age and mortality distribution pdfs from Fig. 3. The ~ symbol in Eq. (5) signifies proportionality, i.e. the right hand side of Eq. (5) should be normalized. The full pdf for the *age at onset* for the PC may then be derived by combining pdfs f the form in Eq. (5), using the point probabilities for N as coefficients.

The pdf for age of the SC, at the *age at onset* for the PC, is more difficult to compute. The SC have the same *age at infection* distribution as the PC, but the variable to add to this to obtain the *age at onset* distribution is not the incubation time of the SC, but the shorter incubation time of the PC. Furthermore, the conditional distributions of this *age at onset* distribution, given the *age at onset* for the PC is needed, as it is the pdf for these distributions that enters in Eq. (1). For this, start by taking aao_u as the pdf for the conditional distribution of the *age at onset* for the PC, given the incubation time u. Multiply this with the Bayes correction factor $i_r(u)/pc_r(a)$ to arrive at i_a, the pdf for the conditional distribution of the incubation time given the *age at onset* equal to a. Using this, the required $sc_r(b|a)$ may be computed similarly to $pc_r(a)$ in Eq. (5), with i_a substituted for i_r. The full pdf $sc(b|a)$ is found by combining the pdfs for the different values of

r, using the point probabilities for *N* as coefficients. The *age at infection* for both PC and SC is depicted in Fig. 5. Note that the SC are, on average, slightly younger than the PC.

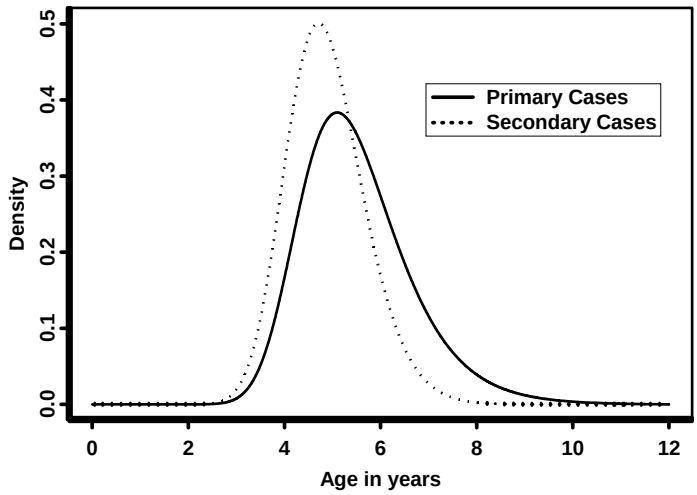

Fig. 5 The age distribution of *age at onset* for primary cases (PC) and secondary cases (SC)

RESULTS

Using Eq. (1) extended to other window sizes and data on herd sizes, the probability of cohort culling efficiency is calculated as in Table 1.

Table 1. Cohort culling efficiency

Window Size	% of secondary cases escaping culling
1 year (EC cohort culling)	1.06 %
1½ years	0.76 %
2 years	0.59 %
No cull	5.58 %

Table 1 demonstrates that the EC cohort culling is effective at a 1% level, and that the risk taken by not culling is 5-6 %. However, the figures in Table 1 are averaged over both herd size and age of the PC. In real cases these figures are known and an appropriate distribution to apply is therefore the specific conditional distributions given herd size and *age at onset* of the PC. In Fig. 6, examples of conditional distributions of *age at onset* for SC given PC drawn, and this reveals a different picture than Table 1. The one-year window will obviously perform poorly for older PC. For PC aged six years or below (i.e. the left hand side of Fig. 6) the majority of the SC are within the one-year window. However, for PC aged 7 years or more (i.e. the right hand side of Fig. 6) bi-modal distributions for the SC are revealed.

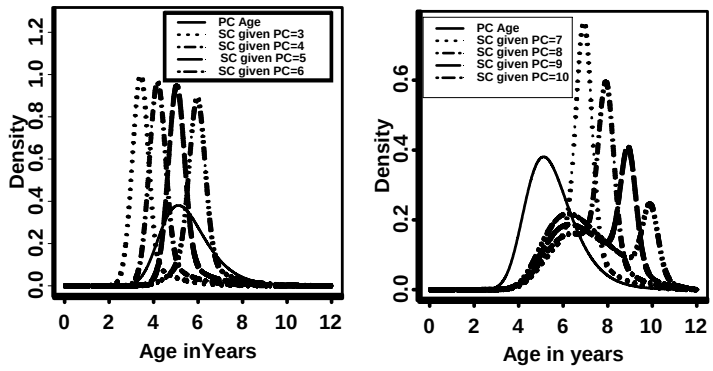

Fig. 6 Conditional distributions of secondary cases (SC) given primary cases (PC)

When results for larger herds are added this increases the probability of presence of SC and performance will be poorer for all window sizes. Nevertheless, the fact that cohort culling works much better for younger PC than older, results in the desire to correct the problem. Assuming that the certainty of removing all SC must be at least 99% under the assumptions of the models, the question as to what should the window size be for each age of the PC needs to be addressed.

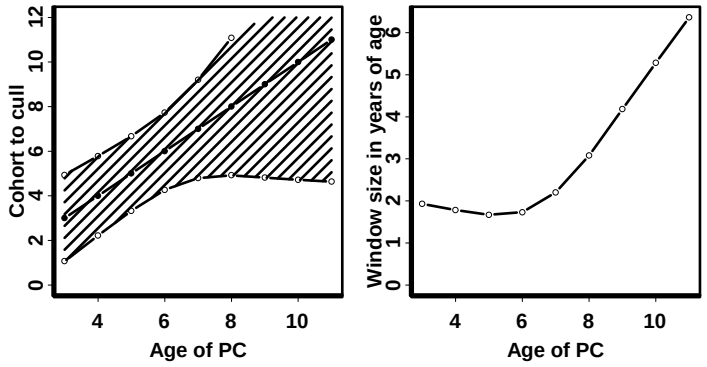

Fig. 7 Symmetric windows corresponding to 99% certainty of secondary case (SC) removal.

Such a window size is easily calculated from the developed framework. In Fig. 7 the window sizes in both asymmetric and symmetric form are shown for a herd of size 250, corresponding to the 85% fractile in the Danish cattle herds. The asymmetric windows are somewhat smaller. A rapid increase in window size is present in both situations from the age of 7 for PC. Using windows as those depicted in Fig. 7 results in a significant proportion of the herd still surviving the cull. While the windows are larger for older PC, the corresponding

cohorts of animals are smaller. On average about 80% of the animals will survive culling despite the wider windows.

DISCUSSION

Age-dependent windows

It was demonstrated (Fig. 7) that no fixed window width of reasonable size will result in a cohort cull efficiency of 99%, and the obvious conclusion is that for Danish herds, the EC cohort cull provides insufficient security. However, widening the windows is not an option. Instead, the obvious conclusion is to advocate age-dependent windows, such that the cohort needing culling depends on the age of the PC. This has been communicated to the Danish authorities, which have incorporated age dependent windows into the Danish legislation for handling BSE cases, as of September 2005. The chosen window sizes are depicted in Fig. 8 and the resemblance to Fig. 7 is striking. The window sizes are slightly smaller than those in Fig. 7, but these are also designed to handle a herd size corresponding to the 85% fractile among the herd sizes in Denmark. Authorities have a different need for round numbers than researchers, and the alternative of using 2-year windows for younger PC have been deemed an overly cautious.

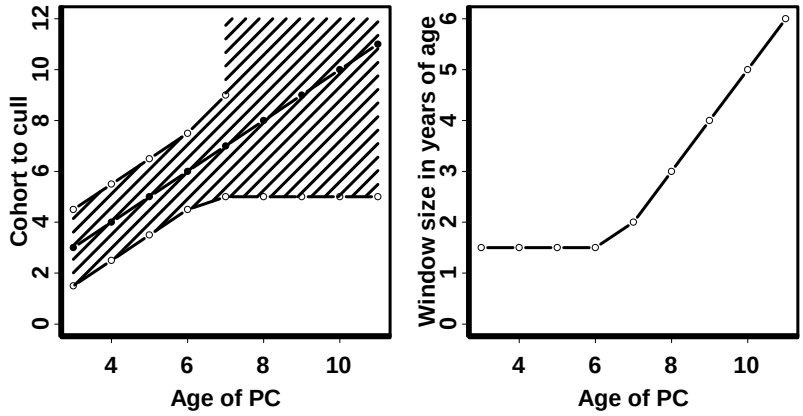

Fig. 8 Windows accepted for handling Danish BSE cases

Case study

In September 2005, a Danish BSE case is confirmed. The *age at onset* is 9 years and 5 months, and the herd size is 330 animals. The animal was moved from herd 1 to herd 2 at the age of five years.

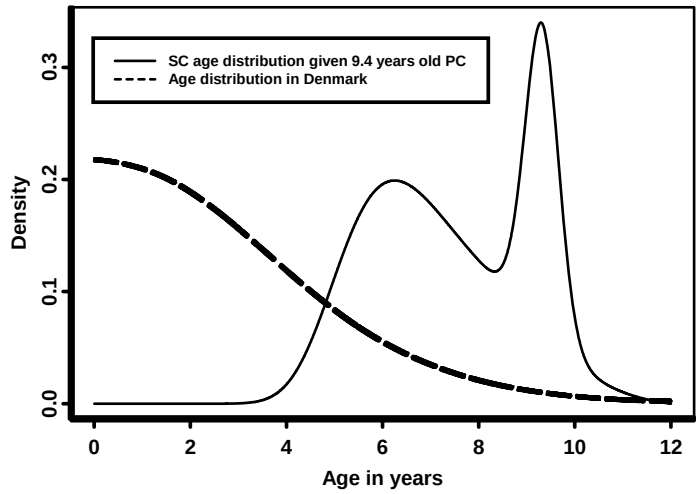

Fig. 9 Age distribution of secondary cases (SC) in the case study and the age distribution for cattle in Denmark

The study investigated whether the EC *cohort culling* was effective, and whether it was likely that the infection has occurred in herd 1 or herd 2. The age distribution of SC given the PC for the case study is depicted in Fig. 9. From the graph, it is demonstrated that the one-year cohort cull method is problematic. The proportion of SC within the one-year window from the PC is only 36%, and computing the culling efficiency reveals that one-year cohort culling only provides a certainty of 81% for removing all SC. The herd size of 330 is above average, so the 5-6% risk when abstaining from culling does not apply here. Even with one-year cohort culling, there is still a 19% risk of new BSE cases. However, using the window sizes from Fig. 8 shows that 94% of the SC are within the age-dependent window and results in a 99% culling efficiency. The probability that the PC was actually infected in herd 1 and not in herd 2 where it was confirmed as a BSE case, may in this framework be translated to the probability that the incubation period is more than 4.41 years. This is readily computed as 0.02 (2%). The infection is therefore likely to have happened in herd 1 rather than herd 2 where it was confirmed. Age-dependent windows were applied in the authorities' handling of this case.

CONCLUSION

The case study shows that situations do occur where age dependent windows for cohort culling are a necessity if acceptable levels of certainty are to be reached. Conversely, the fact that the method is incorporated into Danish legislation shows that the method of age dependent windows provides the required risk levels, while based on assumptions which have been deemed acceptable. While the method does not reduce the number of animals culled as much as the one-year cohort culling, a reduction to 20% is still considered significant. Further research is needed to investigate the validity of the assumptions on probabilities of infection and *age at infection*.

ACKNOWLEDGEMENTS

The author wishes to thank Larry Paisley for providing insight into BSE and its epidemiology and the data upon which to base it, although this paper does not necessarily reflect the views of Larry Paisley.

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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
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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