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The Gareth Davies Lecture

THE 1997/98 OUTBREAK OF CLASSICAL SWINE FEVER IN THE NETHERLANDS: LESSONS TO BE LEARNED FROM AN ECONOMIC PERSPECTIVE

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Early 1986, when I had just returned from a sabbatical leave with dr Roger Morris in Minnesota (USA), the Dutch Veterinary Service asked me to help them prepare a paper on the epidemiological and economic aspects of Foot-and-Mouth disease control strategies. They had been asked to do so by the FAO European Commission for the Control of Foot-and-Mouth Disease that would meet later that year in the UK. I gladly accepted that invitation, not in the least because it gave me the opportunity to get hold of an IBM-compatible PC. I had become devoted to working on such a machine during my stay in the US, but back home discovered them to be very rare in the Netherlands and even not (yet) available in our University. One does not need to be an economist to understand that prices of the ones that were available were very high at the time, and certainly far beyond normal university budget standards. Relative to the losses of an outbreak of FMD, however, the costs of such PCs were low and that convinced the Veterinary Service to let me get one.

More seriously, the project turned out to be very interesting and the start for at least three developments that shaped our future research activities. First, it led to an intensive and long-term collaboration with the Veterinary Service and other disease-control authorities which through their input and funding contributed enormously to the further development of our work in Animal Health Economics. Second, the simple spreadsheet model that resulted from the project (Dijkhuizen, 1989) was succeeded by various projects and activities that now - in close collaboration with the group of dr Roger Morris in New Zealand - have reached the level of both really advanced and very practically-oriented computer simulation work. A framework is now available based on what is called spatial and stochastic simulation in which an ever-increasing range of diseases and disease control strategies can be evaluated and compared (Jalvingh et al., 1998). Third, I got to learn Gareth Davies who chaired the meeting of the FAO Commission where I was asked to present the then available model and its outcome. This contact worked out to be highly determinant for our further activities in this area.

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Over the years, Gareth Davies has been very supportive of a quantitative and integrated epidemiological and economic modelling approach to help improve policy-making in contagious animal disease control. This resulted among other things in me (as a non-veterinarian) becoming a member of the Scientific Veterinary Committee in Brussels. Through this membership it was possible to establish a closer connection between epidemiological and economic modelling research on the one hand, and actual disease control policy-making on the other. It also gave me the opportunity to work with him in Brussels on various interesting projects, the last one of which was focused on animal health and related problems in densely populated livestock areas (Dijkhuizen & Davies, 1995). Unfortunately, this one became reality shortly afterwards when the Netherlands was faced with an outbreak of Classical Swine Fever in one of its most dense pig areas. Undoubtedly, this outbreak has been the biggest and most costly one of its kind so far, and has not only led to increased epidemiological and economic research but also started intense political and public debates about the future of intensive livestock industry in general and disease control strategies in particular. It is therefore that I have chosen this sad but realistic example to be the core of my Gareth Davies lecture and would like to discuss with you the lessons (to be) learned from an economic perspective.

THE ACTUAL OUTBREAK: EXTENT AND ECONOMIC LOSSES

On February 4 1997, Classical Swine Fever (CSF) was diagnosed on a farm in the southern part of the Netherlands. Thus started an epidemic in one of the most densely populated pig areas of the world, with between 2500 and 2900 pigs per km^2 and about 1000 pigs per farm. By the end of October 1997, 420 farms were infected and detected. In the next four months another 9 cases occurred, with the last one on March 6 1998, and it was only in May 1998 that it was evident that the disease was definitely eradicated. Control of the outbreak was carried out within the EU Directive 80/217 from which two main instruments were applied: stamping-out infected herds and movement restrictions. In addition, pre-emptive slaughter was carried out, and included a total of 1286 farms. These measures together led to a destruction of almost 2 million pigs, of which 0.7 million were infected and detected and 1.1 million pre-emptively slaughtered. By far the biggest number of pigs was destroyed because of welfare slaughter, i.e. 9.2 million, of which almost 2.5 million fattened pigs, 5 million weaned piglets, and 2 million young piglets of between 3 and 17 days old. A summary of the figures is given in Table 1.

Table 1. Extent and economic losses of the outbreak

Number of farms infected and detected	429
Number of farms slaughtered pre-emptively	1286
Number of pigs infected and detected	0.7 m
Number of pigs slaughtered pre-emptively	1.1 m
Number of pigs for welfare slaughter	9.2 m
Total economic losses (billion NLG)[a)]	4.7

[a] Exchange rate: 1.8 NLG = 1 US$ and 2.2 NLG = 1 Euro

Total economic losses of the outbreak were calculated to be 4.7 billion Dutch guilders (Meuwissen et al., 1999), as also indicated in Table 1. This total equals about 2.5 billion US$ and 2.1 billion euros. Almost 40% of the losses were related to welfare slaughter, where stamping-out infected farms and pre-emptive slaughter caused 4% and 8% of the total respectively. Costs of organisation (such as extra personnel costs for crisis centre and field tasks) turned out to be 6%. Total losses of the outbreak were about equally distributed among public and private sectors, with the EU bearing 37% (1.7 billion NLG), the Dutch government 10% (0.5 billion NLG), the farmers 28% (1.3 billion NLG) and the related industries, such as slaughter houses, animal traders, feed suppliers and breeding organisations, 25% (1.2 billion NLG).

THE SIMULATED OUTBREAK: 'WHAT ..... IF'

The model

The Dutch disease control authorities as well as the Parliament asked for an evaluation of the efficacy of the control strategies that had or could have been applied during the course of the epidemic. A preliminary analysis, using a deterministic-tree model had provided some initial estimates of the efficacy of pre-emptive slaughter of neighbouring farms (Elbers et al., 1998), but these estimates were influenced by many other factors that had occurred simultaneously and could not be controlled in the analysis. Computer simulation is considered a more appropriate approach for such analyses (Dijkhuizen & Morris, 1997). This was especially so in this case, where a comparison of the simulated and the actual outbreak could serve as a unique basis for model validation.

The conceptual model underlying the analyses was developed in New Zealand and focused on FMD (Sanson, 1993). The model was further developed and modified to suit Dutch and EU conditions, and was also adapted to CSF (Jalvingh et al., 1998). Starting point in the model is data on individual farms, including animal numbers and geographic location. The spread of CSF-virus between farms is simulated from day to day via two possible spread mechanisms: (a) contacts (animals, people, vehicles, material), and (b) local/neighbourhood spread. Once the first infected farm is diagnosed, several control measures can be put in place, such as stamping-out, tracing, movement control and pre-emptive slaughter. Spread and control mechanisms include risk and uncertainty through so-called Monte Carlo simulation, and act spatially by using the geographic location of farms. Key-issue of Monte Carlo simulation is that an input value for a specific variable is sampled from an appropriate probability distribution. This has the disadvantage that multiple runs are required to get a reliable estimate of the average outcome. The major advantage is that these replicates provide insight into the spread in possible outcome and hence into best and worse case scenarios (Dijkhuizen & Morris, 1997). In the CSF-model 100 replicates were carried out for each set of input values. Examples of important input parameters are the interval of infection to detection, the distance and chance of local and contact spread, the size and duration of protection and surveillance zones, and when/where/how pre-emptive slaughter is carried out. In total more than 100 input variables are included in the model. Where possible these inputs were based on the outcome of the first epidemiological analyses of the real outbreak, complemented with experts' estimates. More detailed information on the model is provided by Jalvingh et al. (1999).

Outcome basic simulation

The basic simulation was aimed at simulating the real outbreak as closely as possible. Simulation started on the day the first case was detected, i.e., February 4 1997. The most likely date of infection of the first farm was estimated to be December 23, 1996 (Elbers et al., 1999), and at the time of first detection already 36 more farms were infected. In the model these farms were considered fixed events, and used as starting point for further simulation. Some key-outcome of the basic simulation is summarised in Table 2.

Table 2. Comparison of the real and simulated outbreak

	Real outbreak	Simulated outbreak		
		median (50%)	5%-percentile	95%-percentile
Number of detected farms	429	381	231	1787
Farms pre-emp. slaughtered	1286	741	320	1865
Duration of outbreak (days)	> 365	306	254	>365
Losses (billion NLG)	4.7	2.5	1.8	4.1

Given the restricted time available for the simulation and for the epidemiological analyses to provide the underlying input values, the basic simulation was considered reasonably close to the real outbreak. The median of the 100 replicates of the simulation shows an outcome of 381 infected and detected farms. This is less than the 429 farms in the real outbreak, but still excessive. The median for the number of pre-emptively slaughtered farms deviated much more from reality, despite the fact that the written protocols obtained from the disease control authorities were followed in the model precisely from week to week. One possible reason for the lower number in the simulation is that the total geographic region in the simulated outbreak is somewhat smaller than in reality. It may also be possible, however, that at some stage(s) of the actual outbreak pre-emptive slaughter was carried out more than indicated in the written protocols. The number of pre-emptively slaughtered farms could be increased in the simulation, but not without the number of infected and detected farms becoming too high. Despite the deviation of the median, it is also shown in Table 2 that the real number of pre-emptively slaughtered farms fitted well within the 5%-95% interval of the simulated outcome. The same applied to the duration of the outbreak. The losses calculated in the simulated outbreaks were considerably lower than in reality, although the same calculation model was used (Meuwissen et al., 1999). Besides the smaller size of the simulated outbreaks, there are two major reasons for the differences in extent of the losses: (1) some costs - such as for disease control organisation - were only available for the entire outbreak and hence could not be split up and included in the simulation, where outbreaks differ in size, and (2) average pig prices for 1997 were considered more appropriate for use in the simulation than predicted prices which should then be related to each simulated size of the epidemic.

Evaluation of events and control strategies

To gain insight into the possible impact of various events and control strategies, about 20 different alternatives / scenarios were defined and simulated. Vaccination was not included in the alternatives, as this was not considered a realistic option for such a much exporting country as the Netherlands[2]. For each alternative set of input values 100 replicates were carried out again and the outcome was compared to the basic simulation. A summary of the outcome (median values) for some of the most important alternatives is given in Table 3.

Table 3. Possible impact of various events and control strategies

	Detected farms	Pre-empt. slaughter	Duration (days)	Losses (b NLG)
Basic simulation	381	741	306	2.5
First detection - 2 wks	201	453	298	2.0
Pre-empt. slaughter 4/2+	70	451	164	1.3
Maximum hygiene	40	20	98	0.8

As indicated in Table 3, the outbreak would have been considerably smaller and also at a lower cost if the first case had been detected 2 weeks earlier. Then the most reasonable (i.e., median) outcome would have been 201 infected and detected farms, to 381 in the basic simulation. The number of pre-emptively slaughtered farms then would also have been considerably smaller. There would have been an even bigger impact, had a strict and extensive pre-emptive slaughter policy been applied right from the beginning of the outbreak on February 4 (i.e., slaughter of serious contact farms and farms within 1 km around an infected premise). In reality, only 26 farms were emptied around the first 2 detected cases. Major reason for not continuing was because pre-emptive slaughter was seen as a highly inefficient method to stop the epidemic. Emphasis should be put on tracing possibilities (Terpstra, 1998). Pre-emptive slaughter was started again around the middle of April, when it the outbreak appeared to growing still rapidly. As shown in Table 3 a more strict and extensive pre-emptive slaughter policy from the beginning could have limited the size of the outbreak to 70 farms, even in such a highly densely populated pig area. Also the total number of pre-emptively slaughtered farms would have been much lower than in the basic simulation, and the total duration of the outbreak would have been not longer than about 5 months. Total losses would have been smaller accordingly. Finally a theoretical option is presented in Table 3, assuming maximum hygiene, i.e., no risk of infection through local spread. Could that be realised, then even the currently applied policy would have been strict enough to keep the size (and losses) of the epidemic within acceptable limits.

[2] Research is under way to simulate the possible impact of the newly developed marker vaccines.

LESSONS TO BE LEARNED FROM AN ECONOMIC POINT OF VIEW

Reducing the risk of introduction

The costs of the 1997/98 outbreak of CSF can be considered record high. The previous big epidemic in the Netherlands dates back to 1983/84, when around 350 farms were infected and detected (Davies, 1995). In between, some small outbreaks occurred, i.e., 36 infected farms in 1985, 1 in 1986, 1 in 1987, 2 in 1990 and 7 in 1992. Such an outbreak pattern leads to a loss of between 300 and 400 million Dutch guilders per year. Thinking the other way around, this means that such an amount of money could be spent annually to avoid the introduction of CSF-virus into the country / region. Horst (1998) found the import of livestock and livestock products to be the two major risk factors, together accounting for more than half of the total risk. So, considerable money is available to improve the traceability of animals and animal products. Moreover, a joint effort of countries facing regular outbreaks (and losses) to improve the health status in 'virus-source countries' or even eliminate the major sources of infection could be a very attractive investment from an economic point of view. The first lesson to be learned, therefore, is to consider to increasingly invest in improving the animal health status outside the country's own borders, i.e., in regions / countries that cause a serious threat to the animal health status of others, either directly or in an indirect way.

Limiting the high-risk period

The length of the high-risk period (HRP) is one of the most important parameters that determine the magnitude of an outbreak because it defines the period in which the virus can circulate freely (Horst, 1998). The period begins when the first animal is infected and ends when all eradication measures are in full operation, i.e., the region concerned does not longer involve any risk for other regions. Within the entire HRP the probably most important part begins when the first animal is infected and ends when an infected animal is detected, i.e., the period of undetected infection. The length of this part depends on the alertness and motivation of farmers and veterinarians. Horst et al. (1998) consulted Dutch CSF-experts, who estimated the most likely length of this period to range from 21 days in the Netherlands to 42 days in Eastern Europe. Experts from research were much more pessimistic than those involved in policy-making and field work, and estimated these values to be 28 and 60 days respectively. The most likely date of infection of the first farm in the 1997/98-outbreak was estimated to be December 23 1996 (Elbers et al., 1999), as mentioned before, which means that the period of undetected infection was 42 days in this case. This is much longer than the experts' estimate for the Netherlands, and also made that at the time of first detection already 36 more farms were infected. Simulation showed that a 2-week earlier detection of the first case would have limited the size of the outbreak considerably and would have saved around 500 million Dutch guilders (Table 3). The second lesson to be learned, therefore, is that there is considerable economic scope to develop tests and monitoring systems that help improve the alertness and motivation of farmers and veterinarians to detect the first infected farm as soon as possible. Regular training sessions could also be of help.

Pre-emptive slaughter

Simulation suggests that the epidemic would have been much smaller if pre-emptive slaughter had been used consistently right from the beginning (i.e., February 4) onwards. The effectiveness of pre-emptive slaughter agreed with the perception of many involved in the control of the real outbreak, and it was therefore proposed by the Minister to include pre-emptive slaughter as a standard strategy for future epidemics in the Netherlands (Ministry of Agriculture, Nature Management and Fisheries, 1998). The finding was also in agreement with conclusions from previous outbreaks in Belgium (Vanthemsche, 1995) and Germany (Winkenwerder & Rassow, 1998). Economically, pre-emptive slaughter was not as expensive as feared, because the smaller size of the epidemic led to much lower overall costs. The outcome of the simulation also puts the discussion on emergency vaccination in a better perspective. It is highly questionable whether vaccination (had a marker vaccine been available) could have reduced the size of the epidemic to less than 70 farms, taking into account the fact that already 36 more farms were infected at the time of first detection. A possible advantage of vaccination could be fewer farms to be slaughtered pre-emptively than the 451 currently calculated under the strict pre-emptive slaughter policy (Table 3). That would of course have been a great advantage to the farms involved, and certainly more acceptable to the public. Whether it also would have been economically more attractive highly depends on the acceptance of vaccinated animals by domestic consumers and international trade. More research and negotiations are required to find out more about these issues. For now, the lesson to be learned is that epidemics of CSF can be controlled effectively without vaccination, even in densely populated pig areas. Research is under way to further test and refine this conclusion for various areas and conditions.

Hygienic improvements versus industry restructuring

The 1997/98 outbreak in the Netherlands has drawn a lot of attention in the media and started an extensive debate among the public and in the Parliament about the future of the swine industry. This has led to legislation that reduces the herd size of each individual pig farm by 20%. Moreover, it is proposed to establish regional clusters separated by pig-free corridors of at least 1 km wide. These measures have highly been questioned, and even brought to court. Farmers and related industry strongly feel that the outbreak of CSF has been mis-used by the Minister to settle other issues, such as environmental improvements. This view is supported by a wide group of scientists, who also questioned the efficacy of these measures with respect to future CSF outbreaks (Wageningen University and Research Centre, 1999). Of course, the measures will contribute to a better disease control, but at too high a price and not without additional measures to improve the management in general and the hygienic level in particular. This is in accordance with the simulation exercise, which shows that an improved hygienic level considerably limits the size of CSF-outbreaks and hence makes industry restructuring redundant from this point of view (Table 3). Hygienic measures that reduce the infection probabilities, therefore, should strongly be stimulated. Money incentives as to especially the amount of premium to be paid for future disease control activities are considered essential to realise such improvements (see also next point).

Public versus private money

The big impact of the possible preventive measures in general and hygienic improvements in particular to be taken by the farmers and the related industry supports the question as brought forward by Davies (1996) whether such a big part of the costs of epidemics should be borne by the public sector. In the Netherlands this has led to a study to investigate alternative payment systems (Meuwissen et al., 1997). They suggested a system that provides: (1) a compulsory insurance for losses from stamping-out infected herds to give farmers an incentive to report an outbreak on the farm as soon as possible, (2) trust in the level of compensation, because otherwise the incentive to report an outbreak would yet be lost, (3) a level of compensation that is not too high, to avoid that it becomes attractive to get involved in an outbreak, and (4) a differentiation of premiums to encourage preventive measures and risk-averse behaviour. As also pointed out by Howe & Whittaker (1997) it is likely that the government (i.e., a central authority) remains heavily involved in such a system in order to help guarantee its future. The first step of such a system for pig farms has been put forward by the Dutch Ministry and accepted by the Parliament (Ministry of Agriculture, Nature Management and Fisheries, 1998). Further differentiation in premium related to risk factors is to be expected, as well as extension of the system to other species. Moreover, private activities are under way to set up voluntary insurance systems for related losses not included in the common / compulsory compensation system.

TO CONCLUDE

Much of the vision Gareth Davies expressed in his legislative (i.e., EU) work and scientific publications has - painfully - been confirmed by the 1997/98 outbreak of CSF in the Netherlands. The outbreak also led to full priority for further development and implementation of computerised information systems, such as EpiMAN (Morris et al., 1992), which Gareth Davies helped to initiate to bring closer to EU and Dutch conditions (Jalvingh et al., 1998). Also the close relationship he helped to establish between epidemiological and economic research on the one hand and disease control policy-making on the other, has shown to be very fruitful and mutually beneficial (Van Klink et al., 1999). Within the Netherlands a framework has been put in place for disease control training sessions and continuous improvement of contingency plans that undoubtly will pay off in the control of future epidemics. A much closer collaboration and exchange of experience at the international level should be encouraged, and would be a further realisation of Gareth Davies' vision and mission.

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EPIDEMIOLOGY IN POLICY MAKING

RISK MANAGEMENT AND DECISION ANALYSIS IN ANIMAL HEALTH

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Risk analysis & communication (RAC) is a process used to derive and communicate a qualitative or quantitative estimate of the risk involved in an activity and of the relative merit of various risk mitigating options. It is currently used in many areas of endeavour. In the last two decades, RAC has found applications to animal health issues; it has been the focus of investigators and a preoccupation of many animal health decision-makers mainly in their dealings with cross-border movement of animals (trade) and their products. The main components of RAC are hazard identification, risk assessment, and risk management; each using its own tools and techniques. Hazard identification and risk assessment constitute the investigative phase, risk management (RM) the implementation phase, of the RAC process.

In this presentation we attempt to demonstrate the importance of using sound epidemiological input data in both the investigative phase and in the risk management component of the RAC. To that effect we first review the relationship between RAC and the quality of input data; then introduce a framework for RM; and finally examine how decision analysis (DA) contributes to risk management.

RAC AND THE IMPORTANCE OF QUALITY INPUT DATA

The accuracy of the output of the RM component (a decision about which mitigation option to select) depends to a large degree on the reliability of the input data used in the RAC process. For import risk analysis "input data" refers mainly to data of the disease prevalence and its determinants in the exporting country. The reliability of such data, in turn, depends on the quality of the veterinary services; the surveillance systems in place; and the diagnostic capabilities. In the case of a public health related RAC, reliability of the input data depends mainly on the local conditions of the same factors. In both cases, the quality of these input data depends on the reliability of the findings from the epidemiological investigations of the disease condition under study.

Measuring the risk posed by a biological phenomenon such as a disease can be a rather subjective undertaking. The degree of subjectivity must be reduced as much as possible in order to make the measurement more valid and reliable. The reliability can further be augmented by thoroughly reviewing the information about the disease and its circumstances; i.e., the epidemiology of the disease. The more information that is available about the epidemiology of the agent or the disease, the easier it is also to move from a qualitative to a quantitative approach for the RAC process.

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Several unique issues are associated with utilising population animal disease data when when embarking on a RAC process, particularly when dealing with contagious infectious diseases; they are:

1. Animal populations are aggregated and mobile, therefore, their diseases can be **clustered** and dynamic in time and space.

2. Infectious diseases have different **epidemiological characteristics** than do non-infectious diseases. Hence, when doing a risk analysis involving an infectious disease in an animal population, one needs to account for these differences. Unfortunately, for many of these infectious diseases of livestock, there is a partial or complete lack of epidemiological knowledge, thus introducing uncertainty into the risk analysis process.

3. Screening of infectious diseases of livestock usually requires more than one test. However, the use of a combination of tests, either sequentially or simultaneously, may underestimate the true **prevalence** of a disease when the tests measure similar biologic markers. Methods to adjust for the use of multiple dependent tests need to be incorporated into the prevalence assessment part of the risk analysis procedure.

4. The international requirements of **freedom from infectious animal diseases** mandate the availability of negative outcomes of surveys and surveillance systems. Methods to evaluate negative results arising from these systems--be it at herd, region or country level--need to be developed. Existing approaches, developed mainly in the human and public health sectors and used in animal health, have focused on the detection of cases rather than on demonstrating their absence in a population.

5. The quality of animal health programs and **surveillance systems** in different countries varies more than that of similar systems in human medicine. Animal disease risk analyses need to take this variability in the quality of programmes into account along with the associated uncertainty. Objective methods to assess the quality of animal health programmes become a necessity.

6. Only few **software** exist that can simultaneously evaluate the dynamics of disease transmission and apply the outcomes to risk analysis for infectious animal diseases.

7. Economic models too require scientifically **sound parameters** which, unfortunately, are not usually available.

There is a need to develop approaches that can adjust for or solve the problems pointed up by these issues; only then will epidemiological input data make their full contribution to risk analysis in animal health.

A FRAMEWORK FOR RISK MANAGEMENT

The outcome of the risk management process should be a decision as to which of several risk mitigation options is the most appropriate one to implement. The various release and exposure assessment scenarios utilised in the early part of the analysis forecast the RM options that need to be considered. Likewise, many of the biological assumptions embodied in the risk assessment component of the RAC must be carried over into the RM component and given heed to. In addition, the impact of each option on the "total environment" must also be accounted for, because many segments of society could be affected by the mitigation action selected. In the animal health arena the stakeholders include consumers, the livestock industry, public health officers, local animal health officers, veterinarians, and others. Political and social consequences are a further issue to be taken into account in the evaluation of each of the risk mitigation options. It is against this background that we present the following general framework for the risk management process. Each of the six elements of he framework has a certain role to play in the overall process and some can also be used as inputs in the decision analysis outlined under the next subheading.

1. All the **options** that could be helpful in mitigating the risk under consideration are listed. The hazards identified at the beginning of the RAC are good starting points to identify RM options. Following is an example to illustrate this point.

   Country A wishes to import animal skins to be used for musical instruments from country B in which FMD, anthrax, and Clostridium perferingens are endemic. If FMD and anthrax are considered to be the main hazards, the risk management process should include risk mitigation options for the agents of at least these two diseases.

2. The specific **purpose** for each of the options identified in the previous step is spelled out. One answers the question "what is the potential effect" of this option; is it complete avoidance of a risk; is it mere risk reduction; or is it strictly to gain a monetary advantage (minimising the losses or maximising the benefits)? The example below serves to illustrate this.

   Risk mitigation options in the above example could be a) a ban on the importation of the skins (avoidance of risk); b) a requirement to disinfect the skins in country A (risk reduction); or c) a decision to import and then treat the skin in country B (monetary advantage).

   Each option is evaluated by balancing three criteria against each other; they are: the estimated risk (the output of the risk assessment component); the acceptable risk (the output of regulatory guidelines or of professional or expert judgement); and an opportunity for gain--particularly a gain associated with trade.

3. The **impact** of each of the options identified in step one is appraised in relation to political, sociological and, at times, psychological issues. This may require input from various stakeholders affected by the risk mitigation action, but is an important precaution to ensure that the decision will have a chance to be implemented successfully. The following imaginary situation illustrates this point.

A ban on the importation of the skins mentioned above could be construed by a particular interest group as discriminating against a certain kind of musical expression which relies on the availability of these specific skins.

Furthermore, the impact appraisal can also serve as a cost entry and/or a probability entry in the decision tree discussed below.

4. The nature of the **uncertainties** associated with each option needs to be delineated and prioritised. It is important to conduct a thorough investigation to identify these uncertainties and, if possible, to quantify them. The real or estimated values can then be used as a probability entry in the DA model. Literature reviews and expert interviews are a good sources of information at this point.

   There is uncertainty about the effectiveness of the disinfection procedure used in the risk reduction option proposed in our example. This uncertainty can be expressed as a probability of the agent being able to survive the process.

5. The **costs and time delays** associated with each of the options will have a major bearing on the feasibility of the proposed risk mitigation intervention; they can also make a contribution as a cost factor in the decision analysis as illustrated in the example below.

   Treatment of the skins in country B could be considered prohibitively expensive due to high labour costs and because of the excessive duration of the treatment.

6. Finally, the magnitude of the **risk reduction** associated with each of the options needs to be elucidated taking into consideration the elements mentioned in steps 2 & 3. Here, epidemiological tools such as relative risk and attributable risk calculations can play a major role (see below).

   The amount of reduction in the anthrax spores achieved by using different disinfection methods can be compared by means of a relative risk ratio which can then be used in the DA process.

DECISION ANALYSIS AND RISK MANAGEMENT

Decision analysis (DA) offers a structured process for organising information and incorporating uncertainty into a decision making process. Several disciplines have utilised this tool to assess options and to arrive at a decision as to the most appropriate option. According to Clemen (1996) decision analysis is "a scientific discipline that describes an approach to decision making under conditions of complexity, with inherent uncertainty, multiple objectives and different perspectives of the problem". Economists and engineers commonly use this tool in their conventional evaluation of alternatives. Decision analysis does not provide solutions, rather it is a source of information that provides insight into the situation by clarifying the uncertainty, objectives and counter points. Therefore, decision analysis is an aid to a decision-maker to justify a particular intervention.

Three approaches to decision analysis are commonly used: pay-off tables, influence diagrams and decision trees. Pay-off tables are often used by economists; influence

diagrams are structured displays of decisions, uncertain events and outcomes--they provide a snapshot of the decision environment at a given point in time. Computer software users have a tendency to use influence diagrams to do decision analysis.

The decision tree is a display of the decision analysis which represents the options and their associated outcomes; it shows more detail than the other two structures. We will focus on the decision tree as a model to show how decision analysis can be used in risk management. Decision analysis as applied to RM involves the following steps:

1. The risk mitigation **options** which have previously been identified constitute the branches of the decision tree; they should be mutually exclusive and independent of each other.

2. For each option all potential **outcomes** are identified--ignoring one of the possible outcomes of an option can lead to erroneous conclusions.

3. A **cost** is assigned to each of these outcomes. The cost can be a loss or a gain in income. Social and/or political values can be incorporated at this stage of the process as part of the cost.

4. A sensible **probability** value is assigned to each of the outcomes identified in step two. These probabilities can be real values and may be obtained from the literature, from experts, or they can be estimations based on opinions. A probability can be computed either by means of a deterministic or a stochastic procedure, depending on the interest and the sensitivity of the desired outcome.

5. For each outcome the probability value is multiplied with the associated cost and the products are summed to obtain the **utility value** of each option. The option with the lowest loss value (if losses are considered) or with the highest gain value (if gains are considered) would be considered as the option of choice.

6. Finally, the analysis is subjected to a **sensitivity analysis**; i.e., steps 3 through 5 are repeated with different assumptions, different probabilities, or variable costs.

CONCLUSION

- Epidemiology can make a significant contribution to the risk analysis & communication process because professionally conducted epidemiological investigations provide reliable input data for this process; ignoring the valuable contribution epidemiology can make, may lead to erroneous outcomes of a risk analysis & communication exercise in the animal health arena.

- The risk management (RM) component of the risk analysis & communication process is not an independent entity. RM relies on the input from the investigative phase of the RAC process and leads it to the intervention phase, namely to the implementation of a risk mitigation action.

- Decision analysis (DA) is one of the tools used within the RM framework; it is the tool par excellence to derive a decision from the RM process. However, DA does not provide the solution, rather it helps to structure the information and to clarify the impact of uncertainty.

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AN APPROACH TO THE EVALUATION OF A CLASSICAL SWINE FEVER OUTBREAK. THE ROLE OF EPIDEMIOLOGY AND ITS RELATION TO POLICY

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From February 1997 onwards The Netherlands experienced an unprecedented outbreak of classical swine fever. The outbreak lasted well into 1998, and a total of 429 farms were found infected. On another 1289 farms the pigs were killed and destroyed pre-emptively, either because of contacts with infected farms, or because of the presence of an infected farm within a 1 kilometre radius (Ministry of Agriculture, Nature Management and Fisheries, 1997, 1998, Jalvingh et al., 1998b). The impact of the outbreak, on the attention of the Dutch community being drawn to the problem, the consequences for the pig production sector, and the enormous costs involved in the control of it, was very large. Therefore it was deemed necessary to carry out an elaborate evaluation of the outbreak and its control. This evaluation was aimed at identifying elements that were relevant for the prevention of a similar catastrophe, at identifying elements in the control activities that needed improvement, and at proper information of Parliament on results and effectiveness of the outbreak control efforts.

In this paper the approach used for the evaluation of the outbreak is described. The role epidemiology played during the outbreak, but more specifically in the evaluation process is elaborated on.

SHORT OVERVIEW OF THE 1997 OUTBREAK.

On the fourth of February 1997 a diagnosis of classical swine fever was confirmed on a farm in Venhorst, in the south of The Netherlands. The eastern part of the province of Noord-Brabant, where Venhorst is located, is one of the most densely populated pig producing areas. Figure 1 shows a map of The Netherlands with the total area under surveillance during the outbreak.

Soon it was found, that more farms had been infected. In the first 4 weeks of the outbreak 27 farms had to be cleared. The numbers of new infections found per week remained high, and especially during the summer the numbers rose. At the peak of the outbreak up to 26

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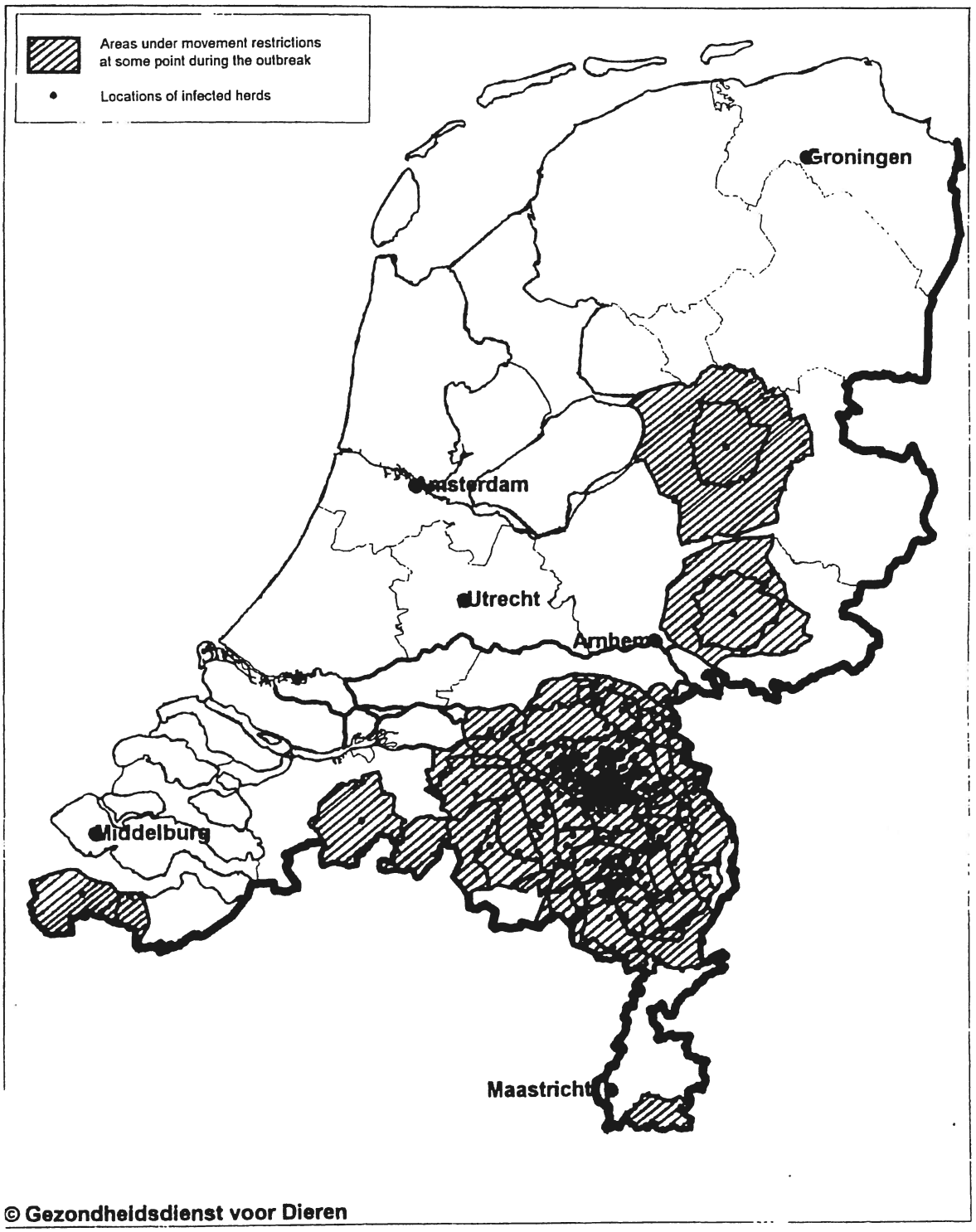

Fig. 1. Map of The Netherlands, showing location and largest extent of area under movement restrictions during the outbreak of classical swine fever in 1997. (Source: National Animal Health Service, Boxtel, The Netherlands.)

infected farms were cleared per week. Figure 2 gives an overview of the total number of infected farms cleared during the entire outbreak.

Pre-emptive slaughter of farms, either contacts or neighbourhood farms of those infected, was done during the larger part of the outbreak. An overview is given in fig. 3. Over 11 million pigs of all ages were eventually killed, either originating from infected or pre-emptively slaughtered farms, or bought out because of animal welfare constraints. Altogether the costs of the outbreak ranged in the billions of Dutch Guilders.

APPROACH

Under the supervision of a steering group, a project team was set up to implement the evaluation of the outbreak. The evaluation of the initial phase of the outbreak started already before the outbreak had entirely ended (Ministry of Agriculture, Nature Management and Fisheries, 1997). A second evaluation document looked back at the entire outbreak (Ministry of Agriculture, Nature Management and Fisheries, 1998).The project team involved three research groups in the process, each with its own area of attention:

Research group fieldwork

Because of the expected amount of effort to be put into the evaluation of the outbreak, and in order to allow for a more objective view on the events that took place during the control activities, it was decided to employ a research group from outside the Ministry of Agriculture, Nature Management and Fisheries to carry out the evaluation of events and decisions. This group, consisting of employees from two consulting companies (Deloitte & Touche and Research voor Beleid), was expected to carry out a thorough analysis of organisational aspects, of events and their background, of decisions made and their arguments and context, and of relations between actions, organisation and circumstances. This group was also responsible for integration of all information generated and for editing of the reports.

Research group epidemiological and economic simulation modelling

A second research group, from Wageningen Agricultural University, was involved in the evaluation in order to design a computer simulation model for the spread of CSF, that should enable analysis of the consequences of measures and events for the development of the outbreak, and of economic aspects of the outbreak and outbreak control measures (Jalvingh et al., 1998b).

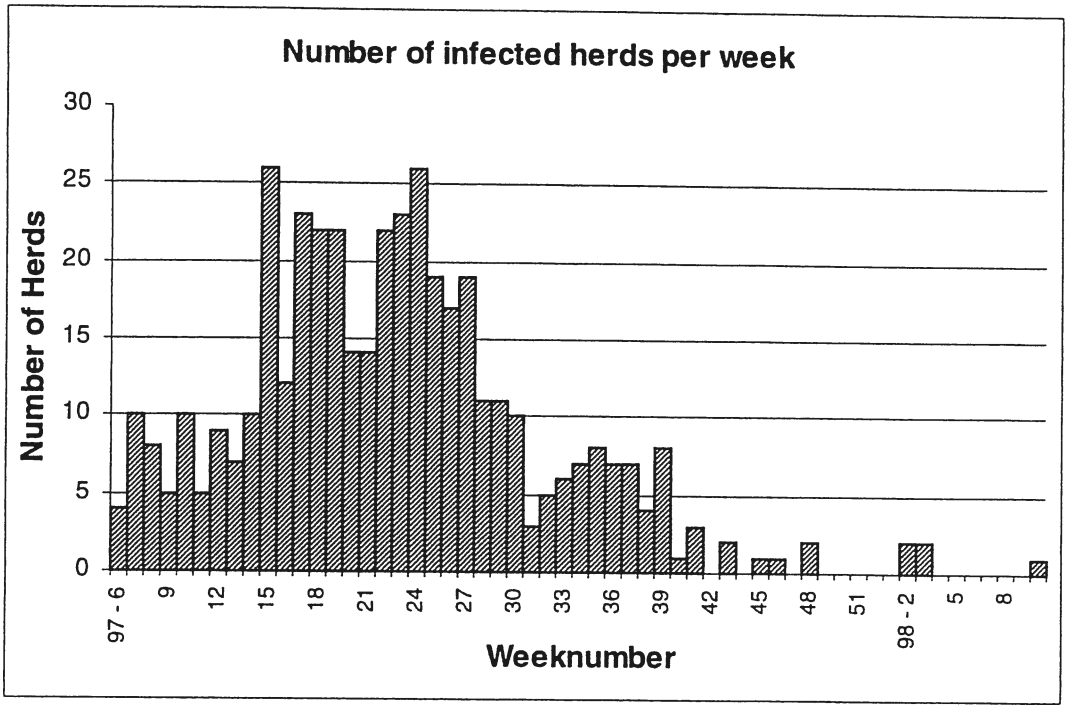

Fig. 2. Overview of the number of farms found infected with classical swine fever during the outbreak of 1997 in The Netherlands. (Source: Crisis Centre CSF Control, Information Department, Uden, The Netherlands)

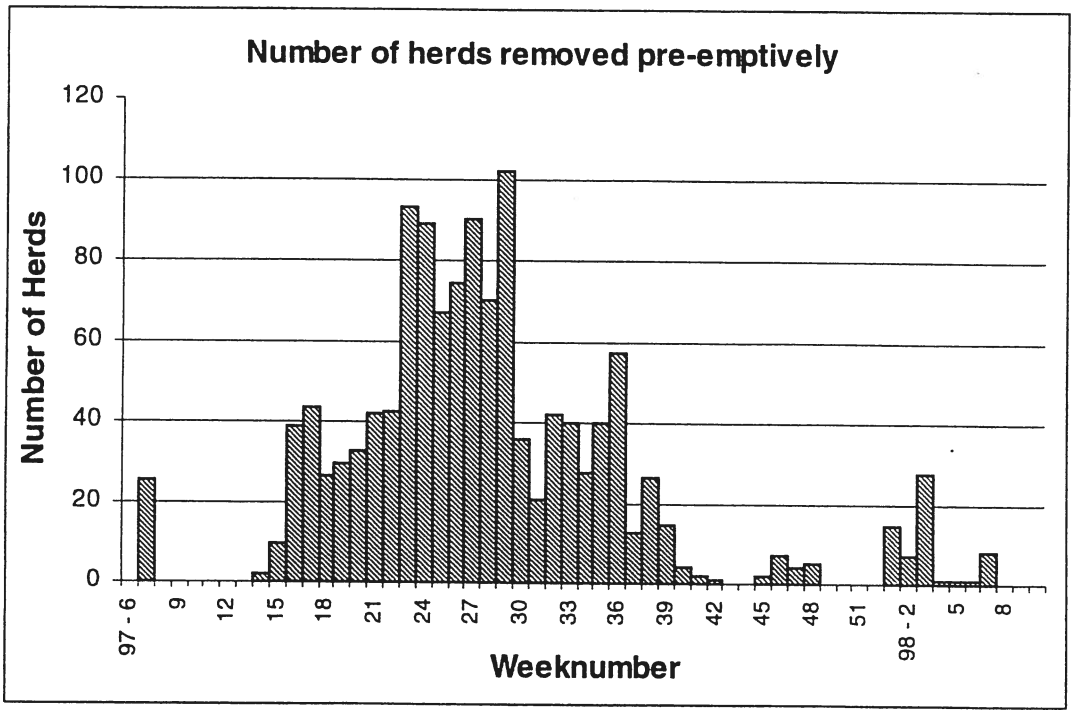

Fig. 3. Overview of the number of herds slaughtered pre-emptively during the classical swine fever outbreak of 1997 in The Netherlands. (Source: Crisis Centre CSF Control, Information Department, Uden, The Netherlands)

Operational epidemiology team

The operational epidemiology team was primarily established to support the on-going control activities, as prescribed in the EC-directive on classical swine fever control (EC, 1980). On the basis of the information brought together by this team surveillance and screening activities were managed, and decisions on control activities, such as clearing of contact farms, were taken, and on-going analyses of events in the outbreak was provided to improve veterinary insight in the disease behaviour (Stegeman et al., 1997).

Figure 4 provides an overview of the organisation of the project and the relations between the groups involved, as proposed in the original plan for the project. In practice, communication lines not always operated as intended. The project team regularly intervened in establishing and maintaining communication lines between groups, passing information from, particularly, the two (veterinary) technical groups to the research group fieldwork.

EXPERIENCES

Research group fieldwork

Through interviews and study of available information and documentation the research group tried to gain insight in the processes that took place throughout the outbreak. For all decisions made during the outbreak, the team tried to document the arguments that led to these decisions. Also the justifiability of both arguments and decisions was analysed, using, where appropriate, information provided by the other two research groups. Bringing the necessary information together was not always easy. Arguments were not always clearly documented. Furthermore, the amount of information generated during the outbreak was vast.

In view of oncoming elections, there was a relatively high pressure on time. The exercise was expected to produce its result before the recess preceding the elections, in order to allow Parliament to react on it. The resulting time pressure demanded a large effort from the group.

For the project team assessment of the progress and activities of the group appeared to be difficult. Drafts were produced regularly of parts of the final report, but an overview of the activities of the group was lacking. Only towards the end of the project period integrated drafts could be presented.

Research group epidemiological and economic simulation modelling

The model used for the evaluation of the control strategies deployed in the CSF-outbreak, the InterCSF model, developed on the basis of the InterSpread model (Jalvingh et al., 1996, Jalvingh et al., 1998a) provided a clear insight in the relative importance of control strategies. The technical experiences and results are reported separately (Jalvingh et al., 1998b).

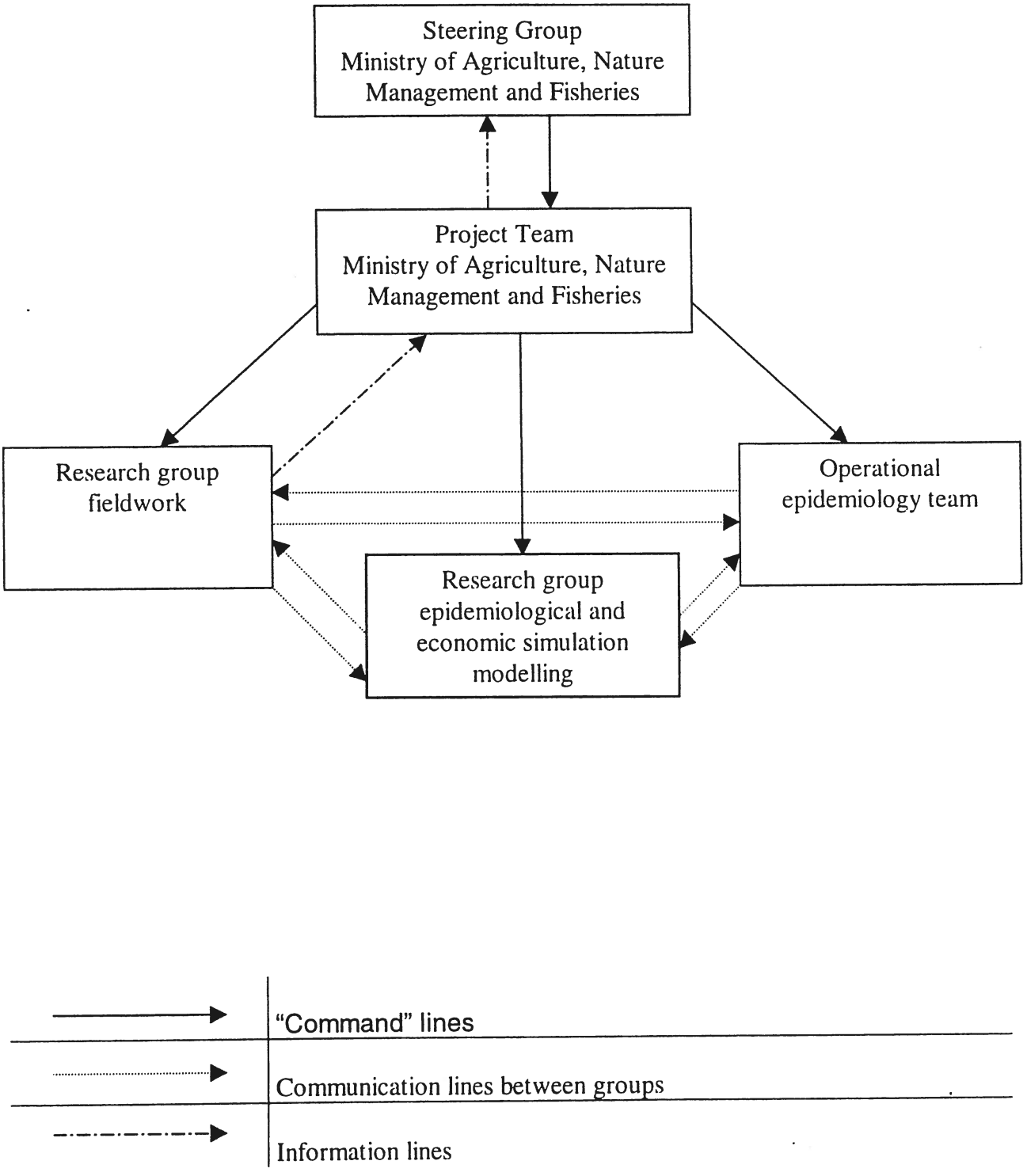

Fig. 4. Organisation chart of the project Evaluation Classical Swine Fever Outbreak 1997, as proposed in project plan.

In order to feed the model, the research group used factual quantitative information on epidemiological events during the outbreak, provided by, among others, the operational epidemiology team (Stegeman et al., 1997), as well as information on decisions and actions provided by the research group field work.

Operational epidemiology team

In the framework of the evaluation the information produced by the team was mainly used to feed the InterCSF model, and the team also assisted in providing argumentation for decisions made during the outbreak, to enable the research group fieldwork to carry out the analysis. The team also assisted in the valuation of the input in the model. For the model to be able to evaluate variations in control strategies, a baseline strategy has to be agreed upon. The project team was involved in the decision on the level of this baseline, on the basis of a proposal done by the research group.

DISCUSSION

The outbreak of classical swine fever in 1997 was the largest ever recorded in The Netherlands. The only comparable outbreak took place from 1983 through 1985, during which period 373 farms were infected (Meuwissen et al., 1997). This outbreak lasted several years, in a period when vaccination as a control strategy was still used. Partly through a masking effect of vaccination, and partly because of designated vaccination areas being too small, this outbreak may have been lingering for such a long time. Probably, the present approach of rigorous stamping out draws more attention of the general public to the problem than the approach followed in the 1983-1985 outbreak. Vaccination is often mentioned in the discussions as a preferable tool. It is however questionable whether such an approach would be cheaper, or would eventually cost less pigs lives. The international discussion, in particular within the EU, on the non-vaccination policy (EC, 1980, Laddomada and Westergaard, 1997), plays a key role in the possibilities of vaccination as a control measure. The availability on the market of marker-vaccines for classical swine fever may prove an important issue in that discussion. Apart from the availability, research is needed on protocols for application; under what circumstances does one decide to use vaccination, which characteristics of the outbreak area (density of farms, distance between farms, size of total population, etc.), of the disease (virulence of virus strain, speed of spread, etc.) are important, how to decide on the size of the vaccination area, how to prioritise in categories or age groups, are some of the questions to be answered in this process. Further development of the simulation model (Jalvingh et al., 1998a, Jalvingh et al., 1998b) may provide information needed to design such a protocol.

The InterCSF simulation model (Jalvingh, et al., 1998) in its present state proved to provide very useful information to evaluate the measures taken in the 1997 outbreak. After calibration of the model in order to reflect as much as possible the actual situation in the real outbreak, control measures could be varied in terms of starting moment, intensity, extent, etc., after which the effect on the course of the outbreak could be simulated (Jalvingh et al.,

1998b). Specifically in calibration of the model, the information provided by the operational epidemiology team was essential (Stegeman et al., 1997).

Stegeman et al. (1997) concluded already, that measures prescribed in the EU-regulations seemed insufficient to end the epidemic in a pig dense region such as the south east of The Netherlands. Additional measures seemed necessary, among which pre-emptive slaughter of contact and neighbourhood herds, reduction of truck movement for the market support systems that were in operation, and a strict hygiene programme. The simulation model showed the possible extent of the effect of the measures (Jalvingh et al., 1998b). These results give rise to the development of new approaches in the control of classical swine fever. For the instrument of pre-emptive slaughter a protocol should be worked out, including criteria on the basis of which it is decided to use it, much comparable to the one for the vaccination strategy.

A large problem for the evaluation of the epidemic was, that for several episodes the basis and arguments for decisions taken have not always been recorded unambiguously. A conclusion of the evaluation was, that clear control plans were needed, as well as regular training with their operation (Ministry of Agriculture, Nature Management and Fisheries, 1998). A clear and effective crisis organisation is necessary, and absolute clarity should be created on organisation and strategy for all involved, and especially for the pig producing sector itself. An important finding was, that information needed for the planning and execution of control measures and the management of the control exercise was not always directly at hand. It often took time to find the information needed and to link up databases where necessary. From the policy-makers complains were heard on the adequacy of the information provided to base policy decisions on at the central Ministry level. A well integrated, up to date information system, capable of providing information in designated formats for the various purposes is highly needed. Such an information system should be based on a solid and reliable central farm registration and management system, linked to a proper identification and registration system, combining records of location and transfers of animals. Preferably the system should also contain information on health aspects and health status of individual herds. Much of the information is in fact available. The challenge is to integrate the databases into one, readily accessible (at least for relevant authorities) system. Reliability is a key quality of the system; in the description of the National Animal Health Monitoring System of the USA King (1988) stated, that decisions will never be better than the information used to take them.

In principle the InterCSF model, used in the present outbreak as an evaluation tool, could be linked to the information system, and be developed further into an online decision support system. The EpiMan system (Morris et al., 1992), to which the model is related, was designed for such a purpose originally in New Zealand. Important to note is, that models are useful tools in the support of policy and decision making, but can never replace sound reasoning. Models do not represent reality, but illustrate possible trends. Through this, they generate argumentation for decisions to be made. The 1997 outbreak of CSF has provided a wealth of material on the basis of which the InterCSF model will be developed and refined (Jalvingh, 1998a).

The role of the quality of hygiene and preventive measures at farm level on the development of the epidemic was recognised in the evaluation. In the modelling the effect could also be shown (Jalvingh et al., 1998b). This implies, that the individual farmer bears a large responsibility. In The Netherlands, after outbreaks of CSF and Newcastle Disease in

1991 and 1992, this notion sparked the project "Animal Health in Motion" (Julicher et al., 1993), aiming at increasing farmers awareness and sense of responsibility (risk-consciousness) for the health situation in his herd. Part of the ensuing project, which started in 1995, were studies into animal health information tools for farmers, animal health monitoring and possibilities of insurance systems for epidemic diseases (Meuwissen et al., 1997). Although the project itself has ended, several activities are still continued, one of which being the further elaboration of a differentiated levy system based on risk factors for all livestock sectors. Possibilities of recovering the cost of disease control from the sectors were discussed by Davies (1996) and Howe and Whittaker (1997). Dijkhuizen (1999) discusses the implications and consequences extensively.

Awareness among farmers of the importance of proper risk management is considered to be very important. In the project mentioned earlier (Julicher et al., 1993), this aspect received a lot of attention. In the framework of organised disease control programmes in several sectors in The Netherlands, awareness of risks and how to deal with them seem to take root. The recently started IBR eradication programme in the cattle sector is an example of this. Jalvingh et al. (1998a) showed the importance of several risk factors for this disease through the application of the InterCSF model. The results of this study, and of another study carried out within the "Animal Health in Motion"-project (Stegeman et al., 1996) are extensively used in extension.

The decision to involve a research group for the fieldwork from outside the Ministry to ensure an objective view on the events, seemed a good one. As mentioned, assessment of progress and activities in the research team fieldwork was difficult. A factor that may have been related to this is the apparent lack of an evaluation framework. Policy making principles and implementation, and as one of the subjects evaluation of policy measures, deserve more attention within the veterinary profession as well as education and research.

The input from both other teams was essential for the evaluation of the outbreak. The use of epidemiological data and the modelling allowed for quantitative comparison of alternative strategies. This was an essential part in the assessment of appropriateness of measures.

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SIMULATION MODELLING TO SUPPORT POLICY MAKING IN THE CONTROL OF BOVINE HERPES VIRUS TYPE 1

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Further integration of markets within the European Union (EU) will facilitate trade between member states. The diversity of animal health status, however, is still a main reason for restrictions on trade of cattle. Part of these constraints for dairy cattle is due to the agent Bovine herpesvirus type I (BHV1), causing infectious bovine rhinotracheitis (IBR). While several countries within the EU have successfully eradicated BHV1 (Denmark, Finland and Sweden) or have an EU approved national compulsory eradication program (Austria), some other countries still have a high prevalence (Belgium, the Netherlands).

In the Netherlands, about 42% of the dairy cows has antibodies against BHV1 and about 85% of the dairy herds has one or more infected animals (Van Wuijkhuise et al., 1993). At the end of 1998, 12.500 farms were certified BHV1-free, of which 7.300 were dairy farms. As with all Alphaherpesvirinae, BHV1 has the property to induce latent infection. Therefore, an animal, once infected with BHV1, must be regarded as a potential source of the virus and can consequently be considered a risk to BHV1-free herd mates (Pastoret et al., 1984).

To avoid (future) losses due to export restrictions, and diminish the on-farm costs of reduced milk production and abortion caused by BHV1 (Wiseman, 1978), plans were developed to eradicate BHV1 in the Netherlands. Gene-deleted marker vaccines and companion diagnostic tests, are considered valuable tools for the eradication of the virus (Van Oirschot & Kaashoek, 1996; Bosch 1997).

To support policymakers in their decisions on the eradication of BHV1 in the Netherlands, simulation models that evaluate both the epidemiological and economic consequences of various control strategies are under continuous development. Questions these models have dealt with so far were 1) which control strategy should be applied to eradicate the virus (Vonk Noordegraaf et al., 1998) and 2) how to control outbreaks after re-introduction in BHV1-free areas. This paper describes two simulation models that were built to deal with these questions, and gives an overview of results that policy makers have used to decide on the eradication of IBR in the Netherlands.

MATERIAL AND METHODS

There is a wide range of modelling techniques available to help perform economic analysis of animal disease and their control (Dijkhuizen and Morris, 1997). The choice of a modelling technique will depend on a number of factors such as 1) the nature of the problem, 2) the resources available and 3) the availability of data. The two models presented in this paper are both dynamic simulation models, meaning that time is taken into account and the outcome is calculated for a pre-defined set of input variables. The first model presented is deterministic, meaning that one set of input variables

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produces one set of output, whereas the second model is stochastic, taking into account random elements (representing risk and uncertainty with respect to spread and control).

Comparing strategies to eradicate BHV1(state transition model)

Model framework: To compare strategies to eradicate BHV1 from the Dutch cattle population, a state-transition approach was applied (Buijtels, 1997). The key factor in this technique is the transition between disease states in which the unit to be modelled (in this case dairy herds) can be.

The population of dairy herds was divided into a number of mutually exclusive disease states, which were characterised by the ability of the virus to spread within a herd and the initial prevalence of infected and infectious cows. These states were based on (1) the reproduction ratio R_{ind}, which is the average number of infected animals caused by 1 infectious animal within a herd (2) the prevalence of gE-positive cows within a herd, within each value of R_{ind} and (3) the expected number of infectious animals in a herd within each prevalence range.

The value of R_{ind} depended on the vaccination strategy applied to the herd. In the model a herd can either not vaccinate (R_{ind}=5.6; Bosch, 1997) vaccinate with inactivated vaccine (R_{ind}=2.6; Bosch, 1997) or with live vaccine (Rind=1.5). These strategies also affect the number of days that infected animals spread virus, and the amount of virus excreted after reactivation of a latent BHV1 infection. Within each value of R_{ind}, five gE-prevalence classes were distinguished: 0%, ≥0% - <20%, ≥20% - <50%, ≥50% - <80% and ≥80%. Within each prevalence range, there were a few classes of expected number of infectious cows per infectious herd, based on a deterministic SIR-model (De Jong, 1995). Infection of young-stock on dairy farms was not included in the model.

The population of herds in the different states are elements of the state vector, and the probabilities of the herds moving to a different state in the next time period are elements in the transition matrix. By multiplying the current state vector by the transition matrix, the development of the infection over time can be calculated. Three herd types, each with a herd size of 50 dairy cattle, were considered in relation to the number of cows purchased each year (open ≥ 2, open <2 and closed).

The probability of herds becoming infected depends on several factors – requiring that a dynamic element be included in the calculation of the transition probabilities. The routes of virus transmission between herds were purchase of infectious cows, purchase of gE-positive cows which reactivate during transport and so-called other contacts. Furthermore, gE-positive cows can reactivate virus on the farm during stressful moments. The probability of non-infectious herds with disease state s and herd type j to become infected in week t ($pi_{js}(t)$) was calculated as:

$$pi_{js}(t) = 1 - e^{-\left(\sum_{s=1}^{14} \left\{\frac{(\gamma_s + \beta_s)}{N_j} + \frac{\alpha_s}{N}\right\} \times x_s(t-1)\right)} + \mathrm{React}_s(t) \qquad (1)$$

where

γ	=	rate of virus introduction by purchase of infectious cows
β	=	rate of virus introduction by purchase of gE-positive cows which reactivate during transport
α	=	rate of virus introduction from other contacts.
$x_s(t-1)$	=	number of herds with state s in week t-1
N_j	=	total number of herds with which herd-type j has animal contacts
N	=	total number of herds in the population
$React_s(t)$	=	probability of reactivation in a herd that is in disease state s in week t

The input values used for the model were based on results of (field) experiments where possible, and estimates of experts when experimental data were not available. Insight into the impact of uncertain epidemiological and economic input factors was provided through sensitivity analysis.

Control strategies: Each strategy had a threshold value of 5% cow-level prevalence in the national population, below which the remaining gE-positive cows were slaughtered. It was assumed that no

reintroduction of virus occurred thereafter. Five strategies were evaluated, varying from voluntary participation in a vaccination program to compulsory vaccination for all herds.

Costs and benefits of a program: Program costs are associated with vaccination, diagnosis, monitoring and early disposal of gE-positive cows. The benefits of a vaccination program were derived as the reduced economic losses due to IBR. Losses caused by IBR include a lower milk production of gE-positive cows, clinical and subclinical losses from infectious cows and outbreaks at artificial insemination (AI) stations. Potential losses due to export bans were not included in the basic calculations.

The weekly calculated costs and benefits were both discounted by an annual interest rate of 4% (i.e. market interest rate minus inflation). The economic parameter we chose to use to further compare vaccination strategies was the 'pay-back period', defined as the number of weeks after the beginning of the strategy at which the cumulative discounted benefits are equal to the cumulative discounted costs of a program.

Evaluating strategies to control outbreaks in BHV1-free areas (stochastic model)

Model framework: To provide insight into the consequences of BHV1 introduction once free, the dynamic, stochastic and spatial infection spread model InterSpread (Jalvingh et al., 1998) was modified into InterIBR. This model simulates the spread and control of BHV1 after re-introduction into a BHV1-free area. Additions and modifications mainly concerned transmission dynamics of infectious disease, which were modelled both within and between herds, and the detailed use of actual farm data to represent the heterogeneous population of cattle farms.

By using stochastic simulation, probability distributions and random elements could be included, to provide insight into variability of outcome due to risk and uncertainty (e.g. with respect to number of animals sold, duration till detection of the infection and distance of animal contacts). This made it necessary to execute multiple runs with the same set of input values, each run representing a simulated outbreak of BHV1 after re-introduction. In this way, events could turn out more or less favourable than the most likely case. A brief schematic representation of the general framework of InterIBR is shown in Figure 1.

In the initialisation phase, a dataset of farms was loaded into the model, and parameters were assigned to the various spread and control mechanisms. Simulation of a BHV1-outbreak started with introduction of the virus on a pre-defined farm type, after which both transmission within and between farms were simulated. On a weekly base, between-farm contacts off infected farms that may carry virus were simulated, where probabilities of transmitting BHV1 by each of these contacts were steered by the spread within a farm. Next to an infection spread module, monitoring and control measures were implemented to simulate detection of the infection and subsequent control activities.

Farm data: The simulation model contained a dataset of 25 thousand fictive, individual, uniquely identified cattle farms, each with its own characteristics. These herd characteristics were 1) farm type, 2) herd size, 3) open versus closed, 4) yearly number of animals sold for life and 5) geographic location. Each farm in the dataset was assigned a fictitious location (x and y co-ordinate) at random, representing the Dutch situation with an average farm density of 1.5 farms per square kilometre.

Information on cattle farms was obtained from the Dutch Identification and Recording (I&R) system. Summary information on the Dutch cattle farms, used to generate a representative dataset for the simulation model, is presented in Table 1. Four types of farms were distinguished: dairy, beef, veal and miscellaneous (such as suckler herds). Within each farm type, various classes of herd size were distinguished. To get more insight into the potential risk of farms that trade frequently, a special group of miscellaneous farms was defined (miscellaneous 100+), which sells over 100 animals for life per year. Each simulation starts with introduction of infection on a pre-defined farm type.

Transmission within farms: To simulate the on-farm spread of BHV1, a deterministic SIR-model, using the concept of basic reproduction ratio (R_0), was applied (Anderson and May, 1991; De Jong, 1995). With this model, dynamic transitions of animals between the states susceptible

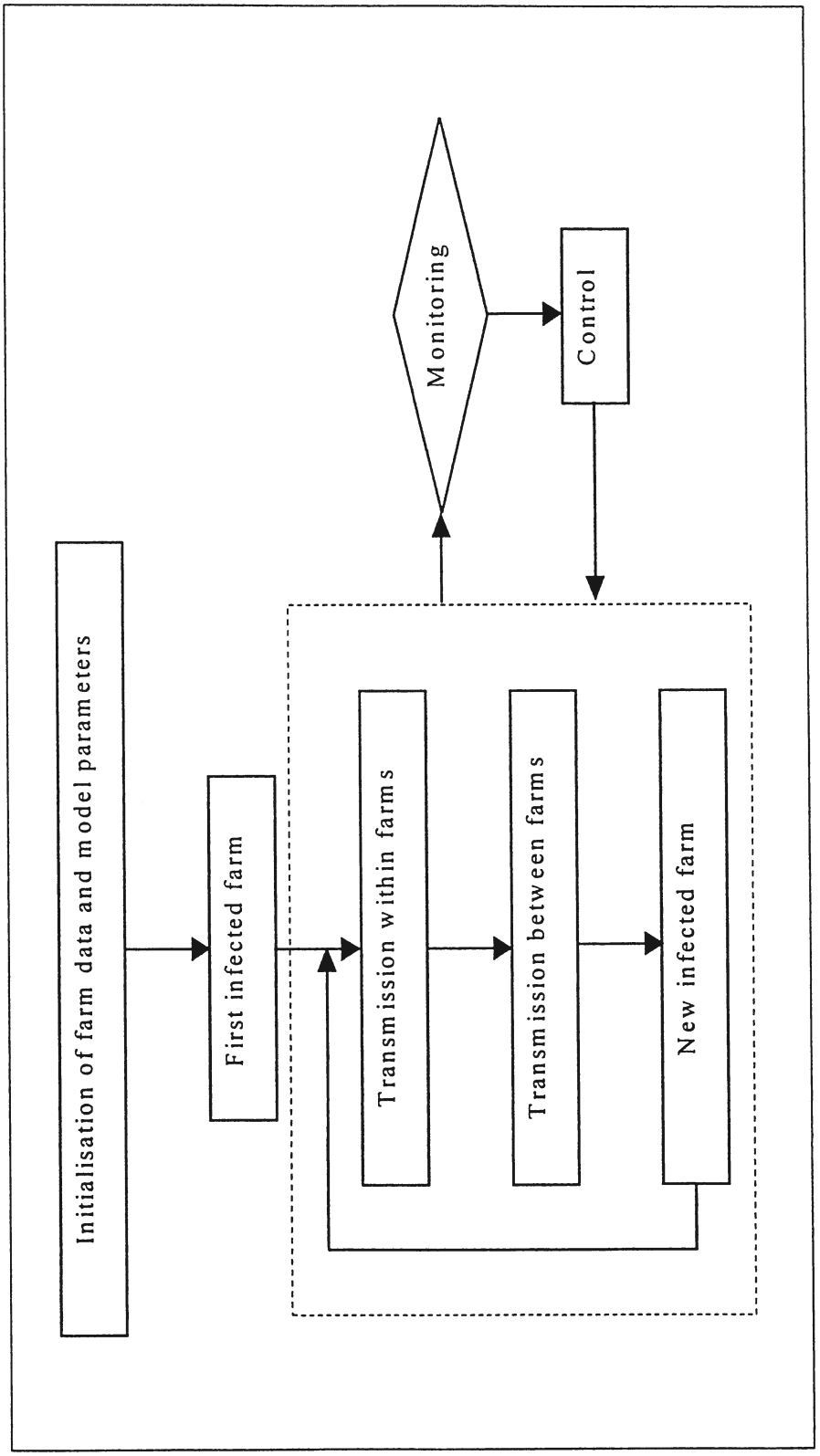

Fig.1. Schematic representation of the general framework of InterIBR.

Table 1. Summary information on Dutch cattle farms (October 1996), used to generate the dataset of individual farms.

Farm type (FT)	Herd size (HS)	% of total herds	% herds closed [b] per FT x HS	% of herds per FT x HS combination per class of number of animals (all ages) sold for life (per year)							
				0	1-2	3-6	7-10	11-15	16-25	26-50	>50
Dairy	<30[a]	11.3	34.0	2.4	4.8	13.8	19.7	27.6	25.1	6.0	0.6
Dairy	30-70[a]	30.3	38.0	1.0	0.8	2.1	2.8	6.6	29.1	52.7	4.9
Dairy	71-100[a]	8.8	34.3	0.5	0.5	0.7	0.8	1.7	6.6	59.7	29.5
Dairy	>100[a]	4.0	23.2	1.2	0.7	0.9	1.3	1.1	3.5	25.3	66.0
Beef	<100	8.9	29.1	100	0	0	0	0	0	0	0
Beef	100-200	0.4	4.4	100	0	0	0	0	0	0	0
Beef	>200	0.2	2.0	100	0	0	0	0	0	0	0
Veal	<100	0.7	1.6	0	0	0	0	0	0	0	0
Veal	100-200	0.3	0	0	0	0	0	0	0	0	0
Veal	>200	1.1	0	0	0	0	0	0	0	0	0
Miscellaneous	<30	26.8	32.2	37.1	24.2	21.4	8.1	4.3	2.8	1.4	0.7
Miscellaneous	30-70	4.9	16.4	11.8	8.7	14.7	14.3	16.3	18.1	11.8	4.3
Miscellaneous	71-100	0.9	13.3	13.0	7.9	6.8	7.1	10.2	15.0	24.1	15.9
Miscellaneous	>100	1.4	5.8	13.8	10.3	8.6	5.1	4.0	9.3	19.8	29.1

[a] based on number of cows > 2 years

[b] no purchase of animals

(S=seronegative), infectious (I=excreting BHV1) and recovered (R=seropositive) were simulated daily, using the true mass action formulation given by De Jong (1995).

Transmission between farms: InterIBR distinguished three routes of transmission between farms: 1) animal contacts, 2) local contacts and 3) professional contacts. Weekly, for each infected farm, the average number of infectious and latently infected animals on that farm was used to assess the risk of transmission to other farms by each of these routes. The simulation processes of infection spread were based on Monte Carlo simulation.

1- For each infectious and latently infected herd, the weekly number of animal contacts was derived stochastically by a Poisson distribution (Vose, 1996). For each animal contact, the distribution over the states S, I and R on the farm was used to assess randomly the state of the animal sold. Next, for each animal contact, a destination farm was selected, based on the animal contact-structure between farm types and the probability distribution for distance classes.

2- For all farms j within a certain radius (default 1 km) of infectious farm i, each week the probability of becoming infected by local contacts was calculated as:

$$p_{c,t,i \rightarrow j} = c_l \times \{1 - e^{-(I_{i,t} \times d_i \times (w_c \times R_{0,within}))}\} \qquad (2)$$

where

$p_{c,t,i \rightarrow j}$	=	probability of infecting at least 1 animal on farm j by local contacts with infectious farm i in week t
c_l	=	catch-on-factor for receiving virus through local contacts
$I_{i,t}$	=	average number of infectious animals on farm i in week t
d_i	=	herd density within a certain radius of infectious farm (no. farms / km^2)
w_c	=	scaling factor for $R_{0,within}$
$R_{0,within}$	=	reproduction ratio within a herd

3- As with animal contacts, a Poisson distribution was used to stochastically determine the number of professional contacts in a certain week. In the model, for each professional contact a destination farm is selected, after which it is determined whether the contact results in BHV1-transmission using:

$$p_{p,t,i \rightarrow j} = c_p \times \{1 - e^{-(I_{i,t} \times w_p)}\} \qquad (3)$$

where

$p_{p,t,i \rightarrow j}$	=	probability of introducing virus on farm j by professional contact with infectious farm i in week t
c_p	=	catch-on-factor for receiving virus through professional contacts
$I_{i,t}$	=	average number of infectious animals on farm i in week t
w_p	=	weighing factor for professional contacts

Monitoring and control: After detection of an infected farm, infection control mechanisms were activated, which could affect the infected farm, neighbouring farms and contact farms. Three control strategies were explored: a so-called basic strategy (strategy I), a strategy with fast removal of infected cattle (strategy II), and a strategy with vaccination of infected farms (strategy III).

In the basic strategy, monthly bulk milk tests and half yearly serological tests were carried out on dairy farms and miscellaneous farms respectively. No monitoring was implemented on beef and veal farms. As soon as a farm is tested positive, no animal contacts on and off the farm were allowed, and extra hygiene measures were taken to prevent transmission. Furthermore, after detection, animal contacts on and off the infected farm were traced. Traced herds, and herds within a 1-km radius were put under surveillance for a 4-week period. Infected farms did not need to cull their positive animals immediately, but should do so within five years.

Strategy II differed from the basic strategy such that all infected animals on detected farms were removed within 4 weeks after the on-farm spread of BHV1 had ended. Strategy III implemented, in addition to the basic strategy, a half yearly vaccination program for all animals on infected farms.

Costs of infection and control: For each iteration, InterIBR generated output to calculate economic consequences of re-introduction of BHV1. These are related to 1) losses due to infection and 2) costs of control. Losses due to infection include reduced production (milk or growth), extra feeding costs, abortion and mortality, taking into account clinical and subclinical infection. (Wiseman et al. 1978; Hage et al. 1998). Costs for control are related to serological tests, vaccination, culling, and open places after culling.

RESULTS AND DISCUSSION

Comparing strategies to eradicate BHV1 (state transition model)

Some results of the state-transition model, comparing strategies to eradicate BHV1 in the Netherlands, are shown in Table 2. The first column displays the number of weeks before the national prevalence of gE-positive cows reached the culling threshold value of 5%. Compulsory vaccination (Strategy II) leaded to a prevalence of 5% after 288 weeks. Strategies III, IVa and IVb, which assumed exceptions from compulsory vaccination, resulted in a prevalence of 5% after about 240 weeks of the vaccination program. Preceding Strategy III with two years of voluntary participation in the vaccination program (Strategy V), prolonged the period to 5% prevalence by 71 weeks.

The total costs (of the vaccination program, incurred in the period presented in the first column) are shown in the second column. It appeared that a compulsory vaccination program for all herds (Strategy II) incurred by far the highest costs – whereas Strategy V (with two years of voluntary participation) incurred the lowest. When a national prevalence of 5% gE-positive cows was reached, the model assumed that the last positive cows in the population had to be detected, so that they could be culled. The costs of testing and culling are not included in the second column of table 2. Detection was done by testing serum of all cows in the non-certified herds – resulting in the costs presented in the third column of Table 2.

Table 2. Epidemiological and economic outcomes of different vaccination strategies for infectious bovine rhinotracheitis in the Netherlands.

	Weeks until national prevalence of gE-positive cows is 5%	Costs to 5% (Million Dfl)	Costs for program-required culling (Million Dfl)		Pay-back period (weeks)
			Testing	Cow losses	
Strategy I	Does not lead to eradication of IBR				
Strategy II	288	320	25.9	56	598
Strategy III	241	225	6.0	55	405
Strategy IVa	241	219	6.0	55	397
Strategy IVb	242	217	5.9	56	394
Strategy V	312	197	5.5	51	400

Strategy I	Voluntary program, 30% or 50% participation.
Strategy II	Compulsory vaccination for all farms.
Strategy III	As II, exemptions for certified herds.
Strategy IVa	As III, exemptions for youngstock on closed herds.
Strategy IVb	As III, exemptions for gE-negative animals on closed herds.
Strategy V	2 years as I with 30% participation, followed by III.

Because Strategy II did not include certification, all cows in the population had to be checked, incurring large costs of Dfl 25.9 million. The costs of culling of the last 5% gE-positive cows in the population are presented in the fourth column. At the time a national prevalence of 5% gE-positive

cows was reached, the percentage of herds with a within-herd prevalence between 1-50% varied from 16-21% and the percentage of herds with a within-herd prevalence higher than 50% varied from 1-2%.

The pay-back period, earlier defined as the number of weeks after the beginning of the strategy st which the cumulative discounted benefits were equal to the cumulative discounted costs of a program, is presented in the fifth column of Table 2. Preference is given to a vaccination strategy with a short pay-back period. Strategy II had by far the longest pay-back period, and is therefore economically not attractive. Strategies IVa and IVb turned out to be the most attractive from this point of view.

Evaluating strategies to control outbreaks in BHV1-free areas (stochastic model)

Due to stochastic processes and heterogeneity of farm characteristics, a wide variation in the expected number of secondary infected farms could be seen when applying the basic strategy (Table 3), as shown by the various percentiles ($x_{0.25}$ - $x_{0.99}$).

Table 3. Mean, some percentiles and 'maximum' number of secondary infected farms after first introduction of BHV1 on a certain farm type, when applying the basic monitoring and control strategy.

	First introduction on				
	Dairy farm	Beef farm	Veal farm	Miscellan. 100+	Miscellan.
Mean	0.9	0.1	0.2	21.6	1.4
$x_{0.25}$	0	0	0	11	0
$x_{0.50}$	0	0	0	21	0
$x_{0.75}$	1	0	0	30	1
$x_{0.90}$	2	0	0	38	5
$x_{0.95}$	3	0	1	43	7
$x_{0.99}$	6	1	3	56	14
Max.	31	12	20	89	23

The mean number of secondary infected farms showed that, after first introduction of BHV1 on a dairy, beef or veal farm, virus will be transmitted on average to less than one other farm. Re-introduction on a miscellaneous 100+ farm had much more impact, with on average 21.6 secondary infected farms. The percentiles were based on the total number of iterations, however the maximum number of infected farms shown in the table is the outcome of only one 'extreme' iteration. Therefore, it has to be kept in mind that this maximum is not the absolute maximum number of infected farms that can occur (based on the model assumptions), but is just an illustration of a possible 'worst-case scenario'.

Applying the basic strategy, first introduction on a dairy farm had a 99% probability of causing 6 secondary infected farms at most. In case of first introduction on a miscellaneous 100+ farm, the 99% percentile was 56 infected farms. When BHV1 was introduced on a beef or veal farm (which only sell animals for slaughter), there was a very high probability that no virus was transmitted to other farms. For all farm types with first introduction, there turned out to be a small probability of having a big increase in the number of secondary infected herds.

However, not all secondary infected farms counted in Table 3 suffered a major outbreak. On average 55% of the infected farms became infected by purchase of one or more latently infected animals, without the other animals on the farm becoming infected. Other infected farms suffered a minor (10%) or major outbreak (35%).

Table 4 shows the mean and some percentiles of the expected economic consequences of BHV1 re-introduction, caused by losses due to infection and costs of control. The mean costs varied from about Dfl 1000 after first introduction on a beef farm, to Dfl 300.000 when re-introduction occured on a miscellaneous 100+ farm. As shown by $x_{0.95}$, $x_{0.99}$ and max., the few percent worst-case scenarios resulted in large economic consequences of re-introduction, with a maximum of Dfl 929.000 after first introduction on a miscellaneous 100+ farm.

Table 4. Mean, some percentiles and 'maximum' of total economic consequences (X1000 Dfl) after first introduction of BHV1 on a certain farm type, applying the basic monitoring and control strategy.

	First introduction on				
	Dairy farm	Beef farm	Veal farm	Miscellan. 100+	Miscellan.
Mean	44	1	5	300	22
$x_{0.25}$	16	0	0	159	3
$x_{0.50}$	36	0	2	297	8
$x_{0.75}$	61	0	5	438	22
$x_{0.90}$	86	0	5	538	68
$x_{0.95}$	110	1	9	611	108
$x_{0.99}$	186	20	81	765	154
Max.	408	227	245	929	349

Compared to the basic strategy, application of strategy II (rapid removal of infected animals) and III (half yearly vaccination of infected farms) had no significant impact on the total number of secondary infected farms. This is because infected farms that were detected, were not allowed to have any off-farm animal contacts in the basic strategy. This seemed to be sufficient to exclude the risks for other farms. However, total costs were increased on average by 10% for strategy II, whereas a strategy with rapid removal of infected cattle resulted in a doubling of total costs.

Table 5 shows the outcome for the basic strategy and the results of some sensitivity analysis, after first introduction on a dairy and miscellaneous 100+ farm type. When doubling the risk of transmission by local and professional contacts, the number of infected farms and total costs only increased slightly. In the basic scenario, all farms within a 1 km radius of a detected farm were put on surveillance for 4 weeks. Leaving out this control measure only had a minor effect on the number of secondary infected farms, whereas the total costs due to re-introduction of BHV1 decreased about 30%, because of the reduced costs for testing all these farms.

Doubling the frequency of monitoring on miscellaneous farms to 4 times a year, reduced the mean and in particular the higher percentiles of the number of secondary infected farms. After first introduction on miscellaneous 100+, the 99% percentile of number of secondary infected farms was reduced from 56 to 36. As a consequence, total costs of an outbreak were reduced, however, the yearly costs for standard monitoring increased by Dfl 5.42 million. Less frequent bulk milk testing resulted in an increase in the size of an outbreak, both after introduction on a dairy farm and on a miscellaneous 100+ farm.

Of the other scenarios in Table 5, the biggest effect on the size of an outbreak could be seen from a 10% non-compliance with the ban on live sales off infected farms and farms on surveillance ('illegal animal transports'). Although only a small economic effect could be seen, the number of secondary infected farms accordingly increased three-fold, which can be explained by the increased purchase of latently infected animals.

Table 5. Mean, 95% and 99% percentile of number of secondary infected farms and economic consequences (x1000 Dfl.) for the basic strategy and some alternative scenarios, with first introduction on a dairy or miscellaneous 100+ farm.

	First introduction on					
	Dairy			Miscellan.100+		
	Mean	$x_{0.95}$	$x_{0.99}$	Mean	$x_{0.95}$	$x_{0.99}$
Basic scenario						
Number of infected farms	0.9	3	6	21.6	43	56
Total losses and costs	44	110	186	300	611	765
Double risk local contacts						
Number of infected farms	1.0	3	7	22.1	46	57
Total losses and costs	46	114	186	307	616	788
Double risk professional contacts						
Number of infected farms	1.0	4	7	22.6	44	56
Total losses and costs	46	118	186	307	627	766
No surveillance zone						
Number of infected farms	0.9	3	6	22.1	45	56
Total losses and costs	36	98	154	197	439	530
Serological tests 4 times a year						
Number of infected farms	0.7	2	3	15.1	33	36
Total losses and costs	41[1)]	98[1)]	126[1)]	203[1)]	385[1)]	462[1)]
3-monthly bulk milk tests						
Number of infected farms	1.5	5	13	28.1	58	78
Total losses and costs	60[2)]	144[2)]	257[2)]	372[2)]	738[2)]	930[2)]
10% 'illegal' animal transports						
Number of infected farms	3.8	14	50	67.7	152	217
Total losses and costs	49	131	247	354	750	843
10% more selling of animals						
Number of infected farms	1.0	3	7	23.3	57	74
Total losses and costs	45	114	192	321	675	852

[1]Excluding the increase in yearly standard serological monitoring costs from 5.42 to 10.85 million Dfl.
[2]Excluding the decrease in yearly standard bulk milk monitoring costs from 9.97 to 3.32 million Dfl.

FINAL REMARKS

The models presented in this paper were both used to support policy makers in their decisions on the eradication of BHV1 in the Netherlands. Integration of an epidemiological and economic component, provides insight into both the infection-control effectiveness and cost-effectiveness of various control strategies of BHV1.

Building these models, assumptions had to be made to create a simplified mathematical representation of the real-world system of virus spread within and between cattle farms. Furthermore, estimates had to be made for economic and epidemiological parameters because reliable data were not always available. When evaluating the results given by the models, these assumptions should be kept in mind. However, once available, simulation models provide a flexible tool to quantify the impact of changed parameters on the complex system of transmission dynamics and economics.

Important differences between the two models concern modelling technique and the assumptions made to model transmission of the infection. The model used to compare strategies to eradicate BHV1 used the deterministic approach, where a given set of input values produces one single outcome. Furthermore, only dairy farms with average herd size were taken into account, and contacts between

herds were random. In the model to evaluate strategies to control outbreaks in BHV1-free areas, stochastic elements were included, thereby providing insight into variation of outcome and probabilities of worst-case scenarios. Also, other farm types were included and more heterogeneity between farms was considered, like herd size, geographic location and animal contacts.

Future research will focus on the application of the stochastic model InterIBR, to support decision making during the current BHV1 eradication program in the Netherlands. Data that come available from the BHV1 monitoring program will be used for further estimation of underlying parameters and external validation of the model. Given the pattern so far, the model will be used to predict the future pattern of the eradication.

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QUALITY ASSURANCE

USING LOGISTIC REGRESSION TO MODEL THE SENSITIVITY AND SPECIFICITY OF A TEST AIMED AT IDENTIFYING DAMS CARRYING BOVINE VIRAL DIARRHOEA VIRUS (BVDV) INFECTED FOETUSES

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Diagnostic tests play a major role in the medical decision making process. One way of evaluating and expressing the performance of such tests is by estimating those operational parameters called sensitivity (Se) and specificity (Sp). As described in most introductory epidemiologic texts, a straight-forward way to obtain estimates of sensitivity and specificity is by using a 2 x 2-way table, with rows denoting outcome of the test (positive/negative), and columns denoting the true state of nature (diseased/non-diseased). Within this arrangement, an estimate of Se is achieved by calculating the proportion of test positives among those truly diseased, and for Sp, the proportion of truly healthy among test negatives.

In a recent study one objective was to estimate the sensitivity and specificity of an indirect BVDV antibody ELISA (SVANOVA Biotech, Sweden) when used to identify dams pregnant with foetuses persistently infected with bovine viral diarrhoea virus (BVDV) (Lindberg et al., 1999). Trading such dams (hereafter denoted PI-C) is an efficient way of spreading BVDV between herds, and should therefore be prevented. PI-C's are seropositive and virus negative, and therefore qualitatively indistinguishable from other seropositive cattle. However, it has been shown that there is a quantitative difference, in that their antibody titres tend to be higher than for dams with healthy foetuses. This difference is probably a result of the dam being continuously boostered by the PI foetus throughout the pregnancy. In the above-mentioned ELISA, antibody titres are quantitatively reflected as optical density (OD) values. Consequently, a high OD value in the dam indicates a risk of the foetus being PI. This finding has been used in Sweden on an empirical basis to prevent trade of PI-C's as well as in connection with interventions in infected herds. The cut-off value used to indicate a positive PI-C-status has been 1.0 (0.8 in some areas) whereas the regular cut-off value used to classify blood samples as antibody positive is 0.20.

In order to evaluate Se and Sp for the PI-C test, a couple of questions had to be addressed. To start with, there was a need to investigate how Se and Sp were affected by the time in gestation when the dam was tested. Persistent foetal BVDV infections are only established

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when susceptible dams are infected before 120 days of pregnancy, so a test performed before this stage of gestation can not provide any information. Furthermore, based on field observations, it was suspected that the test would show a better overall performance in late pregnancy. Although sensitivity and specificity should be regarded as inherent characteristics of a test which are unaffected by changes in prevalence (given that the test is used in a population which is comparable to the one in which it has been evaluated), there is an additional aspect on this matter. Se and Sp may also be affected by the "strength of the signal" which is to be detected by the test. One example is the increased sensitivity of antigen tests for canine heartworm with increasing worm burdens (Courtney et al., 1988). Using modelling terminology, one may regard this as an inter-action between the disease condition and a covariate, in this case the degree of worm infestation. In the BVDV study, the covariate in question is gestational stage.

Another factor which needed accounting for was the type of specimen used. Both milk and serum samples are used in routine. However, serum is regarded as more stable, both from a practical and an analytical point of view. The former refers to its robustness with respect to mishandling in the field, and the latter to the fact that the OD value in serum is not affected by stage of lactation (i.e. milk yield), as is the case when milk is used as specimen (Niskanen et al., 1989). Another "problem" in the study in question was that clustering could be expected, since the data used consisted of individual measurements on animals from several herds.

The character of the problem, in connection with the features of the data, made it logical to use a modelling framework, where covariates can be included and clustering can be accounted for. The method of choice was logistic regression, which can be used to obtain smoothed estimates of sensitivity and specificity (Coughlin et al., 1992). In such a model, the dependent variable is the dichotomized outcome of the test and the true disease status (the gold standard) is included as an explanatory variable. Depending on type of test/type of disease other covariates may be included, as well as interactions between those. Furthermore, using the framework of generalized linear models, random effects can be included.

This paper compares and discusses the results of the modelling approach with the outcome of a traditional 2 x 2-table calculation, in terms of point- and interval estimation.

MATERIAL AND METHODS

Data extraction and editing

The data set used consisted of 2,162 cow-calf pairs in 126 herds. All cows had been tested for antibodies to BVDV with a positive result during their pregnancy, and their offspring had been subjected to a test for both antibody and virus. The gold standard used was the calf's virological status, i.e. whether BVDV had been isolated from its serum or not. Viruspositive calves were found in 281 records.

The cow-calf pairs were found by targetting herds which had been subjected to BVDV clearance between January 1994 and February 1997. The clearance protocol routinely followed in Sweden starts with a screening on all animals over 10 weeks of age in the herd, followed by testing of all calves born during the subsequent year (Alenius et al., 1997). For

all calves tested (i.e. both for calves found to be virus positive and -negative) during a herd's follow-up period, the identity of the mother was retrieved from the official pedigree recordings. Thereafter, all records were selected where the mother had; a) been tested for antibodies during the pregnancy in question and b) had a positive antibody result. All data concerning results from BVDV analyses (serological and virological) were retrieved from the database of the Swedish BVD scheme. For herds where all selected records consisted of only virus negative *or* virus positive calves, all records were excluded.

The final data set included the following variables for each record; cow identity, days pregnant at testing, the result of the antibody test (reported as an OD value), specimen used (milk or blood), lactation number (age indicator), breed, season when the test was performed and the BVDV status of the calf (the gold standard). The variable "days pregnant at testing" was categorised into six groups corresponding to number of months pregnant at testing. Months 1-3 were merged since a test result during that period could not be expected to have any diagnostic value, i.e. a persistent infection may not yet have been established in the foetus. Months 8 and 9 were merged in order to avoid modelling problems due to lack of observations within certain covariate combinations.

Twelve dichotomous variables were created to indicate whether a dam was positive or negative with respect to a certain cut-off value. These twelve variables were later used as dependent variables when modelling sensitivity and specificity at each respective cut-off, and to denote the test result (positive or negative) in the 2 x 2 tables. The cut-off values used ranged from 0.5 to 1.6, with increments of 0.1.

The prevailing custom within the Swedish BVDV scheme is to cull virus positive animals without a re-test if their clinical status support a persistent infection. In all other cases, persistent infection should be confirmed to avoid the culling of healthy but transiently infected individuals. In this data set, 41 of the 281 virus positive calves were confirmed to have a persistent infection by re-testing, with a result confirming their status in all cases. Furthermore, 315 virus negative calves were retested, and confirmed to be virus negative. From a gold standard point-of-view it should however be noted that the group of virus positive calves which were not retested may have included some transiently infected individuals.

Diagnostics

All test results reported to the BVD scheme database are based on samples analysed at the National Veterinary Institute within the routine BVDV diagnostics. Samples submitted in routine are discarded after the requested analyses have been performed and results have been reported to the scheme database, but can be re-tested if problems with the assay are detected before that.

The level of antibodies to BVDV in sera and in milk samples is determined in an indirect ELISA (SVANOVA Biotech, Uppsala, Sweden) (Juntti et al., 1987 and Niskanen et al., 1989). A standardisation procedure used for this assay is described in the paper by Lindberg and colleagues (1999). One objective with the standardisation is to allow comparability of reported results over time.

Detection of BVDV from sera is performed with an immunoperoxidase test, essentially

according to Meyling (1984). The routine procedure in connection with clearing herds from BVDV is to perform virus isolations only on samples which are antibody negative or weakly antibody positive (OD ≤ 0.20). With the assay in question, young animals should not be tested before 10-12 weeks of age in order to avoid maternal antibodies interfering with the virus isolation.

Statistical methods

Logistic regression models: Twelve logistic regression models were constructed (Se/Sp models), where the dependent variable for each model was the dichotomous test result with respect to a given cut-off value (from 0.5 to 1.6). The test result was regarded as positive (Y=1) if the OD value was larger than or equal to the cut-off value, and negative (Y=0) otherwise. The general model used is represented by the following formula:

$$\text{Logit Pr}\,(Y=1|X_1 \ldots X_k) = \alpha + \Sigma\, \beta_k X_k + \mu_m Z_m + \varepsilon_{km}$$

where the dependent variable Y is the dichotomous test result at a specific cut-off value and X_k denotes the explanatory variables. Z_m is the random effect included to account for dependence between observations on individuals from the same herd and ε_{km} is the remaining unexplained variation.

The gold standard (BVDV status of the calf; 0= virus negative, 1= virus positive) was included as one of the explanatory variables (X_1) in the model. In addition to the gold standard, candidate explanatory variables in the initial model were age (expressed as lactation number) and breed of the dam, gestational stage at testing, specimen used and season at testing. The variables were included on basis of their potential effect as confounders (age, breed, season) or effect modifiers.

The data were analysed using the SAS macro GLIMMIX (Littell et al., 1996). The full model was reduced using a stepwise backwards elimination procedure where the explanatory variables were tested for significance ($p< 0.05$) at each step on basis of Wald's test. Possible interactions between the gold standard and all candidate variables in the model were tested for and included if significant. In addition, the interaction between type of specimen and gestational stage (in practice corresponding to stage of lactation) was also tested for. It was decided to use the same set of explanatory variables in all 12 models, even though lactation number was found to be significant only in the models where the cut-off ranged between 0.8 and 1.0. The overall fit of the model was assessed by means of the Hosmer-Lemeshow test (Hosmer and Lemeshow, 1989).

The output from the model is reported as a log-odds. The odds can be written as $(p/(p-1)$, where p is equal to Se when X_1 (the gold standard) =1, and to 1- Sp when X_1=0. The inverse link of the least square means was used to obtain the probabilities in question in terms of the original scale. Confidence intervals were also computed on the logit scale and converted by the inverse link function.

Cross-tabulation: Twelve 2 x 2 tables were constructed, one for each cut-off level used in the logistic regression models. The tables were further stratified on the 6 pregnancy month categories, resulting in 72 two-way tables. Point estimates of sensitivity and specificity with their associated exact binomial confidence limits were calculated for each table, using the

statistical package Stata (Stata Corp., Texas, USA).

Evaluation of test performance: Test performance was evaluated by the construction of response operating curves (ROC). The ROC curve is a plot with the true positive fraction (Se) on the Y axis, and the false positive fraction (1-Sp) on the X axis. It is used for tests measured on a continuous scale and gives an image of test performance over the whole range of possible cut-off levels. A good test will describe a curve which lies close towards the upper left hand corner (Se close to 1 and (1-Sp) close to 0). As a comparison, a test which describes a straight line will have a discriminatory power which is equal to that of tossing a coin. In the present study, each ROC-curve was drawn from 12 points corresponding to the cut-off values ranging from 0.5 to 1.6, moving from right to left along the curve.

RESULTS

Descriptive statistics for the variables used in the logistic regression models are given in tables 1a-b.

Table 1a. Distribution of virus positive- and negative calves over candidate explanatory variables, $n = 2,162$

Variables	**Categories**	**BVDV status of calf**	
		Virus positive *(%)*	Virus negative *(%)*
Months pregnant at testing	1-3	25 *(1)*	721 *(33)*
	4	21 *(1)*	206 *(10)*
	5	33 *(2)*	204 *(9)*
	6	52 *(2)*	192 *(9)*
	7	58 *(3)*	211 *(10)*
	8-9	92 *(4)*	347 *(16)*
Specimen	Milk	48 *(2)*	441 *(20)*
	Blood	233 *(11)*	1,440 *(67)*
Lactation number	1	9 *(0.5)*	37 *(2)*
	2	69 *(3)*	441 *(20)*
	3	87 *(4)*	487 *(23)*
	>3	116 *(5.5)*	916 *(42)*
Season at testing	January – March	88 *(4)*	738 *(34)*
	April – June	115 *(5)*	709 *(33)*
	July – September	36 *(2)*	115 *(5)*
	October - December	42 *(2)*	319 *(15)*
Breed	SRB[a]	145 *(7)*	910 *(42)*
	SLB[a]	114 *(5)*	859 *(40)*
	Beef breeds	2 *(0.1)*	12 *(0.5)*
	Others	20 *(0.9)*	100 *(4.5)*

[a] SRB; Swedish Red and White
SLB; Swedish Holstein

Final Se/Sp-models

The final models included significant interactions between the gold standard and

gestational stage at testing, and between the latter and type of specimen. In addition to the variables involved in the interactions, the models included lactation number. The effects of breed and season were not significant. Furthermore, the effect of lactation number was only significant in the models corresponding to the cut-off range from 0.8 to 1.0.

Test performance in relation to gestational stage at sampling

The influence on test performance by stage of pregnancy at sampling was reflected in the model by a significant interaction between the calf's BVDV status and gestational stage at testing. This feature is visualised by the response operating characteristic (ROC)

Table 1b. Distribution of virus positive and –negative calves over the test result (i.e. the dependent variable) at each investigated cut-off.

Cut-off	Test result	BVDV status of calf		Cut-off	Test result	BVDV status of calf	
		+	-			+	-
0.5	+	262	1,563	1.1	+	167	484
	-	19	318		-	114	1,397
0.6	+	258	1,398	1.2	+	133	343
	-	23	483		-	148	1,538
0.7	+	249	1,235	1.3	+	102	234
	-	32	646		-	179	1,647
0.8	+	234	1,026	1.4	+	78	158
	-	47	855		-	203	1,723
0.9	+	212	828	1.5	+	50	107
	-	69	1,053		-	231	1,774
1.0	+	189	642	1.6	+	40	70
	-	92	1,239		-	241	1,811

curves in Fig. 1, which also give a visual comparison of the performance as estimated by the logistic regression, and for that estimated in the traditional way. The graphs are limited to pregnancy months 6-9, which proved to be the period in which the test performs the best. In very late pregnancy, all cases were classified in the same way at the lowest and highest cut-off values (only correctly or only incorrectly). In such situations, the logistic model will fail to give estimates of p. However, since the practical implication of this is a sensitivity of 0 or 1 (depending on which end of the scale this occurs), those were the values used to draw the graphs.

Difference in point- and interval estimates

The magnitude and direction of the differences in estimates between the logistic regression method and the traditional method are shown in Fig. 2a-d. The results presented are limited to pregnancy months 5-9. Furthermore, only cut-offs ranging from 0.7 to 1.1 are shown, which is the range of interest when using the test in the field. Differences in point estimates of Se

and Sp both show a systematic pattern. For Se, the absolute deviation increases with higher cut-off values ; increasingly positive in month 5, and increasingly negative for months 6-9 in pregnancy. For Sp, the difference is very small, except in months 8-9, where the traditional method yields a constantly higher estimate. Regarding difference in width of confidence intervals, the deviation for estimates of Se is minor (month 8-9/cut-off 0.7 is an artefact; this is where the logistic regression failed to give estimates since all cases were correctly classified). However, for Sp, the traditional method shows a negative difference for all months and cut-offs.

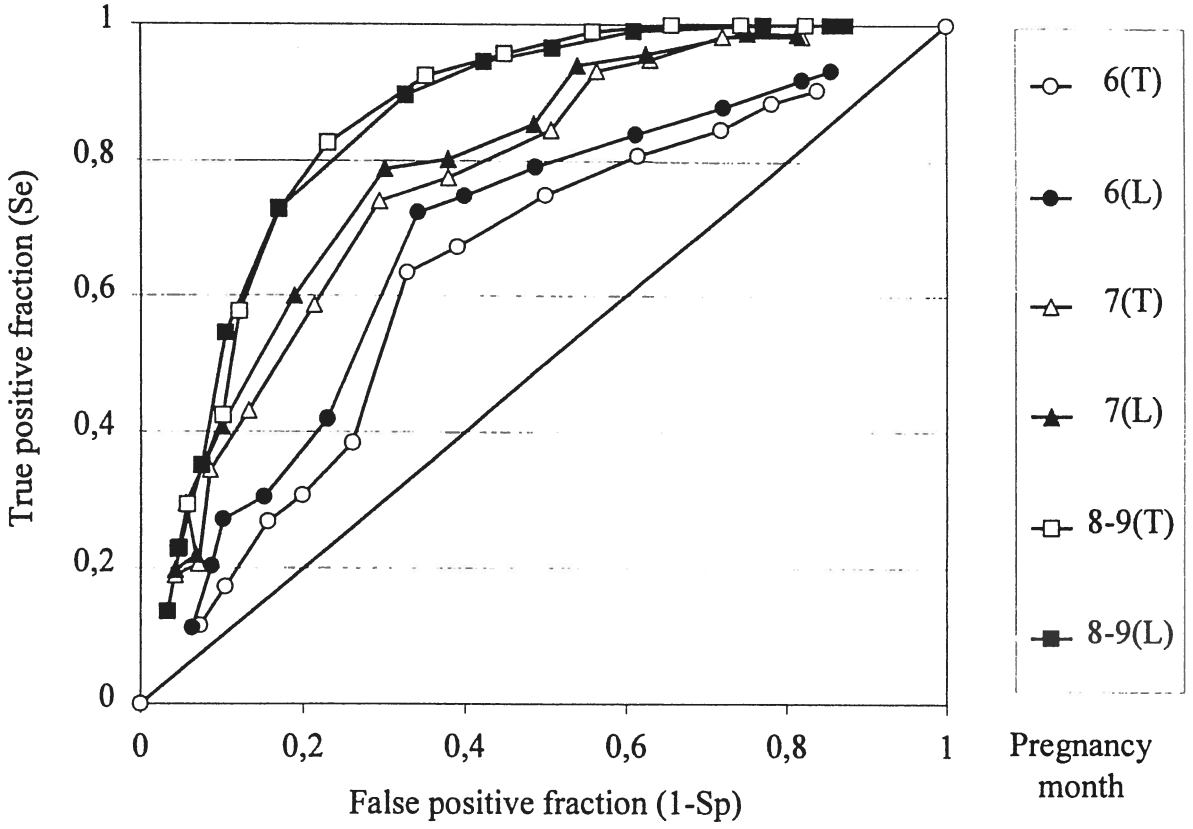

Fig. 1 ROC curves demonstrating test performance in relation to gestational stage at testing for months 6-9. Estimates yielded by logistic regression = (L) and traditional calculation = (T). As comparison, the straight diagonal line indicates the performance of a 'test' which is based on a random process, e.g. tossing a coin.

DISCUSSION

Theoretically, the two methods used in this paper should give identical point- and interval estimates for Se and Sp within each pregnancy month if there was no significant effect of other covariates, and no clustering within herds. In other words, the simple stratifed 2 x 2 table analysis is comparable with a fixed effect model with no other covariates than the gold standard and the effect modifying variable 'gestational stage at sampling'. To get identical results, this would also require that Se and Sp were modelled separately. That is, for the Se model, only data from dams carrying virus positive calves would be used, and similarly, only data from dams with healthy calves would be used for the Sp model.

Generally speaking, there will be two reasons for the detected differences in point- and interval estimates. At first there is the inclusion of other covariates in addition to the gold standard, gestational stage and their interaction. The additional variables here are specimen and its interaction with gestational stage, and the main effect of lactation number. Secondly, it is the inclusion of a random effect of herd. Since mean and variance are related in binomial data, both parameter estimates and variance may be affected (Lam et al., 1996).

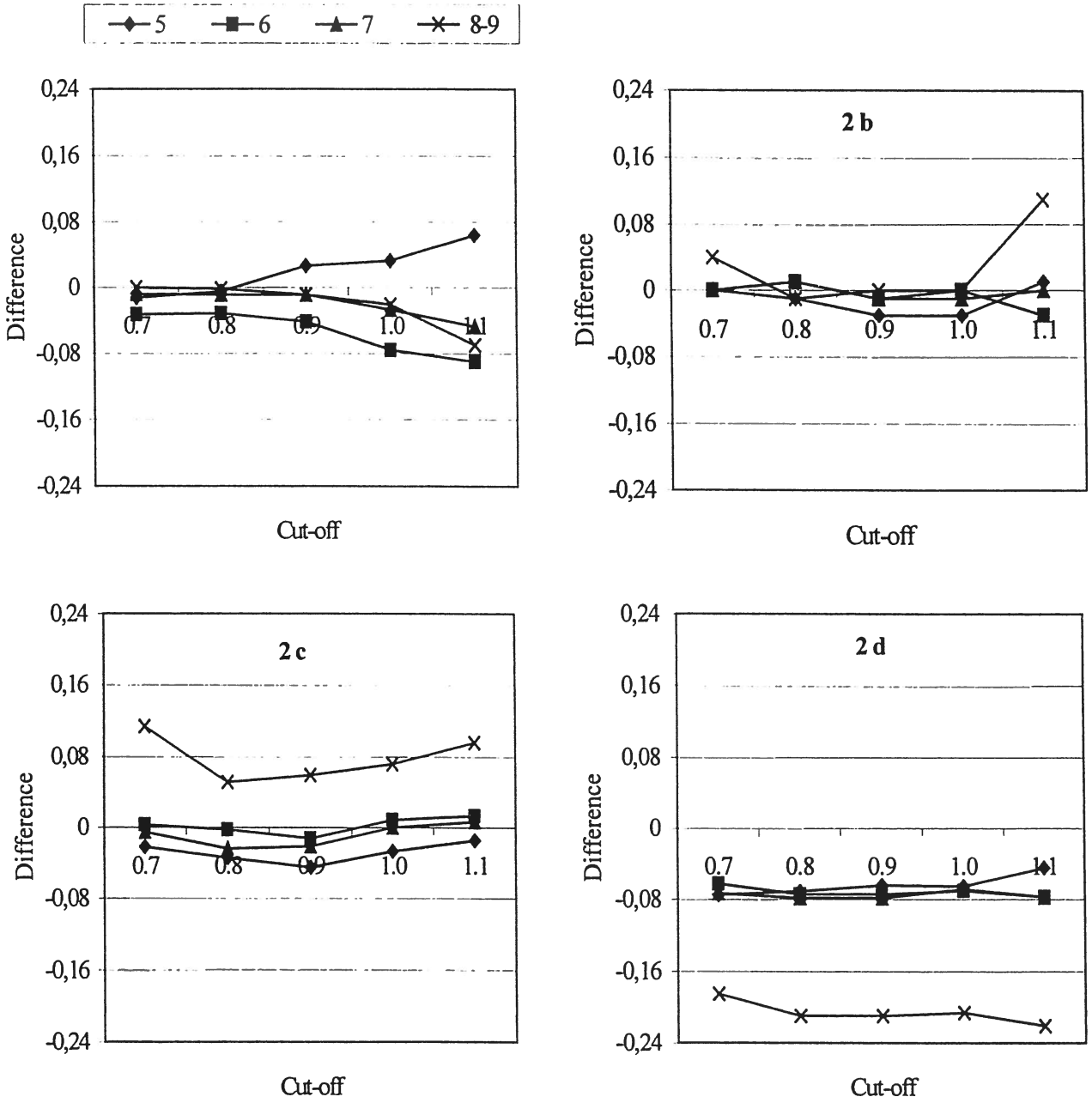

Fig. 2. Difference (T-L) between estimates achieved with traditional calculation (T) and those obtained with logistic regression (L), restricted to pregnancy months 5-9 and to cut-offs ranging from 0.7 to 1.1. 2a) difference in point estimate of Se, 2b) difference in width of confidence interval, Se, 2c) difference in point estimate of Sp, 2d) difference in width of confidence interval, Sp.

The deviations in point estimates of Se and Sp both show a systematic pattern, although different. For Se, the traditional method gives a deviation which is increasingly negative for the last four months in pregancy, indicating underestimation of Se with respect to the logistic model, whereas there is an overestimation of Se in early pregnancy. For Sp on the other hand, modelling seems to have little effect on the point estimates, except in the last months of pregnancy (months 8-9), where the traditional method consistently overestimates Sp. To properly disentangle the source of these patterns, one would have to perform the same modelling exercise, with and without a random effect, and exclude one covariate at a time - something which was not done for this occasion. It is, however, possible that one reason for the effect on estimates in late pregnancy is to be found in the interaction between type of specimen and stage of lactation (reflected by gestational stage in the model). As the lactation approaches its end, the concentration of antibodies in milk will increase, and consequently also the OD value. There is a sigmoidal relationship between the antibody concentration and the OD value in the higher regions, and therefore the OD value will increase relatively less if already high, like in dams with infected foetuses. This may result in a smaller difference in mean OD value in PI-carrying dams as compared to dams with non-infected calves at the end of the lactation, if milk is used as a specimen. It may however be noted that only blood was sampled in the month 9, since all dams in this study where dry at that time.

Interestingly, the deviation pattern for width of confidence intervals differs markedly between Se and Sp. For Se, there is no obvious systematic difference. The confidence interval for Sp, on the other hand, is consistently underestimated with the traditional method. This is to be expected, and is probably related to the inclusion of a random effect of herd. Clustering with respect to the OD value in this case is more likely to be found among the 'non-diseased' part of the population, and therefore the inclusion of a random effect should mainly affect Sp. The biology behind this is the following; PI-carrying dams have their high titres due to the fact that they carry infected foetuses - this condition affects their immune system in the same way irrespective of herd of origin. Therefore, these dams will be very similar between herds with respect to OD value. Dams with healthy foetuses on the other hand will have an OD value which reflects at what stage of infection the herd was in when the tests were taken. Thus, such dams ("the non-diseased population") will tend to cluster with respect to the OD value. Inclusion of a random effect of herd will inflate the standard error for Sp. Thus the confidence interval will be underestimated with the traditional method, as compared to the logistic model.

Examples of estimation of Se and Sp by the use of logistic regression seem to be scarce in the veterinary medical literature. A search of MEDLINE where the key words logistic regression, sensitivity, specificity and veterinary were alternatively used did not result in any articles where this method had been employed. However, papers where logistic regression was used to develop models to predict presence or absence of disease (like a diagnostic tool in itself) were rather common (e.g. Reeves et al., 1989, Hoffman et al., 1992, Reid et al., 1995, Fecteau et al., 1997 and Furr et al., 1995 and 1997). Such an analysis is however not to be confused with the method described here. One paper uses logistic regression to investigate reasons for false positive results, after calculating Se and Sp in the traditional way (Sischo and Burns, 1993). In this case, the dependent variable 'false positive' is already evaluated against a gold standard test. Thus, the gold standard is not included in the model, only reasons for misclassification. Yet another paper uses logistic regression to determine the optimum cut-off for an ELISA. Se and Sp were thereafter calculated in the traditional way, in relation to the estimated optimum cut-off level (Paré et al., 1995).

It has been discussed whether Se and Sp should be evaluated within the same model. Clinical factors may affect these parameters differently which would imply that the best way of modelling them would be to fit separate models for Se and Sp, after stratifying by disease status (Coughlin et al., 1992). This was, however, not considered to be the case in the situation under investigation. Furthermore, there is a certain value of being able to model both Se and Sp within the same model, at least when there are additional covariates. With this approach one incorporates into the model information about the covariates from individuals who were both positive and negative by the gold standard, thus maximising the information available. Another feature with the current modelling approach which could not be achieved with the traditional method is the inclusion of a random effect. Theoretically, one could have made a post-hoc correction of the standard error used in the exact binomial confidence intervals, e.g. by using an intraclass correlation adjustment for the effect of herd, or by multiplying with the square root of the variance inflation factor (McDermott et al., 1994). However, considering the binomial nature of these data, such a measure would still not have been able to adjust the possible bias in point estimates (Lam et al., 1996). Thus, there is no post-hoc method at hand for analysing these data.

It should be noted that the method used here to estimate Se and Sp can also be used to yield estimates of positive- and negative predicitive values (PPV and NPV). The only strategical difference with respect to the Se/Sp model is that the true state of nature and the test result switch position. In other words, the disease status is used as the dependent variable, and the test result is included as one of the explanatory variables. Other covariates can of course also be included. PPV is given by setting the test result equal to the code for a positive test (usually 1), and consequently NPV is given when the test result is negative.

SUMMARY

The model-based approach differs from contingency table methods in that it provides a means to adjust for numerous explanatory covariates and to account for independence between observations, given binomial data. Thus it proved to be an excellent method in the present study, since there were good reasons to assume that gestational stage would be an important effect modifier, that choice of specimen could matter since the OD value in milk is affected by stage of lactation and that the stage of infection would cause individuals within herds to be more similar with respect to antibody levels. The results from this study show that using the traditional method would have resulted in biased estimates, as compared to the output from the logistic models. This was particularly obvious at the end of pregnancy which also is the specific period in which the test should be used. Another conclusion is that the effect of including a random effect is restricted to estimates of Sp, which can be explained by factors pertaining to the disease in question. Finally, knowing that the combination of discrete data and clustering can only be handled correctly within a modelling framework, there is no alternative to random effect logistic regression for estimating Se and Sp based on the current data.

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SAMPLE STRATEGIES TO SUBSTANTIATE FREEDOM FROM DISEASE: A THEORETICAL APPROACH

M. ZILLER*, T. SELHORST, J. TEUFFERT & H. SCHLÜTER

In recent years, numerous legal regulations have been introduced to decide whether an area or a certain holding can be declared as being free from a definite disease. Several different situations have to be distinguished from each other. The status "free from disease" can be granted initially, it can be recovered after rehabilitation or it can remain valid after a period of observation. Beyond it, the regulations in the European Community and in various Member States are heterogeneous and specific cases have been regarded separately. Therefore, requirements can be found for the non-existence of infected animals. In other cases, sample constructions are prescribed based on a percentage of the population, or based on a detailed plan of sample sizes. Also clear aims have been formulated, sometimes in the form of prevalence-thresholds.

In spite of these differences, the common aim of sampling investigations should be the possible recognition of a certain prevalence level at a definite time, depending on the disease and the specific situation. In this paper, we deal with the strategies of surveys to substantiate freedom from disease for a whole territory.

A homogeneous distribution of infected animals cannot be assumed, when considering highly contagious diseases. So, in the case of infection, a relatively high prevalence of infected animals will be observed within an infected herd, while the prevalence of infected herds within a large territory remains smaller. For this case, Cameron and Baldock (1998b) recommended two-stage sampling. Such strategies are flexible enough to realize monitoring and observation programs with very different parameters.

Thus, the calculation of sample sizes becomes more complicated. The classical standards, e.g. collected in the tables of Cannon and Roe (1982), were developed for easy, one-stage samples and perfect tests. The theoretical generalization regarding real testing by Cameron and Baldock (1998a) introduces a simple way to evaluate multi-stage sample sizes. In this paper, we present the theoretical foundations of these calculations. Therefore, each stage has to be regarded separately. The "interface" between both stages is characterized by the test-error probabilities of the following step. Thus, the sample sizes of each step can be evaluated.

Furthermore, we apply these results to a data example of *Brucella melitensis*. We take special care of the herd-size situation in Germany, characterized by many small sheep holdings and a few large ones. Such a distribution of herd sizes is typical for different animals in various countries. Whilst Cameron and Baldock (1998b) investigated the case of homogeneous herd-size distribution, the simulation study

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of Selhorst et al. (submitted) applied their results to the more general situation for the first time. We also demonstrate that our calculation principles can be used in this case, too.

In addition, the simplicity of the evaluation of sample sizes opens up the possibility of optimizing costs or other relevant variables, by choosing the appropriate sample strategy, each of them ensure the same α-level for the first stage.

SAMPLE STRATEGIES

In order to prove that a territory is free from disease, it is sufficient to show that no consequences of a supposed infection occur. In the case of contagious diseases, safeguarding different low-level prevalence-thresholds, amongst herds as well as within herds, is suited to indicate freedom during a certain time. If, for any reason, animals were already infected, the prevalence would exceed those thresholds soon. In general, the threshold for testing within herds should be greater than that for testing at herd level.

The situation within an infected territory is demonstrated in Fig. 1. Infected holdings are clustered, but only a low herd prevalence exists. Within an infected holding, a much higher prevalence can be expected instead.

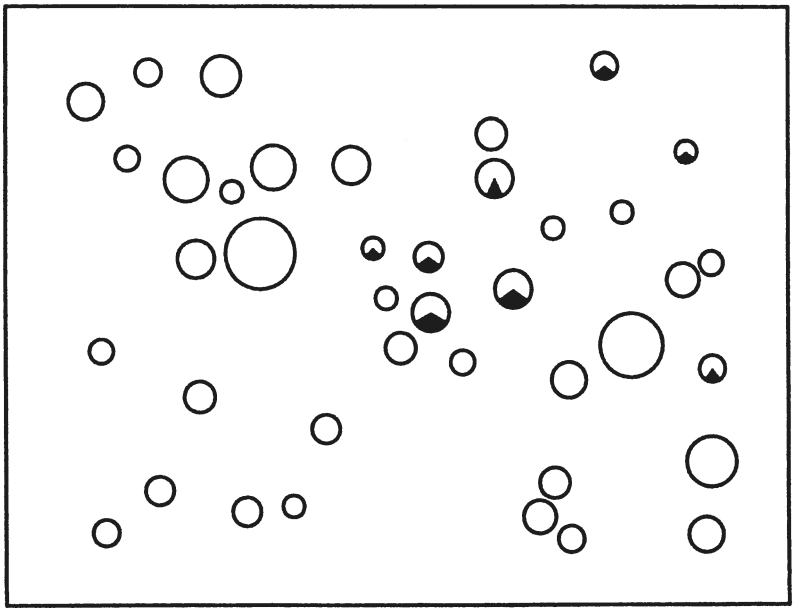

Fig. 1. Scheme of an infected territory.
Area of the circles - herd sizes.
Black segments - infected percentages.

In order to discuss the different strategies we regard both stages of the whole process separately. Firstly, a random sample of holdings has to be selected. Secondly, for each of these holdings it has to be decided whether it is infected or not. What is to be done in this second stage characterizes the strategy. Cluster samples were proposed by Teuffert and Lorenz (1995), while Cameron and Baldock (1998b) and Selhorst (submitted) suggest limited samples. In the following we compare three possibilities for the second stage.

Figure 2 represents a schematic comparison of the principles under consideration.

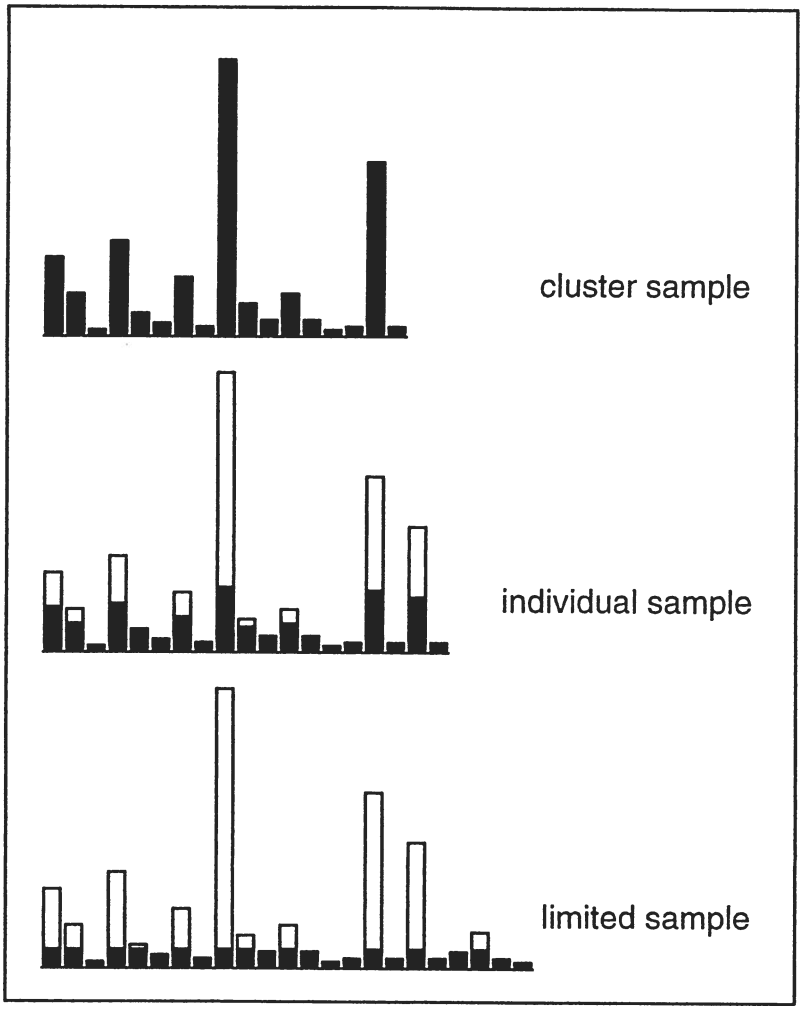

Fig. 2. Scheme of sample strategies.
Total bars - herd sizes.
Black parts - sample sizes.

Cluster Sample

The easiest case is the testing of all of the animals within the herd. By assuming a perfect test we can be sure of deciding correctly.

Individual Sample

Arguing in the same manner as before, a prevalence threshold can be prescribed for testing within a herd. If it were infected, test-positive animals should be found in an appropriate sample. The size of it has to be calculated, in order to ensure a chosen error level of e.g. α_2=0.05. Therefore, the sample size must be chosen dependent upon the herd size. But for each tested herd the same α_2-level is valid.

Limited Sample

Supposing the same prevalence threshold as for the individual sample, a fixed sample size valid for each tested holding can be predefined arbitrarily. If a holding has less animals, all of them have to be tested.

In this case, knowledge of the herd-size distribution is essential. With this knowledge, the necessary number of herds can be calculated in order to ensure the corresponding α_1-level. Depending on the chosen sample size within the holdings and the special herd size distribution, the total number of tested animals can differ in this strategy. Therefore, it can be chosen to minimize a given cost function.

SAMPLE SIZES

For a long time, approximate solutions described by Cannon and Roe (1982) had been the basis of calculating sample sizes, supposing a homogeneous distribution of prevalence and a perfect test. Generally applying the hyper-geometric distribution was problematic due to the large amount of computation time required. This problem has been overcome by the availability of much more powerful computers.

The integration of real testing into sample size calculations was the other great problem and for the same reason. Apart from that, the parameters for testing have not been known exactly. Consequently, in times of quicker computers this more general situation has found more interest.

About one year ago Cameron and Baldock (1998a) published a formula suitable for the calculation of sample sizes using test-sensitivity (Se) and test-specificity (Sp). Let d be the number of true positives in a population of size N. The probability of having x test-positives in a sample of n animals is then:

Eq. (1)

$$P(T^+ = x) = \sum_{y=\max(0,d-N+y)}^{\min(d,y)} \frac{\binom{d}{y}\binom{N-d}{n-y}}{\binom{N}{n}} \sum_{j=0}^{\min(x,y)} \binom{y}{j} \bullet Se^j \bullet (1-Se)^{y-j} \bullet \binom{n-j}{x-j} \bullet (1-Sp)^{x-j} \bullet Sp^{n-x-y+j}.$$

On the basis of this formula more complicated sampling strategies can be evaluated. Cameron and Baldock (1998b) themselves applied it to two-stage sampling using further combinations and approximations based on an average herd size. Unfortunately, in many practical cases a large number of herds are fairly small and vice versa. For this general situation Selhorst et al. (submitted) developed a simulation model evaluating sample strategies while minimizing costs.

In the following, we describe the theoretical basis of exactly calculating the necessary sample sizes for two-stage sampling plans, in order to ensure a low level prevalence threshold. Let an area be regarded as free from disease when the prevalence of infected herds is less than π_1. We now consider the procedure of checking a holding for whether it is infected or not as being a whole. We call it "herd-test". Thus, we can estimate sensitivity and specificity for this herd-test. And consequently we can calculate the sample size at herd level using Eq. (1) and setting

$$p(T^+=0) < \alpha. \qquad \text{Eq. (2)}$$

The estimation of herd-test sensitivity and herd-test specificity is the main key to evaluating the complete sampling plan. For simplicity we assume a perfect test deciding whether an animal is infected or not. In this case the herd-test specificity is always 1. A generalization of this assumption does not affect the principles of the following calculation.

In the three regarded examples the herd-test sensitivity results in:

Cluster Sample

Because all of the animals in the herd are tested by a perfect test, the herd-test sensitivity equals 1.

Individual Sample

The herd-test sensitivity is complementary to the error level of the individual within-herd test. We assume a sampling procedure within the holding to ensure a prevalence of less than π_2 at an error level of α_2=0.05. Thus, the herd-test sensitivity yields 0.95.

Limited Sample

This case is more complicated. The probability of false-negative detecting a holding can only be calculated using the formula of total probability. For that, the knowledge of the herd size distribution has to be taken for granted. Supposing the same prevalence threshold π_2 as for the individual sample, let n_f be the fixed sample size valid for each tested holding. If a holding has less animals, all of them have to be tested.

The herd-test sensitivity then remains the probability of choosing a sample of n_f test-negative animals out of a holding with a prevalence greater or equal to π_2. In a holding with less than n_f animals, this mistake cannot occur because all of the animals will be perfectly tested. Thus,

$$Se = \sum_{h>n_f} P(T^+ = 0 / N = h) \cdot P(N = h). \qquad \text{Eq. (3)}$$

While P(N=h) is known as the herd-size distribution, the other factor can easily be obtained by applying the hyper-geometric distribution. Choosing $d(\pi_2)$ as the lowest number of test-positive animals for that the within-prevalence is greater or equal to π_2, one gets

$$P(T^+ = 0 / N = h) \geq \frac{\binom{h - d(\pi_2)}{n_f}}{\binom{h}{n_f}}. \qquad \text{Eq. (4)}$$

Thus we can estimate the herd-test sensitivity for the limited sample. For each choice of n_f we get a new sampling plan, these differ from one another by the total number of animals to be tested and therefore by the total costs.

Substituting these sensitivity results into Eq. (1) and Eq. (2) respectively, all sampling plans ensure the required significance level for the first stage.

APPLICATION TO DATA

In order to demonstrate our results we have chosen an imaginary survey to substantiate freedom from *Brucella melitensis* for Germany. According to the EC-directive 91/68/EEC (1991), for maintaining the status "free" it is sufficient to prove at the α_1=0.05 level that the prevalence of infected herds is less than 0.002. We now apply the regarded three strategies to this problem. The following figure illustrates the special herd size situation in Germany 1996 according to the "Statistisches Bundesamt" (Federal Office of Statistics).

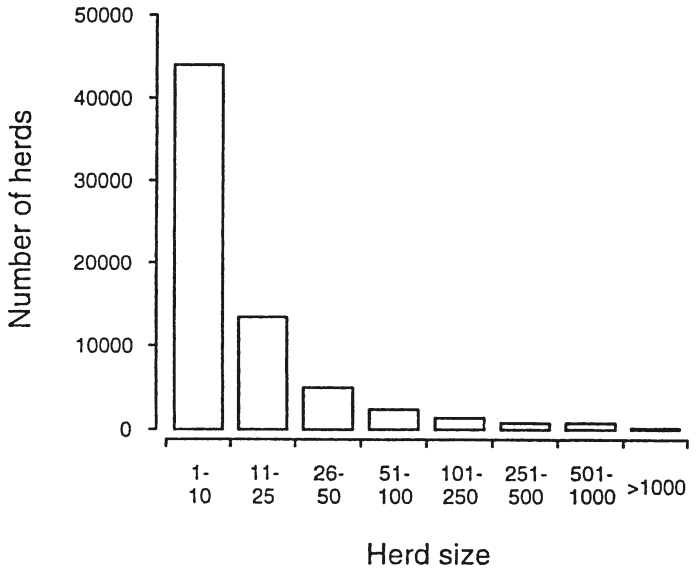

Fig. 3. Herd size distribution in classes.

A total number of 68,767 holdings existed at the end of 1996 in Germany. About 64 % of them contained 10 animals at the most, and only 246 holdings with more then 1000 animals could be found. Considering these facts, a cluster sample procedure is not only very expensive, as can be seen later, but also inefficient and unjust to the holders of large herds.

Given the herd-size distribution, the prevalence-threshold $\pi_1=0.002$ and the maximum error probability $\alpha_1=0.05$ at herd level, we assume the following parameters for the second stage within the holdings: prevalence-threshold $\pi_2=0.01$, maximum error probability $\alpha_2=0.05$. The evaluation of the sample-sizes for the different strategies yield the following results.

Cluster Sample

According to Eq. (2) with Se=1 and Sp=1, a total of 1476 holdings have to be investigated. In the case under consideration, this would mean testing almost 50,000 animals. The costs arising would exceed 375,000 DM.

Individual Sample

Table 1 lists the individual sample sizes and the costs for each herd-test for several examples of herd sizes. With a sensitivity of 0.95 at herd level, Eq. (2) yields a sample size of 1553 holdings, leading to about 31,000 tested animals and costs of about 265,000 DM.

Table 1. Sample sizes and costs for selected individual samples.

Herd size	Sample size	Total costs per herd
		[DM]
5	5	74.00
10	10	105.75
20	19	162.90
50	48	347.05
100	95	645.50
200	155	1044.50
300	189	1260.40
500	225	1489.00
1000	258	1698.55
5000	290	1901.75

Limited Sample

The sample-limit of animals to be tested within one holding can be chosen arbitrarily. Otherwise it can be connected to the task of optimizing a secondary cost function. In our example we want to try to minimize the real costs for the whole survey. These costs consist of three parts (Selhorst et al., submitted), costs for visiting the holding, for taking trials and for diagnostics. All of them are specified for different quantity classes.

In Table 2 for several choices of n_f the estimated sensitivity, and with that the number of herds and of animals to be tested and the total costs, have been listed. There is a range of sample-size limits n_f between 6 and 9 in which the costs do not differ very much.

So, in comparison with the other strategies, about 2000 holdings have to be tested using limited samples. The total costs of little more than 150,000 DM for such a survey are nevertheless much lower than when applying another one of the discussed strategies.

Table 2. Calculated parameters for the limited sample strategy.

Limit of sample size within a holding	Herd-test sensitivity	Number of herds to be tested	Mean number of animals to be tested	Mean of total costs
				[DM]
1	0.2142	6886	6886	342495
2	0.3914	3769	7399	210510
3	0.5214	2830	8013	173672
4	0.6065	2433	8675	160649
5	0.6644	2221	9308	155469
6	0.7054	2092	9898	153620
7	0.7365	2003	10441	153207
8	0.7620	1936	10949	153523
9	0.7836	1883	11422	154227
10	0.8027	1838	11864	155083
11	0.8189	1802	12279	156157
12	0.8328	1772	12668	157327
13	0.8450	1746	13029	158488
14	0.8556	1725	13380	159808
15	0.8652	1706	13708	161067

DISCUSSION

The result of our theoretical considerations is the possibility of calculating sample sizes for different survey strategies in a rather simple way. In all cases we consequently applied the hyper-geometric distribution and the law of total probability, in order to evaluate sampling plans ensuring the significance level for the first stage.

By regarding both layers of a two-stage sample and calculating the necessary sample sizes separately, a large simplification of the problem could be achieved. The interface of both stages can theoretically be reduced to two variables - sensitivity and specificity at herd level.

In the same way, a generalization of this method is imaginable. When e.g. a fattening holding contains large pens, the same problem as discussed appears within this holding. Investigating only one holding of this kind, all of the strategies presented are practicable within it. Surveying a territory containing many holdings of this kind, a three-stage sample strategy could be taken into consideration. For sample size calculation all three stages have to be regarded separately. According to the committed strategy, sensitivity and specificity for the appropriate stages have to be estimated from the bottom up. After that sample sizes can be calculated from the top down.

The three regarded sample strategies in our example differ in their expense as well as in their consequences. A cluster sample strategy of course results in the statement of highest certainty but also in highest costs. While the individual sample allows a result with clearly defined confidence for each

investigated holding, the strategy of limited samples is least expensive. A general statement of confidence for single holdings is impossible here but the α_1-level for the first stage is guaranteed.

Certainly, other questions remain. A survey of the kind considered should discover a centre of infection before clinical symptoms can become manifest. Otherwise, when regulations or political restrains require large surveys, a cost-optimized strategy safeguarding all predefined parameters would be the best solution. Last but not least, ethical questions may be dominant. When animals have to be slaughtered for testing, a strategy should be chosen which minimizes the total sample size.

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91/68/EEC (1991) Council directive on animal health conditions governing intra-community trade in ovine and caprine animals.

THE USE OF EXPERIMENTAL DESIGN METHODS FOR SENSITIVITY ANALYSIS OF A COMPUTER SIMULATION EXPERIMENT

K D C STÄRK, D U PFEIFFER*

The development of computer simulation models involves a series of steps including design, verification and validation. Sensitivity analysis can be used as part of model verification and validation, where it is used to provide insights into model behaviour. More generically, the objective of a sensitivity analysis is to explore the sensitivity of a system to parameter variations (Frank, 1978). It allows the identification of influential input variables in a simulation model. Frank (1978) defines parameter sensitivity as the effect of parameter changes on the behaviour and outputs of a model. Parameter sensitivity is important because it helps identify factors that need to be determined most accurately and possibly require further research. More specifically, we wanted to identify the most influential input variables in the simulation model INTERSPREAD-SF. The main output of this model is the forecast of the average number of infected properties and the duration of a classical swine fever (CSF) epidemic. These outcome parameters are influenced mainly by the disease-spread mechanisms and the control measures in place. Provided that the latter is kept constant, the influence of the remaining factors can be explored.

Statistical methods are available to measure the effect of a change in one factor on the output (Kleijnen, 1979). The use of experimental designs supports a structured approach to sensitivity analysis (Hunter and Naylor, 1970). Particularly, fractional factorial designs are widely used in sensitivity analysis (Kleijnen, 1979, 1987). The results from the experiments can be used to construct a 'meta-model' using regression analysis (Kleijnen, 1987). The term meta-model is adequate because the empirical relationships between input and output parameters of the underlying simulation model are described (Kleijnen, 1979). The coefficients of the meta-model reflect the importance of a factor (Kleijnen *et al.*, 1992).

MATERIAL AND METHODS

A simulation experiment based on a 3^{k-p} fractional factorial design (Cochran and Cox, 1992; Montgomery, 1997) was performed using INTERSPREAD-SF (32-bit version, Stern, 1997). This stochastic model simulates the between-farm spread of CSF using different transmission mechanisms and user-defined control strategies. Only local spread and movement-related spread mechanisms were considered in this experiment. Local spread is a not-fully-understood conglomerate of transmission pathways acting over a limited distance around an infected farm. The simulation was conducted using the farm characteristics and geographical distribution of a farm population from a densely-populated pig area in Northern Germany (Stärk et al., 1998a). The farm density was 2.05 farms/km^2 in an area of 1417 km^2.

The simulation of transmission required the following 5 input variables: 'time to clinical signs' (Factor 1, CLIN), 'time to diagnosis' (Factor 2, DIAG), 'local spread' (Factor 3, LOCAL), 'number of movements'

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(Factor 4, MOVE) and 'probability of infection by movement' (Factor 5, INF). The factors were used on three levels each (low, medium, high). The medium level was defined first and the low and high level were calculated symmetrically by adding or subtracting 25% of the medium value. The values used for the factors under study are given in Tables 1-3.

The variable CLIN represents an approximation of the incubation time and was derived from experimental data using moderately virulent CSF virus strains (Dahle & Liess, 1995). To account for biological variability, this variable was represented as a probability distribution. The probability of ending the incubation time on a given day was calculated from the cumulative probability curve of the respective distribution. The same approach was used for variable DIAG, which represents the estimated time from onset of clinical signs to diagnosis. This interval was estimated from outbreak data (Stärk, 1998).

For the variable LOCAL, distance-dependent probabilities of occurrence were estimated using expert opinion. To simulate the movement contacts, both the number of contacts on each day were estimated and the probability of transmission if a contact occurred. As not all types of conveyors bear the same risk of transmission, movement contacts were grouped according to risk: very high (transport of susceptible animals), high (people and trucks with animal contact), medium (other contacts with pig farms).

Table 1. Input distributions of factors 1 and 2 (type of distribution including mean and standard deviation)

	Level 1[a]	Level 2	Level 3
Factor 1: Time to clinical signs	LogNormal (8,1.5)	LogNormal (12,2)	LogNormal (16,2.5) truncated 0-28
Factor 2: Time to diagnosis	LogNormal (8,7.5) truncated 0-28	LogNormal (12.8,10) truncated 0-28	LogNormal (17.6,12.5) truncated 0-28

[a]All probability distributions generated using @RISK v. 3.5 (Palisade Corporation, New York), approx. 1100 runs (relative change <1.5%).

Table 2. Input values for factor 3: Local spread[a]

Distance to infected farm	Level 1	Level 2	Level 3
0-0.10 km	0.03	0.04	0.05
0.11-0.25 km	0.01125	0.01500	0.01875
0.26-0.50 km	0.0075	0.0100	0.0125
0.51-1 km	0.00225	0.00300	0.00375

[a]Local spread was simulated for up to 28 days after a farm became infected.

Table 3. Input values for factors 4 and 5

	Risk category	Level 1	Level 2	Level 3
Factor 4: Number of expected movements per day	Very high	0.019	0.025	0.031
	High	0.053	0.070	0.088
	Medium	0.150	0.200	0.250
Factor 5: Probability of transmission by one movement	Very high	0.71	0.95	1.00
	High	0.56	0.75	0.93
	Medium	0.38	0.50	0.63

A basic control strategy was used including two radial restriction zones with a radius of 3,000 m and 10,000 m, respectively. Within the radial zones, movement control and surveillance was applied. Farms, which had experienced a very-high risk contact, were pre-emptively depopulated.

A 3^{5-1} fractional factorial design with 81 factor combinations was created using STATISTICA for Windows v.5.1 (StatSoft Inc., Tulsa). Ten simulation iterations over a maximum of 381 days were run using INTERSPREAD-SF for each of the factor combinations. The average number of infected properties (IP) and the average duration of the epidemic in days were used as dependent variables. In order to approach normal distribution, the number of IPs was $\log_{10}$-transformed for further analysis.

The results were analysed using STATISTICA for Windows v.5.1 (StatSoft Inc., Tulsa). Linear and quadratic main effects as well as two-way linear interaction terms were assessed using analysis of variance (ANOVA). Pareto diagrams were plotted for visual inspection of the standardised effect sizes of the different factors. Regression coefficients of the significant effects were calculated.

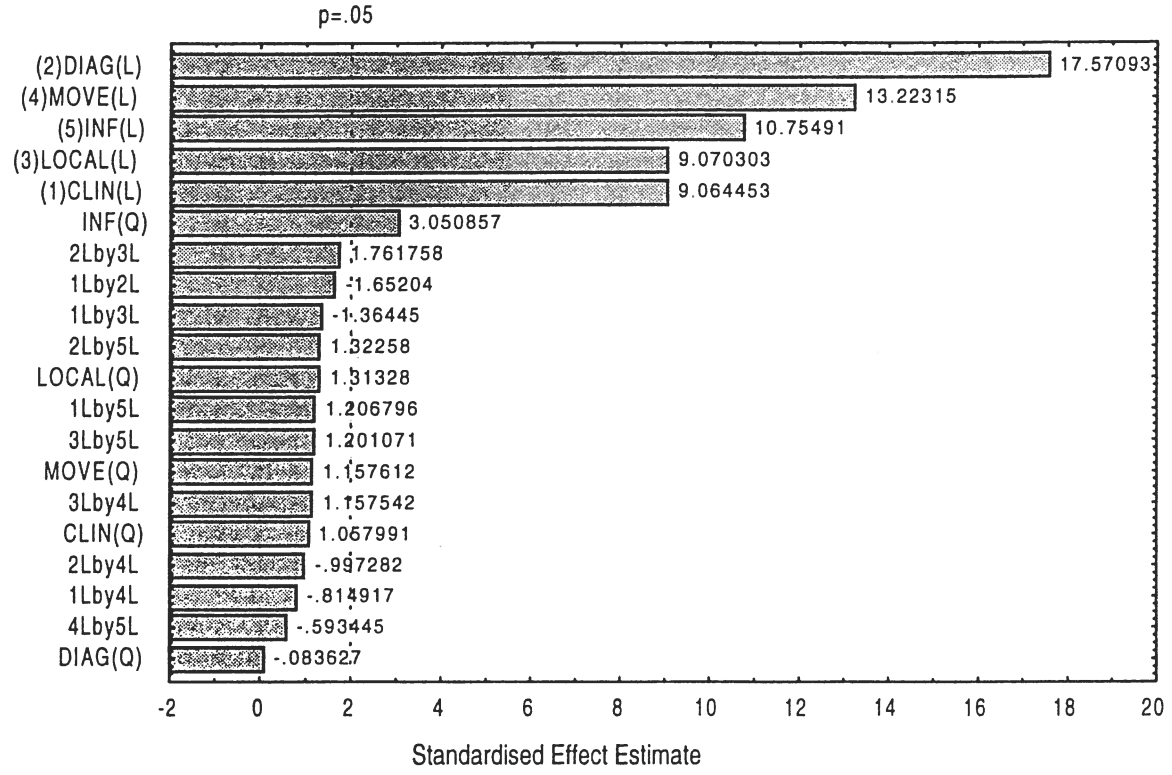
RESULTS

With the exception of INF, only linear main effects were significantly associated ($p \leq 0.05$) with the log of the average number of IPs (Fig. 1A). A simple linear-main-effects model was considered sufficient to explain the results of the sensitivity analysis. Of the main effects, DIAG had the largest influence on the outcome, followed by MOVE, INF, LOCAL, and CLIN (Table 4). This model had an adjusted R^2 of 0.89.

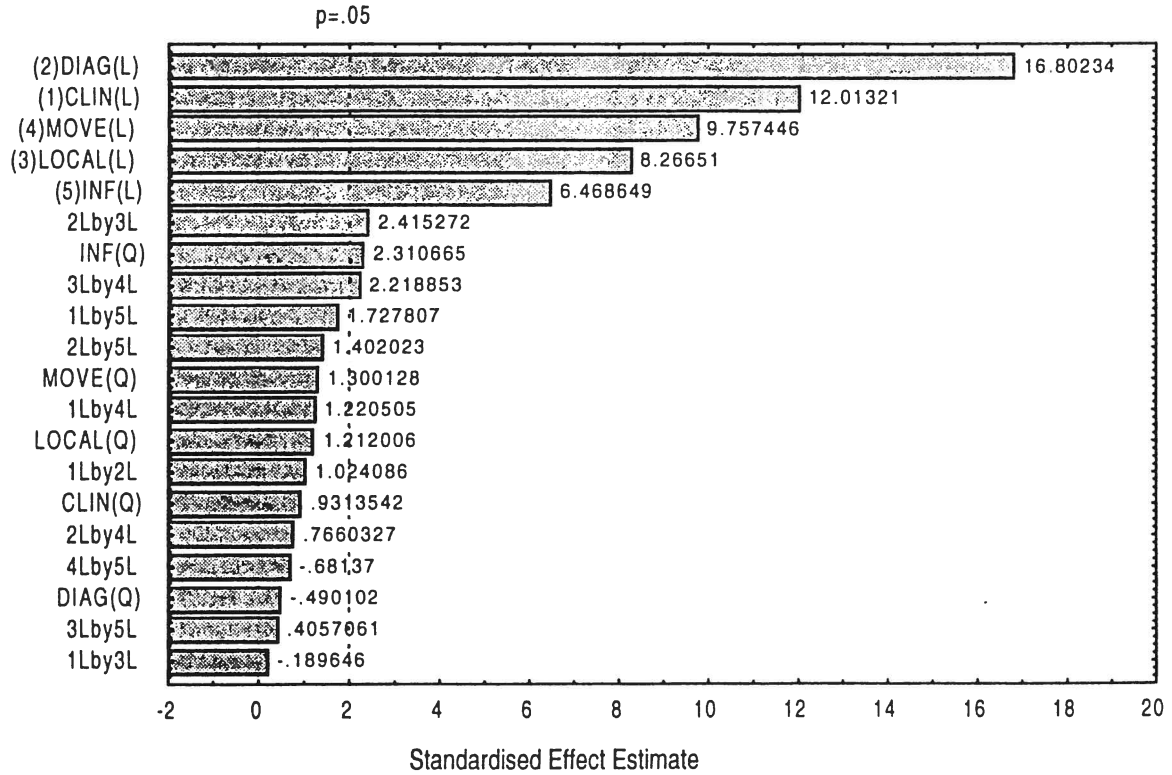
Table 4. Regression coefficients for model with dependent variable = $\log_{10}$(average number of infected properties). R^2 adjusted=0.89, n=81, DF=75

Factor	Coefficient	Std. Error	T	P	95% C.I.
Intercept	1.64	0.02	75.69	0.000	1.60-1.69
DIAG	0.43	0.03	16.11	0.000	0.38-0.48
MOVE	0.32	0.03	12.13	0.000	0.27-0.38
INF	0.26	0.03	9.86	0.000	0.21-0.31
CLIN	0.22	0.03	8.31	0.000	0.17-0.27
LOCAL	0.22	0.03	8.31	0.000	0.17-0.27

Similarly, mainly linear main effects were significantly associated ($p \leq 0.05$) with the average number of days that the epidemic lasted (Fig. 1B). Again, a linear-main-effects model was built. DIAG was the most influential variable with CLIN, MOVE, LOCAL and INF following in this sequence (Table 5). The model had an adjusted R^2 of 0.87.



A)



B)

Fig. 1. Pareto diagrams of standardised ANOVA effects calculated from 81 factor combinations.
L = linear effect, Q = quadratic effect.
A) outcome variable = $\log_{10}$(average number of infected properties)
B) outcome variable = average duration of epidemic (days).

Table 5. Regression coefficients for model with dependent variable = average duration of epidemic in days. R^2 adjusted=0.87, n=81, DF=75

Factor	Coefficient	Std. Error	T	P	95% C.I.
Intercept	170.78	2.98	57.31	0.000	164.84-176.72
DIAG	56.26	3.65	15.41	0.000	48.99-63.54
CLIN	40.23	3.65	11.02	0.000	32.96-47.50
MOVE	32.67	3.65	8.95	0.000	25.40-39.95
LOCAL	27.68	3.65	7.58	0.000	20.41-34.95
INF	21.66	3.65	5.93	0.000	14.39-28.93

DISCUSSION

Sensitivity analysis based on meta-models requires a profound knowledge of the underlying simulation model and the real world (Kleijnen *et al.*, 1992) as the potentially influential factors to be studied have to be selected by the investigator. In the experiments described here, all factors directly related to the probability of local and movement-related disease spread (MOVE, INF, LOCAL) were included plus two additional factors with an indirect influence. The time to first clinical signs (CLIN) is a characteristic of CSF virus strains. As the detection of classical swine fever largely depends on the occurrence of clinical signs, this factor is significant. The time until diagnosis (DIAG) depends on the disease awareness of farmers and the efficiency of veterinary services. The sooner the diagnosis occurs, the faster control measures are put into place and further disease spread is prevented. Unfortunately, both these latter factors are difficult to obtain from field data. The results of this experiment show, that it is particularly important to have a good estimate of DIAG, because this is the most influential factor with respect to both output variables. When performing a sensitivity analysis with INTERSPREAD-FMD on foot-and-mouth disease, Jalvingh et al. (1996) additionally explored the influence of farm density and the size of the area within which the simulation took place. These were considered fixed in this experiment as they are not disease-dependent but can be controlled by the analyst. The high R^2 of the regression models demonstrate that the most important parameters affecting model variability have been included.

The three levels used for each factor in this experiment were calculated such that the low and the high levels were located symmetrically around the medium level. This is not consistent with other descriptions of sensitivity analysis suggesting the use of standardised realistic ranges (Kleijnen *et al.*, 1992). However, the 'true' ranges of all factors are not known at this stage, but it was possible to establish an 'educated guess' based on empirical data and field experience. A similar approach was used by Jalvingh et al. (1996) who also varied a medium-level value by +/- 25%.

Hunter and Naylor (1970) have pointed out the importance of using a sufficiently large number of runs when performing simulation experiments. The effect of the number of runs on the results provided by INTERSPREAD was first assessed by Sanson (1993). He recommended using at least 5 iterations to obtain a reasonably stable output in terms of the average number of IPs. Jalvingh et al. (1996) however postulated a sample size of at least 50 runs. The latter is correct if one is interested in describing the epidemic curve and not only in the average number of infected farms. In the case of the current analysis, although the shapes of the epidemic curves are highly variable if only 5 runs are used (data not shown), the average number of IPs stabilised quickly and was not significantly different from the figure obtained from large number simulations. The total number of IPs simulated with 5, 10, 25 and 50 runs was 47.6, 27.4, 21.6 and 21.6, respectively. Additionally, in this experiment we were less interested in the actual output values than in the relative importance of the co-variates. Therefore, the 10 runs used in this experiment to calculate the average number of IPs were considered sufficient.

Although fractional factorial designs are extremely efficient, they also have a drawback. Due to the fact that not all possible factor combinations are used, a certain degree of confounding between main effects and interactions is introduced (Cochran and Cox, 1992). As no interaction terms were used in our final models, this problem could be avoided. Another potential problem with using experimental designs, ANOVA and regression analysis is that the underlying assumptions (normal distribution of dependent variable and experimental errors, homogeneity of variances) may not be fulfilled. This will influence the sensitivity of the test statistics although the F-test is considered reasonably robust (Lindman, 1974). In this experiment, we were again not so much interested in the actual value of the coefficients but rather in the relative ranking of the factors. Therefore, this concern appears to be of minor importance. However, the residuals of both models were approximately normally distributed (data not shown). Additionally, a $\log_{10}$ transformation of the average number of IPs was used to improve the fit of the regression model.

As an alternative to the Pareto diagram, Tornado charts could have been used. A Tornado chart is a graphical representation of the correlation coefficients between input variables and output. If the chart is based on Spearman rank correlation coefficients, no assumptions have to be made regarding linearity of the underlying relationship between input and output variables. Although this is an advantage (Vose, 1996), the conclusions drawn from the results would have been no different in our exercise (data not shown).

As no interaction terms were significant, all main effects can be considered to be independent from each other. Under the assumption that the variables DIAG, CLIN, INF and LOCAL were all somewhat related to the virulence of the virus strain involved, their interaction would be biologically plausible. However, the coefficients of their interaction terms were small and some had negative signs. This is indicative of spurious effects. In both models, the largest yet statistically insignificant effect observed for an interaction term was between DIAG and LOCAL.

All regression coefficients in our models had a positive sign as they logically should have. This is reassuring because coefficients with 'wrong' signs are indicators of computational or conceptual errors (Kleijnen, 1992). The magnitude of the coefficient is directly proportional to the importance of the factor. Collecting more information on important variables is crucial for generating accurate simulation results. In the CSF case, DIAG was most influential. Both the number of farms that became infected in an epidemic as well as the duration of the epidemic were strongly influenced by this factor. However, this factor is difficult to estimate as the onset of clinical signs as well as the time of infection are not known for most infected farms in real outbreaks. Consequently, the probability of diagnosis is difficult to estimate for any given day. In such a case, principles of risk analysis could be applied (Kleijnen, 1992). The factor under consideration is then replaced by a probability distribution of input values that could be based on expert opinion. INTER-SPREAD-SF uses this approach. The figures used for DIAG and CLIN represent results produced by sampling from lognormal distributions that were transformed into daily probabilities. The lognormal distribution has been shown to be a good description of the distribution of incubation periods of infectious diseases (Sartwell, 1950).

The factor CLIN (probability of onset of clinical signs) can be established under experimental conditions. However, the situation in the field may be different, and due to the significant influence of this factor on the duration of a CSF epidemic, it would be worthwhile to thoroughly analyse field data with particular emphasis on this parameter.

Another influential factor in the CSF models was the number of movements per day (MOVE). Although this figure can be obtained from surveys (Nielen et al., 1996), the classification of the contacts into risk categories is subjective (Stärk et al., 1998b). It is even more difficult to estimate the probability of infection associated with any one of these movements (INF). Both these factors were particularly influential on the average number of IPs. Large data sets from recent European CSF outbreaks can provide more information on this parameter as demonstrated by Stegeman et al. (1998).

Not much information is available on the risk of local spread (LOCAL), partly because this is a conglomerate of risk factors acting over a relatively short distance around an infected farm and it is only poorly

understood. More information is becoming available from analyses of field data currently under way in Europe (e.g. Staubach et al., 1997; Stegeman et al., 1998). However, the results of our experiments suggest that this factor is of minor importance in its effect on the output of INTERSPREAD-SF.

The results of this experiment demonstrate that experimental design methods can be applied relatively easily as part of the verification and validation of simulation models. Information can be gained on the significance of input variables as well as on the structural validity of the model. Verification and validation, both are very important steps in the development of simulation models before the latter can be used to reliably solve real-world problems.

ACKNOWLEDGMENTS

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SURVEILLANCE SYSTEMS

MANAGING LIVESTOCK DISEASE DATA:

THE DISEASE AND VECTOR INTEGRATED DATABASE (DAVID).

T P ROBINSON* AND J S HOPKINS

The Disease And Vector Integrated Database (DAVID) is a geographical information system for managing field data on tsetse, trypanosomiasis and livestock. The programme is supplemented by a field guide, which provides sample data recording sheets that can be modified to meet individual requirements. Where possible, the programme uses established coding systems, such as those adopted by the Office International des Epizooties (OIE). The DAVID programme is used to enter these data in a computer, to verify the data, to query the data and to produce output reports in the form of tables, graphs and maps.

A key feature of the database is that all data are temporally and geographically registered, enableing data from tsetse, trypanosomiasis and livestock surveys to be integrated. For a particular country the programme is customised by providing administrative overlays; national veterinary area overlays; lists of livestock inspection sites (with designated codes); lists of diagnostic methods used; lists of tsetse sampling sites; lists of tsetse sampling methods used; and lists of tsetse and trypanosome species present. These details are then used to provide checks for data entry, and filtering options for data output.

Data output is in the form of tables, graphs or maps. Tabular output (*e.g.* details of drug use and tabular census data) may be presented in reports and analysed further in spreadsheets or statistical packages. Typical graph output includes packed cell volume (PCV) histograms, monthly trypanosomiasis incidence, or monthly tsetse catches. Typical map output includes livestock distributions, mean PCV maps, trypanosomiasis prevalence maps, and tsetse distribution maps.

The DAVID Project has been supported, both financially and institutionally by many national and international organisations. The programme is currently being tested operationally in at least sixteen institutions in nine African countries (Appendix A), and has been widely disseminated (Appendix B).

Development of DAVID has been driven by: a) the development team's field experience in Southern Africa, b) lessons learned during the development of the precursor to DAVID; the Integrated Tsetse and Trypanosomiasis Database (ITTD); c) consideration of other database programmes used in veterinary departments in some African countries; and d) recommendations arising from a variety of international seminars and workshops (Appendix B).

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DATABASE DESIGN

The database was designed using Select Software Tools' SELECT SE (v. 7.0) tool set. The database is fully relational resulting in a high level of data integrity and very little data duplication, makeing data storage and retrieval very efficient. The programme is a 16 bit application written in Visual Basic (4.0) and compiled to run under the Windows 3.1x, '95 or NT operating systems. Data are held in Microsoft Access (2.0) database files. Tabular output is generated within Visual Basic. Graph output is generated using the Graphics Server (5.0) ActiveX and GIS map files (vector and raster) are produced and stored in IDRISI for Windows (2.0) format. The programme is compiled for distribution and installation using the InstallShield Express Professional.

The data in the database are held in two or more Microsoft Access (2.0) data files: one which holds globally defined configuration variables ('global' column of Table 1); and one or more country-specific data files that hold nationally selected and defined configuration variables; and national data sets on livestock, disease and vector distributions ('national' column of Table 1).

Table 1. Details of the global and national settings used to configure DAVID for a particular country. Those national settings with arrows in front of them are selected from previously defined global settings; those that are bulleted are defined specifically in the national set-up.

Module	Global	National
GIS	Countries	
	Datums	→ Datums
	National Area Types	→ National Area Types
	Veterinary Area Types	→ Veterinary Area Types
		• Locations
		• National Areas
		• Veterinary Areas
Livestock	Species and Type	→ Species and Type
Census	Farming Systems	→ Farming Systems
Disease	Species	→ Species
Trypanosomiasis	Veterinary Disease	→ Veterinary Diseases
	Diagnostic Methods	→ Diagnostic Methods
	Drugs	→ Drugs
	Inspection Site Types	→ Inspection Site Types
	Stratification Types	→ Stratification Types
		• Inspection Sites
Vector	Species	→ Species
Tsetse	Sampling Methods & Systems	→ Sampling Methods & Systems
	Stratification Types	→ Stratification Types
		• Sampling Sites

DATABASE CONFIGURATION

Before use, the programme needs to be configured for a particular country. Since much of the data entry is achieved by making selections from drop-down lists, these so-called 'look-up tables' must be created before data can be entered. There are four areas in which look-up table maintenance is required: GIS, livestock, disease and vector, as summarised in Table 1. The GIS options include the selection of administrative boundaries, co-ordinate systems *etc.*, and apply to livestock, disease and vector data. Some aspects of the configuration span these areas (*e.g.* inspection sites are relevant to both livestock and disease data) while others are specific (*e.g.* diagnostic techniques for trypanosomiasis). Some of the information held in look-up tables is relevant to many countries; this is held in the global database. National options are selected from the global database and copied to the national databases. Other options that are country-specific are managed directly within the national databases. Access to the look-up tables depends on the authority level of the operator, and is controlled by usernames and password protection. Only operators with the highest level of authority (regional data managers) are permitted to modify the global settings; operators of intermediate authority (national data managers) are allowed to modify national settings; while staff of the lowest authority level (data entry personnel) may not change any of these settings.

DATA ENTRY AND VERIFICATION

Data are entered in the DAVID programme from specifically adapted forms that are used in the field: there are separate forms for livestock census data, trypanosomiasis survey and surveillance data and for tsetse survey data. Whereas livestock census and disease survey data are collected by inspection sites, tsetse data are collected by sample sites. These are uniquely coded for each country and are associated with accurate locational and other attribute information. All results that are entered are then related to these sites.

Wherever possible, data are held in look-up tables and are selected from drop-down lists during data entry. When this is not possible, checks are introduced to ensure that sensible data are entered and that data are entered in valid formats. For example, when the positions of inspection or sample sites are entered it is checked whether they fall within the administrative boundaries of the selected country. This is achieved using the GIS functionality of DAVID to check point data (inspection site position) against polygon data (national boundary).

One of the biggest problems in information management arises when inaccurate data are entered; either as a result of inaccurate data being collected in the field, or from erroneous data entry. The use of drop-down lists and various checks in data entry reduces the risk of this, but much data can only be checked within certain bounds. The most robust way to ensure that accurate data are entered into databases is to enter them twice; on the grounds that the chance of entering the same wrong data twice is very small.

In DAVID data can be entered twice or individual data records can be inspected without having to re-enter complete data sheets (a method that is restricted to users with a high level of authority). During double data entry the new data are constantly checked against the old, and any discrepancies are highlighted. These are then checked against the data sheets to determine which entry is correct.

OUTPUT – THEORY

The end result of efficient data management must be that data are compiled into information that facilitates decision-making. This can be direct and immediate, for example in identifying outbreaks of trypanosomiasis so that appropriate action can be taken, or it can be indirect, for example comparing new diagnostic tests with old established ones in order to determine their sensitivity and specificity.

Querying is a standard function of databases and involves selecting and processing particular records depending on related characteristics. In database programmes such as Microsoft Access this could be done using query wizards or directly with Structured Query Language (SQL) commands. However, due to the complexity and volume of data held in DAVID, and the fact that operators should not be expected to be familiar with composing queries, the process of extracting information for reports is greatly facilitated by the use of appropriate menus and windows.

There are three types of report that can be produced in DAVID: tables, graphs and maps. Tabular reports output summary data in rectangular arrays; comma separated files that can be imported into word-processor documents for presentation, or into databases, spreadsheets or statistical packages for further analysis. All reports essentially start as summary arrays but the programme provides the option to send these summaries directly to graphs or GIS maps. Graph output is generated using the GraphicsServer control, a flexible graphing tool that allows data to be presented in many two- and three-dimensional graph types. GIS map output is generated in IDRISI for Windows format. This allows for more detailed GIS analysis to be carried out directly on the output, though the DAVID programme has its own map viewer in which these maps can be looked at, modified and printed.

Querying data to produce reports is very similar regardless of the format, though different types of query are appropriate to the different formats and to the different modules. The first step in generating a report is to specify its geographical bounds. GIS polygon data pertaining to administrative boundaries, *e.g.* provinces, districts *etc.* are used to select the required data. Data can also be grouped by operational area or by veterinary area. If maps are being produced the geographical extent and the desired pixel resolution is also specified.

The next step in producing a query is to specify the time period for the report. Most maps simply need a start date and end date. For some graphs, however, a more detailed time series must be given, for example to produce a graph of monthly trypanosomiasis incidence, the time series that defines the horizontal must be specified. Other filters are then create, more specific to the livestock, disease and vector modules, such as which inspection sites or sample sites to include; which diagnostic methods or sampling techniques to include; and which species of livestock, disease organism or vector to include.

Some reports are qualitative, for example presence or absence of different species of tsetse, while some are quantitative, for example the number of flies of a particular species caught per trap day or the prevalence of trypanosomiasis. Whilst maps tend to be used to show the results of surveys over a specified time period, graphs tend to be used to present data as a time series, often appropriate to surveillance or monitoring. The map and graph outputs that are available in the programme are summarised in Table 2.

Table 2. Summary of output types available from the three modules of DAVID.

Module	Table	Map	Graph
Livestock (Census)			
Distribution: numbers	×	×	×
Distribution: TLU	×	×	×
Disease (Trypanosomiasis)			
Qualitative prevalence	×	×	
Apparent prevalence / incidence	×	×	×
True prevalence	×	×	
Packed Cell Volume	×	×	×
Proportion anaemic	×	×	×
Vector (Tsetse)			
Distribution	×	×	
Flies per trap-day	×	×	×
Flies per stop	×	×	×
Sex ratio	×	×	×

Most of the outputs listed in Table 2 are self-explanatory, particularly those for livestock and vector, though some of the disease outputs require a fuller explanation.

Livestock

Livestock census data are output either as numbers per unit area, for individual species or types, or as tropical livestock units (TLU), if it is required to combine the census results for different species. Livestock species and types are assigned a value in tropical livestock units (TLU); a measure that has been developed to standardise the impact of grazing animals. For example, whereas an ox has a TLU value of 0.7, a goat has a TLU value of 0.1. This means that one ox is equivalent to seven goats in terms of grazing impact. 1 TLU is approximately equivalent to 250 kg of live weight.

Vector

The results of tsetse surveys can be mapped simply as a distribution, indicating presence or absence of the different species found, and combinations thereof. For individual species it is also possible to produce maps and graphs of: a) flies per trap-day (number of flies caught per day of trapping using stationary methods such as Epsilon traps); b) flies per stop (number of flies caught per stop using mobile methods such as fly-rounds); and sex ratio, a simple estimate of the proportion of the total flies that are male.

Disease

Herd-health may be inferred through frequency histograms of the packed cell volumes (PCV) of individual animals. Whilst PCV distributions can be compared from area to area they cannot be mapped as such. Both graphs and maps, however, can be produced of the mean PCV, and of the proportion of anaemic animals, *i.e.* those having a PCV below some threshold that is determined by the user.

One available output is 'qualitative prevalence': an indication of whether infected animals were diagnosed during a survey. If infected animals are diagnosed, then there is no doubt that the disease is present. However, if infected animals are not diagnosed, the certainty with which it can be said that the disease is absent depends on the sample size, the size of the population and the assumed underlying prevalence in the population.

The sample size required to detect at least one positive animal, with a specified level of confidence, is given by[1]:

$$n = \left(1-\left(1-p_1\right)^{1/d}\right)\left(N-\frac{d}{2}\right)+1$$

where n = required sample size, p_1 = probability of finding at least one case in the sample, d = (assumed) number of diseased animals in the population, and N = population size.

For example, of a population of 120 animals, with an assumed prevalence of 25%, 11 must be tested to detect at least one positive animal with probability 0.95 The DAVID programme has three classes in the qualitative prevalence output: a) positive, b) negative with a sufficient sample size to be confident that the disease is absent, and c) negative with an insufficient sample size to be confident of this. The desired probability level, and the assumed underlying prevalence are determined by the user.

The apparent prevalence, AP, is simply:

$$AP = \frac{d}{N}$$

The incidence, an estimate of the number of new cases, is calculated in the same way over a specified time period.

If we restrict the output to results based on a single diagnostic method, for which we have reasonable estimates of the sensitivity and specificity, then we can estimate the 'true prevalence', TP, as:

$$TP = \frac{AP + sp - 1}{se + sp - 1}$$

where se = sensitivity (ability correctly to distinguish positive cases), and sp = specificity (ability correctly to distinguish negative cases).

We can then obtain the standard error, SE, of the estimated apparent or true prevalence using the formula[2]:

$$SE = \sqrt{\frac{\left(1-\left(n/N\right)P(1-P)\right.}{n}}$$

where P = either apparent (AP), or true (TP) prevalence.

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[1] Source: **Thrusfield, M.** (1997) *Veterinary Epidemiology*, revised 2nd edition, Blackwell Science. Page 187.

[2] Source: **Putt, S. N . H., Shaw, A. P. M., Woods, A. J., Tyler, L. and James, A. D.** (1987) *Veterinary epidemiology and economics in Africa - A manual for use in the design and appraisal of livestock health policy* ILCA Manual Number 3, Reading, England. Page 53.

OUTPUT – EXAMPLES

Figures 1 and 2 are examples of output in graph format. Figure 1 shows a packed cell volume histogram for animals sampled in the Eastern Province of Zambia during January, 1995. Figure 2 shows the monthly incidence of trypanosomiasis, in sentinel herds in the Eastern Province throughout that year.

Figures 3 and 4 are examples of map output, as observed using the DAVID viewer. Unfortunately it is not possible to reproduce these in colour here so these figures are more to demonstrate the format of the map output than the actual results. Figure 3 shows the results of trypanosomiasis surveys in the Eastern Province of Zambia in 1995. The map shown is one of qualitative prevalence as described above. Figure 4 shows the results of a series of tsetse surveys in Kwazulu/Natal in South Africa: pixels in which no flies were caught are indicated, and those in which *Glossina austeni*, *G. brevipalpis*, and both species were caught are distinguished.

PERSPECTIVES

At present the Disease And Vector Integrated Database is being tested operationally in the Regional Office of the RTTCP, its member countries Mozambique, Malawi, Zimbabwe and Zambia; and in South Africa, Ethiopia and Kenya (Appendix A). Much international interest has been shown in the DAVID Project (Appendix B), and it has been strongly recommended that there is a wide scope for further development. Particularly important developments include:

- Managing data for a variety of livestock diseases and vectors
- Managing drug use and resistance data
- Managing animal production data
- Enhancing GIS functionality
- Developing network functionality (for integrating large data sets)
- Developing training materials (courses, exercises)
- Developing WWW site for dissemination of upgrades, documentation, training materials and data
- General enhancements (*e.g.* on-line help, improved output)

Some of these developments are currently being implemented under funding by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. What is required, however, in the perspective of our increased understanding of the problem of information management for decision-support in the control of livestock diseases, is a conceptual re-design of the programme that would increase its flexibility and reduce the dependence on external aid organisations to implement future developments. Figure 5 provides a schematic illustration of how this might be achieved.

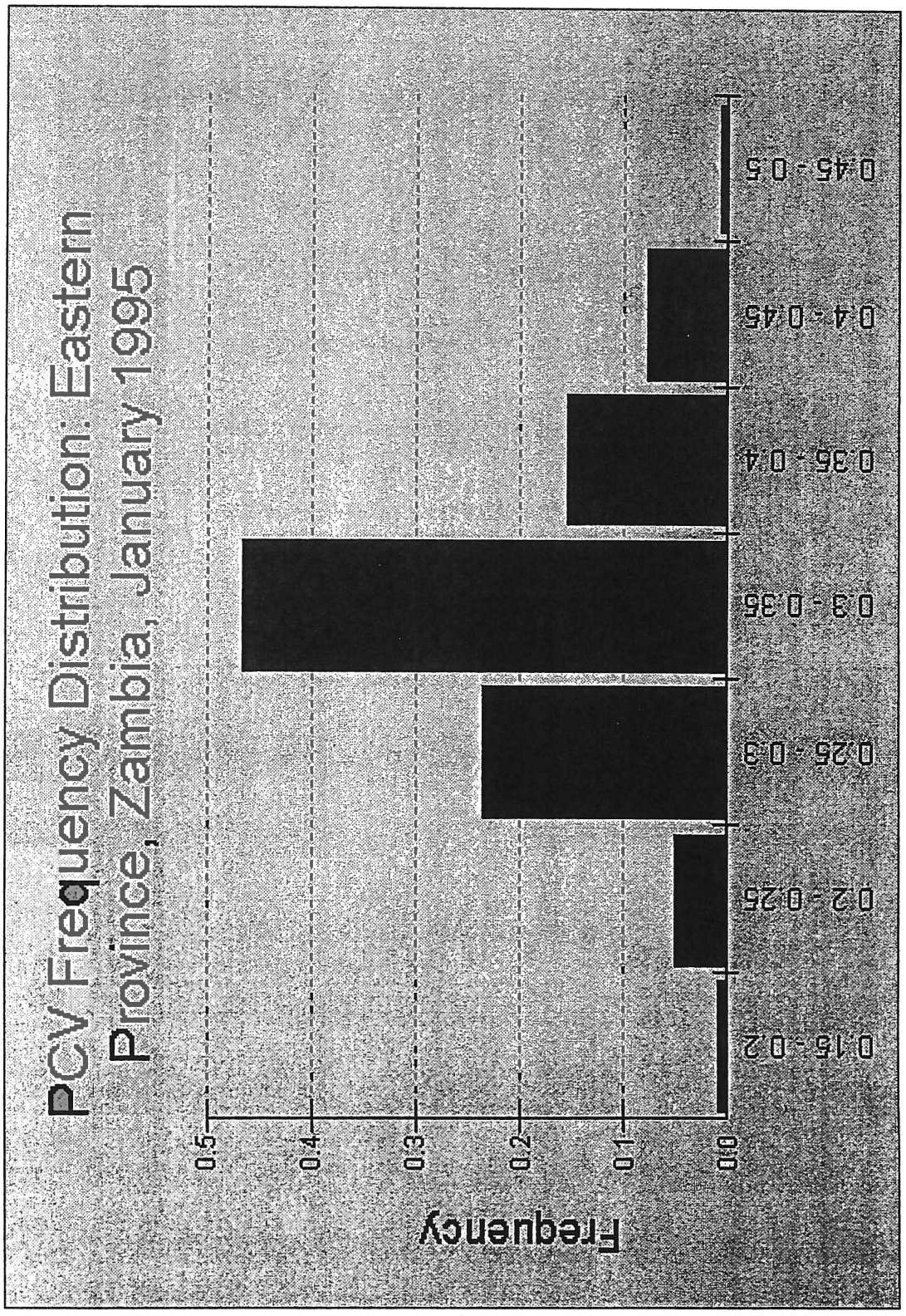

Figure 1. Packed cell volume (PCV) histogram for animals sampled in the Eastern Province of Zambia during January, 1995.

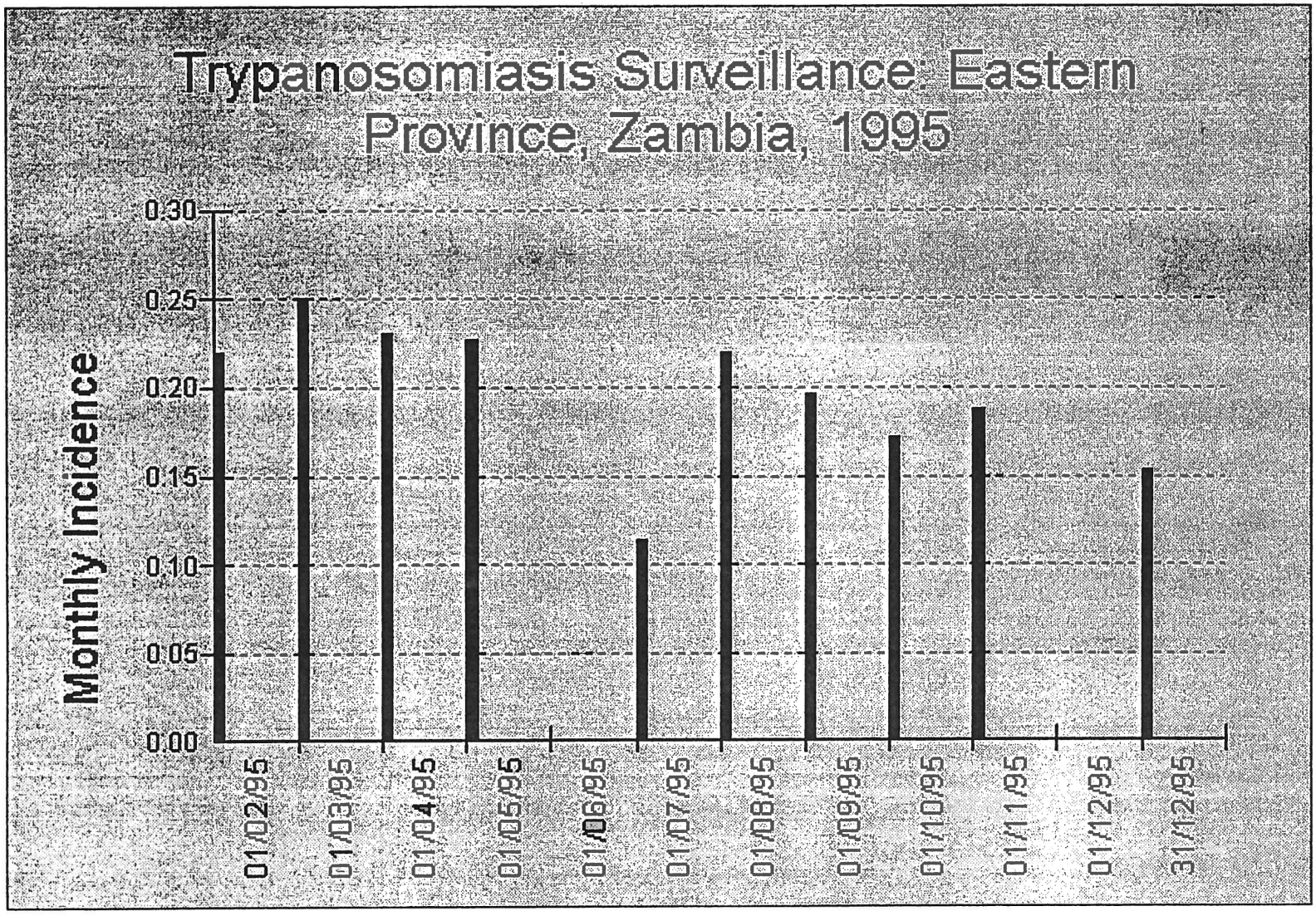

Figure 2. Monthly incidence of trypanosomiasis, in sentinel herds in the Eastern Province, during 1995.

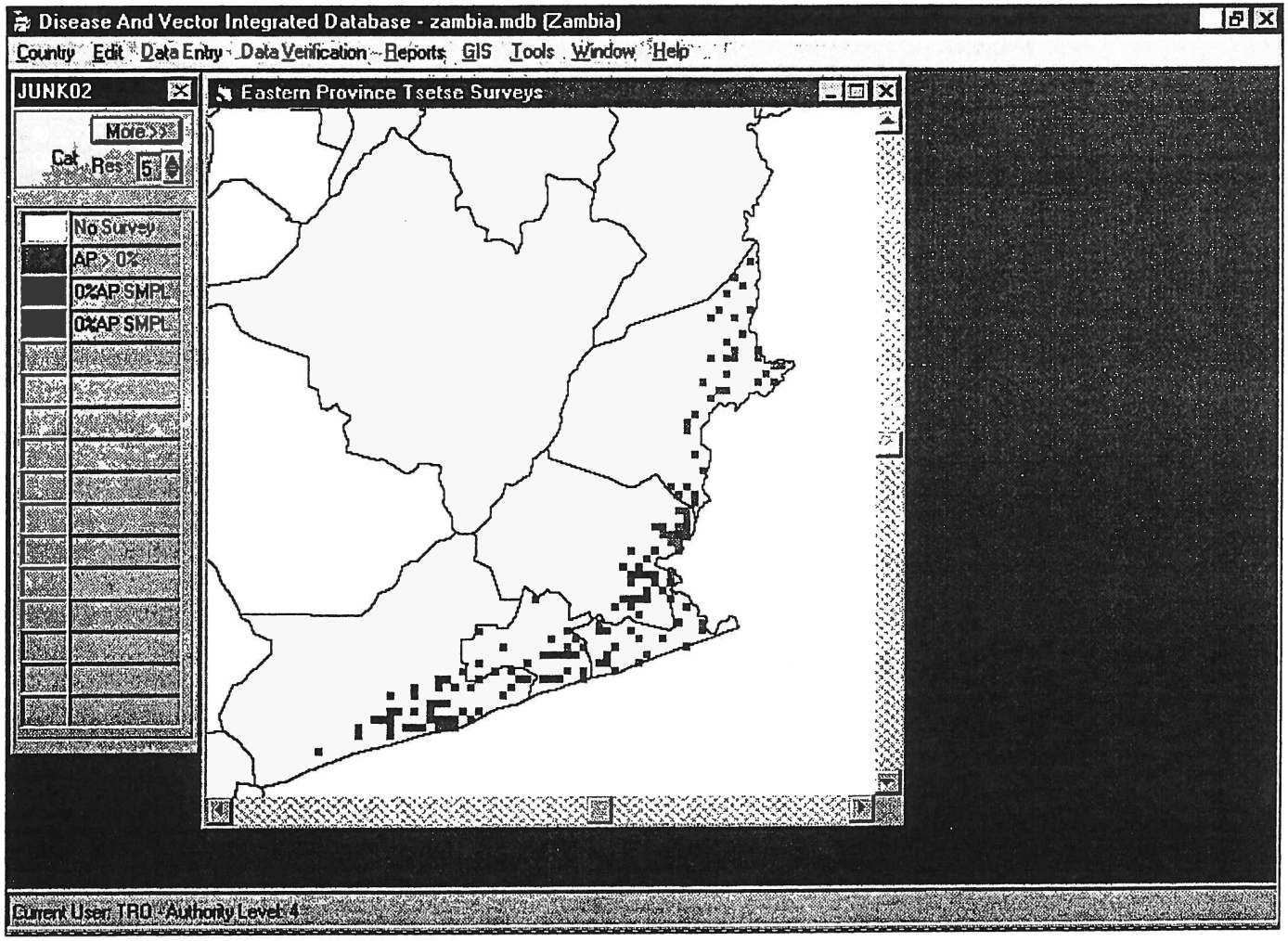

Figure 3. Qualitative prevalence of trypanosomiasis surveys in the Eastern Province of Zambia during 1995. The three categories distinguished are: a) apparent prevalence more than 0, b) apparent prevalence less than 0, but too small a sample to be sure of a negative result, and c) apparent prevalence less than 0, with a sufficiently large sample to be 95% confident of this.

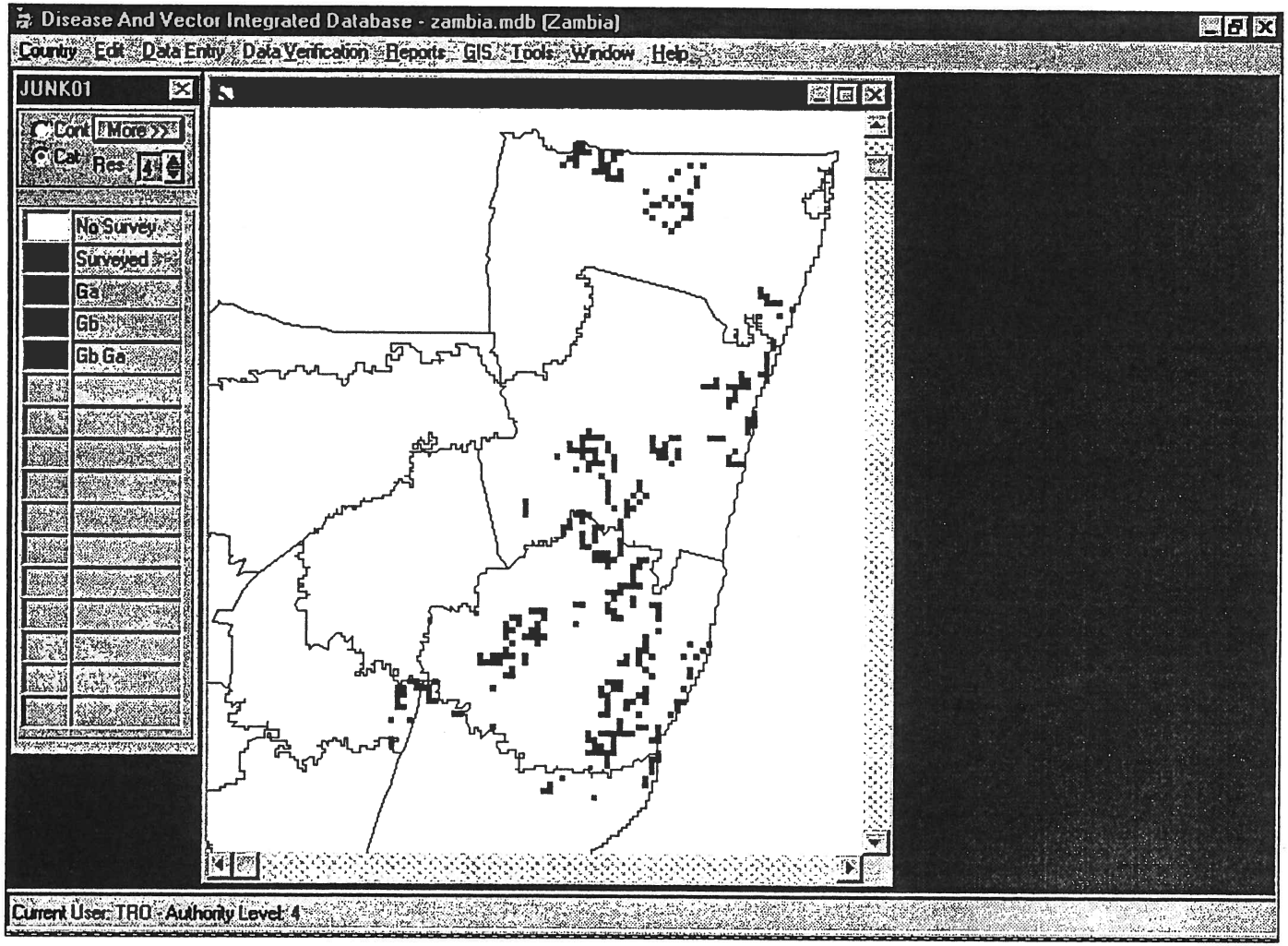

Figure 4. Tsetse surveys in Kwazulu/Natal in South Africa: pixels in which no flies were caught are indicated, and those in which *Glossina austeni*, *G. brevipalpis*, and both species were caught are distinguished.

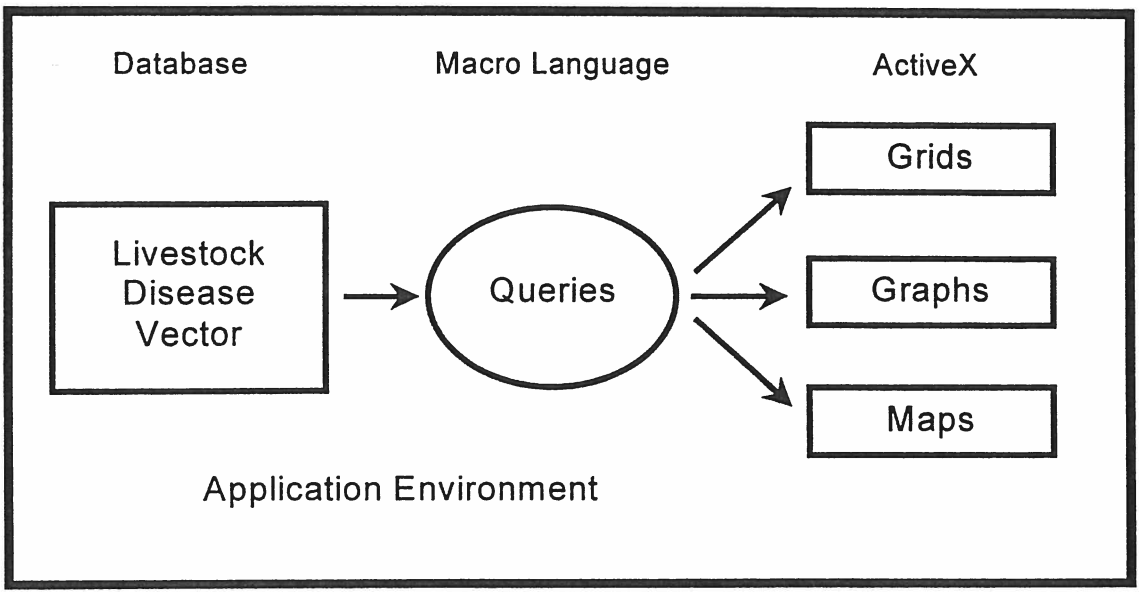

Figure 5. Conceptual framework for DAVID development.

It is proposed that the programme be developed as a 32-bit application, the most appropriate environment being Visual Basic 5.0 (or later). Forms for data entry and verification would be programmed within this. The relational database structure needs to be re-designed, based on the existing structure but streamlining features that have been introduced since the beginning of the project. This would be done in Access 7.0 (or later). This relational database structure would be fixed to allow merging of data from different systems and a common data interchange format would be developed to facilitate this. Clearly this will need to be able to absorb data that have been entered in the existing format.

ActiveX technology should be used to generate outputs so that the ActiveXs can be developed independently of the DAVID programme. At present the only ActiveX used is the GraphicsServer, for graph output, but ActiveXs could be incorporated that generate tabular/gridded output, and map output. The current developers, Belgian Graphic Interface (details below) produce a mapping ActiveX called the DMX that currently deals only with vector format data but could be adapted to meet the requirements of the DAVID project.

Central to the application, and a means by which enormous flexibility could be introduced, would be a macro language used to generate queries. The standard queries listed above would be programmed and provided with the application. In addition to these any user could write their own macros to generate queries, either from scratch, or by modifyingexisting ones. Users who are unable to do this could commission the work to the developers, or indeed to any other programmer. An appropriate macro language such as the freely available Tool Command Language (TCL) would be adopted to meet this requirement.

Further funding will be sought with which to implement these recommendations. If this can be achieved we will be able to provide an invaluable information management tool, that is sustainable, not only to national and regional tsetse and trypanosomiasis control programmes, but to livestock services in general.

ACKNOWLEDGEMENTS

The current release of the Disease And Vector Integrated Database (Version 2.0) is the result of seven years of collaborative work between the development team, staff of the Department of Veterinary and Tsetse Control Services (DVTCS) in Zambia (some of whose data are used in examples), SADC's Regional Tsetse and Trypanosomiasis Control Programme (RTTCP), FAO and IAEA. The tsetse survey data in KwaZulu/Natal have been used here by kind permission of Dr Errol Nevill, Onderstepoort Veterinary Institute (OVI), who conducted these surveys and has collaborated closely in the development of DAVID to date.

We would also like to acknowledge the contributions made to be project by: Mr Zimba Chitalu (ChizSoft); Dr Bob Connnor (RTTCP); Dr David Rogers (TALA, University of Oxford); Dr William Wint (ERGO); Drs Jan Slingenbergh and Brian Hursey (FAO/PAAT); Dr Peter Kerby (DFID); Drs Martyn Jeggo and Udo Feldmann (FAO/IAEA); Dr Mark Eisler (University of Glasgow/ILRI); Dr Martin Warnes (Pestwach, Bristol); and Dr Tom Downing (ECU, University of Oxford).

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Programme development work is currently being undertaken by Belgian Graphic Interface (BGI), Ch. De Wavre 1635, 1160 Bruxelles, Telephone: 02 / 675 56 73, Fax: 02 / 672 56 37, Email: info@bgi-sa.com, WWW: www.bgi-sa.com.

Appendix A: DAVID Installations

Location	Installation and comments
South Africa	• Onderstepoort Veterinary Institute (installed under FAO) • Allerton Veterinary Office, KwaZulu/Natal (installed under FAO)
Zimbabwe	• RTTCP Regional Office • RTTCP Zimbabwe
Mozambique	• DINAP (installed under FAO) • Veterinary Faculty (installed under FAO) • RTTCP Mozambique (installed under FAO)
Malawi	• RTTCP Malawi
Zambia	• RTTCP Zambia • Central Veterinary Research Institute (CVRI) • ASVEZA (Belgian Veterinary Project)
Kenya	• International Livestock Research Institute (ILRI) • Kenyan Trypanosomiasis Research Institute (KETRI)
Zanzibar	• Diagnostic data imported on behalf of FAO/IAEA
Cameroon	• Diagnostic data imported by FAO/IAEA
Ethiopia	• Science and Technology Commission (installed under FAO/IAEA)

Appendix B: International dissemination of the DAVID Project

- Southern Rift Valley of Ethiopia Tsetse Fly Eradication Project (IAEA Project ETH/5/012) GIS meeting. Addis Ababa, 20-24 April, 1998.
- Southern African Development Council (SADC) representatives and Onderstepoort Veterinary Institute (OVI). Pretoria, 20 November, 1997.
- XXIV Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC). Maputo, Mozambique, 29 September to 3 October, 1997.
- International Symposium on Diagnostics and Control of Livestock Diseases using Nuclear and Related Techniques "Towards Disease Control in the 21st Century". IAEA/FAO, Vienna, 7 to 11 April, 1997.
- RTTCP Information Management Workshop, Harare, 24-26 March, 1997.
- FAO headquarters, Rome, October 1996.
- Regional Tsetse and Trypanosomiasis Control Programme (RTTCP), Harare, October 1996.
- International Livestock Research Institute (ILRI), Nairobi, October 1996.
- Food and Agriculture Organisation (FAO) Programme Against African Trypanosomiasis (PAAT) panel of experts, Nairobi, October 1996.

Appendix C: Acronyms

AHP	Animal Health Programme
ASVEZA	Belgian assistance to the Veterinary Services in Zambia
BGI	Belgian Graphic Interface
CTVM	Centre for Tropical Veterinary Medicine
CVRI	Central Veterinary Research Institute
DAVID	Disease And Vector Integrated Database
DFID	Department For International Development
DINAP	Department of Animal Health and Production (Mozambique)
DVTCS	Department for Veterinary and Tsetse control Services (Zambia)
ERGO	Environmental Research Group Oxford
EU	European Union
FAO	Food and Agricuture Organisation of the United Nations
GIS	Geographical Information Systems
IAEA	International Atomic Energy Agency of the United Nations
ILRI	International Livestock Research Institute
ITTD	Integrated Tsetse and Trypanosomiasis Database
KETRI	Kenyan Trypanosomiasis Research Institute
OIE	Office International des Epizooties
OVI	Onderstepoort Veterinary Institute
PAAT	Programme Against African Trypanosomiasis
PCV	Packed Cell Volume
RTTCP	Regional Tsetse and Trypanosomiasis Control Programme
SADC	Southern African Development Council
SQL	Structured Query Language
SRVETFEP	Southern Rift Valley of Ethiopia Tsetse Fly Eradication Project
TALA	Trypanosomiasis And Landuse in Africa

A QUANTITATIVE APPROACH IN DECLARING A COUNTRY FREE OF A DISEASE

L AUDIGÉ[1], M G DOHERR[1] AND M SALMAN[2]

The 1995 World Trade Organisation agreement on the application of Sanitary and Phytosanitary Measures requires scientific validation in order to declare a country or a region free of a disease.

Several approaches have been presented to determine if a country is free from a specific disease (Cannon & Roe, 1982; Garner et al., 1997; OIE, 1998; Cameron & Baldock, 1998; Jordan, 1997). Most of the assumptions for these approaches are statistically valid but lack practicality. Some of these approaches, for instance, assumed that the tests for these diseases are perfect (Cannon & Roe, 1982) or the prevalence of the disease is fixed within and among diseased herds (Garner et al., 1997). For the evaluation of herd-level level tests, the minimum expected prevalence on diseased herds instead of the possible range of expected prevalence has been considered (Garner et al., 1997; Cameron & Baldock, 1998). More recently, a method has been proposed accounting for the variability of disease prevalence within herds, but it is restricted to the herd level (Jordan & McEwen, 1998).

Typically surveys aimed at substantiating freedom of a disease are designed in order to identify at least one diseased herd given that the herd prevalence is above a predefined threshold (e.g. 0.1%). Such surveys in a disease-free region usually anticipate an outcome with negative results. A negative result says, in fact, that the true herd prevalence is very likely lower than this threshold. Such conclusion should in fact account for many factors other than just the survey result, such as the veterinary infrastructure, diagnostic capabilities, evidence of the presence of the disease agent, clinical history of the disease, and effective disease control measures in the country. Thus, the survey result should not conclude the outcome. An approach to count for these factors should be considered to quantify our confidence in declaring a country free of a disease for a given survey result. The process should incorporate as much as possible a quantitative assessment so that it can be standardised between countries and diseases.

Audigé and Beckett (1999) proposed to assess the validity of animal health surveys using stochastic modelling. Their approach can be used to determine the efficacy of a disease detection system in a country. The approach, however, neither provides the confidence limits of the estimated likelihood ratios nor counts for factors that influence the survey outcome.

This paper presents a quantitative approach to estimate the probability that a country is free from a disease for a given survey characteristics and result.

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DESCRIPTION OF THE APPROACH

Surveys as diagnostic systems

A survey can be considered as a diagnostic system aimed at correctly identifying the presence or the absence of a disease in a country or a region. Therefore, both validity and accuracy of the survey results need to be evaluated. The method used in this paper has been further developed based on the modelling approach presented by Audigé and Beckett (1999). We summarise their approach below:

A first step involves the estimation of an overall herd-level test sensitivity and specificity, which are derived from two probability distributions of the number of test-positive animals expected from disease-free and diseased herds, respectively. These distributions are dependent on the individual-animal test sensitivity and specificity, herd sizes, the disease prevalence in diseased herds and the number of animals sampled per herd.

In a second step, probability distributions for the number of test-positive herds expected in a situation of freedom from disease and under various levels of herd prevalence are specified and used to determine survey properties in terms of its sensitivity and specificity, and receiver operating characteristic curves are drawn. These distributions can be used in two ways:

- A threshold number of test-positive herds can be chosen so that the result of the survey will be interpreted as "negative" if the observed number of test-positive herds is below this number. The survey result will be interpreted as "positive" otherwise. This approach poses the challenge of choosing the "correct" threshold.

- Alternatively, these distributions can be used to calculate likelihood ratios for each possible survey result. The model assesses the likelihood ratio of the observed survey result assuming that the country is affected by a disease to that with the assumption that the country is free of this disease. This approach is more powerful for the interpretation of survey results.

The aim of conducting the survey, however, is to derive a conclusion from the survey result taking into consideration the specific factors that can influence this result. In a diagnostic test, we refer to this conclusion as the negative and positive predictive value of test results (Smith, 1995; Fletcher et al., 1996). In a statistical term it is the posterior probability for a conditional probability. In the context of this paper, we are concerned about the probability of the absence of the disease, i.e. the negative predictive value. In order to emphasise this approach, we have made the terminology more specific. The negative predictive value or post-survey probability of freedom of disease can be derived from the following formula:

Post-survey probability = (Pre-survey odds x LR) / (1 + Pre-survey odds x LR)

where,

LR = Likelihood ratio of the probability of observing a survey result in the absence of the disease, to the probability of observing the same result in the presence of the disease at a predefined prevalence level.

and,

Pre-survey odds = Pre-survey probability / (1 – Pre-survey probability)

with,

Pre-survey probability = Probability of freedom of disease prior to the application of the survey

The concept to estimate the likelihood of freedom of a disease is summarised in Figure 1.

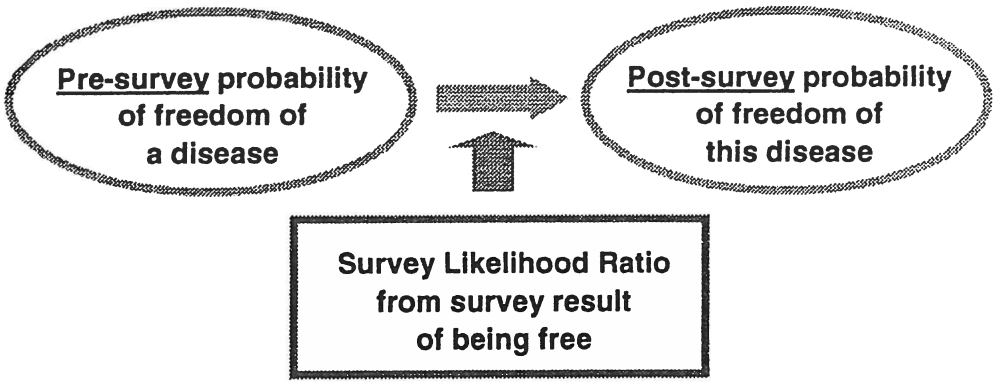

Fig. 1 Concept to estimate the likelihood of freedom of a disease

Stochastic modelling

In order to consider this approach, several changes have been made to the model of Audigé and Beckett (1998), that now allows:

- To have only a few diseased animals in the hypothetical herds, assuming that the disease is not highly infectious. The number of diseased animals within diseased herds can be directly specified instead of being calculated from the herd size and a level of prevalence.
- To assess the validity of surveys involving a large population (e.g. several tens of thousands of herds) and a large sample of herds (e.g. several thousands).
- To provide a probability distribution for the likelihood ratio (for a given survey result).
- To provide a probability distribution for the post-survey probability from the formula presented above where pre-survey probability and LR are specified as probability distributions, thus accounting for the uncertainty in the final output.

Estimation of the pre-survey probability

In the context of disease diagnosis at the individual-animal level, the pre-survey probability can be obtained from the estimated overall prevalence level of the disease among

the population in which the test was performed (Pre-survey probability = 1 - overall prevalence). Applying the same method to conclude a survey result is not suitable since the overall prevalence level of the disease in the population (in this instance country population) is an unrealistic entity. Therefore, an alternative approach is needed.

We propose that the pre-survey probability of freedom of disease is estimated or assessed from a set of criteria, which would contribute to the identification of the disease assuming it is present. These criteria are, then, sorted and summarised statistically to reflect the pre-survey probability. This approach would require standardisation and a thorough evaluation of these factors that can influence the existence of the disease in a particular country. A preliminary list of these criteria (likely incomplete) is presented in Table 1. The method currently under evaluation is presented in a poster at this meeting (Doherr et al., 1999). The assessment of the pre-survey probability could lead to a scale such as proposed in Table 2. Once a prior probability is obtained, a probability distribution is used to model the uncertainty that experts can have in the estimation of that probability. This probability distribution might be a Beta distribution (because it is appropriate to model probabilities) or a BetaPert distribution (because it is appropriate to model expert opinion).

Table 1. Preliminary list of criteria that may be used to assess the pre-survey probability of freedom of a disease in a country.

Non disease-specific criteria	Disease-specific criteria
• Veterinary infrastructure	• History of disease
• Veterinary education	• Current disease status
• Animal population	• Specific disease surveillance / control activities
• Animal movement / trade	• Targeted research activities
• Diagnostic laboratories	
• Disease surveillance / control activities	

Table 2. Proposed scale for the quantitative assessment of the pre-survey probability of freedom of disease

Freedom from disease	Point estimate of pre-survey probability
Extremely unlikely	0.01
Very unlikely	0.05
Unlikely	0.20
Rather unlikely	0.40
Rather likely	0.60
Likely	0.80
Very likely	0.95
Extremely likely	0.99

APPLICATION OF THE APPROACH (IBR IN SWITZERLAND)

Background

Infectious Bovine Rhinotracheitis (IBR) is one of the diseases targeted for eradication in Switzerland (Anon., 1995a). Despite sporadic few cases recorded since 1994, Switzerland is declared officially free from the disease. A comprehensive review of the IBR eradication

campaign in Switzerland has been recently completed (Riggenbach, 1998). We summarise here the principal historical components.

IBR was observed for the first time in Switzerland in March 1974 in the canton of Fribourg and several outbreaks were recorded in autumn and winter of 1977 and 1978 in the cantons of Appenzell and St Gallen. IBR was made reportable in June 1978 (Swiss Ordinance of Epizootics on June 9 1978). By the end of 1978, 366 herds with clinical cases were identified. Within these herds, more than 50% of the animals were sero-positive. Measures to control the disease were taken at the cantonal level. In July 1982, national measures were initiated to eradicate the disease in Switzerland. Until 1993, all cattle > 2 yrs from all Swiss herds were tested annually using an ELISA. Sero-positive animals were considered as virus carriers and slaughtered.

From 1994 to 1997, annual surveys were conducted on a representative sample of approximately 4600 to 5000 herds to substantiate freedom from IBR. The sample size was chosen in order to be 99% confident that at least one diseased herd would be selected if the herd disease prevalence was 0.1% (Anon., 1995b). The selected herds represented about 8% of the 62000 cattle herds in Switzerland (Anon., 1996). Blood samples were taken from all cattle > 24 mo of age and, when there were < 5 such animals, younger animals were sampled in addition. Pooled milk samples from each 5 lactating cows were taken instead of individual blood samples when possible. Sera and milk samples were tested using an ELISA (IBR Checkit® Trachitest, Dr. Bommeli AG, Liebefeld-Bern). In 1998, the procedure was changed to sample only 5 randomly selected animals > 2 yrs from each selected herd. These samples were tested with ELISA as in the previous annual surveys. None of the above surveys indicated the presence of the disease among the sampled herds.

All aborting cows and clinically suspect cattle are tested for antibodies against the IBR virus in one of 17 regional laboratories accredited to perform IBR testing in Switzerland. Test-positive samples are confirmed at the reference laboratory for IBR in Zurich. Testing of animals because of import/export or exhibitions is also routinely performed. A total of 147 animals were confirmed IBR positive by serum neutralisation test between 1995 and 1998 (Stauber, Institute of Virology, Zurich, pers. comm.). These cases included animals that were tested for importation to Switzerland, however, the actual proportion is unknown.

Assessment of the probability that Switzerland is free of IBR from the 1998 survey result

We have used the approach introduced in this paper to estimate the probability that Switzerland is truly free of IBR using the 1998-survey result.

The modelling process involved 3 steps:

Step 1: evaluation of the herd-level test sensitivity and specificity: This step has been fully described by Audigé and Beckett (1999). The input data and assumptions for the model were the following:

- Herd sizes: The distribution of herd size was obtained from the actual number of cattle over 2 yrs of age in herds sampled in 1997 (these data were not available for 1998). We ignored herds with < 5 cattle and used a combination of discrete and uniform distribution as described in Audigé and Beckett (1998) to derive the herd size distribution.

- Number of animals sampled per herd: Blood samples were taken from 5 cattle > 2 yrs in each of the herds in the survey.

- Characteristics of the individual-animal test: Sera were analysed using the ELISA IBR Checkit® Trachitest (Dr. Bommeli AG, Liebefeld-Bern). Based on published literature (Bommeli et al., 1980; Bommeli & Kihm, 1982; Rosskopf et al., 1994), we choose to model the variability of ELISA sensitivity and specificity by two BetaPert distributions. Minimum, most likely and maximum values were set to 94%, 98% and 99.5% for the sensitivity, and to 81%, 98% and 99.5% for the specificity, respectively.

- Within-herd disease prevalence: Within fully susceptible herds, it is believed that the IBR virus would spread rapidly following the introduction of an infectious cattle, resulting in an animal prevalence of more than 50% (Hauser, Swiss Federal Veterinary Office, pers. comm.). Consequently, we modelled this disease prevalence using a BetaPert distribution with minimum, most-likely and maximum values set to 50%, 70% and 100%, respectively.

The distribution of the number of test-positive animals expected in diseased and IBR-free herds is presented in Figure 1. From this figure, the cut-off number of positive animals per herd to declare the herd as IBR positive has been chosen to be 2. Consequently, point estimates of herd-level sensitivity and specificity were 98.2% (1 – (0.016 + 0.002) = 0.982) and 97.3% (1 – (0.024 + 0.002 + 0.001) = 0.973), respectively.

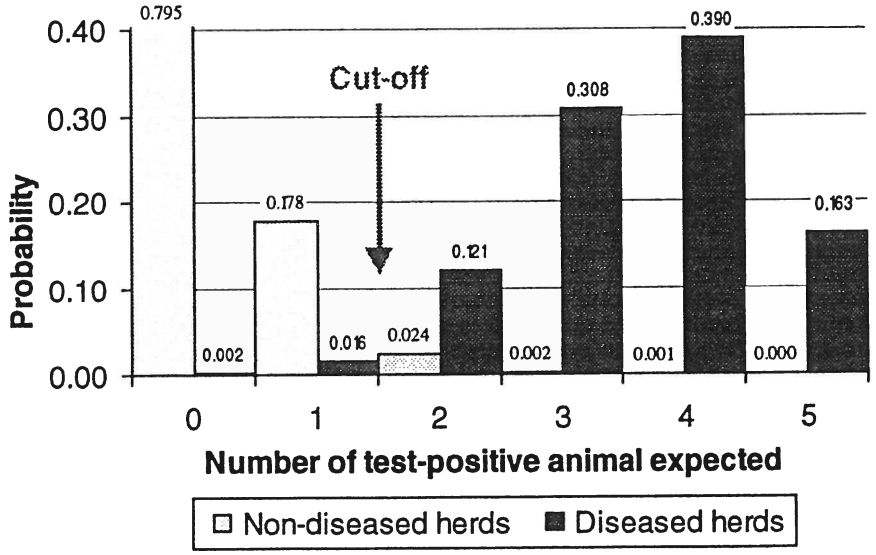

Fig 1. Probability distribution of the number of test-positive animal expected from non-diseased and diseased herds, respectively.

Step 2: Estimation of the likelihood ratio: For the second step simulation, the input data and assumptions were the following:

- Population of herds: the population of cattle herds in 1998 was estimated from the Swiss official statistics of 1997 that recorded 55883 herds (Bohnenblust, Swiss Federal Statistical Office, pers. comm.).

- Sample size of herds: The number of herds sampled during the survey of 1998 was 4672.

- Herd disease prevalence: The herd disease prevalence was the threshold of 0.1%, under which the Swiss Federal Veterinary Office considers Switzerland as "free from IBR" (Anon., 1995b). This threshold corresponds to a number of 56 diseased herds in the current population of 55883 herds.

Probability distributions of the number of test-positive herds expected in a situation of freedom from IBR, and assuming that 56 herds are diseased, are presented in Fig 2.

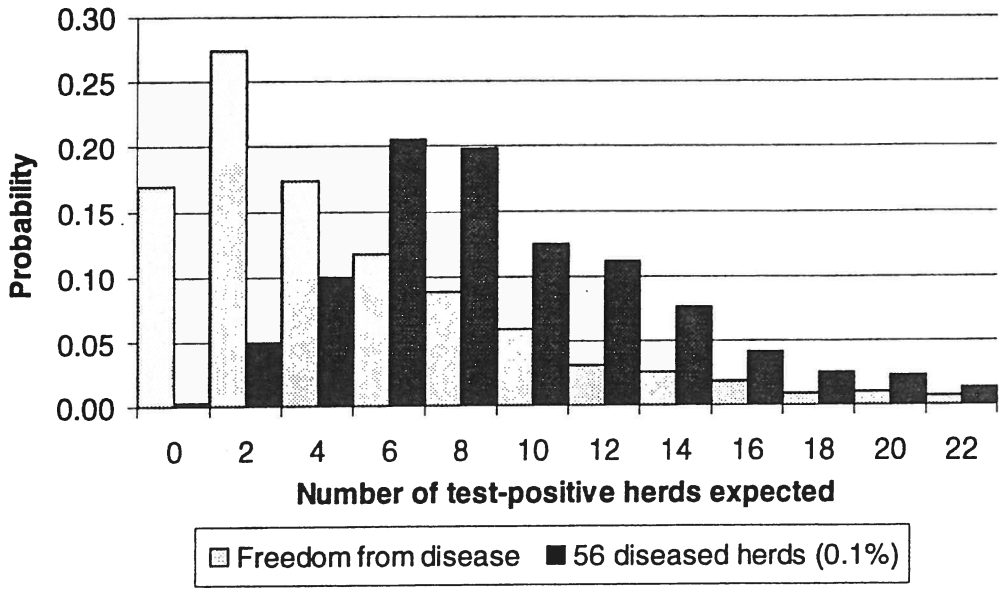

Fig 2. Probability distributions of the number of test-positive herds expected in a situation of freedom from IBR, and assuming that 56 herds are diseased, respectively

In the actual survey, no positive herds were identified. The point estimate of the likelihood ratio for zero test-positive herds is 56.7, which means that we were 56.7-times more-likely to observe no test-positive herds if Switzerland was free of IBR than if 0.1% of cattle herds were diseased.

Third step: Probability that the Switzerland is free of IBR: The probability that Switzerland is free of IBR (posterior probability) is derived from the formula defined previously, i.e. from our estimates of the pre-survey probability and the LR. Stochastic simulation using 1000 iterations was performed to derive the probability distribution of the LR and the post-survey probability with the computer software @Risk (Palisade corporation, Redmont, USA). The probability distribution of the LR is presented in Figure 3.

From the review of the history of the disease summarised earlier and the results of earlier surveys, monitoring and control activities, it could be assessed that Switzerland is likely to be free from IBR (i.e. with no herds with more than a single sero-positive carrier animal in the current context). From Table 2, this corresponds to a pre-survey of disease freedom probability of 80%.

This pre-survey likelihood is prone to uncertainty associated to our subjective assessment. Not only this likelihood could be low or high, but we could also be uncertain about that assessment. For IBR, due to fairly good reporting and diagnostic capabilities, we

may be rather confident about the quality of the data provided. For the purpose of this presentation, we have used a BetaPert distribution because it is flexible to use and appropriate to model expert opinion (Vose, 1996). Minimum, most-likely and maximum values were set to 75%, 80% and 90%.

The distribution of the post-survey probability of freedom of IBR is presented in Figure 4.

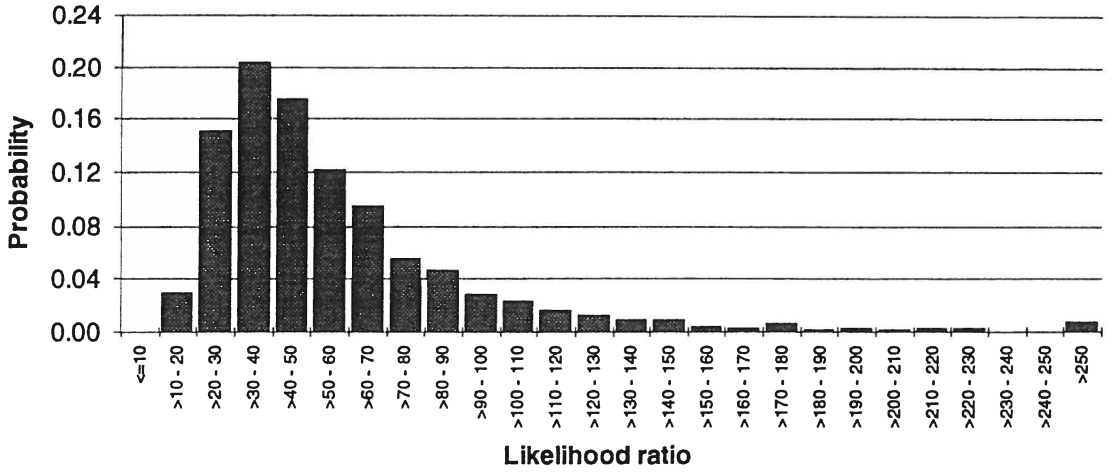

Fig 3. Probability distribution of the likelihood ratio of observing zero test-positive herds

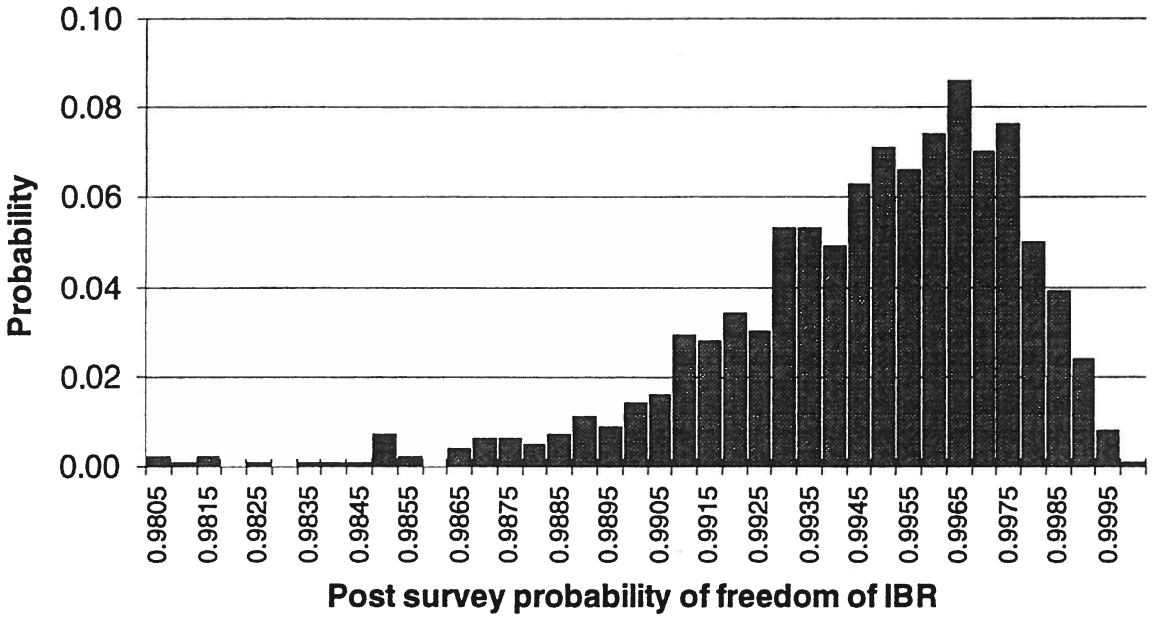

Fig 4. Probability distribution of the post-survey probability of freedom of IBR in Switzerland

While the pre-survey probabilities was subjectively assessed between 75% and 90%, the post-survey probability is estimated to be at above 99.3% in 90% of the iterations. Thus we

can see that the result of the 1998 IBR survey has significantly increased our confidence in believing that Switzerland is free of IBR. Back to the subjective scale used (Table 2), the interpretation of this result would be that Switzerland is extremely likely to be free of IBR.

CONCLUSION

We have presented an innovative quantitative approach to assess from findings of survey systems the degree of certainty in declaring a country or region free of a disease. This approach takes into consideration the survey's methodology, including testing procedures, and factors influencing our prior knowledge of the disease in a country. The method is, however, still under development and will require collaboration between research groups involved in this area to achieve a high degree of standardisation and acceptance. We believe that it has a valuable application in the current decision making process of animal health authorities for movement of animal and animal products.

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PROBLEMS IN MAPPING SPATIAL DATA LINKED TO ARTIFICIAL BOUNDARIES

C. STAUBACH[1], M. ZILLER[1], K. TACKMANN[2], T. MÜLLER[2] AND H. SCHLÜTER[1]

The construction of disease maps has been a central part of descriptive epidemiology throughout its history. Mapping first used for communicable diseases in an attempt to identify the sources of infection, and to describe the rate of spread (Snow, 1854; Howe, 1989). The ideal data for this kind of descriptive epidemiological procedure would be disease information obtained at the finest resolution and highest sample size possible. In practice, disease data are frequently mapped on the basis of artificial boundaries such as administrative units since the data may only available on this scale or because it is desirable to summarize data with a more accurate resolution at a higher (administrative) level (Walter & Birnie, 1991; Rushton, 1998).

In wildlife population surveys, administrative structures are often the only feasible way to map samples. Just in a few studies each sample is connected to exact coordinates (Kitron et al., 1991; Staubach et al., 1998). One of the most popular map types in geographic epidemiology is the choroplethic map. Choroplethic maps assign a shading or colour to geographic areas (defined by their boundaries) to visualize the variable of interest. The assigned hatching pattern or colour is based on a class interval or continuous scale deduced from a descriptive statistic of the aggregated data. Especially in situations where the sampling size per spatial unit is small, dot maps and proportional symbolic maps may be more helpful for visualization. Nevertheless, all manipulation and analysis is fundamentally relies on the given set of zonal units, and this cannot be overcome without access to individual data records (Gatrell, 1994; Burrough & McDonnell, 1998).

The simple classification into different prevalence ranges that is frequently applied causes some problems: (i) Spatial boundaries are artificially chosen and not relevant to disease spread. (ii) The sample size is often not taken into consideration when spatial data are presented. Therefore, confidence limits may overlap with those of neighbouring prevalence ranges and prevalence differences between neighbouring units could thus be random. (iii) When data are stratified, sample sizes in some units or strata may be too low to obtain reliable prevalence estimates. (iv) Mapping surveillance data in this manner may also lead to false interpretations of disease clusters or disease-free areas. Furthermore, all relationships observed between variables will only hold for this particular aggregation of the data, a phenomenon which is well-known as ecological fallacy (Fotheringham & Wong, 1991; Fotheringham & Rogerson, 1993; Pfeiffer & Morris, 1994; Smans & Estève, 1996; Haining, 1998).

Particularly in medical epidemiology, different spatial filters, smoothing methods and parametric regression techniques have been suggested for the solution of these problems (Carrat & Valleron, 1992; Pfeiffer & Morris, 1994; Elliott et al., 1995; Haining, 1998). They do

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frequently not regard all sampled informations as parameters of a hypergeometric distribution (Carrat & Valleron, 1992; Elliott et al., 1995; Smans & Estève, 1996).

By using surveillance data of *Echinococcus multilocularis* and rabies infections of foxes, classical swine fever (CSF) and pseudorabies virus (PRV) infections of wildboars, we compared various smoothing methods such as pycnophylactic interpolation and kriging. In addition, we examined new approaches such as a moving average filter which has been modified for sampled data, and a statistically controlled successive unification of neighbouring units.

MATERIAL AND METHODS

Disease data

The study area comprised of a region in the eastern part of Germany and is situated between 11.7°-14.7°E and 51.3°-53.4°N and covers approximately 29,530 km^2. The Federal State of Brandenburg is divided into 1700 administrative units (municipalities) with an average area of 17.4 km^2. Municipialities may have enclaves with the same identification number which are not directly spatially linked to the main adminstrative unit. The topographical map consists of a total of 1908 geographic areas. For interpolation purposes, a layer of point features representing the centroid of each spatial unit was created in the Geographic Information System (GIS).

Disease data consist of the numbers of diagnosed positive and negative results directly linked in the GIS to the spatially defined administrative units. If enclaves of municipalities existed, the positive and negative results were subdivided proportionally to the area of each unit.

The data base, sampling frame and investigation procedure of the disease data is described elsewhere. We used summarized surveillance data of the parasitic infection of foxes with *Echinococcus multilocularis* between the years 1991 and 1995 (Tackmann et al., 1998) and data of a serological survey on antibodies against rabies virus following oral vaccination of foxes in the year 1995 (Stöhr et al., 1994; Schlüter & Müller, 1995) as examples. The spatial distribution of PRV infections of wildboar based on a serological survey of the year 1993 (Müller et al., 1998). Furthermore, data of a recent outbreak of CSF in wildboar in the northwest of the Federal State of Brandenburg was examined between the years 1995 and 1997 (Kern et al., 1999).

Table 1 summarizes the statistics of the period prevalence data in the region compiled unit by unit.

Table 1. Summary statistics of example data sets

	E. multilocularis 1991 - 1995	Rabies-serology 1995	PRV-serology 1993	CSF 1995 - 1997
No. of spatial units	1908	1908	1908	476
No. of pos. results	437	1905	119	211
No. of neg. results	14443	449	1245	11077
No. of units with samples	1428	775	370	273
Mean prevalence[a]	0.018	0.814	0.101	0.016
Standard deviation	0.074	0.318	0.255	0.062
Maximum prevalence[a]	1.000	1.000	1.000	0.800

[a]Prevalence are individually calculated for each spatial unit

Spatial Analysis

Kriging: The geostatistical technique 'kriging' comprises of an optimal interpolator whose estimates are unbiased and have a known minimum variance. The technique is based on the theory of regionalised variables and utilises the spatial structure of the data. It involves the construction of a variogram and the fitting of an appropriate model. The kriging interpolation estimates by local weighted averaging, where the weights are determined by the variogram and the configuration of the data (Isaaks & Srivastava, 1989; Oliver & Webster, 1990). Kriging has been used frequently in earth science and environmental applications, but it can also be used to describe the geographic variations in the prevalence of a disease over a certain area (Carrat & Valleron, 1992; Pfeiffer, 1994).

The semivariogram of the prevalences at the centroid locations of the spatial units was estimated using the standard formulation. Ten percent of the average sample spacing of 96 km was chosen as lag to calculate the sample variogram up to half of the maximum distance between two sampling points (142 km). The spherical model was used to fit the experimental semivariogram. The map was generated by estimating the value at each node of a regular grid with an arbitrarily chosen cell size of one km^2 and the borderline of the Federal State of Brandenburg as barrier theme (Isaaks & Srivastava, 1989; Oliver & Webster, 1990; Carrat & Valleron, 1992).

Pycnophylactic interpolation: This method interpolates a continuous surface from data given by irregular geographic polygons. In an iterative process the bivariate histogram (polygon times hight of the variable) is converted to a spatial mesh of arbitrary fineness. The volume of the bivariate histogram for each spatial unit is constrained within the zone boundaries with very small residuals depending on the cell size and numbers of iteration (Tobler, 1979).

A cell size of one km^2, two smoothing-steps per iteration and a convergence limit of 0.01 was chosen to produce the disease distribution maps of the example datasets.

Moving average filter: The moving average filter technique (Burrough & McDonnell, 1998; Rushton, 1998) was modified for application to sampled data (i.e. positive and negative results were exactly averaged) and the algorithm was adapted for data based on polygons.

$$\hat{p}_i = (1-w)\cdot p_i + \frac{w}{NoNU(i)}\cdot \sum_{(i,j)\in NU} p_j$$

$$\hat{n}_i = (1-w)\cdot n_i + \frac{w}{NoNU(i)}\cdot \sum_{(i,j)\in NU} n_j$$

where NU is the set of pairs (i, j) in which each unit i is neighbour of cell j, and p_i and n_i are the numbers of positive and negative results for each cell i. $NoNU(i)$ is the number of neighbours per cell i, and w represents the weight of influence of the neighbourhood cells.

For this study a weighting factor of one third was used and the moving average filter scanned the data sets three times. Spatial units with sample sizes below eleven were labelled, so that areas with unreliable results could be easily identified.

Statistically controlled successive unification of neighbouring units (STACSUNU): This algorithm can be used, if the sample results are spatially structured (i.e. the number of positive and negative results are available for each spatial unit) and the neighbourhood relation of the spatial units is known.

The principle of STACSUNU is based on the assumption that neighbouring spatial units whose sample results do not justify a statistical distinction can be unified. Therefore a maximum error level α has to be chosen. For all spatial analysis in this study α was set to 0.05.

The algorithm is carried out cyclically. Definite unifications are executed in each cycle following the same rules. The successive fusion of neighbouring units stops, if no more unifications had to be carried out in the previous cycle. In this terminal stage all generated neighbouring spatial units differ at the same specified α-level.

In each cycle the spatial units are first put into order (i.e. numbered) by decreasing sample sizes as the main criterion and decreasing numbers of positive results (i.e. prevalence) as secondary criterion. Then the sample results are successively compared for each spatial unit with those of their neighbours with larger order characteristic (i.e. larger numbers). For this purpose the exact Fisher-test (Yates, 1934) yielded a probability value (p-value) for each neighbour. If in one or more cases p are larger than α, the corresponding pair with the largest p-value is spatially unified and the positive and negative results are separately added up.

Since all neighbouring spatial units differ on a prespecified error level, an unclassed choroplethic map, i.e. with a continuous shading or hatching pattern directly proportional to the magnitude of prevalence in the spatial units was chosen (Gale & Halperin, 1982; Smans & Estève, 1996).

Software: Raster- and vector-based data were analysed in the Spatial Analyst Module of ArcView (ESRI, Redlands, USA). The semivariograms were estimated using the geostatistical software VARIOWIN 2.2 (Pannatier, 1996).

RESULTS AND DISCUSSION

In order to demonstrate the spatial analysis methods described in this paper only maps concerning the distribution of *E. multilocularis* are shown (Figs. 1 - 6). Figure 1 shows the municipalities and districts of the Federal State of Brandenburg and the city of Berlin, indicating that it had not been sampled. Furthermore, to demonstrate the effect of the techniques in a better way, the northwestern part of Brandenburg is displayed at higher magnification (Figs. 2 - 6). Figure 2 illustrates the period prevalence of *E. multilocularis*-positive foxes calculated for each spatial unit separately in a choropleth map. All maps were classified with the same continuous shading proportional to the prevalence value (i.e. white: 0.0 until black: 1.0).

A large number of epidemiological studies utilize explorative spatial data analysis to describe geographical distributions of very different types (e.g. virological and serological prevalences, incidences, biological marker proportions). Health data are often collected at the scale of geographic areas, because even the step from the smallest administrative unit to exact coordinates of the sampled animal is enormous and only in a few cases necessary, (e.g. for spatial analysis of habitat and microclimate; Kitron et al., 1991; Staubach et al., 1998). Therefore, the data are often mapped independently for each spatial unit (Fig. 2). In spite of efforts to reach a sample size as large as possible, the sampling sizes per spatial unit are often very low (Tab. 1).

This leads to several problems in the explorative analysis of data: (i) If extreme situations are observed in areas with smallest sample sizes, they may attract an interest which is not justified by the data (Fotheringham & Rogerson, 1993; Elliott et al., 1995). A possible solution in this case would be to avoid choroplethic maps and distribute points of different colour for positive and negative results randomly in each spatial unit (e.g. dot maps) to visualize the sampling distribution in a better way (Gatrell, 1994; Müller et al., 1998; Tackmann et al., 1998). Another method consists in the aggregation of the data on a higher spatial level (e.g. districts or countries), but this reduces the sampled spatial information and may lead to false interpretations

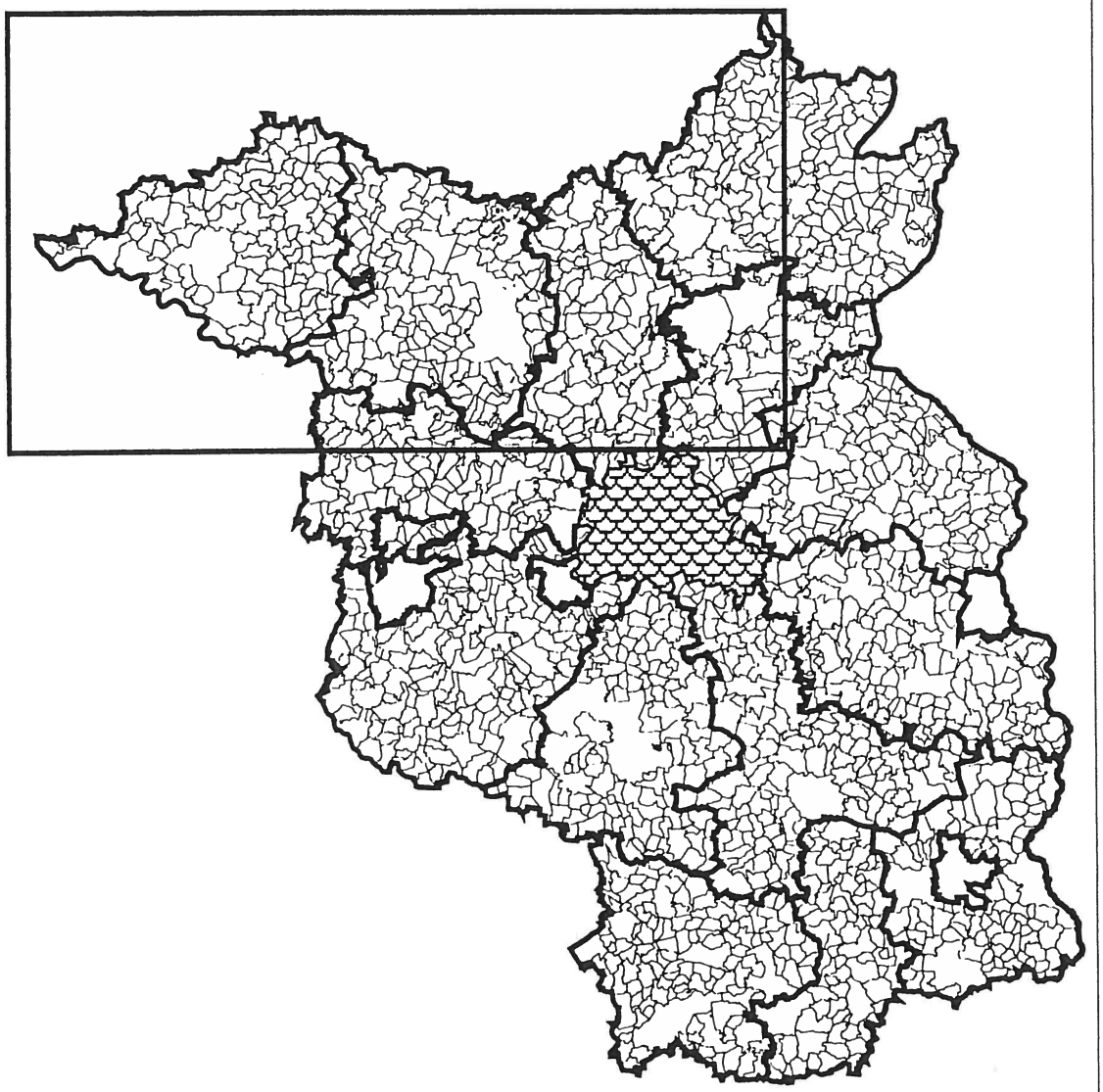


Fig. 1 Spatial units and districts of the Federal State of Brandenburg

Shading patterns of selected prevalence values as examples of the continuous legend (applied to all following figures)

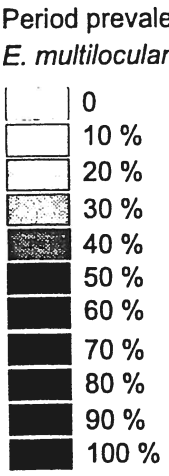


Fig. 2 Prevalence individually mapped for each spatial unit

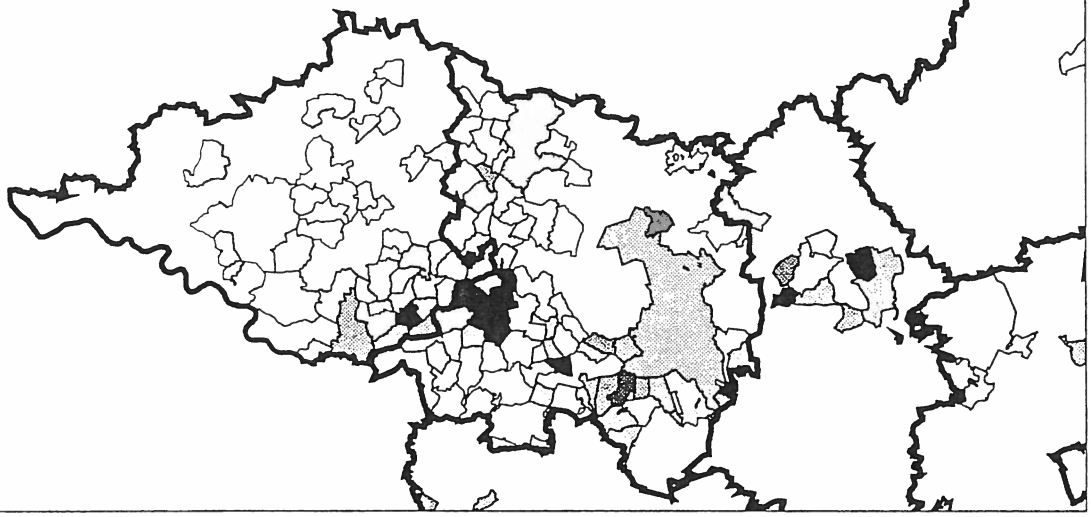


Fig. 3 Kriging

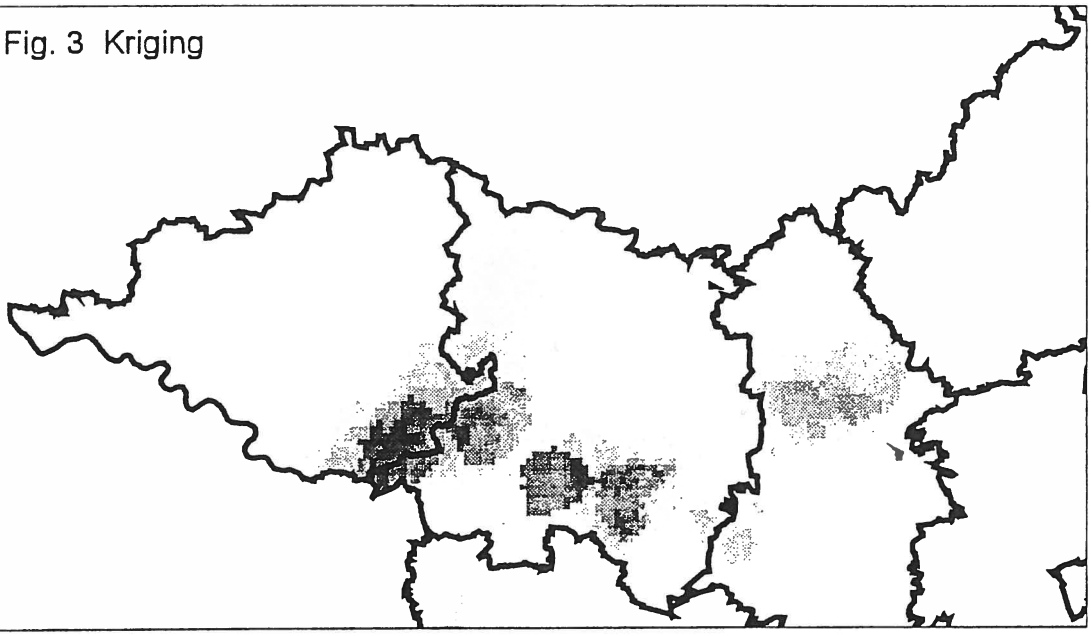


Fig. 4 Pycnophylactic interpolation

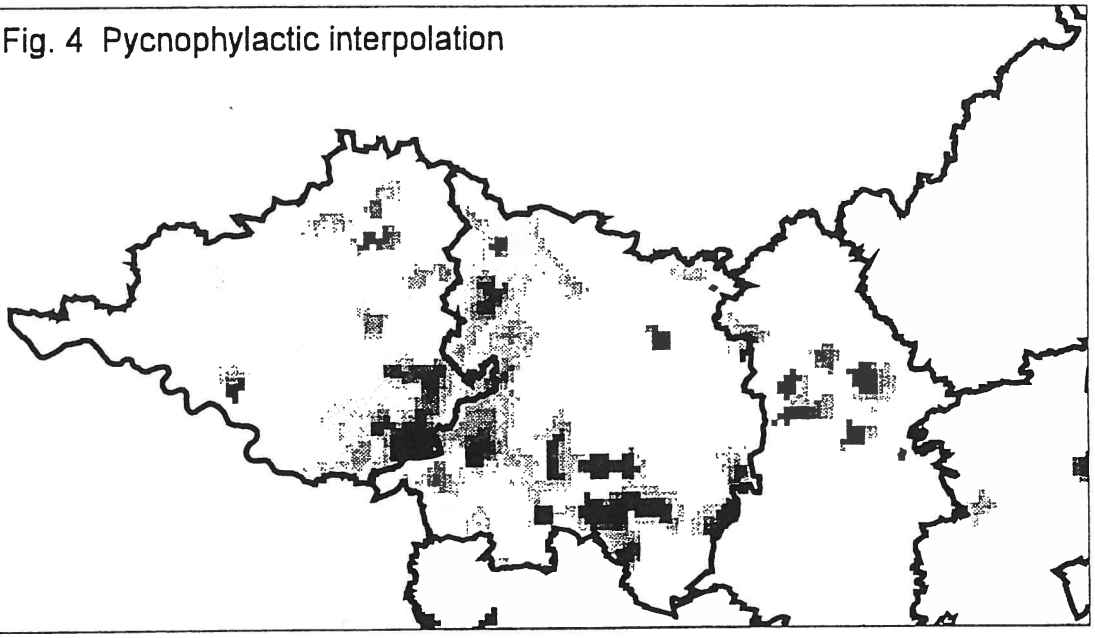


Fig. 5 Modified moving average filter

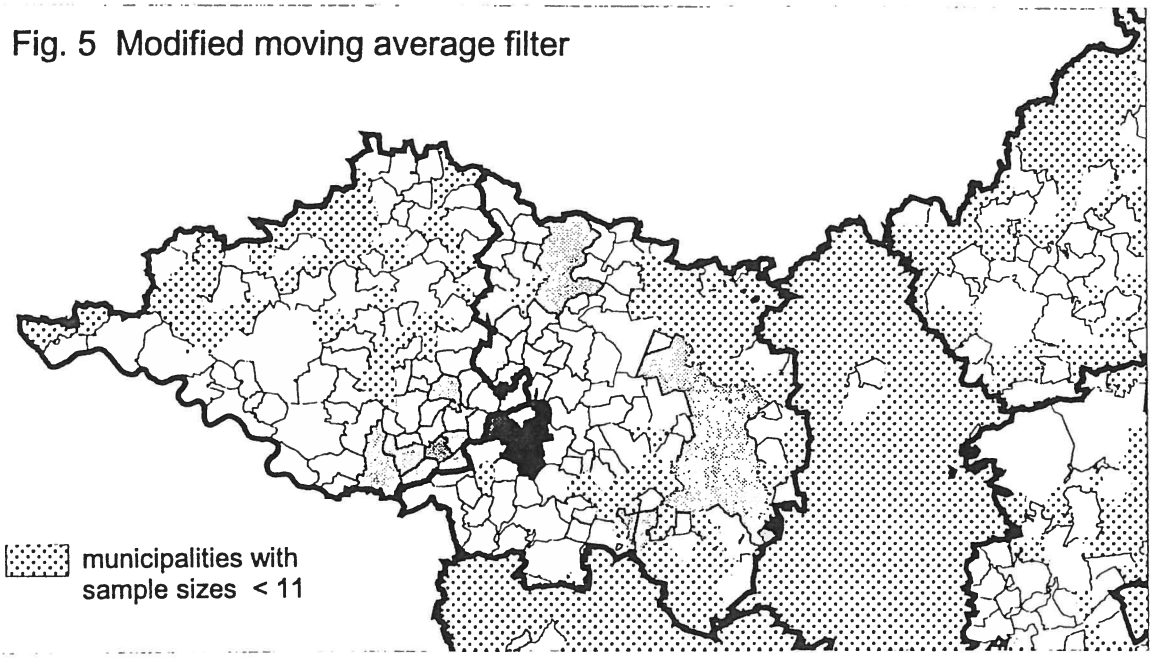
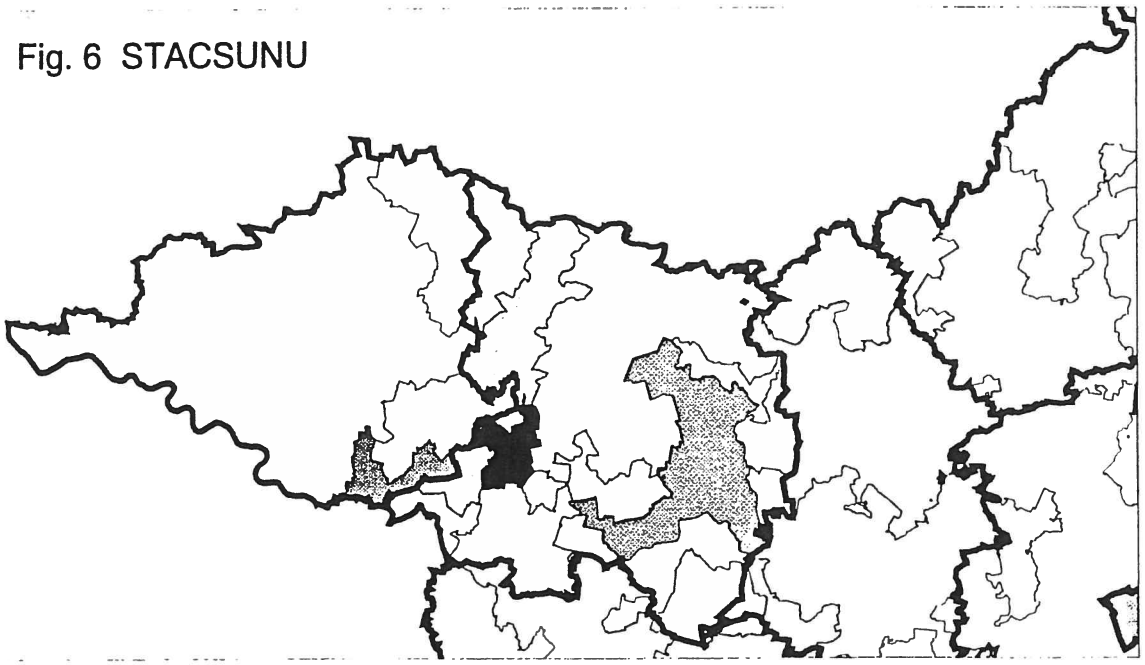


Fig. 6 STACSUNU



of disease clusters or disease-free areas (Schlüter & Müller, 1995). (ii) Choroplethic maps where the sampled ratio per spatial unit is mapped without consideration of the whole sampled information as parameters of a hypergeometric distribution may lead to false interpretations of prevalence differences between neighbouring units. Therefore, these explorative techniques do not allow to conclude whether one area is really different from another spatial unit at a certain confidence level. This problem could be circumvented by producing error maps which show the minimum and maximum confidence limit of the dataset per spatial unit. (iii) The modifiable area unit problem - i.e. the process which generates the positive and negative results is not influenced by the spatial distribution of the artificial boundaries (Fotheringham & Wong, 1991; Fotheringham & Rogerson, 1993; Pfeiffer & Morris, 1994). Therefore, also sudden transitions between levels of two neighboring areas may occur in a choroplethic map which are related to the aggregate nature of the data and the particular configuration of zonal boundaries, and do not show the real distribution of the variable of interest.

For these reasons and to enhance statements about locations on the basis of relationships about events in locations nearby, different spatial filters and smoothing methods were developed and adapted to geographic epidemiological problems. Figures 3 and 4 show the results of kriging and pycnophylactic smoothing, respectively, as examples for interpolation methods. It was not possible to fit an appropiate model for all example data sets without removing extreme values (prevalence = 1.0). Furthermore, no reliable results could be obtained for the spatial distribution of PRV results by the kriging method. The algorithms of kriging and pycnophylactic interpolation estimate the interpolated values for the new geographical areas (i.e. grid cells, polygons) from ratios which aggregate the data in a first step and therefore loose parts of the collected information (Carrat & Valleron, 1992; Elliott et al., 1995; Haining, 1998). If the sample size is large, this loss can be neglected. The modified moving average filter used in this study took the complete sample information into account by correct averaging (Fig. 5). If the sampling frame is sparse - as it is often the case in data sets from wildlife population surveys -, the variable of interest will often be obtained at different confidence levels. The algorithm of STACSUNU takes the whole infomation content of the sample into account to produce maps with new boundaries which fulfill statistical criteria (Fig. 6). Furthermore, STACSUNU is independent of special model assumptions and the final map displays a more adequate data representation. STACSUNU may be useful for the first step of descriptive and explorative spatial data analysis.

The final step of map generation involves the careful selection of colour or shading scales to transform the data set into an informative picture. The need to group data into classes lead cartographers to develop different statistical, graphical and iterative techniques of defining the optimum class interval (Gale & Halperin, 1982). In consequence, different portraits of the data can be produced from the same variable of interest depending on the method used in selecting class intervals (Muller & Honsaker, 1978). The unclassed choroplethic map in which the intensity of the colour is proportional to the prevalence value in the geographic area, solves this problem and determines the shading variation in the most objective way. For our purpose of explorative data analysis, even the critics concerning the representation of data when the distribution is highly skewed (Gale & Halperin, 1982), can be positively utilized: outliers are appropriately emphasized and the remaining areas shaded in a relatively uniform manner. It should be noted that mechanisms of human visual perception tend to overemphasize small difference in colouring or hatching (Smans & Estève, 1996). However, the sampling results of neighbouring units resulting from STACSUNU differ significantly in any case.

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SCRAPIE

UNDERSTANDING THE EPIDEMIOLOGY OF SCRAPIE

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Scrapie is a transmissible spongiform encephalopathy (TSE), a category of diseases which includes bovine spongiform encephalopathy (BSE), kuru and new variant Creutzfeld-Jakob disease (vCJD). TSEs are unusual in many respects, not least because the nature of the infectious agent remains controversial; a leading hypothesis is that these diseases are caused by the presence of an abnormal form of the prion protein (PrP) referred to PrP^{Sc} (Caughey & Chesebro, 1997). TSEs have long incubation periods, have no associated immune response, cause progressive deterioration of neurological function, and are incurable and invariably fatal. Understanding of the epidemiology of TSEs is constrained by incomplete knowledge of many aspects of the biology of these diseases and by the limited availability or accessibility of epidemiological data (Woolhouse & Anderson, 1997).

Scrapie is perhaps the best known of the TSEs and the epidemiology of this disease has been reviewed in detail elsewhere (Hoinville, 1996). There are 4 components to a quantitative understanding of scrapie epidemiology: demography; population genetics; pathogenesis; and transmission. These interact in complex and sometimes counter-intuitive ways and recently developed mathematical models (Stringer et al., 1998 & Woolhouse et al., 1998) have proved useful as aids to the interpretation of field data and as guides to the expected long term impact of control measures.

EPIDEMIOLOGY

Field data

Scrapie has been recognised in the UK for over 200 years. Since the disease became notifiable in 1993, 2032 cases have been reported in sheep and goats in Britain (data from MAFF, correct to July 1998), corresponding to an incidence of less than one case per 100,000 per year. This is generally regarded as a gross underestimate of the true incidence due to widespread under-reporting (Morgan et al., 1990) and several surveys are now under way to provide better estimates.

Scrapie has been reported in almost all counties in Britain. The disease is over-represented in England, relative to the sheep and goat population, and under-represented in Wales. This may reflect differences in scrapie epidemiology or may merely reflect differences in reporting rates.

Some information is available on the age distribution of scrapie cases. In 1993-7 80% of cases

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occurred in sheep or goats between 2 and 5 years old. The mean age of cases was 3.8 years. No cases were reported in animals less than 1 year old (though such cases can occur) and the oldest case was in a 15 year old animal.

Better epidemiological information is available on a number of outbreaks in individual flocks (e.g. Hunter et al., 1996). Incidences exceeding 20% per year have been reported, although lower values may be more typical; in many flocks with scrapie only 1 or 2 cases occur per year. Even in flocks of only a few hundred animals the duration of a scrapie outbreak may be as long as a decade or more. The age distribution of cases in most outbreaks is comparable to that for the national flock.

Demography

Demography is important to understanding scrapie epidemiology for several reasons. First, the disease can be maternally transmitted (see below) and hence lambing patterns influence the distribution of cases. Second, the incubation period of the disease (approximately 2 years) is long with respect to sheep life expectancy (4 years or less); this means that only a fraction of infected sheep survive to show clinical signs although subclinically infected sheep are believed to be capable of transmitting the disease. Third, because of the long duration of an outbreak, there is considerable turnover of the sheep population during its course, influencing numbers of infected and susceptible sheep.

Complete demographic records for the British sheep (and goat) flock are not available. The national flock comprises more than 40 million animals in more than 100,000 individual flocks. Only limited information is available on age structure but approximately 50% are lambs, most of which are slaughtered for meat (data from MAFF). The geographic distribution of sheep, however, is well recorded. For certain breeds, some information on numbers, distributions, pedigrees and ages of sheep flocks is recorded by the breed societies.

In contrast, complete demographic and pedigree data are available for a number of individual flocks for where such records are kept. For one such flock of Cheviot sheep survival analysis gave a mean life expectancy of 3.5 years with the mortality rate increasing with age, although this was not a commercial flock and the results may not be typical (Woolhouse et al., submitted).

Population genetics

Scrapie is also unusual in that the genetics of susceptibility to disease are better understood than its epidemiology. Susceptibility to scrapie infection is influenced by polymorphisms at the PrP locus. A large number of PrP alleles have been identified in sheep and goats but the most important involve amino acid substitutions at codons 136 (valine, V, or alanine, A), 154 (arginine, R, or histidine, H) and 171 (R, H or glutamine, Q) (Hunter et al., 1996; Dawson et al., 1998). Of the 12 possible haplotypes only 5 have been found to date: VRQ, ARQ, AHQ, ARR and ARH. If the other haplotypes never occur this could indicate constraints on the structure of the prion protein. All of the 15 possible genotypes from these 5 alleles have been reported. However, not all alleles are found in all breeds of sheep and the relationship between genotype and susceptibility itself varies between breeds (Dawson et al., 1998). In general, VRQ/VRQ homozygous sheep are highly susceptible, the ARQ allele is associated with some susceptibility, and the ARR allele is associated with resistance. For Cheviot sheep (Hunter et al., 1996; Woolhouse et al., submitted), VRQ/ARQ heterozygotes are approximately 30% as susceptible as VRQ/VRQ homozygotes and all other genotypes are resistant, i.e. they do not succumb to clinical scrapie.

The true status of 'resistant' genotypes, however, remains uncertain. These genotypes do not acquire clinical scrapie but it remains possible that they can become subclinically infected and, if so, that they can act as carriers. Until recently, the absence of a diagnostic test for subclinical scrapie precluded investigation of this possibility but now a test involving a tonsillar biopsy is available (Schreuder et al., 1998) though not yet in routine use. This test can detect infection in Swifter sheep with susceptible genotypes as early as 4 months after exposure and all sheep in which preclinical infection was detected went on to develop clinical scrapie. In contrast, no infection was detected in sheep with 'resistant' genotypes for at least 17 months after exposure. These are preliminary results based on small sample sizes but they are consistent with the view that certain genotypes are indeed resistant to scrapie infection.

Pathogenesis

An important characteristic of TSEs is a long incubation period. The average incubation period is of the order of 2 years for scrapie in sheep, 5 years for BSE in cattle, and is unknown but may be even longer for vCJD in humans. Experimental studies of scrapie in sheep and mice have shown that the incubation period depends on the route of entry (e.g. intracerebral injection, subcutaneous injection, or oral) (Bruce et al., 1991). Patterns of pathology, however, are less variable. The scrapie agent is first detectable in the spleen and lymph nodes. The infection then spreads to the central nervous system and infectivity accumulates relatively rapidly in the brain prior to the appearance of clinical signs. Pathology involves the development of spongiform lesions in brain tissues together with the accumulation of amyloid plaques and fibrils composed of PrP^{Sc}. At some stage infectivity can also appear in placental tissues.

In mice, the incubation period of scrapie varies between lines. There has been no direct experimental comparison of incubation periods in sheep of different genotypes. For an outbreak of natural scrapie in Cheviot sheep the age of cases was greater for VRQ/ARQ heterozygotes than for VRQ/VRQ homozygotes (Hunter et al., 1996). However, this does not necessarily imply that the incubation period is longer for heterozygotes. Other possible explanations include lower susceptibility, which results in a greater average age at infection; and a reduced likelihood of maternal transmission, which results from a lower probability of infection in the ewe (Woolhouse et al., 1998). But the possibility of genetic variation in incubation period relates to the status of 'resistant' genotypes; these genotypes could, in principle, acquire infection but have an incubation period considerably greater than their life expectancy so that they never develop clinical scrapie, although there is no direct evidence that this is the case.

Transmission

It is clear that scrapie can be transmitted both vertically, from ewe to lamb, and horizontally, from sheep to sheep either directly or indirectly. It is not possible – except in special circumstances which do not apply to scrapie in sheep – for an outbreak of an infectious disease to be maintained by vertical transmission alone and several analyses (Hoinville, 1996; Woolhouse et al., 1998) have suggested that most scrapie cases are indeed acquired horizontally.

Evidence for vertical transmission comes from studies of cohorts of lambs born into a flock where scrapie is endemic (Hourrigan et al., 1979); approximately 10% of lambs removed at birth went on to develop scrapie, although the frequency of susceptible genotypes was not known so this is a lower estimate of the vertical transmission rate. In another study (Hunter et al., 1996) 40% of lambs born to dams which went on to develop scrapie themselves developed scrapie, although as some may have become infected horizontally from other sheep this is an upper estimate of the vertical transmission rate. It has also been shown that the risk of developing scrapie is associated

with the infection status of the parents (which may simply reflect genetic predisposition) but may be more so with the dam than the sire (suggesting vertical transmission), although the difference is small (Hoinville, 1996). The route of vertical transmission is uncertain although embryo-transfer experiments suggest that transmission from dam to embryo may be possible (see Hoinville, 1996). Scrapie agent has not yet been isolated from sheep milk or colostrum.

Evidence for horizontal transmission also comes from the cohort studies referred to above (Hourrigan et al., 1979); lambs removed at intervals after birth acquired scrapie at a rate corresponding to 26% per year. Several studies of natural outbreaks have reported comparable transmission rates (e.g. Woolhouse et al., submitted). The route of horizontal transmission, however, remains uncertain. Scrapie agent has not been detected in sheep faeces (although it is present in the intestine), urine (nor in the kidneys) or saliva (although it may be present in the salivary glands and nasal mucosa) but it has been found in expelled placental tissues (see Hoinville, 1996). The agent could then be subsequently ingested, experimental infection by the oral route is routine. It is possible that excreted agent may persist in the pasture or elsewhere in the environment for several years, although there are no reliable quantitative data. Other potential routes of horizontal transmission include scarification (e.g. via shared scratching posts).

Both clinically and preclinically infected sheep have been shown experimentally to be infectious. It is generally considered that infectiousness increases during the incubation period, although there is in fact no good evidence that this is the case, at least for vertical transmission (Hoinville, 1996) and, clearly, the issue cannot be resolved until the route or routes of exit of the agent are known. In principle, infectiousness, and changes in infectiousness through time, could be influenced by sheep genotype but there is no evidence to date that this is the case.

Susceptibility to infection could be related to age, as is indicated for BSE in cattle (Anderson et al., 1996). However, the data from cohort studies do not suggest any marked effect over the first 20 months (Hourrigan et al., 1979).

Outbreak dynamics

Scrapie outbreaks have a long duration, as much as a decade or more even in flocks of only a few hundred sheep (e.g. Hunter et al., 1996 & Woolhouse et al., submitted). There are many more infections than clinical cases because only a fraction of infected sheep survive the incubation period. The ratio of infections to cases has been estimated at 2:1 (Woolhouse et al., submitted) and may approach 3:1 in flocks where lambs are sold for meat. Several analyses have concluded that most transmission is due to preclinical infections (Woolhouse et al., 1998; Ducrot & Calavas, 1998). One consequence of this is that, especially in the early stages of an outbreak, scrapie may occur only as preclinical (and so undetected) cases. This situation may exist for several years (subject to stochastic effects when numbers of infected animals are small), which may explain instances where the first clinical scrapie cases have occurred several years after a flock was closed (e.g. Hunter et al., 1996). The duration of an outbreak depends on a number of factors, including the horizontal transmission rate (a low rate results in a prolonged outbreak with a low incidence of cases) and the existence of any environmental reservoir of infectivity.

A scrapie outbreak, unsurprisingly, leads to strong selection against susceptible genotypes and a reduction in the frequency of susceptibility alleles (Woolhouse et al., 1998). In a closed flock, unless 'resistant' genotypes may act as carriers, the outbreak will end once the density of susceptible sheep falls below a threshold value corresponding to epidemic 'fade out'. Note that this threshold is not 0, scrapie may be eliminated before susceptibility alleles are eliminated.

However, in principle, selection could continue if infectivity can persist either in an environmental reservoir or in carrier genotypes.

This selection against susceptibility alleles raises the question of how scrapie can persist in the national sheep flock in the long term. There are several possible explanations. First, susceptibility alleles may confer some selective advantage in the absence of scrapie, although no such effect is known. Second, susceptibility alleles may be linked to a trait favoured by breeders, a possible explanation of reports of high incidences of scrapie in certain selected lines. Third, it is possible that alleles conferring resistance to some scrapie strains confer susceptibility to others (Goldmann et al., 1994), leading to frequency dependent selection. Finally, it is possible that scrapie is not truly endemic in Britain; an epidemic in a large metapopulation of flocks in which individual outbreaks can last many years and do not completely eliminate susceptible animals is expected to have a very long time course.

CONTROL

Control measures

A number of different scrapie control measures have been attempted or considered; these include reducing vertical transmission, reducing horizontal transmission, selective breeding or culling, and the slaughter of preclinically infected sheep.

A reduction in vertical transmission can, in effect, be achieved by slaughtering all lambs born to clinically (or, using recently developed diagnostics, preclinically) affected dams. This, however, is expected to have limited impact because most infections are thought to occur through horizontal transmission (Woolhouse et al., 1998).

A reduction in horizontal transmission is, in principle, more effective but is difficult to achieve in practice because of uncertainty as to transmission routes. Decontamination of affected pasture using disinfectant and a long rest period has been attempted in Iceland (Sigurdarson, 1991) but the effectiveness of this procedure is uncertain. It is not known how other husbandry practices (e.g. stocking densities, lambing practices) affect horizontal transmission rates although ongoing risk analyses should provide useful information.

The control measure of greatest interest in the UK is selective breeding or culling. This can be achieved most efficiently by genotyping individual sheep, but this option is precluded on a large scale by the currently high cost of the procedure. The alternative is to breed or cull particular lines with known resistant or susceptible phenotypes respectively, although this is considerably less efficient.

With the advent of preclinical diagnostic tests (Schreuder et al., 1998) it may become practicable to identify and slaughter preclinically infected sheep. The efficiency of this measure depends crucially on the sensitivity of the test (Woolhouse et al., 1998). Preliminary results are encouraging: infections have been detected as little as 4 months into the incubation period (Schreuder et al., 1998).

On the other hand, the invasive nature (tonsillar biopsy) and costs of the test makes its widespread application unlikely, at least in the short term.

There is increasing recognition of the desirability of eliminating scrapie from the national sheep and goat flock. Unfortunately, this is likely to prove extremely difficult using the methods outlined above. A major problem is time scale. Reducing transmission rates or selective breeding are effective only over long time periods, at least several years (Woolhouse et al., 1998). Selective culling or the slaughter of preclinical cases are, in principle, effective over shorter time periods but this would require screening of very large numbers of animals, which is unlikely to become logistically feasible or affordable in the immediate future. More drastic options include the slaughter of entire flocks (or slaughter on even larger scales) and restocking with scrapie-resistant sheep. However, this would still involve some crucial uncertainties: whether infectivity persists in the environment and for how long; whether 'resistant' sheep can acquire subclinical infections and act as carriers; whether 'resistant' sheep are resistant to all scrapie strains.

Origins of TSEs

There is considerable interest in the epidemiological and evolutionary relationships between TSEs, especially scrapie, BSE and vCJD. There is now good evidence from 'strain typing' bioassays (incubation times in a panel of mouse lines; lesion profiling of brain pathology) that BSE and vCJD are caused by the same strain of agent but both are distinct from all strains of scrapie which have been typed so far (Bruce et al., 1997). As well as infecting cattle and humans, BSE is also capable of infecting a wide range of species (including ungulates, carnivores, rodents and primates). In contrast, there is little evidence that scrapie can 'jump' species (except to laboratory rodents) and there is no evidence that scrapie has infected humans directly. However, this does not in itself imply that scrapie and BSE are unrelated as TSEs have been shown to change their characteristics on passage between different host species (Bruce et al., 1991).

Nevertheless, at present there is no good evidence that BSE was derived from scrapie; an alternative hypothesis is that it arose from the recycling in cattle feed of rare cases of sporadic BSE (analogous to sporadic CJD in humans, which remains the most common human TSE in the UK). Epidemiological analysis has not been especially helpful here; it is extremely difficult to project back to the origin of the BSE epidemic, not least because of suspected high levels of under-reporting up to mid-1988 (Ferguson et al., in press).

Of great concern is the possibility that BSE may be passed to sheep. Sheep can be experimentally infected (including orally) with BSE (Foster et al., 1996) and surveillance for natural BSE cases in sheep is under way, though no such cases have been reported to date. One approach to determine whether sheep scrapie or cattle BSE are associated with contact with (BSE-affected) cattle or (scrapie-affected) sheep respectively, though this would be evidence for the existence of cross-species infection but not its direction.

Future work

The preceding discussion highlights a number of fundamental uncertainties regarding the biology of scrapie which demand urgent attention. Many of these are currently being addressed by ongoing research programmes (notably by MAFF and the Institute of Animal Health) including epidemiological surveys, risk analyses and experimental studies of pathogenesis and transmission.

The parallel development of mathematical models of the dynamics of scrapie infection (e.g. Stringer et al., 1998) is proving useful as an aid both to the interpretation of field data (Woolhouse et al., submitted) and to the design of control programmes (Woolhouse et al., 1998).

However, efforts to control scrapie are proceeding in advance of this improving understanding. In this context, a number of issues are urgent priorities for research. These include: elucidation of the mechanisms of horizontal transmission; comparisons of the susceptibility of different sheep genotypes to different scrapie strains and to BSE; determination of the infection status of 'resistant' genotypes; persistence of infectivity in environmental (or biotic) reservoirs; and the relationship between scrapie and BSE.

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HERD HEALTH PROGRAMMES

MONITORING THE IMPACT OF SUBCLINICAL INFECTION ON PERFORMANCE BY SEROLOGICAL TESTING AND SLAUGHTER EVALUATION

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An integral part of maintaining a high herd health status in swine operations is monitoring and detection of infection before its impact becomes apparent. For many of the classical swine diseases, a shift from acute disease outbreaks, with major death losses or a marked decrease of productivity, to predominantly subclinical infection, without obvious signs of disease, has been observed. Pathogens like porcine reproductive and respiratory syndrome virus, swine influenza virus and *Mycoplasma hyopneumonia* are prevalent in a large percentage of swine herds in the United States, often without causing obvious signs of disease. Nevertheless, these subclinical infections can cause production losses through decreased weight gain and feed efficiency in nursery to finishing pigs and suboptimal reproductive performance in sows (Baysinger et al., 1997). Several studies have evaluated the financial impact of acute disease outbreaks in swine operations (Miller and Kliebenstein, 1983; Mullan et al., 1994; Poulson et al., 1993; Rougoor et al., 1996). The economic consequences of subclinical infection, on the other hand, are much more difficult to measure.

The objective of this study was to evaluate the effectiveness of herd health monitoring to assess the economic impact of subclinical infections. Two of the most commonly used methods for herd health monitoring are serological testing and slaughter evaluation. Both methods are easy to apply in the field and are relatively inexpensive. The ability of these two methods used for herd health monitoring to predict growth performance in nursery to finishing pigs and reproductive performance in sows was evaluated.

MATERIALS AND METHODS

Farms

This study was conducted on 7 swine farms in Illinois in 1996 and 1997. All farms had a herd size of at least 100 sows and were farrow-to-finish operations. The farms had

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a history of infection with at least one of the monitored pathogens, but no signs of a clinical disease outbreak at the time of the study. In 1996, 3 University research farms were included in the study. Two of these farms could be followed up in 1997. In 1997, one additional research farm and 3 commercial farms were added to the study.

Sampling procedures

Each farm was visited 4 times per year, with a 6-8 week interval between visits. On each visit, blood samples were obtained from a cross section of pigs of different age groups. In a given year, for each farm 2 cohorts of pigs were followed from nursery to slaughter. The initial cohort size was 30 in the first year, and was increased to 35 in the second year. Cohorts were assembled from nursery pigs 6-8 weeks of age. Each pig was identified with an ear tag. At each visit, these pigs were weighed and blood samples were taken. Figure 1. illustrates the sampling scheme on each farm.

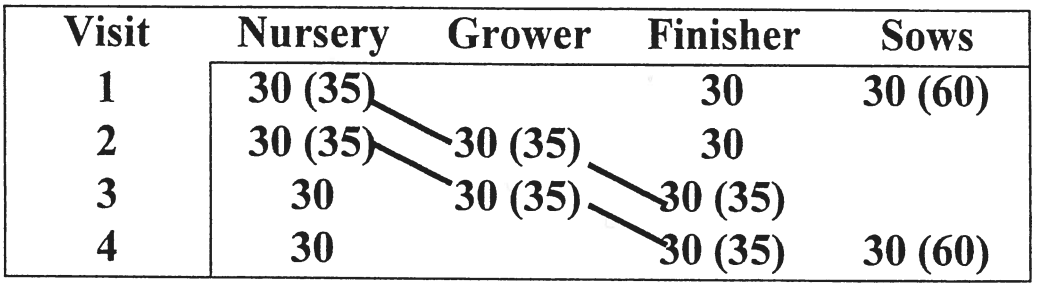

Fig. 1. Sampling scheme for each farm monitored. Numbers indicate number of pigs sampled from each age class on each visit. Arrows indicate individually identified pigs that were followed longitudinally

At slaughter, carcasses of cohort pigs were examined for gross pathology. The skin was evaluated for mange, the lungs for pneumonia and the liver for parasite infestation. The snouts were cut on the height of the first molar tooth to evaluate for atrophic rhinitis. The distance between the nasal septum and each of the lower turbinates was measured. Atrophy of the upper turbinates and deviation of the nasal septum were recorded as present or absent. A combination score was calculated from these measures to evaluate the degree of atrophic rhinitis present.

Data on reproductive performance of sows was obtained from the production records of the farms. Litter size, number of stillborn, average weaning weight, preweaning mortality and interfarrowing interval were recorded for each of the monitored sows.

Serological Testing

The infectious agents monitored serologically were porcine reproductive and respiratory

syndrome virus (PRRSV)[3], swine influenza virus (SIV)[4], transmissible gastro-enteritis virus (TGEV)[5], pseudorabies virus (PRV)[6], *Mycoplasma hyopneumoniae*[7] and *Actinobacillus pleuropneumoniae*[8]. If a farm tested negative for one of the monitored infections on 2 subsequent visits, the testing for this disease was discontinued on that farm. These pathogens were selected for monitoring because of their potential impact on productivity, and because a serological test was available that is economical and feasible under practice conditions.

Statistical Methods

Average daily weight gain was calculated for the pigs that were followed from nursery to slaughter as the difference between weights recorded at the first (6-8 weeks of age) and the last farm visit (22-24 weeks of age), divided by the time between these visits. A multiple linear regression analysis was used to evaluate the influence of subclinical infection on weight gain[9]. The outcome variable was average daily weight gain. Predictors were serological test results for all of the monitored diseases at the first, second, and last visit. Serological test results were entered into the analyses as titer values or ELISA S/P ratios rather than positive and negative values. Thus, more precise quantitative information was used and correlations of higher serological titers with performance could be detected even when all animals were seronegative or seropositive for a pathogen. The results of the slaughter evaluation were also included in the model as predictors. Covariates included to control for confounding were cohort effects, weight at the first farm visit, and sex.

To evaluate the impact of subclinical infection on sow productivity, multiple linear regression analyses were conducted. The outcomes were interfarrowing interval, number of liveborn, number of stillborn, preweaning mortality, and weaning weights for the litters preceding and following the serological testing. The average weaning weight per piglet was calculated as the weaning weight of the litter divided by the number of piglets weaned. Preweaning mortality was calculated as the number of piglets born alive plus (or minus) the number of piglets fostered on (or off), minus the number of piglets weaned. All reproductive measures except interfarrowing interval were recorded for the farrowing before and the farrowing after the serological testing. The interfarrowing interval was calculated from the farrowing before to the farrowing after the serological testing. Each farm was analyzed separately. In all of these models, serological test results for each of the monitored infections were used as the predictors. Parity was included as a covariate, because it has been described to influence reproductive performance (Whittemore, 1993).

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[3] ELISA, IDEXX, positive cutoff = S/P ratio ≥ 0.4
[4] Hemaglutination Inhibition, positive cutoff = titer ≥ 40
[5] Serum Neutralisation, positive cutoff = titer ≥ 64
[6] Differential ELISA for gI deletion vaccines, IDEXX, positive cutoff = S/P ratio ≥ 0.7
[7] ELISA, positive cutoff = S/P ratio ≥ 0.6
[8] Complement Fixation, positive cutoff = titer ≥ 8
[9] PROC REG, SAS Institute, Cary, NC

Regression models were tested for the assumptions of normality, homogeneity of variance and independence of residuals, and for influential observations (Neter et al., 1985). The model of variable selection was initial forced entry of all predictors, with stepwise backward elimination. In order to control for confounding in the model, the p-to remove criterion was set to 0.2. The level of significance was set to α=0.05.

A negative association with weight gain was predicted for increased severity of lesions found at slaughter, as well as for higher antibody levels at 16 and 24 weeks. No directional predictions were made for titers at 8 weeks because these could be due either to infection or to maternal antibodies. Predictions for reproductive performance of sows were that higher serological titers were associated with a decreased number of liveborn, an increased number of stillborn, a higher preweaning mortality, a lower average weaning weight and a longer interfarrowing interval. No directional predictions were made for vaccinated animals unless the serologic test could differentiate between antibodies to vaccine and wildtype virus.

RESULTS

Predictors of average daily weight gain found significant on the monitored farms and their effect size (β) are summarized in Table 1. On almost all of the farms there was a consistent negative association of serological test results at 16 and 24 weeks of age with average daily weight gain. It differed between farms, however, which of the pathogens had an impact on performance. The magnitude of the effect of high antibody levels to a pathogen on weight gain was also different among the farms. The detected differences in average daily weight gain between animals seropositive and seronegative for the monitored infections ranged from 18 to 116 grams per day.

Of the variables monitored at slaughter, only evidence of lung pathology was consistently associated with decreased weight gain. Skin scores were positively correlated with weight gain on one farm and negatively correlated on another. Liver scores were also associated with decreased weight gain on one farm and with increased weight gain on another. Snout scores had a negative association with weight gain on one farm and a positive association on two farms.

Significant predictors of reproductive performance of sows are summarized in Table 2. PRRSV was associated with decreased reproductive performance on 5 of the 7 farms. Sows seropositive for PRRSV had on average between 0.5 and 0.9 stillborn piglets per litter more and a 3 to 10 days longer interfarrowing interval than seronegative sows. A consistent negative impact on reproductive performance across farms could not be found for any of the other monitored pathogens.

Table 1. Summary of effect sizes (β) of significant predictors of average daily weight gain on the study farms

	Farm (year)								
	1 (1996)	**1 (1997)**	**2 (1996)**	**3 (1996)**	**3 (1997)**	**4 (1997)**	**5 (1997)**	**6 (1997)**	**7 (1997)**
Regression model R^2	**0.67****	**0.40****	**0.55****	**0.55****	**0.53****	**0.37****	**0.62****	**0.54****	**0.46****
PRRSV (8 weeks)	sero-	sero-				sero-	781.8*		
PRRSV (16 weeks)	negative	negative			-663.6**	negative		-45.45*	
PRRSV (24 weeks)								-54.55**	
SIV (8 weeks)									
SIV (16 weeks)				-0.45*	-0.45*				
SIV (24 weeks)		-0.45*							
TGEV (8 weeks)			0.45*			sero-	0.91**		
TGEV (16 weeks)						negative			
TGEV (24 weeks)					-1.82**				
APP (8 weeks)	sero-	sero-		sero-	sero-	sero-	sero-		
APP (16 weeks)	negative	negative		negative	negative	negative	negative		
APP (24 weeks)									
Mycoplasma (8 weeks)	not	sero-	not	not	-40.91*				
Mycoplasma (16 weeks)	tested	negative	tested	tested					
Mycoplasma (24 weeks)									-63.64**
PRV (8 weeks)								-295.5**	sero-
PRV (16 weeks)	not tested as farms had no recent history of being quarantined for PRV								negative
PRV (24 weeks)									
Skin score			-36.36**	68.18*					
Snout score			27.27*			-18.18**	31.82**		
Liver score				-86.36**	50.0**				
% Pneumonia				-6.82*					
Scaring			-50.0*						
Pleuritis								-43.64*	

* significant at α=0.05, ** significant at α=0.01

Table 2. Summary of significant effects of serology results on reproductive performance measures

	PRRSV	SIV	TGEV	APP	Myco-plasma	PRV
Lifeborn 1	6-97 ↓	6-97 ↓	6-97 ↓			6-97 ↓
Lifeborn 2	5-97 ↑*					
Stillborn 1	3-96 ↑ 4-97 ↑ 5-97 ↑***	5-97 ↓****				
Stillborn 2	3-97 ↑	1-96 ↓				
Deaths in nursery 1			2-96 ↑** 6-97 ↓	2-96 ↑	6-97 ↑	
Deaths in nursery 2		1-96 ↓ 3-97 ↑	1-97 ↑ 3-96 ↓ 6-97 ↓			
Weaning weight (average) 1	2-96 ↑ 5-97 ↓*		1-97 ↓ 2-96 ↑**		6-97 ↓	
Weaning weight (average) 2						
Interfarrowing interval	3-97 ↑ 4-97 ↑ 5-97 ↑*		3-97 ↑ 6-97 ↓			

Numbers indicate farm and year; arrows indicate direction of the association
1 = Farrowing before serological testing
2 = Farrowing after serological testing
* = Second group of sows on farm 5 were vaccinated for PRRSV and SIV
** = Sows vaccinated for TGEV
*** = Depending on vaccination status, strong increase only in non-vaccinated sows
**** = Depending on vaccination status, decrease only in vaccinated sows

DISCUSSION

The herd health monitoring system used in this study can be used to compare prevalences of antibodies across age groups in order to detect which groups are predominantly infected. It can also detect changes in prevalence over time within an age group. Although there were no apparent disease signs reported on any of these farms at the time of our study, serological test results and slaughter evaluation were associated with performance on most farms.

The effect of a specific pathogen on performance can not be generalized across farms. The magnitude of the impact of an infection on performance is influenced by the strain of the pathogen prevalent on the farm, co-infection with other pathogens and management practices. Thus, the statistical analyses were performed separately for each farm. The more independent statistical tests are performed in a study, the more likely it is to find a significant result by chance when in fact there is no association. The significance of one single variable that enters one of the models should therefore not be overinterpreted. Instead, the focus should be on factors that consistently influence average daily weight gain on several of the monitored farms.

Serological surveillance was more effective in predicting average daily weight gain than slaughter evaluation. Lung pathology was the only measure recorded at slaughter that was consistently associated with decreased weight gain. The results for other slaughter evaluation measures were inconsistent.

The association of subclinical infection with the recorded measures of reproductive performance was less consistent than the associations observed for weight gain. With few exceptions, the number of piglets born alive, the number of stillborn and the interfarrowing interval were negatively affected by higher antibody levels of the sows. Preweaning mortality and average weaning weight, on the other hand, were influenced positively by higher antibody levels on several of the monitored farms. It is likely that this positive effect of infection of the sows is due to protection of the piglets through maternal antibodies.

The magnitude of the effect of subclinical infection on performance found in this study is probably underestimated. The individual pig was used as the unit of analysis rather than the pen, cohort or farm. However, infection with a pathogen is likely to decrease the performance of the whole herd or group in addition to the effect on individual animals. In the multiple linear regression analysis, the performance of animals with high antibody levels is compared to animals of the same group with lower antibody levels to the pathogen. A difference in performance can only be detected, if a sufficient variability of antibody levels exists among the pigs of one group. The statistical power to detect an effect of subclinical infection on performance is greatest if the variability of antibody titers is large. Given the design of this study, it is easiest to detect an effect of infection on performance if only part of the group becomes infected. It is thus not surprising that no significant impact of some of the pathogens on weight gain could be found on farms with very low or very high antibody levels in all animals of the group.

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THE OCCURRENCE OF CLINICAL OUTBREAKS OF ENZOOTIC PNEUMONIA IN CALVES IN TEN DANISH DAIRY HERDS DURING THE WINTER 1996-97: DESCRIPTIVE RESULTS

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Ten Danish dairy herds were selected for a longitudinal study on enzootic pneumonia which lasted from December 1996 to May 1997. Clinical outbreaks occurred in nine herds, and in two herds the outbreaks reoccurred. Nine of a total of eleven outbreaks occurred in two distinct time periods where the temperature varied around zero. This might suggest that the outdoor climate plays a triggering role in the development of a clinical outbreak of enzootic pneumonia. The results revealed that BRS virus and/or corona virus were present in all outbreaks. However, deaths only occurred in herds with other health problems, and in one herd, infection with BRS and corona virus most likely occurred without any calves being observed diseased.

INTRODUCTION

Enzootic pneumonia is a multifactorial disease complex which is commonly seen during winter in dairy and beef calves (Roe, 1982; Andrews, 1992a). It is primarily younger calves which are affected (Stott et al., 1980; Blom, 1982; Virtala, 1996). The incidence and mortality risk vary from year to year, and e.g. Stott et al. (1980) found an incidence risk of treatment for respiratory disease of 22.3% and a mortality risk of 2.6% corresponding to a case fatality of 8.6%.

Several risk factors have been identified, among them insufficient colostral supplementation (Kimman et al., 1988), purchase and mixing of young calves, early weaning, and climatic conditions (Andrews, 1992a; Roe, 1982). It has also been found that the impact of enzootic pneumonia on the individual varies, e.g. Blom (1982) found that calves suffering from uncomplicated enzootic pneumonia at an early stage in life had an unaffected growth rate, while calves with a complicated infection would have a reduced growth rate subsequently.

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Gunn and Stott (1997) calculated that an average case of pneumonia would cost £ 20.58 seen in contrast to a cost of £ 32.92 for a case of diarrhoea. Sischo et al. (1990) found that calf diseases (almost entirely diarrhoea and pneumonia) on average represented 4% of the cost of all disease. The major part of the costs was attributed to an increased mortality risk (54%), while expenses for more labour (16%) and drugs (21%) were less - but still - important.

Although many studies have been carried out, the epidemiology of this disease complex is still not that well understood. Andrews (1992a) and Roe (1982) have focused on the multi-factorial nature, and Andrews (1992a) has listed several vira, mycoplasms, and bacteria as causes of enzootic pneumonia. During the last two decades, studies have focused on the importance of bovine respiratory syncytial virus (BRS virus) in the aetiology of calf respiratory disease (e.g. Stott et al., 1980, Pirie, 1981; Baker, 1993; Larsen, 1998).

In 1994, a large Danish study was initiated regarding the epidemiology and pathogenesis of BRS virus infection. The aim of this epidemiological study was: 1) to assess the incidence and mortality risk associated with outbreaks of enzootic pneumonia in conventionally raised dairy calves, 2) to identify the causal agents involved in each outbreak 3) to identify subclinical infections by use of serology, and 4) to study the impact of the outdoor and indoor climate on the occurrence of outbreaks of enzootic pneumonia.

MATERIALS AND METHODS

Two Danish veterinary practitioners and five dairy herds from each pratice area were selected for the study. The herds were selected based on compliance and production system (conventional and indoor-rearing). In each herd, ten calves of the age one to nine weeks were selected for a virologic and serologic study. The study period lasted from December 1996 to May 1997, and during that period, all medical treatments were recorded.

Definition of an outbreak

In case of clinical signs of respiratory disease the farmer called the veterinarian, who would carry out a clinical examination of the calves. For each individual calf the following parameters were recorded: general condition, frequency and type of coughing, frequency and type of respiration, presence of nasal discharge, rectal temperature, and treatment. An outbreak of enzootic pneumonia was defined as more than 20% of the animals from the same age group suffering from clinical respiratory illness.

Sampling

For assessment of subclinical infections in the ten selected calves, screening blood samples for serology were taken at the commencement (December 1996), midway (March 1997), and at the end of the study period (May 1997).

In case of an outbreak, the veterinary practitioner took samples for virology on this day (day 0) by means of nasal swabs from the initially ten selected calves. If an outbreak was confined to an older age group than the selected calves, which was the youngest in the herd,

samples were also taken from a subset of the diseased calves. Two swabs were inserted approximately ten cm into each nostril and left for at least one minutte. Subsequently, each swab was placed in a cryotube filled with 1.5 ml of phophate-buffered saline, pH 7.2 (PBS). In addition, heparized blood samples were taken from the same calves on day 0, and plain serum samples on day 0, 7, and 21. These results will be presented elsewhere.

Virology

The nasal samples were tested for the presence of BRS, corona, para-influenza type 3 (PI-3), and adeno virus antigens as previously described (Meyling, 1982; Uttenthal et al., 1996). Furthermore, the samples were cultivated for presence of bovine virus diarrhoea (BVD) virus.

If a virus was detected in connection with a clinical outbreak, it was decided to classify the herd as being infected with this particular virus.

Serology

The screening blood samples were analysed for presence of antibodies against BRS (IgG1), corona, PI-3, adeno, and BVD virus as previously described (Uttenthal et al., 1996; Uttenthal et al., in preparation). Only results from herds without clinical outbreaks will be presented here. If the antibody titre rose four-fold from one screening to the next in at least one calf, the herd was classified as being infected with the particular virus sometime during the period between the two screenings.

Necropsy and laboratory analyses

To clarify the cause of death, the heart, lungs, lever, and spleen from casualties among the ten selected calves were sent to the Danish Veterinary Laboratory for necropsy and analyses necessary for a causal diagnosis, including macroscopic and microscopic pathology, bacteriology, and virology. The veterinary practitioner autopsed all other dead calves, and in case lesions of pneumonia were found, the before-mentioned organs were sent to the Danish Veterinary Laboratory for further examination.

Outdoor and indoor climate

Data on hourly measured relative humidity and average temperature were obtained from a climate station situated nearby the ten herds. Average measures for each day were calculated to reduce the degree of interdependence between the hourly measurements. Next, the averages for different seven day periods were compared statistically by use of an analysis of variance. The average measures for the periods are presented as least square means (SAS, 1987). A data logger (Control One International SA, Belgium) placed above the calf boxes recorded the temperature and relative humidity once an hour in each of the herds.

RESULTS

Outbreaks and virology

A summation of the occurrence of clinical outbreaks is presented in Table 1. The outbreaks were primarily concentrated to two distinct periods during the winter: 1) four outbreaks occurred during 16/12-19/12 1996 and 2) five outbreaks occurred during 14/1-17/1 1997. The last two outbreaks occurred either before or between these periods. In total, there were clinical outbreaks in nine out of the ten herds, and in two herds the outbreak reoccurred. Table 1. shows for each herd the number of calves which were sampled at the day of the clinical outbreak as well as the number of sampled calves from which BRS, corona, or adeno virus antigens were detected. In eight out of the eleven outbreaks, BRS virus antigen was detected. In four of these outbreaks, corona virus antigen was detected as well. In total, corona virus antigen was detected in seven out of the eleven outbreaks. Adeno virus antigen was detected from one nasal swab in one clinical outbreak only, and here, BRS virus antigen was detected as well. PI-3 virus antigen was not detected at all.

Table 1. Clinical outbreaks of enzootic pneumonia in calves in ten Danish dairy herds during the winter 1996-97: date of outbreak and results of virology

Herd No.	A	B	C	D	E	F	G	H	I	J
Date of 1. outbreak	Dec16	Dec30		Jan14	Jan14	Dec7	Dec18	Dec19	Dec17	Jan17
BRS virus	4/5*	0/15		6/12	1/16	1/15	0/19	6/15	0/11	2/15
Corona virus	0/5	14/15		0/12	11/16	0/15	6/19	0/15	11/11	8/15
Adeno virus	0/5	0/15		0/12	0/16	1/15	0/19	0/15	0/11	0/15
Date of 2. outbreak		Jan17						Jan16		
BRS virus		1/17						1/5		
Corona virus		3/17						4/5		

*: The number left to the oblique stroke represents the number of sampled calves from which virus antigens were detected. The number to the right represents the total number of calves which were blood sampled from each herd at the day of the clinical outbreak.

Table 2. Results of necropsy and laboratory analyses of five dead calves

Calf No.*	Necropsy	Microbiology and other laboratory results
G-1524	Purulent broncho pneumonia	Salmonella dublin in lever, lung, and spleen Actinomyces pyogenes in lung
G-1533	Necrotizing broncho pneumonia	BVD virus isolated in lung Actinomyces pyogenes in lung
G- 1881	Mucopurulent broncho pneumonia	Actinomyces pyogenes in lung
J-946	Myocardial degeneration necrotizing broncho pneumonia with abscessation	Low amount of selenium in heart and lever (0.07 mg/kg): Obs pro nutrional muscular dystrophia**. Staphylococcus aureus in lung
J-940	Mucopurulent broncho pneumonia	BRS and corona virus antigens detected in lung

*: The letter in the calf number indicates the herd from which the calf originates. **: The Danish Veterinary Laboratory uses the following reference values: for selenium in the heart 0.07-0.15 mg/kg and in the lever 0.25-0.50 mg/kg.

Mortality risk, necropsy, and laboratory results

Deaths among the initially selected calves occurred in two of the ten herds. In herd G, three calves died and in herd J, two calves died. This implies that the mortality risk due to enzootic pneumonia among the selected calves varied in the herds from zero (0/10) to thirty percent (3/10), yielding an average of five percent (5/100). The results of necropsies and associated laboratory examinations on the five dead calves are presented in Table 2.

Serology in herd without outbreak

Only one herd had no clinical outbreak. Therefore, it was of interest to study the results of the serological screenings which had been sampled in this herd. Nine out of the ten selected calves showed a four-fold rise in antibody titres against corona virus when comparing the initial screening to the midway screening, while one calf showed a four-fold rise in titre against corona virus, when comparing the midway to the final screening. Regarding BRS virus, seven out of the ten calves showed a four-fold rise in antibody titre when comparing the initial screening to the midway screening, while no rise in titre was observed when comparing midway and final screening. These results suggest that the calves went through an infection with both corona virus and BRS virus during the period December 12th to March 25th.

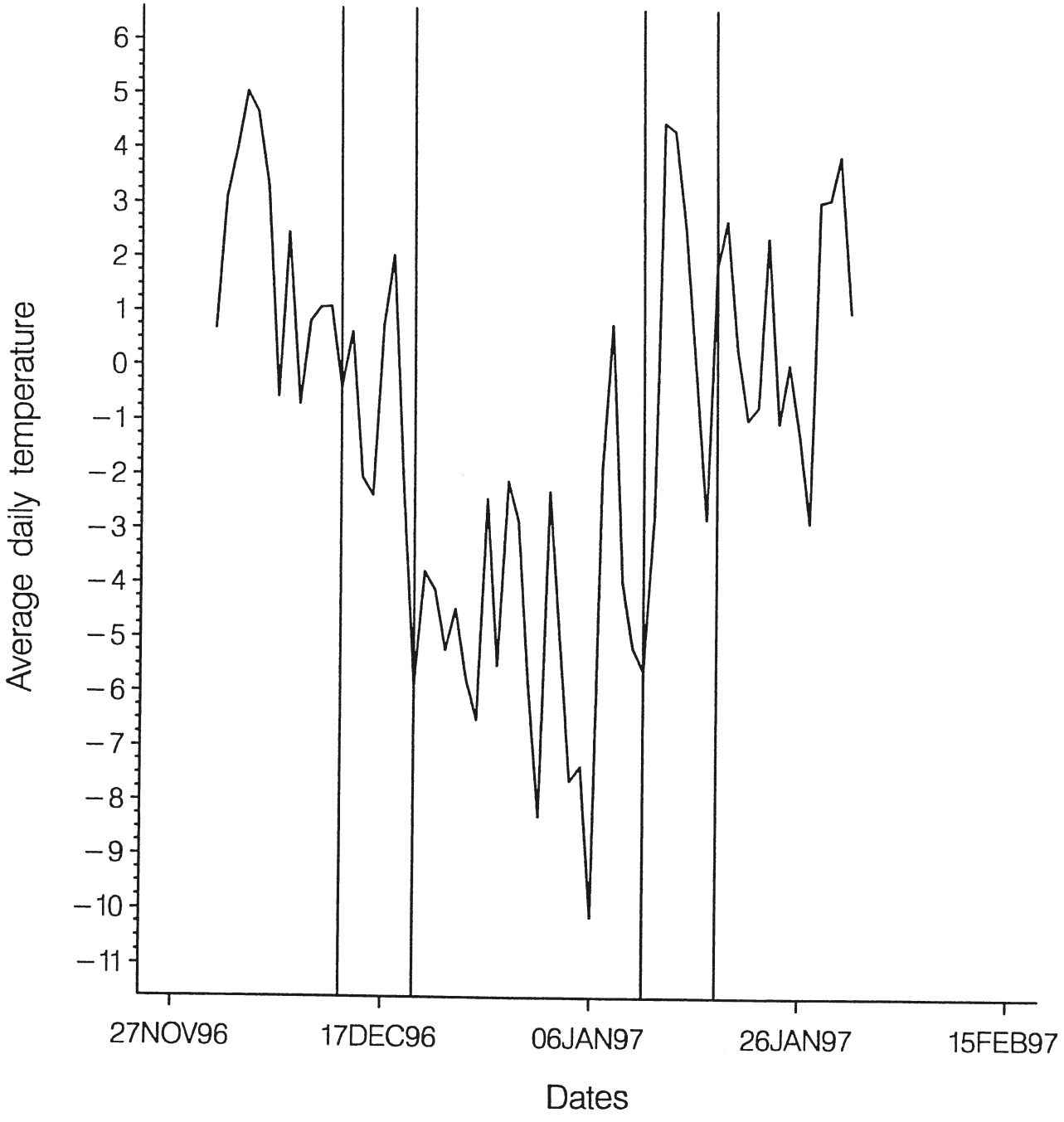

Figure 1. The outdoor temperature during the period 1/12 1996 - 31/1 1997. The lines indicate the two seven day periods where the majority of the clinical outbreaks of enzootic pneumonia occurred during winter 1996-97.

Effect of outdoor and indoor climate

Figure 1. presents the temperature during the period 1/12 1996 -31/1 1997. A time period was defined as either seven days prior to an outbreak or seven days without an outbreak, since experimental infections had revealed that severe, clinical symptoms may not evolve until three to seven days after exposure to BRS virus (Larsen, 1998; Tjørnehøj, in preparation). On Fig. 1. the two seven day periods where the majority of the outbreaks occurred are indicated with each two vertical lines. A summation of the hourly measured outdoor temperature during these two time periods as well as three time periods in between without clinical outbreaks is presented in Table 3. From Table 3. and Fig. 1. it appears that the temperature during the two periods with outbreaks varied around 0°C (-0.5°C and 0.0°C), while the average temperatures during the two time periods between the outbreak periods were significantly lower (-5.1°C and -6.7°C). During a 7-day period occurring after the second group of outbreaks, the average temperature was close to zero (-0.2°C), similar to the temperature during the two outbreak periods. There was no statistical difference in relative humidity between the time periods, and the average relative humidity was high in all five periods.

Table 3. A summation of the hourly measured outdoor temperature and relative humidity during two 7-day periods with clinical outbreaks and three 7-day periods without outbreaks of enzootic pneumonia among calves in ten Danish dairy herds

Time periods	Temperature	Rel.humid*.	H_0: lsmeans(i)=lsmeans(j). P-value for				
Outbreak+/-	mean (°C)	mean (%)	difference in temperature between periods				
			1.	**2.**	**3.**	**4.**	**5.**
1. + 13/12-20/12	-0.53	92.0		<.01	<.01	0.66	0.77
2. - 20/12-27/12	-5.10	90.2	<.01		0.22	<.01	<.01
3. - 31/12-7/1	-6.66	89.9	<.01	0.22		<.01	<.01
4. + 11/1-18/1	0.02	90.9	0.66	<.01	<.01		0.89
5. - 20/1-27/1	-0.16	92.5	0.77	<.01	<.01	0.89	

*: There was no difference in mean relative humidity between the periods.

Table 4. The indoor climate in each of the ten dairy herds: minimum, average and maximum of temperature and relative humidity during the study period December 1996 - May 1997

Herd No.	Temperature (°C) min average max	Relative humidity min average max	Climate compared to the other herds based on the average measures*
A	-10.6 7.0 22.9	29.7 73.2 97.3	Medium temperature, low humidity
B	-8.3 4.9 20.3	33.7 77.1 93.0	Low temperature, medium humidity
C	0.2 9.8 20.3	41.8 87.7 100.0	High temperature, high humidity
D	-1.6 8.9 21.4	41.0 79.5 100.0	Medium temperature, medium humidity
E	0.6 8.2 19.7	54.3 92.7 100.0	Medium temperature, high humidity

Herd No.	Temperature (°C) min average max	Relative humidity min average max	Climate compared to the other herds based on the average measures*
F	-6.5 5.8 18.5	42.8 79.7 100.0	Low temperature, medium humidity
G	**-9.5 4.7 18.6**	**33.3 83.6 100.0**	**Low temperature, high humidity**
H	1.8 11.8 23.2	37.3 76.5 95.0	High temperature, low humidity
I	6.1 15.2 24.9	42.3 72.6 91.5	High temperature, low humidity
J	**-0.8 7.7 18.3**	**42.7 81.9 97.0**	**Medium temperature, medium humidity**

The only deaths due to enzootic pneumonia occurred in the herds marked with bold.*: The average measures for temperature and relative humidity were divided in to three groups: the three lowest, the four mediums, and the three highest.

A summation of the monitoring of the indoor climate for the entire study period December 1996 to May 1997 is presented in Table 4. A wide variation of climatic conditions was found in the ten herds. The two herds in which deaths due to respiratory disease occurred are marked with bold. In one of these herds, the indoor climate was almost like the outdoor climate, because the calves were reared in a non-insulated barn. In the other herd, there was a medium temperature and humidity. These calves were reared in an old, insulated cow stable. Herd C was the only herd which did not encounter a clinical outbreak, and here, a high temperature and humidity was seen.

DISCUSSION

Incidence risk

Enzootic pneumonia was very common in the ten study herds during the winter 1996-97, since clinical outbreaks were observed in nine herds, and in the tenth herd a subclinical infection most likely occurred. A high morbidity risk has also been observed in other studies (Stott el al., 1980; Blom, 1981; Virtala et al., 1996). In a similar study in eight calf herds, Blom (1981) found that during a nine month period, the incidence risk for enzootic pneumonia was 29% on average, but varied among the herds from 14-43%.

Virology

BRS and corona virus were found most commonly - either alone or in combination. Adeno virus was isolated only once. PI-3 virus was not detected at all. Only BRS and corona virus will be discussed here.

BRS virus has been pointed out, e.g. by Stott et al. (1980) and Andrews (1992a), as one of the most important viral causes of enzootic pneumonia. Kimman et al. (1988) found that BRS virus was involved in the majority of 21 Dutch outbreaks, and Pirie et al. (1981) found that BRS virus was involved in the majority of clinical cases in a British study on outbreaks of enzootic pneumonia. In a Danish study on the prevalence of antibodies to BRS virus, it was seen that 79% of the calves and cows had antibodies to BRS virus (Uttenthal, in preparation). This implies that BRS virus is endemic in the Danish dairy population. This is in accordance with Stott et al. (1980) who in an English study found that 70% of the calves had been

infected with BRS virus by nine months of age.

Corona virus is known primarily for its ability to cause diarrhoea, and only secondly for infections of the upper respiratory tract (Hall et al., 1992). Furthermore, in a major English study, only BRS, PI-3, and BVD virus were significantly associated with respiratory disease (Stott et al., 1980). Therefore, it can be discussed whether the findings of corona virus in nasal swabs were occasional or causal associated with enzootic pneumonia. On the other hand, if several animals were showing clinical symptoms of enzootic pneumonia, and corona virus was isolated from the nasal cavity of these animals, a causal relation was likely. In accordance, Martin et al. (1998) concluded in their observational study, that corona virus most likely is linked causally to enzootic pneumonia, but not as a major pathogen. To study the impact of corona virus further, detailed analyses of the serological data will be carried out in the next part of the project.

In the present study, the farmer called the veterinary practitioner upon signs of enzootic pneumonia. Hereafter, the selected calves as well as other diseased calves were sampled to obtain information about the viral agents involved in the outbreak. In experimental studies it has been observed, that the highest levels of virus is shed between three to five days after inoculation and before the onset of more severe respiratory symptoms (Larsen, 1998; Tjørnehøj, in preparation). Thus, antigen-negative nasal samples may be obtained from BRS virus infected calves exhibiting severe symptoms at the time of the sampling, because the infection at this time is beyond the virus-shedding phase. Furthermore, it is well accepted that several virus infections impair the natural host defence system and hereby allow the establishment of a subsequent bacterial infection (Barbuik, 1988). Accordingly, the true incidence of BRS virus infections may be underestimated when assessed on the basis of detection of virus antigen in nasal samples. Therefore, the presence of BRS virus in a nasal sample in a diseased calf is regarded as a true positive result.

Serology in herd without outbreak

Only in one out of the ten herds no clinical outbreak was observed during the winter, and in this herd the majority of the selected calves seroconverted to BRS and corona virus between December 1996 and March 1997. Most likely, a "silent" infection occurred during that period. In aggreement, Stott et al. (1980) and Kimman et al. (1988) found that a respiratory infection could pass entirely unnoticed. When comparing housing system, management, and indoor climate no specific conditions could be addressed to explain the silent infection in the herd. The risk factors might not have been dominant enough to establish a sufficient cause of enzootic pneumonia to provoke an outbreak.

Mortality risk, necropsy, and laboratory results

In this study, the mortality risk associated with respiratory disease in the selected calves varied between herds from zero to thirty percent, yielding an average of five percent. These results are similar to a study in eight Danish dairy herds where Blom (1982) found that the mortality risk due to respiratory disease varied from 0-11.6%, yielding an average of 4.5%. Contrary to this, Stott et al. (1980) and Virtala et al. (1996) reported a lower incidence, 2.6% and 2.2%, respectively, while Willadsen et al. (1977) found a higher mortality risk (9%).

The variation in mortality risk occurs probably because there is a dependency on risk factors like concurrent disease, degree and type of secondary bacterial infection, prior herd exposure, and type of management and housing system.The deaths in this study only occurred in the two herds where other health problems were noted, i.e. presence of BVD virus, Salmonella dublin, and low selenium status. The case fatality of respiratory disease *per se* may be low, but the presence of concurrent disease might alter the case fatality dramatically. Blom (1982) found a strong statistical association between neonatal disease and the risk and case fatality of respiratory disease. The case fatality altered from 11.6% for a calf with respiratory disease only, to 26.7% for a calf with respiratory disease and prior neonatal disease. Blom (1982) explained this association as a result of low immunoglobin status, which is predisposing for both kinds of diseases. Likewise, Kimman et al. (1988) found that the incidence and severity of respiratory disease was inversely related to the level of specific maternal antibodies. Furthermore, low selenium status may be associated with a delayed lymphycystic response which increases the calf's susceptibility to respiratory disease (Andrews, 1992b). A long term immuno-suppressing effect is also seen in relation to BVD virus infections, which hereby may predispose the calf to respiratory disease (Stott et al., 1980; Eddy, 1992). In accordance, an experimental study by Broderson & Kelling (1998) showed, that concurrent infection with BVD and BRS virus resulted in more severe signs of disease and more extensive lung lesions than did either infection alone. As BVD virus gets eradicated from an entire population, like the current Danish cattle population, it may be of interest to investigate whether the course of enzootic pneumonia gets altered or not.

Outdoor and indoor climate

The outbreaks occurred in two distinct time periods primarily, in mid-December and mid-January. Stott et al. (1980), Roe (1982), and Blom (1982) also observed that the majority of respiratory illness occurred in short periods. In a survey regarding the presence of virus in 491 healthy, English calves, Stott et al. (1980) found BRS virus in the nasal cavity in 1% of the calves, and PI-3, rhino, adeno, and entero virus more commonly (12%, 4%, 4%, 17% - respectively). This might lead to a hypothesis that certain elements in the outdoor climate may be involved in triggering an infection. Based on the present study it may be hypothesized that outdoor temperatures constantly below zero to some extent protect against the development of an outbreak of enzootic penumonia. During a seven-day period after the second group of outbreaks, the temperature again varied around zero, without any outbreaks. The reason why no outbreaks occurred during this period may be that the calves had just developed immunity against the involved agents. Martin et al. (1975), Roe (1982), and Andrews (1992a) have focused on large temperature fluctuations as risk factors. Martin et al. (1975) were able to predict the mortality risk for californian calves during winter from meteorological information on temperature, precipitation, and wind, and these authors found that the mortality risk increased in cold, wet, and windy weather. All in all, no definite conclusion can be drawn from the present study, and the hypothesis regarding a protective impact from constantly low temperatures will need further clarification.

It may be hypothesized that the indoor climate may be a risk factor for enzootic pneumonia by modulating the course of an outbreak. Roe (1982) has focused on the need for proper ventilation to remove airborne micro-organisms and animal by-products as water vapour and ammonia. Blom (1981,1982) found, that high relative humidity and large temperature

fluctuations were associated with a higher incidence of enzootic pneumonia. The effect of the indoor climate could not be elucidated in the present study, because too few herds were included and because nine out of ten herds had clinical outbreaks.

Prevention of enzootic pneumonia

The present study suggests that the outdoor climate might be involved in triggering an outbreak, but this information can only be used by the farmer as a warning of when to pay special attention to the calves.

BRS virus is endemic in the Danish dairy population, so even though for the time being it is not known to which degree airborn transmission occurs, it seems unrealistic that a farmer can avoid the entrance of this and other vira associated with enzootic pneumonia. The possibilities of a vaccine against BRS virus shall not be discussed here, but awaits results from ongoing studies on vaccine efficacy.

As Roe (1982) has pointed out, in considering risk factors for enzootic pneumonia it is necessary to determine those factors which are under the farmer's control. By changing the manageable factors, i.e. allocation of sufficient colostrum, providing proper feeding and rearing conditions, an outbreak may not be avoided, but the severity may be reduced. This is in accordance with the theory of sufficient and necessary causes in relation to multifactorial diseases (Martin et al., 1987; Thrusfield, 1995).

CONCLUSION

The results suggest that the climate might play a triggering role in the development of a clinical outbreak of enzootic pneumonia. Furthermore, the study indicates that the incidence risk of enzootic pneumonia may be high in Danish dairy calves, while the case fatality may vary substantially across herds. Finally, the results revealed that BRS and/or corona virus were present in all outbreaks. However, deaths only occurred in herds with other health problems, and infection with BRS and corona virus may occur without any calves being observed diseased.

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ESTIMATION OF MILK PRODUCTION LOSSES DUE TO ACUTE MASTITIS IN DAIRY HERDS

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According to Sandholm *et al.*, (1995) mastitis may cause severe economic losses resulting partly from decreased milk production and partly from increased management costs. The largest losses result from a direct drop in milk production which invariably accompanies the infection, decreased milk revenue because milk contaminated with antibiotics is not marketable, and in many instances premature culling of the animal. Losses to the farmer may be serious if the disease occurs early in lactation (Al-Omar, 1997). Direct treatment costs are usually comparatively minor. In a herd, the impact of mastitis on overall production and profitability is difficult to assess, both because of the complexity of the disease and because many factors other than mastitis influence milk production (Asby *et al.*, 1975).

The first appraisals of the real effect of mastitis were that of Minett (1930) who concluded that diseases of the cow's udder were of major economic importance to the dairy industry. Janzen (1970) reviewed the literature regarding the economic losses due to mastitis attributable to losses in milk yield, changes in milk composition, costs of drugs and therapy and herd replacement costs. The losses due to reduction in milk yield were found to be great, though accurate measurements of the reduction were lacking.

Many attempts have been made to measure the effects on milk output of both sub-clinical and clinical mastitis in individual cows or individual quarters (Janzen, 1970; Morris, 1973; O'Donovan *et al.*, 1960). So far, the most common approach has been to take routine records of cow yields on large numbers of cows and then to compare yields of mastitis-affected and non-affected cows. Another common technique has been to compare the yields of affected and unaffected quarters of the same cow (Martinez, 1987). A difficulty of this approach is that production in the unaffected quarter may increase to compensate for the reduction in the affected quarter, especially in cases where the yield of one quarter has been entirely or almost entirely eliminated. A different ideal method of comparisons between twin pairs following artificial infection of the one twin does not appear to have been used (Martinez, 1987).

According to Crist *et al.* (1996), Blowey and Edmondson (1995), Eberhart *et al.* (1987) and Schneider and Jasper (1964) and many others in the scientific literature, damage in the udder caused by mastitis bacteria and the toxins that they produce causes the count of somatic cells in milk to rise, with a corresponding drop in milk yield.

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Cell counts may be carried out on milk taken from an individual quarter, cow or from the bulk tank. An increased bulk tank somatic cell count (BTSCC) has been related to increased herd infection prevalence which results in decreased milk production (Rice and Bodman, 1997). In most economic studies the BTSCC was used as the major indicator for the level of mastitis (Schaknaad and Dijkhuizen, 1990). However, with the advent of accurate cell counting and statistical techniques it was possible to make comparisons between quarters in the same cow, between cows or between herds with low and high infection levels, relating the differences in milk production to cell counts (Schultz, 1977).

This study makes use of a technique to estimate an individual cow's expected milk production on a daily basis, from retrospective data. Deviations of the actual recorded yield from the expected milk production can then be calculated. A negative deviation (milk loss) would be expected to be related to elevated somatic cell counts (SCC). Using this technique, the relationship between acute rises in SCC and milk loss are studied in much greater detail and with more accuracy than before as a means to estimate the milk loss due to acute mastitis.

MATERIALS AND METHODS

Study population

Data from a single large dairy herd were used in this study. Data were collected during 5 years from the beginning of 1993 to the end of 1997. The herd is maintained at around 206 intensively managed dairy cows, with Holstein as the main breed. All of the cows were kept in a loose housing system. The average milk yields were 6,718.6 kg per full lactation, 6,512.9 kg per standard lactation (305 days) and the average daily milk yield was 21.49 kg. The average individual SCC was 246,740 cells per ml.

Data collection

The data were originally stored using the DAISY programme (the Dairy Information System, Esslemont, 1993). Data collection was handled by the DAISY office, Department of Agriculture, University of Reading. Health, milk production, farming practices and milk quality data were collected. The interval period for milk recording ranged from 28 days to two months. The total number of records included in this study was 9,199 milk records from 1,129 lactations of 419 different cows.

Data analysis

The main objective of this study was to quantify the relationship between raised SCC, used as an indicator of acute mastitis, and loss of milk output. The data were exported from DAISY to a new dairy information programme currently in development at the University of Reading. This programme contains tools to calculate the deviations of SCC and milk yield from mean and expected levels respectively. The basic approach was then to use linear regression analysis to quantify the relationship between an acute rise in individual SCC and milk loss. The analysis was carried out using the software, Statistix® for Windows, version 1.0 (Analytical Software).

Definition of milk loss: Milk loss was calculated for each milk recording as the expected milk yield for that day minus the actual milk yield recorded that day.

The expected daily milk production of a cow was derived from a seasonally-adjusted herd lactation curve of the form described by Wood (1969).

$$Y = aT^b e^{-cT} m_i \qquad \text{Eq. (1)}$$

Where:

Y is daily milk production T days after calving;
a, b and c are least-squares regression estimates of the curve parameters;
m_i is a seasonal adjustment factor for production in the ith month of the year.

The parameter a was adjusted to reflect the actual milk recordings from the same cow-lactation, producing a herd lactation curve shape adjusted to the performance of the individual cow in the whole lactation. For the purpose of calculating milk loss on any recording date, the expected yield curve was fitted without using the recording on that date. This produces an expected yield curve without the influence of any factor that might have had a short-term effect on production on that date.

The difference between the expected and actual production on any date was used as a measure of short-term variation in milk production that might be explained by mastitis among other factors. This difference, which was coded MILKLOSS, was used as the outcome variable in the linear regression analysis.

It should be emphasised that this variable would only be sensitive to short-term influences on production. A chronic problem, for example poor forage, chronic mastitis or other chronic disease, would depress production for a number of recordings, and therefore the whole of the expected lactation curve would also be depressed.

Explanatory variables: Acute deviations from expected milk yield in an individual cow could result from several causes, including sub-clinical and clinical mastitis, other health problems and short-term disturbances in nutrition. There was no recorded information about possible nutrition problems, which therefore could not be considered in this study. Although health problems such as retained placenta, ketosis, milk fever etc. can have an acute effect on milk yield, these events were rarely recorded in the data as compared to the occurrence of raised SCC. An acute increase in SCC above baseline levels, taken as an indicator of acute mastitis, was therefore the only explanatory variable considered in the regression model.

Increases in SCC above baseline levels were calculated as the difference between the SCC on the day of milk recording and the average SCC for the cow and current lactation in question, using the following equation:

$$\text{Increase in SCC} = \text{SCC} - \text{average lactation SCC} \qquad \text{Eq. (2)}$$

This difference, which was coded DISCC, was used as the explanatory variable in the linear regression analysis.

It has been known for many years that cell counts tend to be increased in early and late lactation as a result of an elevation in epithelial cells in colostrum and late lactation milk, i.e. these increases in SCC are not associated with mastitis (Bodoh *et al.*, 1976). In this study all the milk production and SCC measurements were made after the first week of lactation, thereby avoiding any confusion between SCC increases caused by epithelial cells in colostrum and SCC increases caused by mastitis.

In older cows, SCC tends to be increased by epithelial cells after six months of lactation (Bodoh *et al.*, 1976). It is therefore possible that the magnitude of the reduction in milk production associated with any given rise in SCC could be different depending on the stage of lactation. To investigate this, another explanatory variable (coded as LL) was included in the analysis along with terms for any possible interactions between DISCC and lactation stage. LL was a dummy which took the value 0 for recordings up to 200 days after calving, and 1 for recordings taken later in lactation.

RESULTS

The final model was determined through a stepwise linear regression analysis. In the beginning DISCC was included as a continuous variable. However, residual analysis showed that the effect of DISCC was not linear. Because the relationship between DISCC and MILKLOSS was not linear the variable DISCC was divided into 14 categories according to different magnitudes of increase in SCC. These categories were included in the regression model by using dummy variables. As coefficients of some categories were approximately similar it was possible to combine these categories, resulting finally in 6 distinct categories. Each category was associated with a progressively greater milk loss. Table 1 presents the levels of DISCC which were included in the model.

Table 1. Definition of the categories of DISCC levels in the linear model

Category	Increase in SCC over lactation average (10^3 cells per ml)
DISCC0	1-50
DISCC1	51-100
DISCC2	101-200
DISCC3	201-300
DISCC4	301-600
DISCC5	>600

The output of the least square regression analysis of the association between DISCC and MILKLOSS is summarised in Table 2.

Table 2. The least square regression analysis of milk loss due to acute mastitis

Explanatory variables	Coefficient	STD Error	Student's t	p value
Constant	-0.59546	0.044	-13.43	0.0000
DISCC1	0.98901	0.150	6.58	0.0000
DISCC2	1.43724	0.143	10.03	0.0000
DISCC3	1.93141	0.188	10.22	0.0000
DISCC4	2.14453	0.160	13.34	0.0000
DISSC5	2.84889	0.179	15.84	0.0000
			$F = 108.31$	$p = 0.0000$

Table 3. presents the least square regression analysis of milk loss including the terms for possible interactions between DISCC levels and stage of lactation.

Table 3. The least square regression analysis of milk loss due to acute mastitis with the interactions between DISCC and late stage of lactation

Explanatory variables	Coefficient	STD Error	Student's t	p value
Constant	-0.58244	0.04970	-11.72	0.0000
DISCC1	0.90340	0.23038	3.92	0.0001
DISCC2	1.6369	0.26413	6.18	0.0000
DISCC3	1.75660	0.37910	4.63	0.0000
DISCC4	1.67195	0.36927	4.53	0.0000
DISSC5	2.42254	0.30185	8.03	0.0000
LL	-0.06365	0.10990	-0.58	0.5625
DISCC1×LL	0.18621	0.31227	-0.69	0.4891
DISCC2×LL	-0.22415	0.32400	0.62	0.5344
DISCC3×LL	0.27621	0.44458	0.62	0.5344
DISCC4×LL	0.62302	0.41839	1.49	0.1365
DISCC5×LL	0.69257	0.38335	1.81	0.0709
			$F = 49.83$	$p = 0.0000$

The p values in Table 3. suggests that any interaction of stage of lactation with the effect of SCC on milk production is unlikely to be of significance. A partial F test confirmed that the inclusion of the dummy variable for late lactation and all the interaction terms made no significant contribution to the model (p value = 0.4241). The dummy variable for late lactation and all the interaction terms were therefore not included in the final model, which can be represented as follows:

Eq. (3)

$$y = \beta_0 + \beta_1 \times \text{DISCC1} + \beta_2 \times \text{DISCC2} + \beta_3 \times \text{DISCC3} + \beta_4 \times \text{DISCC4} + \beta_5 \times \text{DISCC5}$$

Where:

y = milk loss

β_0 = constant (i.e. milk loss associated with DISCC0)

β_1 to β_5 = coefficients (i.e. additional milk losses) associated with DISCC1 to DISCC5

Using Eq. (3) and the values from Table 3. the estimated milk losses associated with acute elevations of SCC can be calculated and are presented in Table 4. and Fig. 1.

Table 4. The daily milk loss associated with different increases in SCC assumed to result from acute (clinical and sub-clinical) mastitis.

Level of SCC above average (10^3 cells per ml)	Daily milk loss (kg)	Daily milk loss as a % of herd average daily yield [a]
1-50	-0.59	-
51-100	0.39	1.81
101-200	0.84	3.90
201-300	1.34	6.23
301-600	1.55	7.21
>600	2.25	10.46

[a] Based on the average daily milk yield 21.49 kg.

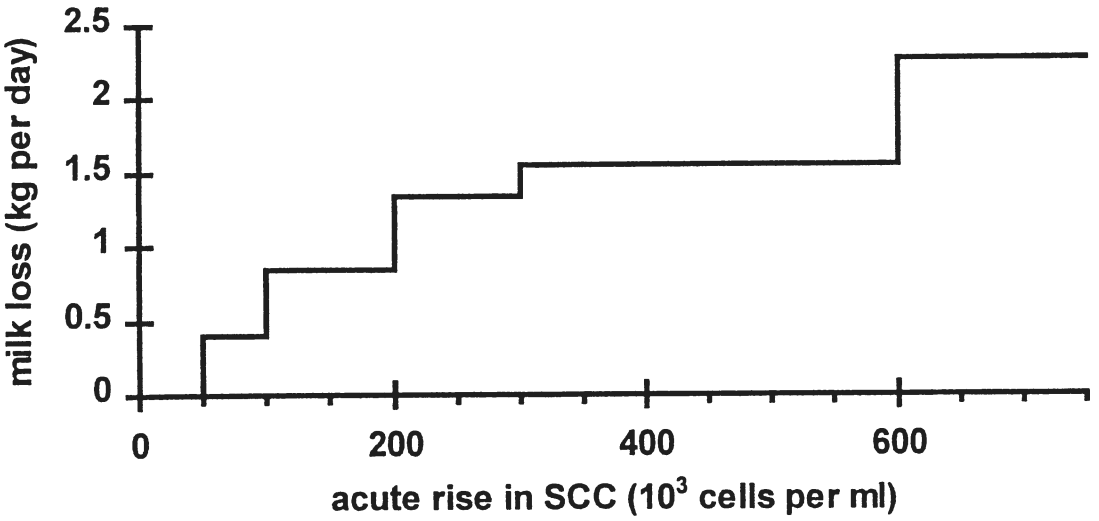

Fig. 1. The daily milk loss associated with acute rise in SCC

DISCUSSION

This paper presents a new method to quantify the relationship between increased somatic cell count and loss of milk output. Because a cow's actual production is being compared with an individually calculated expected yield for that cow on that day, it is suggested that this method will provide the most accurate estimate of the effect of mastitis on the production of the individual cow. Previously comparisons had to be made between different cows, involving problems with matching, or between different quarters of the same cow, involving problems of compensation.

Gray and Schalm (1962) studied the relationship between mastitis and milk yield based on production records and CMT scores in ten commercial Holstein-Friesian herds, involving 1,243 cows over 305 days of lactation. Comparing lactation yields between CMT-negative and CMT-positive groups, there was an average loss of 6%, 10%, 16% and 24.5% for CMT positive groups

with scores of trace, 1, 2 and 3 respectively. Stages of lactation and differences in absolute yield between herds did not significantly affect the relationship of CMT score and milk yield.

Foster *et al.*, (1967) compared 1,258 opposite quarter milkings from 763 cows in 30 dairy herds. These opposite quarter comparisons were well distributed over the lactation period, season of year, and between front and rear quarters of the udder. California Mastitis Test (CMT) reactions (on total quarter milk) of trace, 1, 2 and 3 were associated with average decrease in milk yield of 0.42, 0.95, 1.72 and 2.33 kg per quarter per day, respectively.

Blowey (1986) studied clinical mastitis, which he categorised as mild, acute and peracute. His results suggested no decrease in milk production for mild mastitis and a 10% decrease in the remaining lactation for acute mastitis based on the average daily milk production of 20 litres.

In an American study, Crist *et al.* (1996) reported that the loss of milk yield due to sub-clinical mastitis would be 6% for a SCC 200 x 10^3 cells per ml and 10% for a SCC 600 x 10^3 cells per ml.

This study estimates a daily milk loss of 1.34 kg, or 6.23%, for a SCC from 200 x 10^3 to 300 x 10^3 cells per ml above average up to a daily loss of 2.25 kg, or 10.46%, for a SCC of greater than 600 x 10^3 cells per ml above average (Table 4). These milk losses are in good accordance with those estimated in previous studies.

Along with information from similar studies on chronic mastitis currently being undertaken, these figures would provide herd managers and advisors with an accurate scale of losses associated with different levels of SCC, which could be used in assessing the economic impact of the herd's mastitis problem and allowing them to derive cost/benefit analyses of any suggested interventions.

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OPEN SESSION

CONTROLLING RABIES IN FOXES BY ORAL VACCINATION – LEARNING FROM A SIMULATION MODEL

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Rabies is one of the oldest diseases described in the world. In recent years European countries have made huge efforts to combat this zoonosis (Stöhr & Meslin, 1996; Meslin, 1997). Rabies control measures traditionally focused on population reduction designed to break the infection chain (e.g. gassing, poisoning, trapping and shooting). However, most of the control measures proposed failed to permanently reduce the host population below the critical threshold at which rabies ceases (e.g. Winkler & Bögel, 1992). The development of effective anti-rabies vaccines heralded a new control measure. Initial field studies in the 1970s in Switzerland (Steck et al., 1978) demonstrated the oral vaccination of the fox population to be the measure of choice for successful rabies control. Consequently, over the past two decades, research into the control of sylvatic rabies has chiefly concentrated on developing methods and strategies for the oral vaccination of wildlife rabies vectors (Masson et al., 1996; Schlüter et al., 1997).

Various modelling studies have been conducted to support rabies control and contingency planning (Smith & Harris, 1991; Pech & Hone, 1992). Progressing with the evolution of applied mathematics and computer power, rabies models have changed from mimicking cycles of fox biology (Smart & Giles, 1973) via abstract analytical models (Anderson et al., 1981; Murray et al., 1986) to detailed, complex individual-based models (Voigt et al., 1985; Smith & Harris, 1991). Yet although each model is appropriate for analysing specific questions, none are suitable for tackling all rabies-related problems at the same time Nevertheless, recent questions have arisen at the end of the millennium concerning the systematic eradication of rabies in Western Europe by exhaustive vaccination programmes. To tackle these questions, modelling methods are required which could assist decision-making in future rabies control policy.

We have developed a disease-related, spatially explicit rabies model. It is based on modern modelling techniques and enables comparisons with the findings gained from the literature on rabies modelling (Thulke et al., 1999a). In this paper we will discuss three aspects of rabies epidemiology in particular which are elucidated by our simulation studies. Firstly, although random walks of rabid foxes are thought to play a major role in spatial rabies spread, simulations by Jeltsch et al. (1997) allow a revival of this discussion and point to a different conclusion. The next two items particularly relate to the anti-rabies vaccination of red foxes. Secondly, the effect of heterogeneity in the coverage

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of vaccine bait is often discussed in the context of rabies control. Therefore we applied our model to scenarios with spatially heterogeneous population immunity. Comparison with results by Tischendorf et al. (1998) for the homogeneously vaccinated rabies-fox-system provides further understanding. Thirdly, we extend our investigations concerning the potential persistence of rabies at low, hardly detectable prevalence levels despite the long-term and large-scale vaccination of foxes (Tischendorf et al., 1998). Having discovered this potential source of failure, we tackle the need for ongoing control strategies to eliminate uncertainty about the final proof.

MODEL AND METHODS

The model

We depict the host population spatially throughout 'infection communities'. The concept of an Infection Community (IFC) reflects the establishment of small temporary social fox communities (Storm & Montgomery, 1975; Niewold, 1980) in which contact rates can be assumed high enough (White et al., 1995) to spread a potential infection of one group member throughout the whole group. This eliminates the need to deal with less well-known biological and epidemiological factors, such as the territorial behaviour of red foxes, contact rates, basic reproduction rates or individual differences in incubation period (Anderson et al., 1981; Voigt et al., 1985; Murray et al., 1986; Smith & Harris, 1991). Moreover, because the definition of IFC is not area-dependent, our model output is resilient to varying population densities.

We use a two-dimensional, grid-based model to arrange the IFCs in space. Each cell represents one IFC. Each IFC can have precisely one of the following states (Fig. 1): **S**usceptible (IFCs in which all members are susceptible to an infection), **I**nfected (IFCs in which at least one animal is infected) and **E**mpty (extinct IFCs as a result of disease mortality). The grid of IFCs was exposed to vaccination. To accomplish this, we introduced three new states representing the straight proportional immunisation of IFCs (states **EM**, **SM** and **IM** in Fig. 1). The actual proportion within 'partially immunised' IFCs is defined by the mean immunisation level (IR).

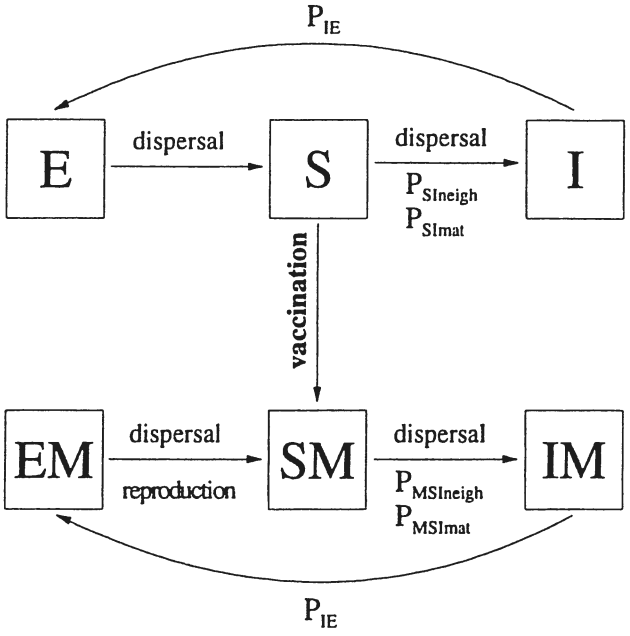

Fig. 1 The six possible states of IFCs. E=empty; S=susceptible; I=infected; M=iMmune after vaccination, i.e. IFCs in state SM are partially immunised according to assumed immunisation (IR). The arrows indicate the possible state transitions. P_{SI}=probability of infection; P_{IE}=probability of disease related extinction within one time-step (after Tischendorf et al., 1998).

Rabies spread is modelled by three mechanisms: 'neighbourhood infection', 'mating' and 'dispersal'. By 'neighbourhood infection' a susceptible IFC is infected by adjacent IFCs with a probability of P_{SI} representing the most local spread of rabies via infection caused by interactions and conflicts between animals of neighbouring IFCs. By 'mating' we refer to the increased spatial activity of itinerant adult males during the rut (Toma & Andral, 1977). Therefore, during 'mating' an infection could symbolically be carried over from up to three IFCs away when evaluating the infection event for a susceptible IFC. The annual dispersal of fox cubs is modelled in an individual-based manner in the time-step of 'dispersal'. The number of dispersing cubs (Allen, 1984) and the distribution of dispersal distances are based on field data (Trewhella et al., 1988; Goretzki et al., 1997). Because immunisation reduces the susceptible part of the host population, i.e. the number of potentially infective contacts, the onset of 'vaccination' linearly reduces the infection probabilities applied for the spreading mechanisms (Tischendorf et al., 1998).

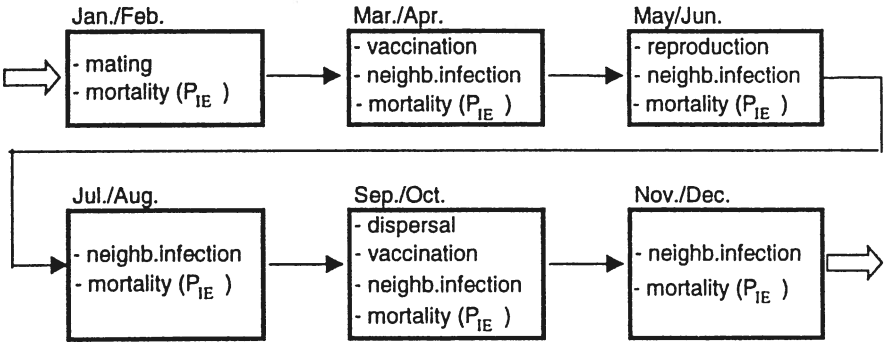

Fig. 2 The schedule of individual processes within the six model time-steps representing one year. Reproduction occurs in the third step because of the delay between birth and effective susceptibility, i.e. when an infection actually can be introduced in the IFC via the offspring.

We use a temporal scale of two months, which is suitable for the annual rhythm of red foxes and the dynamics of rabies infection (Fig. 2).

Simulation experiments

The role of rabid foxes: Jeltsch et al. (1997) identified the specific interaction of short-distance rabies spread (e.g. neighbourhood infections) and rare long-distance spread (e.g. annual dispersal) to be responsible for the formation of the observed spatial wavy pattern of rabies epidemics (Steck & Wandeler, 1980; Macdonald, 1980). Here we use projections of the pattern simulated on a grid of 600x300 cells over up to 600 time-steps, either with the described model or with suppressed 'dispersal' but explicit modelling of random walking rabid foxes in each time-step. We compare the simulated pattern with results from the literature (Källen et al., 1985; Murray et al., 1986).

Heterogeneous immunisation: We compare the probabilities of eradication for simulations based on a homogeneous and spatially heterogeneous immunised population. The differences allow the effect of heterogeneity in the immunisation level to be quantified. The reference data are taken from Tischendorf et al. (1998). Both simulation experiments are conducted on a square lattice of 140x140 cell over 120 time-steps with 'vaccination' (i.e. 10 years of repeated vaccination campaigns). All model parameters were varied over their meaningful range (see Tischendorf et al., 1998) reflecting a whole set of different epidemiological set-ups (cf. Spear & Hornberger, 1983; Fahrig, 1991). For each set-up of parameter values, 100 simulation runs were performed and from the 100 repetitions the frequency of complete eradication was assigned to the parameter configuration. Finally, a measure of success is defined as a function of the mean immunisation level [i.e. IR = 60%-80%; step 2%] in the vaccination area: For each level of immunisation (i.e. value of IR), the relative frequency of

eradication (RFE) was calculated from the respective subset of parameter configurations by averaging their frequencies of eradication (Fig. 3).

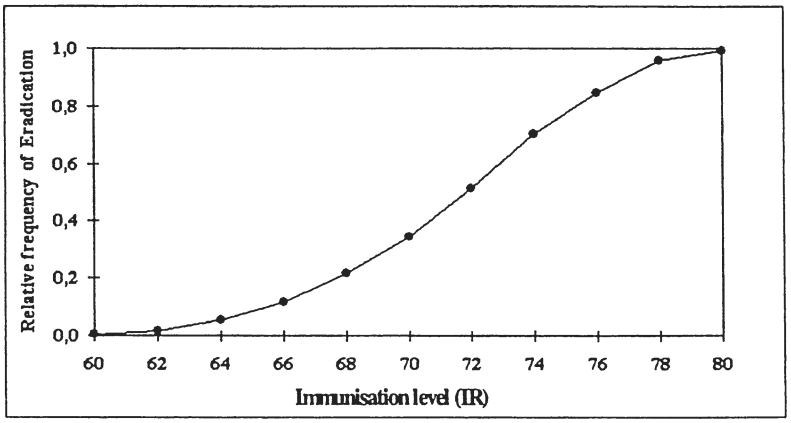

Fig. 3 The relative frequency of eradication (RFE calculated as explained in the text) for the investigated values of mean immunisation level in the homogeneous scenario (after Tischendorf et al., 1998).

The spatial homogeneity in immunisation is achieved by assuming the given value IR to hold for any single IFC, i.e. 70% of the animals in any susceptible IFCs are immune. Now heterogeneity is introduced by an algorithmic selection (Plotnick & Gardner, 1993) of a proportion of all IFCs (parameter no_bait) that are excluded from immunisation. Obviously, no_bait equals 0% in the homogeneous scenario and the value of the parameter determines the total *amount of perturbation* in bait coverage. The selecting algorithm (Plotnick & Gardner, 1993) is governed by a second parameter (frag) which determines the *fragmentation of perturbation* (cf. Fig. 6), i.e. whether it is rather clumped (Fig. 6a) or evenly distributed (Fig. 6c). At the beginning of each simulation run a random pattern of IFCs is excluded from immunisation (Fig. 6a-c). As a consequence of exclusion, the effective immunisation level (IR_eff) for any non selected IFC must be adjusted depending on the assumed mean immunisation level IR, i.e. IR_eff = IR/(1-no_bait/100).

Large-scale and long-term oral vaccination of red foxes: Previous simulation experiments have revealed that the immunised fox-rabies system can eventually harbour the low-level persistence of the disease (Fig. 4) despite prolonged vaccination (Tischendorf et al., 1998). To examine the

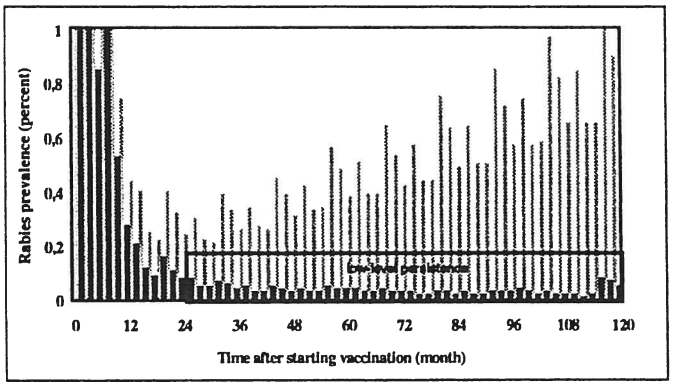

Fig. 4 Definition of low-level persistence by an time-series of rabies cases shown by way of example. After two initial years the overall prevalence in the simulation area neither exceeds 0.2% nor tends to zero despite continuous immunisation of 70%. By contrast, the shaded background shows an epidemic which clearly recovers despite vaccination (IR=64%; after Thulke et al., 1997)

characteristics of the ignored persistence of rabies after termination of vaccination, we conducted another simulation experiment (Thulke et al., 1999b). With all parameter configurations that in our previous simulations (Tischendorf et al., 1998) had produced a low-level persistence of rabies, 100 new runs were performed on a 140x140 cell lattice and vaccination was terminated after 30 time-steps (five years). The development of the new outbreak was reported by the time-series of infected IFC. The necessary model adjustment focuses on the immediately reduction of the immunisation level due to the turnover of the fox population. To deal with this, a six-valued step function was derived from the life statistics for red foxes (Stubbe, 1980) to describe the consecutive reduction in IR, i.e. the remaining percentage of immune animals in the population (for technique see Selhorst, 1996; Thulke et al., 1999b).

RESULTS

The role of rabid foxes: The results on which our discussion is based are presented graphically (Fig. 5) by the number of infected IFCs recorded at different locations. In the case of individually modelled dispersing animals (Fig. 5a), distinguished spatial waves follow after the front wave. Whereas in cases without explicit dispersing animals (Fig. 5b) no regular wavy spatial structure emerges behind the front of the epidemic. The latter mimics the simulation of rabies spread by a diffusion approach (Källen et al., 1985; Murray et al., 1986; Yachi et al., 1989), i.e. by performing each time-step with both neighbourhood infection and the random walking of rabid foxes the disease diffuses from infected areas into non infected adjacencies with no variability over the year.

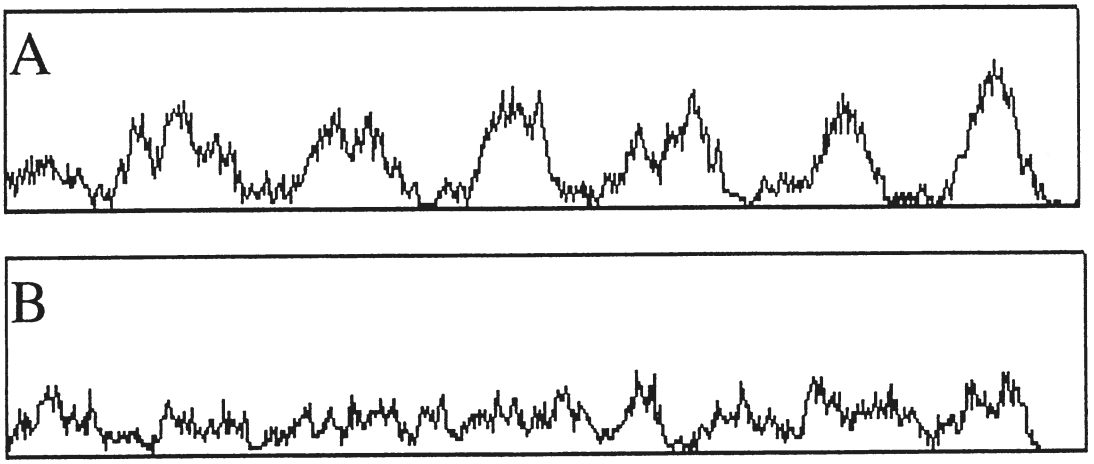

Fig. 5 The one-dimensional projection of a rabies epidemic perpendicular to the spreading direction simulated on a 600x300 cell lattice (after Jeltsch et al., 1997). A) The model includes the individual-based dispersal of foxes once a year. The movement of rabid foxes is aggregated in the probability of standard neighbourhood infection. Snapshot after 120 time steps (20 years). B) The movement of rabid foxes is modelled explicitly by performing individual random walks originating in infected IFCs. By contrast individual-based dispersal is excluded, i.e. the spread of the disease by dispersing cubs is restricted to the standard neighbourhood infection. This version of the model relates to the approach of Källen et al., (1985) and Murray et al., (1986) which neglects the importance of dispersing foxes and suggests only rabid foxes to be the driving force behind the spread of disease. Snapshot after 216 time-steps (36 years).

Heterogeneous immunisation: We quantified failure in immunisation by two measures: the total amount (no_baits) and the degree of fragmentation (frag) of perturbation. We found the relative frequency of eradication (RFE) to rise as the values of fragmentation increase (Fig. 6d). However, two different effects emerge. If the fragmentation value is small (i.e. perturbation appears in coherent clusters, Fig. 6a), the chance of disease eradication (RFE) decreases compared to the homogeneous scenario (Fig. 6d, left). On the other hand, larger fragmentation values (i.e. perturbation appears rather uniformly distributed, Fig. 6c) increases the RFE beyond the value found for the homogeneous reference (Fig. 6d, right). It is noteworthy that in the latter case disease eradication is favoured by perturbation. Both effects intensify when the assumed amount of perturbation (no_bait) increases (Fig. 6d, crossed vs. circled graph).

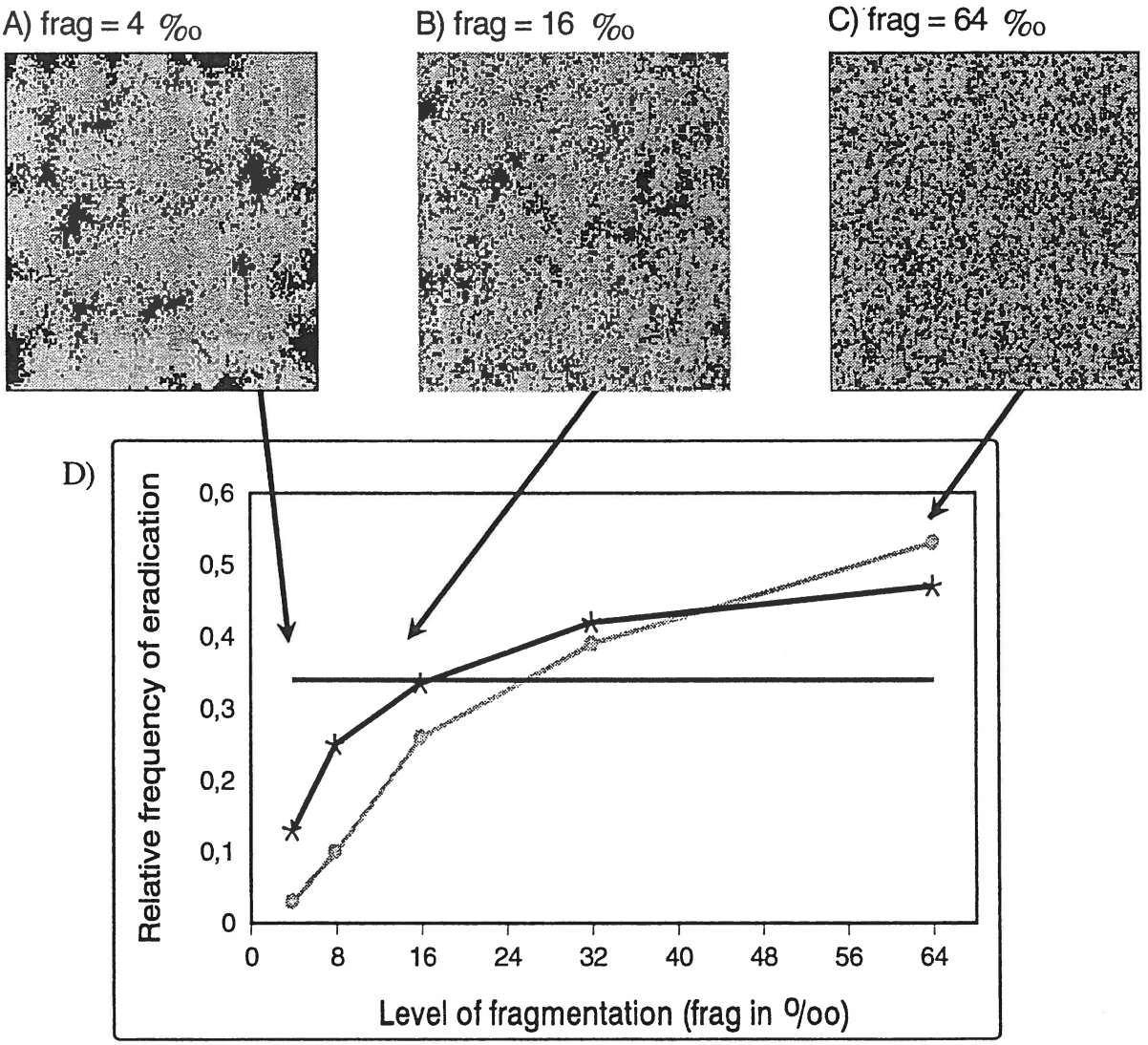

Fig. 6 Consequences of heterogeneous bait coverage for the eradication efficacy of the long-term and large-scale vaccination of foxes. A)-C) Random selection patterns depicted by way of example determining the set of IFCs which are excluded from immunisation in one simulation run. Although the fragmentation of perturbation (frag) increases from A) to C), the total amount remains constant (no_bait = 20%). D) Straight line: RFE value for the homogenous reference (from Fig.3). The other graphs show the RFE values for the increasing degree of fragmentation (frag) for one particular amount of perturbation (black line crossed: no_bait = 10%; grey line circled no_bait = 15%). Mean (overall) immunisation level (IR) remains constant for all simulations and equals 70%.

Large-scale and long-term oral vaccination of red foxes: The potential dynamics of low-level persisting rabies following the termination of a vaccination programme is summarised in a band of time (Fig. 7). This possibility spectrum is spanned by all the time-series resulting from our

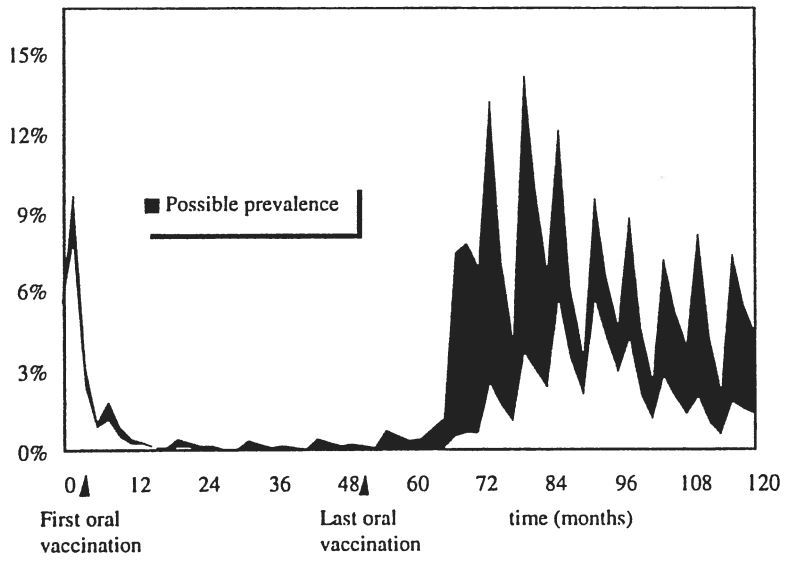

Fig. 7 Spectrum of simulated time-series for overall rabies prevalence in a 140x140 area. Immunisation started in year one on a standard spatial scenario and terminated within the fifth year, i.e. after month 50 (after Thulke et al., 1999b). All parameter configurations that previously proved to be linked to the low-level persistence of rabies were included, i.e. different epidemiological (actual immunisation level) and biological (mean litter sizes, contact rates) circumstances were taken into consideration.

simulations (Thulke et al., 1999b). We found, that during the time-steps of vaccination the bandwidth is small and after the offset of 'vaccination' the variability of the epidemic course increases. More generally, however, at least two years after the last vaccination the lower bound of rabies prevalence spectrum clearly passes the 2% value and tends into regular cyclic dynamics (Fig. 7). Therefore the new outbreak ought to be detectable as was the epidemic before control started.

DISCUSSION

Within this paper we unify particular aspects of rabies epidemiology. Based on rabies models evolving with respect to the scale of the questions addressed (Thulke et al., 1999a), we gained the results from our systematic simulation studies (Jeltsch et al., 1997; Tischendorf et al., 1998; Thulke et al., 1999b; Tischendorf et al., forthcoming).

The role of rabid foxes: The name of the disease stems from rabid foxes as they express abnormal behaviour (Charlton, 1988) and spontaneous aggressiveness (Macdonald & Voigt, 1985). Although it is well documented that only half of all infected foxes exhibit rabid symptoms (Bacon, 1981), rabid foxes are often cited as a major factor in the spread of the disease as they move around randomly during the clinical phase (e.g. Toma & Andral, 1977). Some authors exclusively refer to rabid foxes when they model rabies spread in space (Källen et al., 1985; Murray et al., 1986). The infection is diffused throughout the host population uniformly in time. By contrast, Jeltsch et al. (1997) found the coupling of two transmission processes from different spatial scales to be responsible for the formation of consecutive spatial waves, the typical pattern of rabies spread (Sayers et al., 1977; Hengeveld, 1989). Short-distance neighbourhood infections are the driving force behind disease transmission throughout the year. However, additional rare events (Jeltsch et al., 1997) of long-

distance transmission occur during dispersal, i.e. in autumn infected cubs move a long way compared to the tail of dispersal distance distributions (Trewhella et al., 1988; Goretzki et al., 1997). Without the temporal differences in the spatial scale of rabies spread, the disease pattern loses its wavy spatial structure just behind the front wave (Fig. 5b; cf. Källen et al., 1985; Murray et al., 1986). Källen et al. (1985) refer to varying incubation periods not considered in their model when reasoning the rapidly damped out front wave. Although Murray et al. (1986) added an incubating class to the model, they again found rapidly damped out rabies waves. Consistent with this, we found a damped out front wave with our model as long as we had only incorporated a diffusion-like disease spread by neighbourhood infection (Thulke et al., 1999a). Indeed, we only found the pattern of repeated advancing waves when potential spreading distances changed between the dispersal phase and the remaining year (Fig. 5a).

Rabies control planning needs very efficient (emergency) strategies in cases when a new outbreak occurs within a previously rabies-freed region. The interaction between the time spent until initial detection and possible spatial spread determines the hazard area after an outbreak (Bacon, 1981). Now, if we think about spatial rabies spread in a purely diffusion-like manner (Källen et al., 1985; Muray et al., 1986; Gardner et al., 1990), we would disregard any variability in time. Consequently, with respect to the time-dependence of potential spreading distance over the year, we would exclude useful information from management planning by the diffusion approach. Particularly in view of control planning, we explicitly refer to the importance of coupled short-distance and long-distance transmission events over the year, the basic feature of spatial rabies spread.

Heterogeneous immunisation: Our simulation studies concerning the chance of rabies eradication by the oral vaccination of foxes so far have been based on the assumption of spatial homogeneous control efficacy (Tischendorf et al., 1998). However, heterogeneity is a multifaceted issue of natural features and impedes not only processes in wildlife epidemiology. Enquirers anticipate that vaccination efforts closely depend on the potentially perturbed immunisation level in the fox population. Such disturbances are commonly thought to be related to technical errors, i.e. inaccurate aircraft navigation, wind-shifted bait, bait disruption or hand-baiting (Breitenmoser & Müller, 1997). Consequently, we tried to adjust our simulation results to the potential impact of heterogeneous immunisation.

In order to determine the influence of spatial heterogeneity, we may have used GIS-related approaches. However, any empirical map only represents a singularity of the infinite number of different possible combinations of factors that finally impede bait uptake. If general implications are necessary for further rabies control policy, such a restriction to one particular circumstance seems less helpful. Instead, we generate random spatial patterns of subareas where foxes are assumed to be excluded from immunisation and neglect the respective cause. Judging by the results presented we link a rabies-related quantitative description of heterogeneity (i.e. the amount of failed immunisation and its fragmentation in space) to control performance measure (i.e. the probability of eradication). We found that in general, heterogeneity is not necessarily disadvantageous for successful rabies eradication. However, we found two opposite effects of heterogeneous population immunity depending on whether the amount of failure appears clustered in a few coherent subregions (frag small) or is uniformly distributed over the whole area (frag large).

In detail, if bait coverage fails in coherent subregions (frag low, Fig. 6a) e.g. by vaccine failure due to logistics, then the chances of final disease eradication worsen compared to homogeneous reference (Fig. 6d). This is due to potential microepidemics within the subregions. The uncontrolled disease inside the subregion causes isolated cases in the partially immunised surroundings (cf. the situation on the border between Germany and the Czech Republic over the past few years). But in turn the neighbouring cases reintroduce the disease into the subregion if the population inside it has regrown. This mechanism could increasingly prevent rabies from eradication if subregions became larger (Fig. 6d).

Now we turn to higher degrees of fragmentation (Fig. 6c) e.g. by wind shift. The subregions with failed immunisation are no longer large enough for self-contained microepidemics. However, potential infected foxes within the well immunised part of the total area have a low chance of transmitting the infection unless they contact animals from a non-immunised group. And continued, the solitary (i.e. fragmentation is high) non-immunised fox group will soon be completely infected and die out within a short time-horizon. Therefore, it is unlikely that the disease will be retransmitted into the well immunised surroundings, but will instead cease with the solitary fox group. This phenomenon of 'catching rabies seeds from the immunised area' accounts for the increasing probability of disease eradication if the spatial distribution of baiting failure changes to uniformity. Finally, the probability of eradication exceeds the homogeneous reference value (Fig. 6d) because the positive effect of small uniformly distributed failure in immunisation is combined with the higher immunisation level within the baited area (IR_eff) relative to the homogeneous reference. Nevertheless, the mean level of immunisation (IR) used as a control quality measure in the field is equally determined for all simulation scenarios.

Large-scale and long-term oral vaccination of red foxes: We conducted a simulation study examining the spread of rabies within an immunised fox population (Tischendorf et al., 1998). We found that in the worst case rabies persists within the vaccination area even at an average immunisation level of 70% (Tischendorf et al., 1998). However, practicable surveillance measures would reflect low-level persistent rabies either as sporadic, solitary cases or as an apparently rabies-free area (Thulke et al., 1997). Consequently, field data cannot reliably demonstrate the conclusive success of a vaccination programme. Even if no rabid foxes have been detected, the final termination of control seems an impossible strategic decision owing to continuing uncertainty. Furthermore, if for economic reasons the apparently rabies-free state of a control area results in termination, emergency measurems must still be kept ready for worst-case management. But for how long must the logistics for emergency activities be maintained on standby?

Obviously, emergency measures need not be kept ready longer than the time it would take for a post-vaccination epidemic to recover at a definitely detectable level of prevalence. However, the detection of an uncontrolled rabies epidemic due to typical prevalence levels is guaranteed by practical experience. The time any post-vaccination epidemic needs to recover beyond the respective limits of certain detection is provided by our simulation result. The potential worst-case scenario would definitely be detected by newly recorded rabid foxes within two years after the last vaccination campaign (cf. Fig. 7). The simulation result therefore provides a basis for political management decisions in the context of areas suspected to be rabies-free after long-term, large-scale vaccination. We can thus assure both conclusive proof of whether an eradication programme has succeeded (assuming invasion to be impossible) and the limited need for emergency logistics in the time horizon of two years after the last vaccination. Altogether it is striking that the risk for control outcome due to the inherent low-level persistence of rabies actually reaches zero within a finite time interval.

CONCLUSIONS

Finally we translate the three results shown above into applied statements:
1. The role of rabid foxes for the formation of the typical spatial rabies pattern overestimated and particularly emergency measures should be based on the interaction of diffusive short-distance transmission and rare long-distance transmission events during the time span of dispersal.
2. Small. randomly distributed (i.e. highly fragmented) errors in the vaccination of fox populations (by random wind shift or bait disruption) are insignificant and instead favour the eradication of rabies.
3. A long-term, large-scale vaccination programme can be conclusively proven to have failed or succeeded at least two years after the last baiting campaign.

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EFFECT OF SURVEILLANCE PROGRAMMES ON SPREAD OF BOVINE HERPESVIRUS 1 BETWEEN CERTIFIED CATTLE HERDS

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Infectious Bovine Rhinotracheitis (IBR) is a widespread viral disease of cattle and can cause serious economic losses. The virus is easily transferred by contact and infection is most often introduced into the herd through the addition of purchased animals. The disease might be transmitted by cattle showing symptoms, but also by those not showing symptoms, and by previously infected animals in which the virus reactivates. Prevention of IBR might be accomplished by vaccination. In some countries, however, demands exists towards a non-vaccination strategy, considering health status of im/exported animals and animal products.

From May 1998, a compulsory eradication campaign for IBR has started in The Netherlands. In this campaign two aspects are important, namely to become free and to stay free of the infection. A free herd will receive a certificate. To stay free, in other words, to be sure that a herd deserves this certificate continuously, it should be surveilled on a regular basis. To support policy makers in their decisions about how a surveillance programme should look like, a mathematical model was developed, in which consequences of various strategies can be calculated to see whether or not it prevents spread of infection between herds after introduction of a positive animal in a free herd.

The surveillance might be done on the basis of testing of individual animals (blood or milk samples) or on the basis of testing a bulk milk sample. Therefore, 2 models were made. Using these models, the so-called Reproduction Ratio (R) between herds is calculated. An introduction of infection into one herd will only result in infection of many herds, i.e. in a major outbreak between herds, if each infected herd infects on average more than one other herd. This average number of herds that are infected by a typically infected herd is the Reproduction Ratio (R). When R is smaller than 1, the infection will eventually disappear before that herd causes directly or indirectly infection in many other herds (De Jong & Diekmann, 1992).

The aim of this research is to determine the spread of infection between certified herds with several surveillance programmes. In other words, which aspects of a surveillance programme will lead to an R between herds smaller than 1, even when R within herds (between animals within a herd) is larger than 1.

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MATERIAL AND METHODS

Model description

Model based on individual samples: Roughly, the model consist of two parts (for formulas see Notations), namely:

1. dynamics (course) of infection within a herd: given a certain reproduction ratio between animals within a herd (R), fraction of positive animals (C_0) and the proportion of animals that are replaced by common culling (v).
2. probability of detection: with sampling of x number of animals per herd, every 'Δ' time units, given the dynamics within a herd (see 1.), and the sensitivity (sens) of the used test.

Ad 1. Here, it was assumed that minor outbreaks will not be detected, and need not to be dteected as these minor outbreaks within a herd will **not** result in transmission between herds. Not all introductions will lead towards a major outbreak; only a fraction (1 - 1/R) (De Jong & Diekmann, 1992). This means that in a herd that is BHV-1 free and not vaccinating (R=5.6), 82% of introductions of a positive animal will result in a major outbreak. After an outbreak, positive animals disappear as a result of common culling. To restrict the number of model runs, it was assumed that a quarter of all animals is replaced annually. This parameter, however, can be varied as desired. To simplify the model further, it was assumed that an outbreak takes place at one moment. Consequently, the dynamics start with the number of positive animals after a major outbreak. The size of a major outbreak is the solution to $p=1-e^{-pR}$, that is the fraction of animals that become positive with a given R between animals within a herd (Diekmann et al., 1990).

The R of BHV-1 within (free) herds is dependent on the way they became free. In herds that became free without vaccination, infection will spread relatively rapid (R=5.6). In herds that became free by vaccination with "dead" vaccine R=2.6 and with "live" vaccine R=1.5 (determined in challenge and field trials) (Bosch, 1997; Vonk Noordegraaf et al., 1998) .

Ad 2. Here it was assumed that detection is dependent on the fraction of positive animals per herd, the sensitivity of the test, the number of samples and sampling frequency, and common culling.

Model based on bulk milk samples: This model is similar to the model on the basis of individual samples, namely the two steps: dynamics (course) of infection within a herd, and probability of detection. However probability of detection is not based on a number of samples but on only one bulk milk sample per herd. Therefore, a parameter called threshold was introduced. This threshold is the fraction of positive animals within a herd at which bulk milk will be classified as positive (conversion of antibody titre) (Wellenberg et al., 1997).

For each herd type (either or not free by means of vaccination), model outcomes are calculated with different values for parameters. One by one, parameters were varied, while the others were kept constant. For both models the used default values can be found in Table 1.

Model assumptions

Calculations start from a population of certified herds, that is herds free of BHV-1. Herds became free either by vaccination with live vaccine, dead vaccine, or just culling of (sero)positive individuals, that is without vaccination. Reasons to start calculations with a free

population of herds is as follows. If a surveillance programme is successful in a negative population it will be feasible to stay free if the intake of new certified herds gives sufficient guarantee that new additions are not too often fals-negatively called free. If the programme is not able to prevent spread of infection when it is introduced into one herd, then trying to become free is useless, even when intake is very good.

Table 1. Default values of parameters in the models

Parameter Model	Default value Individual sampling	Default value Bulk milk sampling
Sensitivity	0.7	0.7
Number of samples	20	1
Threshold	-	0.15
Herd size	50	50
Sampling frequency	1 per year	1 per month
Fraction common culled animals ($year^{-1}$)	0.25	0.25
Fraction positive animals that introduce infection in other herds (per year)	0.04	0.04

A surveillance programme is only successful when positive animals in a certified herd are sufficiently quick detected. Positive animals are defined as those animals that are judged false-negative at the intake procedure for a certificate; e.g. by being seronegative, or not being sampled (by e.g. an age limit for sampling), or that became positive by contacts (purchase or contacts). With "sufficiently detected" it is meant that the R between herds should be below 1.

As yet, it was assumed that herds are homogeneously spread over The Netherlands and that spread of infection within a herd is not dependent on herd size. Also, it was assumed that all herds are equally susceptible, and after infection equally infectious. Further, it was assumed that contacts between herds take place randomly, what is (probably) not the case in reality. Probably, the number of contacts will be dependent on herd size. In a study in a limited are of The Netherlands, however, the number of contacts (animal flow and other contacts) was not predictable on the basis of herd characteristics (Nielen et al., 1996).

The model only accounts for animals that are sold "for life", and therefore bought by other herds. Animals that go directly to the slaughterhouse (common culling) do not spread the infection. In the current situation, certified herds will not buy animals from non-certified herds. The multiplication factor in both models is therefore only meant for the fraction of positive animals that introduce infection into another herd. This fraction can, consequently, consist of purchase of infectious animals, purchase of seropositive animals that might reactivate, and all other contacts (contact over fence, neighbourhood contact, vermin, persons (veterinarian, dealer, inseminator), materials, etc.). The fact that the number of transported and sold animals are not equally distributed over the year, but related to calving periods (Nielen et al., 1996), is not accounted for in the model. Over one third of all dairy herds buy annually less than 3 cows, other herds buy more animals varying from 3 tot > 50 (Van Wuijckhuise et al., 1998). This latter study did not mention whether or not purchase is related to herd size.

In both models, it is not accounted for the time between sampling and result of the test and with that measures that are taken to prevent further spread. In these models, therefore, time of

sampling and test result is the same. Real time between sampling and result is neglectable when considering sampling frequency in the default situation (certainly in the model for individual sampling).

Most important, in the model the R between herds is not accounted for measures to be taken in the eradication campaign. In this model, only the infected herd is found (and therefore looses his certificate) and no special attention is paid to "contact" herds. In reality however, there will be taken extra measures in these "contact" herds. As a result of this, the outcomes of the models (R between herds) are probably overestimated.

RESULTS

Results model based on individual samples

From Figure 1, it can be seen that sensitivity hardly has an effect on R between herds. The sensitivity of tests that are used at the animal health service in The Netherlands varies between 70 and 95%, dependent on the used panel (personal communication, J.J. de Wit). Therefore, the choice of 70% is on the safe side. With the chosen default values of a surveillance programme spread of infection between herds can be prevented for all herd types.

Using a criterion of $R < 1$ in a surveillance programme, sampling frequency is not really important when vaccines are used to maintain the herd free (Fig. 1). Apparently, the vaccine is sufficiently effective to prevent spread between herds (R between herds remains lower than 1). However, the more frequent a herd is sampled the lower the R between herds. With no vaccination (Rw=5.6), sampling every 15 months (at least with the default values used) might prevent spread of infection between herds. When the fraction of positives and/or herd size is increased sampling frequency needs to be intensified to keep Rb below 1 (Data not shown).

Increasing the number of samples does not have an effect on the R between herds (Fig. 1). It might be concluded that sampling frequency is more important than sample size.

An increasing herd size always results in an increased R between herds (Fig. 1). Herd size, however, is not really important for herds that vaccinate with live vaccine, when it is decided that for a surveillance programme to be successful R between herds should be below 1. Spread of infection can only be prevented in herds vaccinating with dead vaccine when the herd consists of less than 100 animals. No vaccination in herds larger than 60 animals always results in spread of infection, although this will be quicker with increasing herd size. These conclusions, however, could only be made with the chosen default values. Since default of the sample size is 20 (40% sampling with the default herd size of 50) the effect of herd size is also calculated with a sampling fraction of 40% (in stead of 20 samples). These results (data not shown) were equal to a sample size of 20, and therefore sample size was considered not important. The increasing R with increasing herd size is caused by the fact that the number of positive animals that introduce infection in other herds is larger with larger herds. The default is that 4% of positive animals introduce infection in other herd; 4% of larger herds is absolutely a larger number of positive animals. When increasing this value to 8%, the Rb increases drastically (Data not shown). Taking samples twice a year instead of once a year, results in an Rb below 1, whether or not vaccination took place (Data not shown).

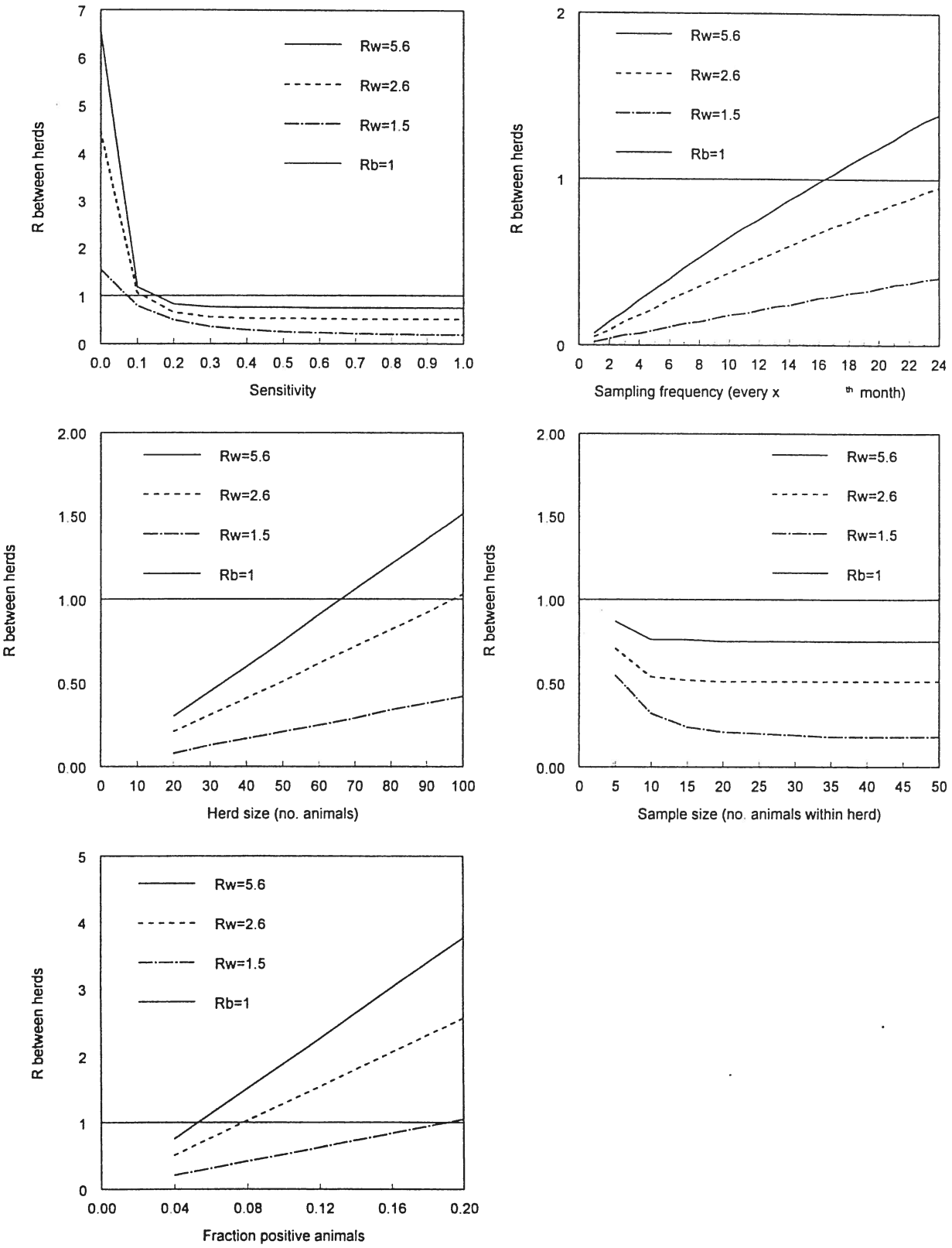

Fig. 1 R between herds with varying resp. sensitivity, sampling frequency, sample size, herd size, and fraction positives (model individual samples) with default values of the other parameters

Increase in fraction of positive animals that introduce infection in other herds results in an increase of spread of infection between herds (Rb) (Fig. 1). When herds do not vaccinate or vaccinate with dead vaccine this always leads to major outbreaks between herds when the parameter is larger than or equal to 0.08. With herds that vaccinate with live vaccine and have less than 20% of positive animals that introduce infection in other herds no major outbreaks occur. Increasing the herd size leads to a large increase in Rb (Data not shown). If the fraction of positives is set at 0% (so no positive animal that introduce infection in other herds) spread of infection between herds can always be prevented; even with very large herd sizes.

Results model based on bulk milk samples

From Fig. 2, it can be seen that also with bulk milk the level of sensitivity does not affect the R between herds very much at the level of existing tests (around 0.7). Only with a sensitivity of 0 and 0.1 major outbreaks can occur. With the chosen default sensitivity of 0.7 major outbreaks are always prevented, even when herd size and/or fraction of positives is increased (Data not shown).

Sampling frequency is not important (at least when it is decided that R between herds should be below 1) for herds that vaccinate with live vaccine (Fig. 2). Apparently, the vaccine is sufficiently efficacious to prevent spread of infection between herds (R between herds always below 1). When vaccinating with a dead vaccine, sampling of bulk milk should be done about every 9 months to see whether a herd is still negative. When no vaccination takes place sampling for testing bulk milk on presence of antibodies should be done every 22 weeks (5 months) to prevent spread of infection between herds (consider the other default values). However, when herd size and/or fraction of positives is increased then the sampling frequency should be increased up to every month to keep Rb below 1 (Data not shown).

The threshold only affects R between herds, when it is above 70% for herds that don't vaccinate (Fig. 2). If an outbreak occurs in such herd, within no time a major part of the animals will become positive. When a live vaccine is used, a threshold of larger than 15% is too low and introduction of infection in such herd will result in a major outbreak between herds. In practice, however, bulk milk already shows seroconversion with 10 to 15% of the animals being seropositive (Wellenberg et al., 1997). Thus, even with an increased herd size and/or fraction of positives, the Rb will be below 1 (with the other used default values).

With all the used default values, herd size does not have a large influence on R between herds. It was seen that R between herds always is smaller than 1, even when the fraction of positives is increased to 8% (Data not shown). In other words, when sampling bulk milk every month, no major outbreaks will occur.

Increase in fraction of positive animals that introduce infection in other herds results in an increase in spread of infection (Fig. 2). However when sampling occurs every month, no major outbreaks will occur, even with "a lot of contacts". When herd size is increased to 100 animals, however, major outbreaks will occur in herds that did not vaccinate (Rw=5.6) and have a proportion of animals that introduce infection in other herds above 10% (Data not shown).

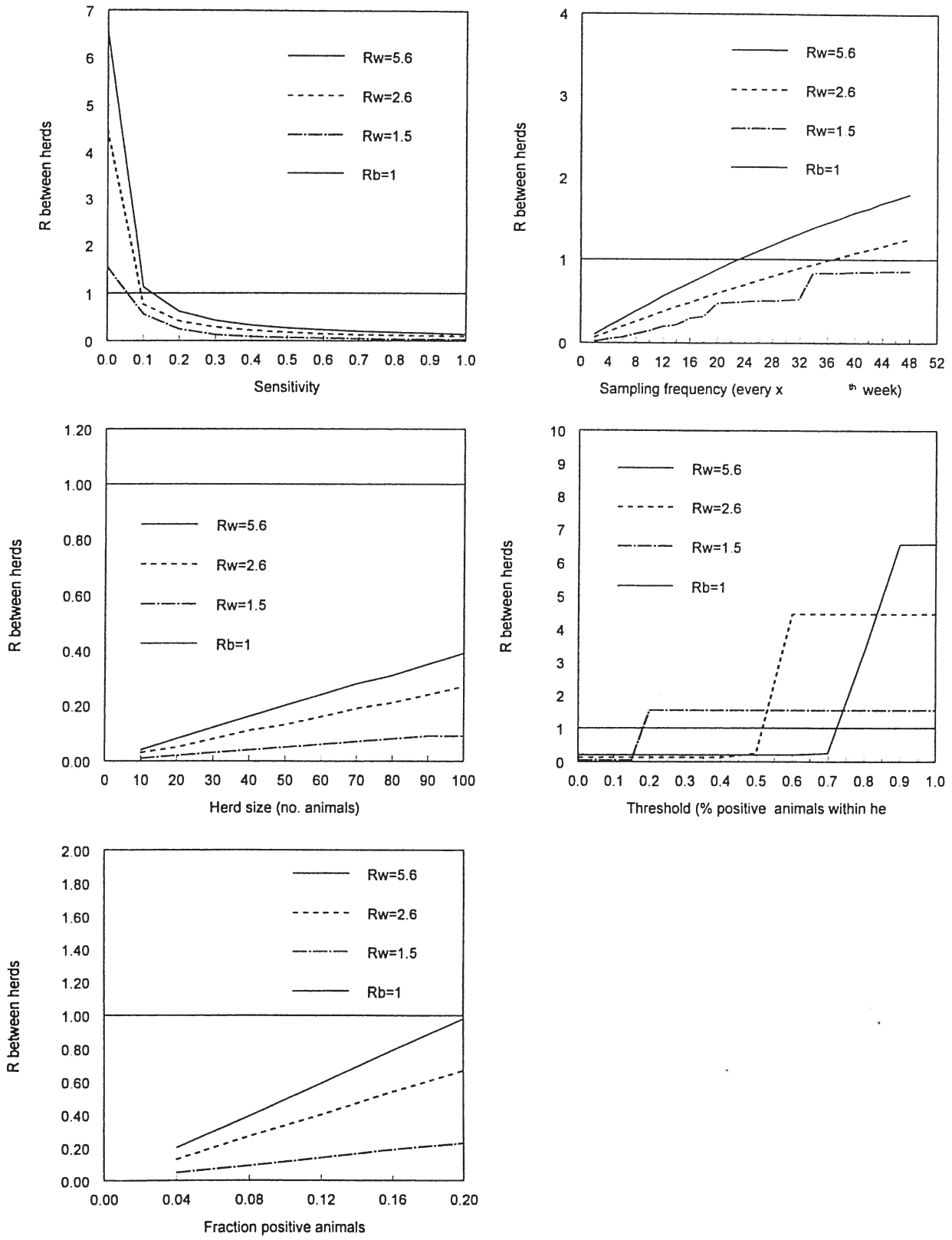

Fig. 2 R between herds with varying resp. sensitivity, sampling frequency, threshold, herd size, and fraction positives (model bulk milk samples) with default values of the other parameters

DISCUSSION

In this paper, models were described in which the spread of a BHV-1 infection between herds is calculated with different aspects of a surveillance programme. It should be emphasised, that the results that were presented deal with the aspect of staying free of BHV-1 once the population is negative. Becoming free of a population with 75% of positive herds, as is in The Netherlands (Van Wuijckhuise et al., 1998), is something completely different and conclusions whether or not vaccination (either with "dead" or "live" marker vaccine) is necessary to become free should **not** be made on the basis of this study. Also, decisions about how a surveillance programme for certified herds should look like when a lot of surrounding herds are still not free (as is the case at the moment in The Netherlands) should be made with care.

When in a BHV-1 free country a major outbreak occurs at an AI centre or trade intensive farm, the spread between herds will probably much different (R underestimated). This aspect was not looked at in the presented models. Neither, it was accounted for the fact that specific measures are taken in an eradication campaign once a herd is found positive. This will result in an overestimation of R between herds. Another overestimation might be present with the value of proportion of positives. With an average herd size of 50 animals and the knowledge that over one third of all dairy farms purchase 2 animals annually (Van Wuijckhuise et al., 1998), the chosen default values of 4% is on the safe side. A value of 0.04 implies that only just the infectious and/or seropositive animals are being sold (which is, for a matter of fact, likely after an outbreak on non-vaccinating farms) and that positive animals always reactivate, which is not absolutely. Therefore, the value of 4% will be rather overestimated than underestimated. Since calculations start from worst possible situations, nothing was added to this fraction for contacts other than purchase of positive animals (contact over the fence, neighbourhood contacts, vermin, persons, materials, etc.).

If R is smaller than 1, only minor outbreaks occur. The number of herds involved in a minor outbreak depends on the R between herds. The expected number of infected herds when the R of a surveillance programme is smaller than 1, is 1/(1-R) (De Jong & Diekmann, 1992). In our study, this will be the number of herds that become BHV-1 positive. From this formula, it can be calculated that decreasing an R from 0.99 to 0.90 dramatically reduces the number of herds that become infected in a minor outbreak. Decreasing it to lower values however, does not result in a very large reduction. The choice of how a surveillance programme will look like depends on measures to be taken on positive farms. If such herds are stamped out then it is best to create a programme with an R between herds as low as possible.

In general, from results from this study it can be concluded that spread of BHV-1 between herds might be prevented when animals within herds are sampled once a year (individual milk as well as blood samples). This frequency needs to be intensified for larger herds and/or herds with extensive contacts with other herds (**sampling twice a year**). When bulk milk is sampled, sampling should be done at least every 5 months. Just like with individual animal sampling, the frequency should be increased for larger herds and/or herds with extensive contacts with other herds (**sampling each month**).

The sensitivity of test is of lesser importance. The existing diagnostic tests are sufficiently good for **surveillance** of certified herds. For receiving a certificate (intake procedure) the sensitivity of tests will possibly be more important, since as few as possible false-negative herds should be given a certificate. However, if with the intake procedure **all** animals within a herd are

sampled individually, the probability that all positive animals are classified false-negative will be very small. Intake on the basis of bulk milk samples is useless since bulk milk only becomes positive with a minimum of 10 to 15% positive animals (Wellenberg et al., 1997).

Further, the sample size (with individual sampling) is of minor importance. Sampling a minimum of 10 animals twice a year is sufficient to prevent spread of BHV-1. The choice between individual sampling and bulk milk sampling for surveillance of certified herds seems to be in favour of surveillance on the basis of bulk milk sampling. An average of 12 samples a year will suffice in stead of twice a year a minimum of 10 animals which is 20 samples. The choice for bulk milk sampling is approved by the fact that with individual sampling, costs are made for taking the samples. Logically, non-dairy farms should take individual samples.

From the results of both models, individual sampling as well as bulk milk sampling, it was shown that especially sampling frequency and fraction of positive animals that introduce infection in other herds are important parameters in the model. Especially, the latter one affects the spread of infection between herds to a large extent. To give a reliable advice concerning surveillance programmes, the value of this parameter in practice should be known.

From the results of this study, it is shown that theoretically a surveillance programme can be constructed that keeps R between herds below 1, even with bulk milk sampling. However, crucial to this is how the herds look like when they are qualified. This quantitative theoretical approach might be very valuable in deciding how a surveillance programme should look like. An additional economic analysis of several strategies might help in decision making.

NOTATIONS

1. Dynamics: course of number of positive animals within a herd:

The extent of number of positive animals within a herd after a major outbreak is the solution to: $C_0 = 1 - e^{-CoR}$. Subsequently, the course of fraction of positive animals ($i(t)$) within a herd in time (t) is dependent on the fraction of positive animals just after a major outbreak (C_0 = fraction positives just after an outbreak, so on a certain point in time t somewhere between 0 en Δ (=sampling time)) and the common culling rate (v):

$$i(t) = C_0 \, e^{-v(t-t_0)} \qquad \text{for } t > t_0 \qquad (1)$$

$$i(t) = 0 \qquad \text{for } t \leq t_0 \qquad (2)$$

The total number of positive animals from time of introduction of infection is determined as follows. Infection starts after t=0 and before t=Δ, since time starts counting at the last sampling moment at which no outbreak occurred (so at t=0). We assume that infection starts somewhere between 0 and t_0, and that this happens randomly. The number of positive animals at a moment t after t=0:

$$i^*(t) = \int_0^t \frac{C_0}{\Delta} (e^{-\nu(t-t_0)})\, dt_0 = \frac{C_0}{\Delta\nu}(1 - e^{-\nu} \qquad \text{for } t < \Delta \qquad (3)$$

$$i^*(t) = \int_0^t \frac{C_0}{\Delta} (e^{-\nu(t-t_0)})\, dt_0 = \frac{C_0}{\Delta\nu}\, e^{-\nu t}(e \qquad \text{for } t \geq \Delta \qquad (4)$$

The cumulative number of positive animals ever being present is defined by:

$$I(t) = \int_0^t i^*(z)dz \qquad (5)$$

From which it follows that:

$$I(t) = \int_0^{\Delta} i^*(z)dz + \int_{\Delta}^t i^*(z)dz \qquad \text{for } t > \Delta \qquad (6)$$

Thus:

$$I(t) = \frac{C_0}{\Delta\nu^2} (e^{-\nu t} - e^{-\nu (t-\Delta)}) + \frac{C_0}{\nu} \qquad \text{for t} > \Delta \qquad (7)$$

NB. Start is at time 0. Infection takes place somewhere between t and t_0. At Δ sampling is done for the first time, so on average there will be a fraction of positives (last formula). Multiplying with herd size (N) this results in the total number of positive animals.

2. Detection: detection of positive animals within a herd:

Dependent on the prevalence (C_0) infection will be detected, after a k number of samples, if sampling is done each Δ time units of x animals (average time between introduction of virus (either by "new" introduction, or reactivation). All this is dependent on test sensitivity and the number of positive animals that disappear through common culling.

The probability of detection is:

$$1 - \prod_{i=0}^{k} e^{-sens\ x\ C_0\ e^{-\nu(\Delta\ -\ t_0\ +\ i\ \Delta)}} \qquad (8)$$

This results in:

$$1 - e^{-sens \times C_0 \frac{1}{\Delta v}(1 - e^{(-k \Delta v)})} \quad (9)$$

The probability of detection before the first sampling (k=0) is 0. If no samples are taken, nothing can be found either. The probability of detecting a positive herd before the first sampling (0<k<1) is the probability as calculated in the last formula. The probability of detection with 1 or more samples (k≥1) is the probability of detection at sampling k minus probability of detection at the previous sampling, k-1.

The spread between herds (at a certain sensitivity, sample size, herd size, sampling frequency, R within herds, common culling, and fraction of animals that introduce infection in other herds) is the sum of probability of detection × the cumulative number of positive animals at time of detection × the fraction of animals that introduce infection in other herds. In other words, this is the **R between herds**.

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ESTIMATING THE INCIDENCE RATE OF WITHIN-HERD SPREAD OF *M. BOVIS*

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Mycobacterium bovis, the causative agent of tuberculosis in cattle and cervids is capable of infecting a broad range of animals including humans. Cattle and cervids may become maintenance hosts while most other animals, although they become infected, act only as spillover hosts (ie. become infected but do not normally transmit the disease) (Morris et al., 1994; Griffin & Dolon, 1995). An understanding of the role of animal-to-animal transmission of *Mycobacterium bovis* is important for an understanding of the epidemiology of this disease.

While there are some reports of factors which affect the between animal spread of *M. bovis*, there is very little information in the published literature about the incidence rate of *M. bovis* infection within infected cervid or cattle herds. This paper presents the analysis of several potential risk factors affecting the within-herd spread of tuberculosis in Canadian cattle and cervids, and estimates the incidence rate of new infections in these herds. The results were based on a retrospective analysis of the 9 tuberculosis outbreaks that occurred in Canada between 1985 and 1994.

MATERIALS AND METHODS

Records Retrieved

A preliminary survey of Agriculture and Agri-food Canada offices in the fall of 1994 indicated that it was likely that adequate records from the 9 tuberculosis outbreaks occurring between 1985 and 1994 could be found in the Regional or District Offices of Agriculture and Agri-food Canada. Consequently all offices associated with tuberculosis outbreaks were visited and the relevant data collected.

Each outbreak investigation started with the identification of a tuberculosis suspect animal, usually at slaughter or post-mortem. Tissues from the initial suspect animal in each outbreak were submitted to a federal laboratory for histology and culture. Skin testing of the herd was initiated if the histology was suggestive of tuberculosis. The herd was usually depopulated only if the culture was positive for *M. bovis*. An investigation was conducted to determine the source and spread of the disease.

Data that were collected during the outbreak investigation were stored in files for the individual farm and data for this study were extracted manually by reviewing all herd and individual animal files and photocopying necessary information. The data used in this study were generally found in one of 2 formats in the outbreak files. The first was test result sheets which included skin test reports, post-mortem reports, histology and culture reports. The second format was an Inspector's Report which included: a history of the herd as it related to the outbreak; the herds which had been

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identified for further testing as a result of their relationship with the farm under investigation; epidemiological information such as subsequent testing information and animal history; and miscellaneous information unique to each situation. The data are described in more detail in a paper describing the analysis of factors associated with the between herd spread of *M. bovis* (Munroe et. al., 1999).

Data

Data used for these analyses were collected on an individual animal basis and were collected only on animals that were in positive/reactor herds. A herd was classified as positive/reactor if one or more animals in the herd were positive or suspicious on a comparative cervical or mid-cervical tuberculin skin test, gross pathological or histopathological test for tuberculosis, or culture for *M. bovis*.

The variables used in the analysis are described in Table1. REACTOR was a dichotomous variable representing the status of the individual animal at the end of the investigation and was either negative or positive/reactor. Negative meant that the animal had been negative on all tests with the possible exception of the caudal fold test. Animals that were caudal fold test suspicious or positive were always submitted to other tests. If an animal was suspect or positive on any test other than the caudal fold test, it was considered a positive/reactor animal. Therefore positive/reactor animals had been suspect or positive on a comparative cervical, mid-cervical, gross pathology, histopathology, or culture test. When a test was administered more than once, the result from the last test administered was used in the classification process.

AGE was a categorical variable with animals assigned to one of three groups: 0-12 months old; 12-24 months old, or 24 or more months of age. SEX was a dichotomous variable with females in one category and males and neutered males in the other. BREED represented the species/breed of the individual animal. It was coded as dairy, beef, cervid, bison, or other. Other included swine, sheep, goats, and zoo animals and any miscellaneous breeds or species not covered by the specific classifications.

OUTBREAK was a categorical variable which represented the location of the outbreak. Generally each outbreak was limited to one province with the Alberta/Saskatchewan outbreak being the only exception.

Definition of a case

An animal was considered to be a case (i.e. new infection) if the investigators were reasonably certain that the transmission of the infection had occurred within the herd being evaluated and within the time period at risk (see below). Consequently, all animals that were positive/reactor in the index herd were excluded (period at risk not known). Similarly positive/reactor animals that had been purchased from an infected herd were excluded (presumed transmission before the animal entered herd being evaluated).

Definition of Time at Risk

Since the probability of transmission of *M.bovis* within a herd depends on the duration of the exposure to the organism, an exposure time was calculated for each animal. To understand the exposure time it is necessary to define 2 terms used in its calculation - the earliest exposure date for the "study" herd and the departure date for each animal.

Table 1. Description of variables used in the unconditional and negative binomial regression analyses of animal to animal transmission of *Mycobacterium bovis* in Canadian cattle and cervids from tuberculosis outbreaks between 1985-1994

Variable(a)	Description	Frequency distribution	Negative animals (%)	Positive/ reactor animals (%)
REACTOR	Animal status	0 = neg	1534 (88.7%)	
		1 = pos/react		195 (11.3%)
AGE	Age category in months	0 = 0-12	365 (99.5%)	2 (0.5%)
		1 = 12-24	301 (86.2%)	48 (13.8%)
		2 > 24	868 (85.7%)	145 (14.3%)
			$p < 0.000$	
SEX	Sex of animal	0 = female	1188 (88.2%)	159 (11.8%)
		1 = male or neutered	346 (90.6%)	36 (9.4%)
			$p = 0.19$	
BREED	Breed or species	dairy	284 (92%)	25 (8%)
		beef	666 (91.1%)	65 (8.9%)
		cervid	556 (84.5%)	102 (15.5%)
		other	28 (90.3%)	3 (9.7%)
			$p < 0.000$	
OUTBREAK	Location of outbreak	Quebec cattle	261 (91.9%)	23 (8.1%)
		Ontario cervid	673 (90.8%)	68 (9.2%)
		Manitoba cattle	47 (94%)	3 (6%)
		Alberta/Sask cer	194 (79.8%)	49 (20.2%)
		Alberta cattle	320 (86.5%)	50 (13.5%)
		Quebec cervid	39 (95.1%)	2 (4.9%)
			$p < 0.000$	

Earliest exposure date: The earliest exposure date was the earliest possible date that the study herd may have been exposed to a potentially tuberculous animal from some "reference farm". The reference farm was the farm which prompted the investigation of the study farm. In the case of sales of animals, this was the date that the potentially infectious animal entered the study farm from the reference farm. In the case of fence line contact or perimeter contact, the earliest exposure date for the study farm was the date that the reference farm was first exposed to potentially infectious animals. If this date was unknown, then the earliest exposure date for the study farm was the date when the reference farm was first known to be a reactor or a positive farm. Study farms which were identified as positive/reactor farms as a result of traceback investigations would have an earliest exposure date only if the likely source of infection (i.e. the reference farm) was known. The earliest exposure date for study farms that co-pastured with a potentially infectious animal was the date that the co-pasturing began after it was known that the reference farm was a positive/reactor farm. There was no earliest exposure date for index farms where the source of infection could not be determined. Farms investigated for other reasons were given an earliest exposure date only if the farm had received animals from or had been in contact with animals that were from a positive/reactor farm.

Departure date: The departure date was the last date that the status of an animal was known, i.e. the date of the last test on an individual animal.

Calculation of exposure time: The exposure time for most animals in the study was the elapsed time in days from this earliest exposure date for the herd to the departure date for the animal. The exposure time for animals that were born after the earliest exposure date for their herd was the time from their birth until their departure date.

Statistical analysis

All analyses were performed using a statistical software package (Stata, Stata Corp, College Station, Texas). The variables used in this analysis are described in Table 1. A chi square test was used to test the unconditional association of the independent variables AGE, SEX, BREED, and OUTBREAK, with the dependent variable, REACTOR. A p-value of less than or equal to 0.2 was designated as the cut off to incorporate the variable into the poisson and negative binomial regression models.

The data were organized into covariate patterns based on OUTBREAK, AGE, SEX, BREED, and farm. For each covariate pattern , the number of REACTOR animals and the number of animal-days at risk were determined. The number of REACTOR animals was the dependent variable and the number of animal-days at risk (ie. exposure time) was included in all regression models as an offset. The appropriateness of the poisson models was checked by comparing the mean number of reactors across covariate patterns with the variance and by a goodness of fit test after fitting the poisson model. In the negative binomial regression models OUTBREAK was included a priori as a confounder and BREED was also evaluated as a confounder. Likelihood ratio tests were performed to determine the significance of the variables which were retained in the model. Outliers were assessed to determine their impact on the coefficients.

Actual and predicted incidence rates were calculated based on outbreak location, breed or species involved, and age (greater than 24 months of age) and are reported as number of new cases of tuberculosis per 100 animal years.

RESULTS

Descriptive statistics

Data used in these analyses were derived from 1534 negative animals from 30 herds and 195 positive/reactor animals from 23 of the 30 herds. (Positive/reactor herds may not have had any positive/reactor animals used in these analyses since index animals and those brought in by purchase were removed.) Thus the average herd size was 58 animals and the average number of positive/reactor animals per herd was 6.5. Observations for the Prince Edward Island bovine, New Brunswick bison, and British Columbia cervid outbreaks were dropped as there were no data for positive/reactor animals other than the index farms. Table 1. gives a brief description of the variables used in the unvariable analysis, a frequency distribution in each level of the outcome variable (REACTOR) and the P-value of the test for unconditional association of each independent variable with the dependent variable.

Unconditional associations

The percentage of positive animals increased as the age category increased with very few positive animals being less than 12 months of age. The percent positive animals in the 12-24 months old and the greater than 24 month categories were very close (13.8% and 14.3% respectively). The p-value for sex of the animal was just below the cut off value required to be considered in the regression analyses. There were 11.8% and 9.4% positive females and males respectively. Dairy, beef and "other animals" had a similar percent positive at 8%, 8.9%, and 9.7% respectively. The cervids had a higher percentage positive (15.5%). The Alberta/Saskatchewan cervid outbreak had the highest proportion of positive animals (20.2%) followed by the Alberta bovine outbreak (13.5%).

Negative binomial regression

When, a poisson regression model was fit to the data the goodness of fit test for the poisson

regression ($\Pi^2 = 319.26$; df =123; p = 0.000) showed that the data did not fit a Poisson distribution. Data fell into 134 distinct co-variate patterns with the mean and standard deviation of the number of reactors (REACTOR) being 1.46 and 4.01 respectively. Thus the assumption in a poisson regression that the variance and mean of the dependent variable are equal was not supported and consequently a negative binomial model was used in all subsequent analyses.

All variables were entered into the negative binomial regression analysis. The p-value for SEX was greater than 0.05 and it was dropped from the model. OUTBREAK was left in the model as a confounder. BREED was left in the model even though its overall statistical significance was >0.05 because the confidence interval for the incidence rate ratio for one category (cervids) did not include 1. Consequently, the variables which remained in the full model were OUTBREAK, BREED and AGE. The results of the negative binomial regression analysis are in Table 2.

Table 2. Results of the negative binomial regression for the risk of transmission of tuberculosis between animals in Canadian cattle and cervids from 1985-1994, using an overestimated exposure time for all animals

Variable	Coef.	Incidence rate ratio (IRR)	SE(IRR)	P-value	95% C.I (IRR)
MATURE					
0 - 12 months		1		0.009	
12 - 24 months	2.03	7.65	6.96		1.29 - 45.43
> 24 months	2.34	10.42	9.21		1.84 - 58.94
BREED					
Dairy		1		0.210	
Beef	0.73	2.08	1.34		0.59 - 7.34
Cervid	1.61	4.99	3.76		1.14 - 21.84
Other	1.76	5.80	6.54		0.64 - 52.90
OUTBREAK					
Quebec cervid		1		0.039	
Quebec bovine	2.08	8.03	8.41		1.03 - 62.52
Ontario cervid	0.50	1.65	1.58		0.25 - 10.82
Manitoba bovine	0.14	1.15	1.42		0.10 - 12.96
Alberta/Sask cervid	1.01	2.75	2.90		0.35 - 25.71
Alberta bovine	1.96	7.13	7.66		0.87 - 58.60
Constant	-12.64			0.000	(-15.40)-(-9.88)

Predicted outcome values and leverage values were calculated for all of the covariate patterns. The predicted values were graphed against the actual REACTOR values. This graph identified three covariate patterns which appeared to be outliers. When the outliers were removed, the overall model chi square was more highly significant (P = 0.004) than the full model (P = 0.013). The coefficient for AGE changed very little and this variable was still significant. There were more substantial changes in the coefficient for the two variables, BREED and OUTBREAK but, since there were no biological or other reasons to remove these observations, they were left in the final model.

Table 3. gives the actual and predicted incidence rates of new cases of tuberculosis positive/reactor animals per 100 animal years in different groups of mature (greater than 24 months of age) animals. Only rates for categories in which some new cases were observed are presented and the groups are characterized by breed and outbreak location. For example, mature dairy cattle in the Quebec bovine outbreak had actual and predicted incidence rates of new cases of reactor or positive animals, of 2.8 and 9.8, respectively, per 100 cow years. Only one outbreak (Ontario) had cases in all 3

breed/species groups and in this outbreak, the observed incidence rate for cervids (IR = 9.3 cases per 100 animal years) was almost twice that of dairy cattle (IR = 5.0) and three times that of beef cattle (IR = 3.1).

Table 3. Actual and predicted incidence rates for tuberculosis in mature (>24 months) Canadian cattle and cervids in tuberculosis outbreaks in Canada between 1985-1994 (expressed as the number of new cases of positive/reactor animals per 100 animal years).

Outbreak location and breed	Actual and predicted incidence rates in different breeds/species					
	Mature dairy		Mature beef		Mature cervid	
	Actual	Predicted	Actual	Predicted	Actual	Predicted
Quebec Bovine	2.8	9.8	10.1	20.4		
Ontario Cervid	5.0	2.0	3.1	4.2	9.3	10.1
Manitoba Bovine			2.0	2.9		
Alb/Sask Cervid					18.6	16.9
Alberta Bovine	4.9	8.7	2	18.1		

DISCUSSION

Every possible effort was made during data collection to assure completeness and correctness of the data. However some difficulties were encountered due to the fact that the outbreaks dated back several years and the investigators who worked on the outbreak were not always available to clarify questionable records.

Only records which had complete data for the variables incorporated in the models were used in the analysis. Thus there was a substantial reduction in the number of herds which were included in the analyses compared to the total number of positive/reactor herds identified.

Methodology

It was impossible to know the exact exposure time for each animal and the method of estimation used has probably resulted in there being an upward bias in exposure time. Using the earliest exposure date assumes that the animal was at risk of becoming infected with *M. bovis* starting on that date. The departure date represented when the investigators knew the status of positive animals and assumes that all positive/reactor animals became infected immediately before testing. It does not take into account the actual (but unknown) date that the animal became positive. The overestimation of the exposure time would lead to an underestimation of the incidence rates.

However, for 2 specific situations, exposure by fence line contact or co-pasturing, the estimate of exposure time may have been an underestimate. In these 2 situations, the earliest exposure date was set to the date when we knew that the reference farm was infected but this was probably some period of time after the reference farm had first become infected. Consequently, there was a period (duration unknown) of exposure which had not been accounted for.

Risk factors

In this study, the age of the animal was found to be a risk factor for being a positive/reactor animal. Increasing age increased the risk. This was also found in a study in Northern Ireland where older animals (cows, heifers, and bullocks) were significantly more likely to fail a tuberculin test than calves (Griffin et al., 1996). Young cervids however may be infected with *M.bovis* yet remain negative to a tuberculin test (Griffin et al., 1994). This would contribute to an apparent increase in risk with age for cervids.

The overall p-value for BREED in the negative binomial regression analysis was 0.21 but the model coefficients indicated that all breed categories were at greater risk of being positive/reactor animals than the comparison dairy group. This is contrary to the view that dairy animals are at greater risk than beef animals (Morris et al., 1994; Jubb et al., 1993). Cervids had an IRR of 4.99 in comparison to the baseline dairy breeds and its confidence interval did not include 1. This supports the view that cervids are more susceptible to tuberculosis than bovines. The apparent statistical significance of BREED may have been reduced by the fact that there was a strong association between BREED and OUTBREAK (ie. outbreaks tended to be dominated by one type of breed/species). However, it was necessary to include OUTBREAK in the model to control for the potentially confounding effects associated with this variable. These potential confounders would include factors such as variability between farming practices in different locations and variability in the techniques used in outbreak investigations in different parts of the country.

Actual & predicted incidence rate

The actual and predicted incidence rates (IR) for tuberculosis in mature Canadian cattle and cervids in outbreaks from 1985-1994 are presented in Table 4. In general the incidence rates in cervids were higher than those in cattle. The data do not clearly indicate if the speed of spread in dairy herds was greater or less than in beef herds. There were 3 outbreaks where rates between dairy and beef animals could be compared and of these, 2 outbreaks had higher incidence rates in dairy cattle than in beef cattle. Consequently, no firm conclusions could be drawn regarding the rate of spread of tuberculosis in dairy versus beef herds.

The predicted incidence rates in the model were similar to the actual incidence rates for cervids. The correlation between actual and predicted rates in the dairy and beef breeds was much more variable and the predicted rates were higher in all but 2 outbreaks (beef cattle in the Quebec cervid outbreak and dairy cattle in the Ontario cervid outbreak). This model was not meant to be a predictive tool for within herd incidence rates of tuberculosis but was designed to give some estimate of the IR of tuberculosis in infected herds.

In a recently published study (Wahlstrom et al., 1998) used a modified Reed-Frost model to study the spread of *M. bovis* within a small number of Swedish fallow deer herds. Data extracted from that paper suggested within herd incidence rates ranging from 0 to 7.7 cases per 100 animal-years. Six of the 7 herds had incidence rates < 1.5 cases/100 animal-years. These estimates are substantially lower than the observed or predicted rates found in this study for cervid herds.

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QUANTITATIVE ANALYSES OF *NEOSPORA CANINUM* SEROLOGICAL DATA OBTAINED FROM DAIRY CATTLE

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Optimal use of diagnostic tests requires an understanding of the distributions of test variables in the target population and choice of appropriate cut-off values. Prevalence values that have been estimated using tests of unknown diagnostic sensitivity and specificity are commonly reported, leading to uncertainty in the accuracy of the results. If populations of infected and non-infected animals can be identified for a particular disease, test parameters can be estimated, but this is problematic because reference tests ('gold standards') are often not available. Here a new approach, which uses the serological status of new-born calves to indicate the infection status of their dams, is used to estimate the diagnostic sensitivity and diagnostic specificity of a test. The test applied is a commercial antibody-detection enzyme-linked immunosorbent assay (ELISA; Williams et al., 1997) for *Neospora caninum* which is a protozoan parasite and is the most commonly reported infectious cause of bovine abortion in the United Kingdom (Anon, 1997). This approach is appropriate because of the high vertical transmission probability of *N. caninum* and because congenitally-infected calves typically have high antibody responses, whereas some adult cattle have fluctuating responses giving false-negative results.

For many infectious agents, seropositivity provides evidence that cattle have been previously exposed, but is not a good indication of current infection. However, cattle which are seropositive for *N. caninum* are likely to be chronically infected and will infect the majority of their offspring (Anderson et al., 1997), and vertical transmission has been found to be the major transmission route in many herds (Paré et al., 1996; Davison et al., 1999a). On-going studies of endemic herds in the United Kingdom have found that 95% of seropositive cows (n = 123) produced congenitally-infected calves when tested with the ELISA used here (preliminary observations).

Serological data for the analyses were obtained from studies of *N. caninum* in British cattle. Optimal ELISA cut-off values were computed from positive and negative reference cattle groups using the 'two-graph receiver operating

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characteristic' (TG-ROC) programme (Greiner et al., 1995), and the values were then compared with 'intrinsic' cut-off values derived by computer-assisted analysis of mixtures (C.A.MAN) from populations of cattle of unknown infection status (Greiner et al., 1994). TG-ROC was also used to calculate other test parameters, including likelihood ratios and misclassification costs associated with false-positive and false-negative results (Greiner, 1996).

MATERIALS AND METHODS

Cattle groups

Dam-calf paired groups: Data were obtained from a study of *N. caninum*-endemic British dairy herds. Paired serum samples were collected from 313 cows and their new-born calves, at calving, in 7 herds; a herd test had been conducted in 6 herds and herd seroprevalence ranged from 7.4% to 18.5% (Davison et al., 1999a). All calves were sampled prior to consumption of colostrum which was confirmed by measurement of serum gamma glutamyltransferase activity (Perino et al., 1993). For the TG-ROC analysis, cows that had seronegative calves were considered to be negative (n = 199) and cows that had seropositive calves were considered to be positive (n = 114).

Normally-calving cattle and aborting cattle groups: Data were obtained from a seroprevalence case-control study of common bovine abortifacients in which serum samples from 418 dairy cows that had calved normally within 1 week (the control group) and from 633 cattle that had aborted were tested (Davison et al., 1999b). The control cows were sampled by randomly-chosen veterinary practitioners who were requested to sample 1 cow on the next 6 farms that they visited, regardless of the clinical history of the herd. Samples from cattle that had aborted were randomly selected from serum banks at the 14 Veterinary Investigation Centres (VICs) in England and Wales. The number of sera obtained were proportional to the annual number of abortion submissions to each VIC in the previous year; the majority of sera had been collected under the national brucellosis control scheme.

Neospora caninum ELISA

All sera were stored at - 20^{0}C prior to testing for specific antibodies against *N. caninum* with a commercial *N. caninum* ELISA (MAST Diagnostics, Mast House, Derby Road, Bootle, Merseyside) (Williams et al., 1997). The results were expressed as percent positivity (PP) values, and the cut-off values recommended by the supplier are: 20 - 25 PP (inconclusive) and > 25 PP (positive).

Data analyses

Descriptive statistics of the data sets were computed with Minitab version 10 (Minitab Inc, 3081 Enterprise Drive, State College, PA 16801-3008, USA). TG-ROC plots were constructed (Greiner et al., 1995) with the ELISA PP values of the positive

cows and negative cows, as categorised by the status of their calves. The cut-off value (d_0) that produced equal values of diagnostic sensitivity and diagnostic specificity (Se = Sp = θ_0) was calculated. An intermediate range (IR) was established for a 95% accuracy level; values below and above the intermediate range are considered negative and positive, respectively. The valid range proportion (VRP) indicates the proportion of the measurement range (MR) that excludes the intermediate range (VRP = [MR - IR]/MR). In addition, TG-ROC was used to calculate logarithmic values of positive and negative likelihood ratios (LR^+, LR^-) for different cut-off values, where an ideal test would have values of $\log(LR^+) = +\infty$ and $\log(LR^-) = -\infty$. True prevalence values (P) were calculated from apparent prevalence values (i.e. seroprevalence at the optimal cut-off value) found in the control cattle and aborting cattle groups, using the diagnostic sensitivity and specificity point estimates at the cut-off value given by TG-ROC (Thrusfield, 1997). These prevalence values were then used to examine the effect of cut-off values on the misclassification costs term (MCT) for different false-negative to false-positive cost ratios (r), as described by Greiner (1996).

'Intrinsic' cut-off values were obtained by analysis of the latent mixture of PP distributions in the control cattle and aborting cattle groups using the programme C.A.MAN (Greiner et al., 1994) and compared with the TG-ROC-derived cut-off values. A maximum of 500 data can be analysed by C.A.MAN, and, therefore, for the aborting cattle group (n = 633) 500 values were randomly selected, without replacement, for analysis. Three sets of 500 data were analysed and the results were compared. C.A.MAN identified sub-populations by their weights (i.e. proportions of values in each sub-population) and mean ELISA PP values. 'Intrinsic' cut-off values were computed by combining sub-populations and repeating the analysis with 2 defined sub-populations (i.e. for a bimodal solution), if appropriate. Prevalence values were then calculated for the control and aborting cattle groups using the cut-off values selected by the methods described above, and 95% confidence intervals (CIs) were computed using Confidence Interval Analysis (CIA; © British Medical Journal, London) with the exact binomial method (Gardner and Altman, 1989).

RESULTS

TG-ROC analysis

Of the cows that were sampled with their calves at calving, 11 of 204 seronegative cows produced seropositive calves and 6 of 109 seropositive cows produced seronegative calves. For TG-ROC analysis, cows were categorised as either positive (n = 114) or negative (n = 199) according to the status of their calves. The median and standard deviation (s) of ELISA PP values were 54.5 (s = 22.2) and 6.0 (s = 7.8) in these positive and negative groups, respectively. TG-ROC analysis with the non-parametric option was used because the data did not have a normal distribution, and the results are given in Table 1. A cut-off value (d_0) of 20 PP gave equal point estimates of diagnostic sensitivity and specificity (Se = Sp = 0.949), as indicated by the intersection point of the 2 graphs shown in Fig. 1; the intermediate range (IR) was 1 and the valid range proportion (VRP) was 0.99, for a 95% accuracy level.

Table 1. Results of non-parametric TG-ROC analysis of a *Neospora caninum* ELISA using data obtained from cows sampled at calving.

Measure [a,b]	Parameter value	95% Confidence interval
θ_0	0.949	
d_0	20	(12, 24)
$IR_{0.95}$	1	(0, 32)
upper limit	20	(12, 44)
lower limit	19	(12, 24)
$VRP_{0.95}$	0.99	(0.70, 1)

[a]ELISA values are given as percent positivity (PP) values.
[b]Equality in the point estimates of diagnostic sensitivity and specificity (Se = Sp = θ_0) was achieved with the cut-off value d_0. The intermediate range (IR), with upper and lower limits, and the valid range proportion (VRP) are given for a 95% accuracy level.

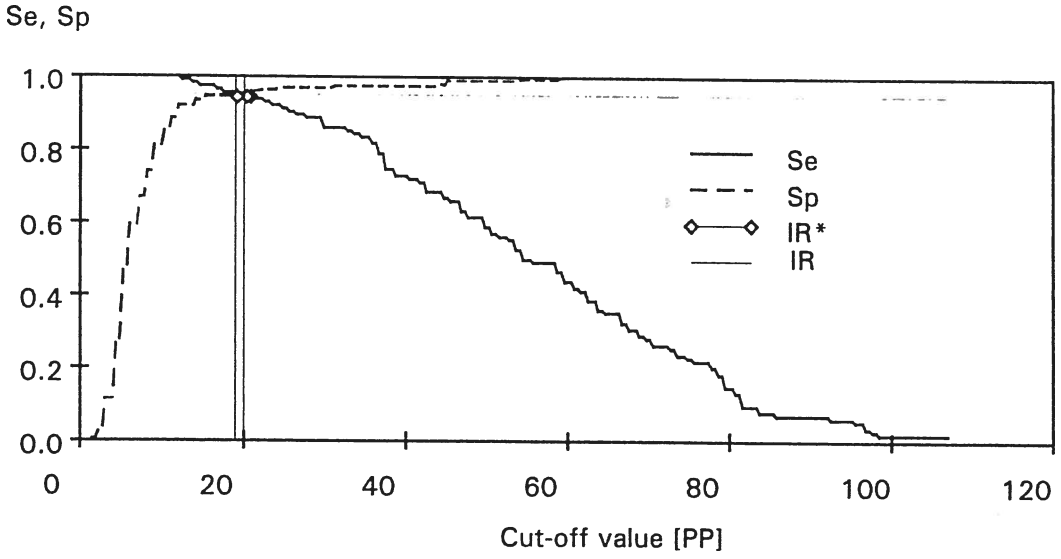


Fig. 1 TG-ROC analysis of a *Neospora caninum* ELISA. The intersection point of the 2 graphs indicates the cut-off value d_0 at which equivalent values of diagnostic sensitivity and specificity (Se = Sp = θ_0) were achieved. IR* and IR are the intermediate ranges for parametric and non-parametric analyses, respectively.

The relationship between the cut-off value and positive (LR^+) and negative (LR^-) likelihood ratios is demonstrated in Fig. 2. Log(LR-) approaches negative infinity ($-\infty$) with cut-off values of 12 PP or less, and log (LR+) approaches positive infinity ($+\infty$) with cut-off values of 60 PP and above.

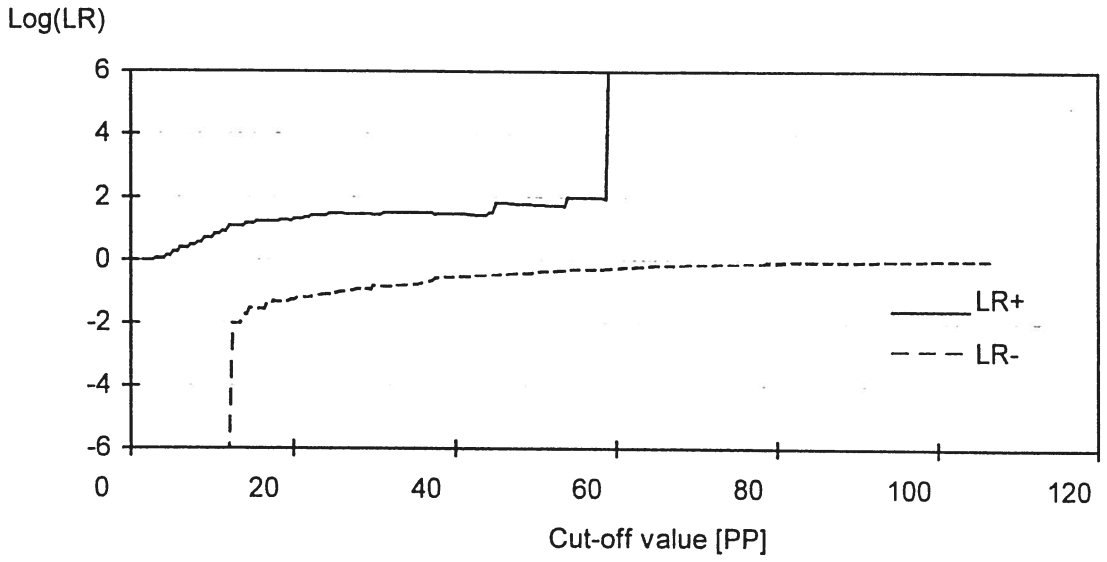


Fig. 2 Positive (LR+) and negative (LR-) likelihood ratios, shown as logarithmic values, as a function of the selected cut-off value.

Corrected prevalence values for the control cattle (1.9%) and aborting cattle (15.3%) groups were calculated from uncorrected prevalence values (shown in Table 2) obtained at the 20 PP cut-off value and using test parameter point estimates of 95%; corrected values were used for calculation of the misclassification costs term (MCT). Results of analyses conducted using r values of 0.5, 1, 2 and 10, where r is the ratio of the cost of false-negative results to the cost of false-positive results, were compared. An example of the MCT analysis is given in Fig. 3 which shows MCT values as a function of the cut-off value when the prevalence is 15.3% and the cost of a false-negative result is twice the cost of a false-positive result (r = 2). In this situation, the minimum MCT value is observed with cut-off values close to 20 PP. However, if the cost of a false-negative result is ten-fold the cost of a false-positive result (r = 10), then a cut-off value of 10 PP gives the lowest MCT value, and this value increases rapidly as higher cut-off values are selected. With a prevalence of 1.9%, the magnitude of the effect of the cut-off value on the MCT was smaller than that observed with a prevalence of 15.3% (data not shown).

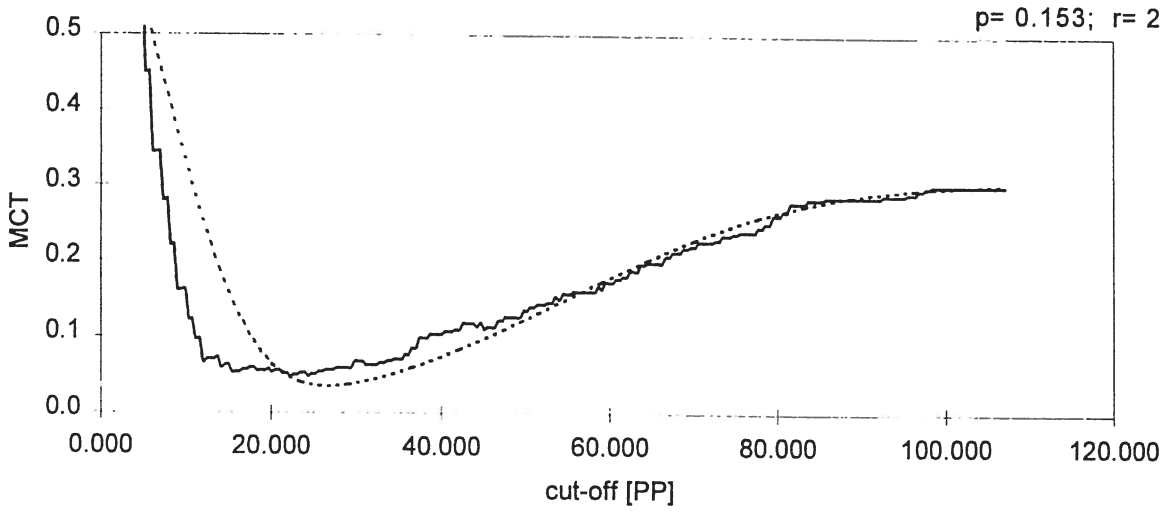
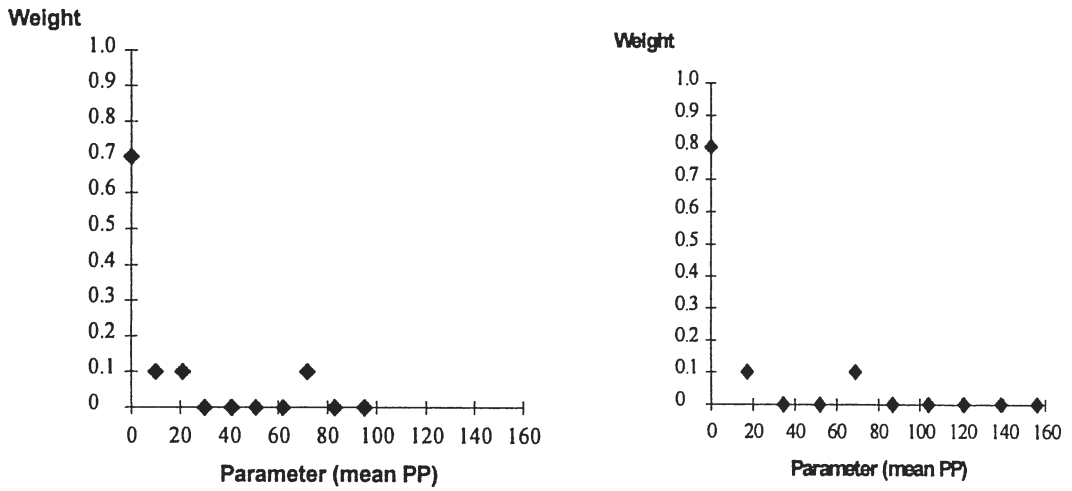


Fig. 3 Misclassification costs term (MCT) as a function of the selected cut-off value computed for a prevalence of 15.3% with the cost of a false-negative result equal to twice the cost of a false-positive result ($r = 2$). The solid line represents the non-parametric analysis (appropriate for this data) and the dotted line represents the parametric analysis.

C.A.MAN analysis

The mixture analysis initially identified a latent mixture of 10 sub-populations both within the control group (Fig. 4a) and within the aborting cattle group (Fig. 4b - comparable results were obtained with the other 2 sets of 500 data). In the control group, 4 subpopulations with positive weights were identified and, after pooling subpopulations, an intrinsic cut-off value of 31 PP was derived from 2 subpopulations with weight = 0.95 and mean PP = 2.9 (subpopulation 1) and weight = 0.05 and mean PP = 56.7 (subpopulation 2). Higher ELISA PP values were found in the aborting cattle group than in the control group. In the 3 data sets from the aborting cattle group sub-populations were pooled to give 2 sub-populations with mean PP values ranging between 5.8 PP and 6.0 PP (sub-population 1) and between 77.8 PP and 79.8 PP (sub-population 2); corresponding 'intrinsic' cut-off values ranged from 44.0 PP to 45.2 PP.



(a) Normally-calving cows (n = 418) (b) Aborting cows (n = 500)

Fig. 4 Results of mixture analysis (C.A.MAN) of *Neospora caninum* ELISA percent positivity (PP) values obtained from a) normally-calving cows (n = 418) and b) aborting cows (n = 500). Each subpopulation identified by C.A.MAN is represented by a diamond.

Estimation of prevalence using different cut-off values

Prevalence estimates were calculated for the control group and aborting cattle group using the cut-off values selected by TG-ROC and C.A.MAN, as described above, and the value recommended by the ELISA kit's supplier. Uncorrected prevalence estimates are given in Table 2.

Table 2. Estimates of *Neospora caninum* prevalence (uncorrected) in normally-calving cattle and aborting cattle obtained using an ELISA with cut-off values derived by different methods.

Cattle group	Prevalence (%) obtained with different ELISA cut-off values, 95% confidence intervals in brackets			
	ELISA kit	TG-ROC	'Intrinsic' value from normally-calving cattle[b]	'Intrinsic' value from aborting cattle[b].
	> 25 PP[a]	≥ 20 PP[a]	> 31 PP[a]	> 45 PP[a]
Normally-calving cattle	5.7 (4, 8)	6.7 (5, 10)	5.0 (3, 8)	3.6 (2, 6)
Aborting cattle	17.5 (15, 21)	18.8 (16, 22)	16.6 (14, 20)	14.1 (11, 17)

[a]Percent positivity.
[b]Computed using C.A.MAN.

DISCUSSION

TG-ROC and C.A.MAN programmes were used to analyse serological data obtained from epidemiological studies of *N. caninum* in British cattle. Cut-off values were computed, firstly from positive and negative reference populations using TG-ROC, and secondly from populations of normally-calving cattle and aborting cattle using C.A.MAN. The reference populations for the TG-ROC analysis were cattle that were sampled at calving in *N. caninum* endemic herds and were categorised as positive or negative according to the serological status of their new-born calves. The rationale for this new approach is that the vertical transmission probability of *N. caninum* is very high and infection is more readily detected in congenitally-infected calves than in adult cattle by serology. Although not 100% of infected cattle will be identified by this method, it is considered to give the lowest misclassification rate for normally-calving cows of currently available methods; no reference ('gold standard') tests are available with a high diagnostic sensitivity and specificity for *N. caninum*.

An ideal test would have test parameters equal to or greater than 95%, an intermediate range (IR) of 0 and a valid range proportion (VRP) of 1 (Greiner et al., 1995). TG-ROC analysis found that this *N. caninum* ELISA approaches these characteristics; a single cut-off value of 20 PP gave diagnostic sensitivity and specificity point estimates of nearly 95% (IR = 1, VRP = 0.99). C.A.MAN analysis showed that the distribution of ELISA PP values differed between the control group and the aborting cattle group with respect to individual PP values and also to the location of the sub-populations on the ELISA PP scale. 'Intrinsic' cut-off values given by C.A.MAN were higher than the TG-ROC value, particularly in the aborting cattle group because of higher 'background' values within this group. However, the cut-off values obtained by the different methods gave comparable estimates of prevalence suggesting that only a small proportion of PP values were within the range of the cut-off values (20 - 45 PP).

The *N. caninum* ELISA is commonly used for both normally-calving and aborting cattle, and these were the sample populations used in the analyses. However, the test parameters may vary if the test was used for other target populations or diagnostic purposes. An advantage of TG-ROC is the possibility to observe the effect of different cut-off values and prevalence values on test parameters. Distinct distributions of *N. caninum* test values in cows and calves were also observed with an immunofluorescent antibody test (Paré et al., 1995). If the ELISA was used to test new-born calves, likelihood ratios would approach ideal values because congenitally-infected new-born calves typically have high antibody responses (e.g. > 50 PP) and non-infected calves have low background values (e.g. < 10 PP) (unpublished observations); the log likelihood ratios approximated to infinity in these PP ranges. For some applications of the ELISA, a higher or lower cut-off value may be chosen to minimise the costs associated with false-positive or false-negative results, respectively, and the misclassification costs term (MCT) provides a quantitative value of these costs for different prevalence values. The interpretation of *N. caninum* serology differs importantly from other bovine disease serology. The analyses described here showed that the ELISA, with a cut-off value of 20 PP, has a diagnostic

sensitivity and specificity of nearly 95% for testing adult cattle, and provide important additional validation of a commercial ELISA which is routinely used by diagnostic laboratories in the UK and elsewhere.

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ECONOMIC ANALYSIS OF ANIMAL WELFARE ASPECTS IN THE BROILER PRODUCTION CHAIN

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In today's society people show a growing interest in the quality of agricultural products and the production process, also towards the broiler production chain. This may include aspects such as animal welfare, food safety and environmental pollution (Den Ouden et al., 1997a). Like the pork production chain (See Den Ouden et al., 1996), the Dutch broiler industry has an integrated character where several members of the production chain (stages) are closely related to each other (Information and Knowledge Center, 1994). Demands concerning quality in animal production may, therefore, ask for an integrated approach, which may result in adaptations in production methods in the early stages of the chain (Den Ouden et al., 1997a).

To make a well-founded decision on what production strategy should be followed, trade-offs have to be made between the (welfare) preferences on the one side and (economic) profitability on the other (Den Ouden et al., 1997a). This paper describes the first results of a project funded by the Dutch Ministry of Agriculture, Nature management and Fisheries and focuses on evaluating several welfare measures, which are (about to be) implemented in the broiler production chain, both on welfare perception and economics. To get the complete picture the project in total consists of 3 stages, a) measurement of welfare perception, b) assessment of economic effects and c) optimization of chain concepts, to improve welfare at minimum costs. This paper gives a broad overview of the first stage of the project and a short outlook to the following stages.

MATERIALS AND METHODS

Welfare aspects in the broiler production chain

Notwithstanding the different standpoints from which the welfare issue can be viewed, in practical terms the appropriate way to treat animals remains strictly a matter of human

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preference. Consequently we need to study the determinants and the mechanisms of welfare choices in society if we are to better identify the standards appropriate to that society. It is not easy to put those choices into a framework of scientific assessment, or to base them on scientific principles (McInerney, 1991). Several methods are known to assess animal welfare. Indicators described in literature include productivity (growth rate, reproductive performance), physiological (heart rate, blood composition), veterinary (injuries) and ethological variables (behaviour observations) (Fraser & Broom, 1990; Smidt, 1983 and Den Ouden et al., 1997a). The fact that these indicators are a) assessed across different stages of the chain, b) often measured under varying experimental conditions and c) are not easy to weight against each other speaks to their disadvantage (Smidt, 1983). Despite the above mentioned methods to measure welfare, we can never actually know what the animals would choose for themselves. Therefore, discussions of welfare unavoidably involve a subjective human judgement on what we believe are appropriate conditions for animals to enjoy or suffer. In this sense it is more correct to talk in terms of "perceived welfare" to avoid any suggestion that we are dealing with something that has been or can be objectively assessed (McInerney, 1991). Within this study it was, therefore, decided to develop a questionnaire, based on (scientific) literature and expert opinion, to evaluate people's welfare perception regarding welfare measures in the broiler production chain. Within this questionnaire the method of conjoint analysis was applied (Metegrano, 1994).

As mentioned earlier, the Dutch broiler production chain is hierarchically organized in several stages (Fig. 1), each with their own specific production characteristics. Discussion with a small group of experts resulted in the following stages to be included in the study: a) the rearing phase of the broiler breeder hens, b) the laying phase of the broiler breeder hens, c) the broiler farm and d) the transportation of broilers to the slaughterhouse.

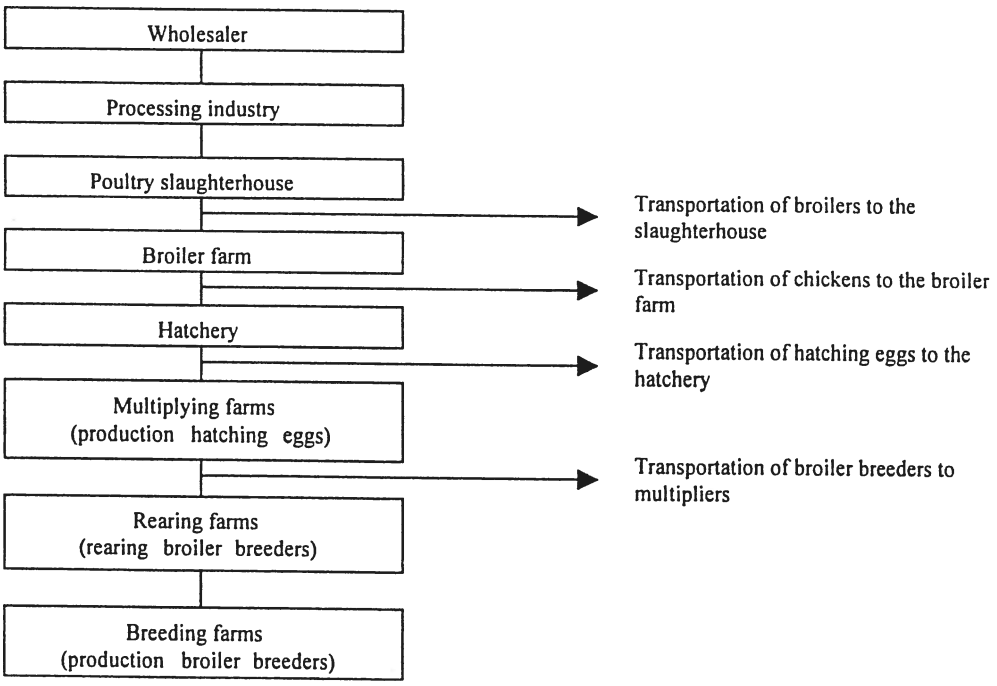

Fig. 1. Dutch broiler production chain (Information and Knowledge Center, 1994)

The welfare concept was covered by several (underlying) welfare attributes, which were presumed to be responsible for observed welfare problems. To account for the variation in the attribute values they were all subdivided into 2 or more (underlying) levels. The attributes taken into account covered among other things the following aspects:

Breed type: Within the current broiler production chain breed type is an important cause of health and welfare problems. Due to the intensive selection of broiler breeder hens on growth (breed type), ad libitum feeding may result in leg problems, reduced (re)production and metabolic disorders (Blokhuis, 1995 and Saxena, 1985).

Feeding practice: Restricted feeding may lead to abnormal behavior (Savory & Maros, 1993 and Savory et al., 1992). Cocks may show more aggressive behavior (Mench et al., 1991), while restricted hens are more active, showing stereotyped behaviour towards feeders and the watering system (Van Niekerk et al., 1988 and Savory et al., 1992). At the broiler farm itself the fast growth causes problems like e.g. insufficient development of the carcass in relation to the bodyweight, heat stress and inactivity (Frankenhuis et al., 1989 and Appleby et al., 1994).

Surgery: To improve reproduction results and prevent lesions during mating the exterior parts of the inside and backside toes of cocks are clipped (Van Rooijen, 1992 and Van Rooijen & Jacobs, 1992), while within the current husbandry systems both hens and cocks are also beak trimmed.

Litter: Another important welfare item might be the availability of litter. Litter is used by broiler breeders for scratching behaviour and dust-bathing. In absence of litter the animals may focus their attention more intensely on the feeders or other substrates, leading to stereotyped behaviour and feather pecking (Blokhuis, 1995).

Litter quality: Besides the presence of litter especially in broiler farms the quality of the litter is also an important welfare issue. Several (skin) disorders in broilers are associated with litter quality. Wet and dirty litter combined with raised ammonia concentrations leads to an increased incidence of breast blisters (Greene et al., 1985 and McIlroy et al., 1987), while Ekstrand et al (1997) reported a significant effect of litter quality on the prevalence of food-pad dermatitis. Reduction of skin lesions is important to both the animal (welfare) as to the farmer (carcass quality) (Bokkers, 1997).

Stocking density: One of the risk factors for wet litter is the stocking density. Besides effects on the litter quality and related health problems, a high stocking density also has a negative influence on the activity of broilers, resulting in several leg problems (Ekstrand, 1997 and Grashorn & Kutritz, 1991). For the broiler breeders the stocking density strongly depends on the applied housing system in the laying phase (Blokhuis, 1995). In general, litter quality may be improved by adequate ventilation at the animal level or activation of the animals (Bokkers, 1997). The latter can be reached by supplying grain in the litter or using alternating lighting regimes (Bos, 1984).

Perches: Often perches are used to rest and wash on (Blokhuis, 1984). Broilers with access to perches may develop stronger bones and are more active (Elson, 1993 and Ekstrand, 1993), while the presence of perches in the rearing phase of broiler breeders may serve as a

training, resulting in a reduction in floor laying during the laying phase (Appleby et al., 1988).

Catching/Crating: An unacceptable percentage (10-30%) of broilers are damaged during catching and placing into transportation units. This leads to economic losses and is also undesirable for the animal welfare (Gerrits et al., 1985). In many countries broilers are still largely caught by hand and then carried by one leg to the transport unit (Gerrits et al., 1985 and Bayliss & Hinton, 1990). To improve this problem, various systems for catching broilers mechanically have been developed (Gerrits et al., 1985). Also the system of loose crates is largely superseded by systems using various modules. Within these systems birds are no longer multi handled or carried for long distances for crating, while catching speeds are comparable with crate systems (Bayliss & Hinton, 1990).

Transportation: Of the stresses the bird is subjected to during transportation, climate (temperature, relative humidity, wind speed) is considered to be one of the most important (Freeman, 1984).

Workshops

To account for a representative sample of the production chain, a broad scale of organizations and industries were approached by mail to take part in the study. Finally 36 respondents out of 57 (63%) gave a response to the invitation and participated in the study. They could roughly be subdivided into 5 groups; a) the research and knowledge centres (8), b) the supplying industry (9), c) the processing industry and sales organizations (10), d) the Dutch Animal Health Service and veterinary practitioners (5) and e) representatives of social (pressure) groups (4). While the consumer ultimately decides what food products are desired in the market (Steenkamp, 1987), their opinion should not be underestimated. In this study the respondents in the last group (e) were supposed to represent public concerns quite well.

Participation in the study implied that respondents took part in a 2,5 hour workshop, organized at the Wageningen Agricultural University. The respondents were asked to focus on the welfare aspects of the shown attributes, leaving the economic aspects as far as possible out of consideration while they are taken care of in the second stage of the project. After that an introduction was given to the method applied, the "adaptive conjoint analysis" (ACA). While this is a fully computer based interviewing technique (Metegrano, 1994) and each respondent was provided with a personal computer, interaction between respondents could be prohibited, so respondents could work independently from each other at their own speed. The respondents were asked to complete four ACA interviews, related to the 4 distinguished stages of the production chain respectively. For those not familiar with the computer, a written hand-out was available containing background information on the questionnaire and a short explanation on how to handle the computer program. The handout also contained a questionnaire which gave the respondents the opportunity to give their opinion on e.g. the interviewing technique, duration of the interview and realism of the material used.

Adaptive conjoint analysis

A product or event can be evaluated as a composition of attributes or characteristics (Fishbein, 1963). The process by which consumers compare and finally select different products or services is complex. While only in a few cases 1 product is preferred in all attributes above all other products, the consumer has to decide which attributes of a certain product are most important to him (Steenkamp, 1985). Conjoint analysis is a technique that can help within this election process while it enables quantification of the importance of attributes of a product or event in relation to the overall assessment of a consumer with respect to that particular product or event (Horst et al., 1996). Basic assumptions of conjoint analysis are: 1) a product can be described according to levels of a set of attributes and 2) the consumer's overall judgement with respect to that product is based on these attribute levels (Steenkamp, 1987). In this study, conjoint analysis is used to elicit the opinion (or perception) of people concerning the impact of certain attributes on animal welfare in the broiler production chain. Conjoint analysis is a so-called "decompositional method". Respondents are asked to rank or rate combinations of attributes or attribute levels ("profiles"). Afterwards regression analysis is used to decompose the scores (utilities) given by the respondent to these profiles i.e. break down the total score into components belonging to the separate attributes (Horst et al., 1996). The method of conjoint analysis is developed from a need to analyze the effect of predictor variables (attributes) that are often qualitatively specified or differently scaled (Hair et al., 1990), as was the case with the welfare attributes. Conjoint analysis is preferred above a compositional method like direct questioning mainly because of the greater realism. It appears difficult for respondents to provide a non-biased score for an attribute, holding everything else equal, which may result in (heavy) biases from direct questioning on the importance of socially sensitive factors (Green & Srinivasan, 1990).

In this study the method of adaptive conjoint analysis (ACA) is applied. ACA is a computer (PC) based system, based on the principles of conjoint analysis. The term adaptive refers to the fact that the computer-administered interview is customized for each respondent. At each step, previous answers are used to decide which question to ask next, so the respondent is asked in detail only about those attributes and levels of greatest relevance to him/her. Therefore, ACA appears capable of dealing with a large number of attributes (Johnson, 1993 and Metegrano, 1994). The respondent's utilities are available upon completion of the interview (Johnson, 1993 and Johnson, 1987). Other advantages of the ACA method is the possibility to check for consistency in respondent answers, while Johnson (1987) also indicates that the respondent's interaction with the computer increases respondent's interest and involvement with the task. A detailed description of the background and estimation procedure of ACA is given in Johnson (1987 and 1993). An ACA questionnaire consists of several sections, each with a specific purpose (Johnson, 1993):

1. Elimination of unacceptable levels is an optional section that can be used to delete attribute levels, if the interview would otherwise be too long. Once an attribute level has been deleted, it receives no further consideration during the interview. This section was not included in this study.

2. Next, the respondents are asked to rank the remaining levels for preference. In this study this implied that levels were ordered according to their (declining) contribution to (perceptional) animal welfare improvement.
3. Having found a relevant set of levels for each attribute, the relative importance of each attribute to a respondent is determined. The questioning is based on differences between those levels the respondent indicates to like best and least. After this section the interview is focused on the most important attributes.
4. A series of paired "product" concepts is composed (each consisting of 2 or 3 attributes differing in one or more attribute levels), to refine the respondent's utilities.
5. Finally the computer composes a series of "calibration concepts" using those attributes determined to be the most important and those levels most salient to the respondent. In this section the welfare attributes estimated to contribute most to the animal welfare improvement were used to describe various farm situations. Information from this section is used to calibrate the utilities obtained in the earlier part of the interview.

The utilities produced by ACA are scaled at the individual level, using each respondent's welfare perception information. To use ACA data with other processing software the data must first be (re)scaled and normalized so that utilities can be compared across respondents. The utilities were scaled in such a way that the sum of the utilities across all levels for a respondent was equal to the number of attributes x 100 (for details on the method see also Metegrano, 1994). After that they were converted into percentages per respondent. To check for the consistency of respondents, ACA also computes the correlation (scale 0-1) between the respondents' expected responses to the calibration concepts, as inferred from the estimated utilities, and the actual responses. This gives the researcher the opportunity to exclude respondents who gave inconsistent answers during the interview (Metegrano, 1994).

Data analysis

After (re)scaling the utilities from ACA software, several analyses were performed to investigate whether the welfare perception between the distinguished groups differed or not. By plotting the number of respondents against the value of the correlation coefficient, a correlation cutoff-value (ccv) of 0.5 was chosen for the analyses. In the analyses, observations were assumed to be independent and no interaction was presumed between the levels of different welfare attributes. As the derived utilities were not normally distributed a non parametric Kruskal-Wallis test was used at $P \leq 0.05$ (Conover, 1980 and Norusis, 1992). For those attribute levels revealing differences in welfare perception between respondent groups, the Mann Whitney U-test was used in pairs to determine which groups differ in perception. Since this was multiple testing of the same data the significance level was adjusted using the Bonferroni test (Norusis, 1992). Applying the Bonferroni test implies that the significance level is divided by the number of comparisons made, so in this study the significance level became 0.05/10 is $P \leq 0.005$.

RESULTS

Consistency

The average correlation values for the interviews "rearing phase of broiler breeders", "laying phase of broiler breeders", "broiler farm" and "transport of broilers to the slaughterhouse" are 0.78, 0.76, 0.74 and 0.89 respectively (Table 1). Using a correlation cut-off value of 0.5 respondents' background did not have a significant effect on the value of the correlation coefficient.

Table 1. Distribution of respondents over the correlation classes for the various ACA interviews (Number of respondents = 36)

	Correlation classes:						
ACA version:	0-0.5	0.5-0.6	0.6-0.7	0.7-0.8	0.8-0.9	>0.9	Mean
Rearing phase of broiler breeders	4	2	1	5	13	11	0.777
Laying phase of broiler breeders	5	1	3	3	13	11	0.764
Broiler breeder farm	7	2	2	3	10	12	0.742
Transport to slaughterhouse	3	1	1	1	2	28	0.887

Ranking

Based on the utilities given by the respondents, attribute levels can be ranked according to their contribution to perceived animal welfare improvement. The results will be worked out in more detail for the broiler farm below, while the results for the other stages of the broiler production chain are summarized in Table 4. As is the case for the broiler breeders, distribution of litter is also indicated as the factor to improve welfare most at the broiler farm (Table 2). Also the use of other less intensive breeds, application of a 16L8D lighting regime and a low stocking density (2 levels in the top 5) are perceived to improve animal welfare. In contrast to the broiler breeders it is preferred not to supply perches. When a ccv of 0.9 is chosen a lower stocking density and the use of less intensive breeds becomes even more important to the respondents, while the utility for improving litter quality by means of a raised floor is reduced to 0.

Table 2. Ranking of the 10 most important welfare attribute levels for the broiler farm and their utilities at different correlation cut-off values (N=29)

Level (attribute)	Correlation cut-off value		
Broiler farm	0.5	0	0.9
Litter is supplied	16.81	16.58	16.58
Breeds less intensively selected for growth are applied	12.48	12.08	14.51
A day/night rhythm is applied / 16L8D	9.92	10.85	8.01
<18 broilers are housed per m^2 or ≤ 35kg/m^2	8.72	9.16	14.04
18-21 broilers are housed per m2 or 36-42 kg/m^2	8.32	8.11	10.50
Litter aerating is applied / "raised floor"	7.84	7.97	0.00
Alternating lighting is applied	7.20	5.87	4.55
No broilers are unloaded during growing period	4.89	4.91	3.65
Grain is supplied in the litter	3.71	2.33	2.93
No perches are supplied	0.90	2.58	2.06

Table 3 shows the ranking of the welfare attributes, based on the underlying attribute levels at a ccv of 0.5. The attribute with the highest median utility for one of its levels (litter) ranks first. Possibly in consequence of the number of levels included in the study and the applied method for ranking the attributes, stocking density is only ranked 5th, while 2 levels of this attribute are considered important (Table 2). Table 4 shows the ranking of the 3 most important welfare attributes, in accordance with the results for the broiler farm in Table 3, for the rearing and laying phase of the broiler breeders and the transportation phase of the broilers respectively. Supply of litter, surgery (beak trimming, toe clipping) and use of breeds less intensively selected for growth were perceived to improve welfare most during the rearing phase of the broiler breeders. As in the rearing phase, litter is considered an important factor in the laying phase too. Besides litter also feed restriction and an available floor space of 1600 cm^2 are perceived to improve animal welfare during the laying period.

Table 3. The welfare attributes for the broiler farm, ranked by the level with the highest median utility value per attribute (Consistency ≥ 0.5, N=29)

Attribute	Level	Percentiles		
		25th	50th	75th
Litter	Litter is supplied	13.66	16.81	22.01
Breed type	Breeds less intensively selected for growth are applied	3.94	12.48	16.02
Lighting regime	A day/night rhythm is applied / 16L8D	5.88	9.92	13.21
Stocking density	<18 broilers are housed per m^2 or ≤ 35kg/m^2	2.86	8.72	14.87
Litter quality	Litter aerating is applied / "raised floor"	0.00	7.84	10.82
Unloading	No broilers are unloaded during growing period	0.00	4.89	9.59
Grain supply	Grain is supplied in the litter	0.00	3.71	8.26
Perches	No perches are supplied	0.00	0.90	5.82

In the perception of the respondents the welfare during transport of broilers to the slaughterhouse can be improved by applying loading modules instead of crates and the use of a waiting room with (mechanical) climate control at the slaughterhouse. Also mechanical catching systems receive a high utility (Table 4).

Table 4. The 3 most important welfare attributes per stage of the production chain, ranked by the level with the highest median utility value per attribute (Consistency ≥ 0.5)

Attribute	Level	Percentiles		
		25th	50th	75th
Rearing phase of broiler breeders (N=32)				
Litter	Litter is supplied	10.23	13.64	16.34
Surgery	(Fe)males are beak trimmed; males' toes and spurs are clipped	2.14	12.81	17.24
Breed type	Breeds, less intensively selected for growth, are applied	4.06	10.79	14.52
Laying phase of broiler breeders (N=31)				
Litter	Litter is supplied	9.90	12.56	17.44
Feeding practice	Birds are fed restricted	0.66	11.84	17.87
Living space	1600 cm^2 living space per animal is available	3.79	10.59	13.95
Transport of broilers to the slaughterhouse (N=33)				
Crating	Loading modules are applied	20.22	25.15	30.86
Climate control at the slaughterhouse	Air conditioned waiting-room is available at the slaughterhouse	12.04	19.13	25.82
Catching	Mechanical catching systems are applied	2.57	15.69	27.96

Background differences

Table 5 shows the median utilities for the attribute levels resulting from a Kruskal-Wallis test at $P \leq 0.05$ for which the respondents, ordered by background, differed in perception. Only a few of these differences could be reduced by means of the Mann-Whitney U-test to a concrete difference between 2 groups, possibly due to the small number of observations. For the broiler farm it was found that members of the "health" group considered supply of perches less desirable than the researchers. In general, also referring to the other stages of the chain, no application of surgery is given a high utility by members of social (pressure) groups, in contrast to researchers and members of the processing industry. Otherwise researchers considered restricted feeding during rearing of broiler breeders much more important than members of social groups did.

Table 5. Median utilities for several welfare measures at the broiler farm, stratified on background of the respondents, resulting from a Kruskal-Wallis test at $P \leq 0.05$ (N=29)

Welfare levels	Research/ knowledge centres	Supplying industry	Processing industry	AHS and veterinarians	Social groups
Broiler farm:	N=8	N=6	N=7	N=4	N=4
Breeds less intensively selected for growth are applied	10.95	2.85	14.16	6.47	16.66
Perches are supplied	5.46	0.00	0.00	-	3.39
No perches are supplied	0.00[a]	4.11	0.90	5.79[a]	0.36

[a] Significant difference (Mann-Whitney U-test, $P \leq 0.005$, 2-tailed asymp. sign.) between researchers and AHS (Animal Health Service) / veterinarians (2* 1-tailed exact sign. = 0.008)

DISCUSSION/CONCLUSIONS

Response

The response rate was with 63% a bit lower than might have been expected following earlier experiences with similar workshops (Horst et al., 1998). The response rate might have been reduced by the fact that most of the approached organizations only delegated 1 representative, while often more were invited. Other reasons (might) have been the required time to take part in the workshop and unfamiliarity with the applied method or the use of computers. Also some people considered themselves too unfamiliar with the subject to take part in the study.

Welfare aspects

Within this study the welfare concept was covered by an arbitrary number of welfare attributes. In order to gain insight into how preferences are determined, it is essential that considerable effort is expended on generating attributes. Selection of the attributes for a preference study can be seen as one of the most important steps within the whole process (Cattin & Wittink, 1982). Too few attributes may reduce realism of the exercise, while too

many attributes may cause an information overload on the respondents, resulting in less reliable utility estimates. While the factors (and underlying levels) in this study were selected within a limited group of experts, this might have led to the exclusion of attributes from the workshop which were considered important by other respondents. To overcome this imperfection within the evaluation respondents could indicate which attributes or levels they had missed. In general the attribute "climate conditions within the housing system" is mentioned as being missing, while also attributes like stocking density (rearing phase broiler breeders), feeding and drinking systems (broiler farm) and management before catching/loading (transport phase) are considered important within the welfare discussion.

Adaptive Conjoint Analysis (ACA)

Conjoint analysis is especially suitable for handling variables that are qualitatively specified or evaluated on different scales (Den Ouden, 1997a) and it seems therefore a suitable method to evaluate the poultry welfare issue. ACA is a "main effects only" model and assumes that there are no interactions among attributes. One should, therefore, remain alert for the possibility of interactions. Usually it is possible to choose attributes so that interactions will not present severe problems and by defining composite variables ACA can also deal with interactions in a limited way (Metegrano, 1994). However, it remains difficult to judge afterwards if interactions really should have been included or not.

Consistency

Although the various interviews are pre-tested both at the Dutch Ministry of Agriculture, Nature Management and Fisheries and Wageningen Agricultural University, part of the differences in consistency among respondents might result from misunderstanding the questions. Other reasons for inconsistency might have been motivation, unfamiliarity with the applied method or computers, or lack of knowledge on the subject. Using a correlation cut-off value of 0.5 (measure of consistency) at most 7 respondents were excluded from the analysis (broiler farm interview). The remaining correlation coefficients were considerably high, namely median values of 0.88, 0.87, 0.87 and 0.98 for the rearing phase, the laying phase, the broiler farm and the transport phase respectively. This indicates that respondents were quite capable of a consistent evaluation of the poultry welfare attributes. Differences in consistency among the various interviews might have resulted partly from differences in the number of attributes between the interviews.

Results

As mentioned by Blokhuis (1995) and Savory et al. (1992) hunger, resulting in necessary restricted feeding, can be seen as one of the main bottlenecks regarding welfare of broiler breeders. Although not a problem in today's floor housing systems, availability of litter and space are important factors to prevent welfare problems among broiler breeders. As can be seen in Table 4 these findings are roughly confirmed by this study. In his final recommendations, resulting from a study on welfare issues on commercial broiler farms, Bokkers (1997) mentions the current breed type as one of the main causes for the current welfare problems on commercial broiler farms. Other aspects mentioned in his

study like stocking density and litter supply also appear in the top 5 of important aspects within the current study (Table 3). Using ACA it is also possible to divide respondents in groups, based on their (professional) background. The differences found in this study between the researchers and processing industry versus the social groups with respect to restricted feeding and surgery can possibly be explained as a cause/result issue. One can reject surgery because of the impact on the animal itself but also judge this attribute based on the results of not applying surgery, like cannibalism and injuries during mating (Blokhuis, 1995). Possibly due to the limited number of respondents (observations) only few differences between groups could be found significant. Also due to the number of respondents and the applied tests the power of the study might be limited. The results, however, can be seen as trends of the perception of the importance of welfare attributes in the broiler production chain in general.

FUTURE OUTLOOK

As can be concluded from a risk factor study by Van Schaik et al., (1998) ACA is an easy-to-use tool to obtain the perception of respondents on a certain object. In this study the importance of several welfare attributes were estimated. Besides ranking these welfare attributes on perception the utility estimates will also be used as weight factors (see also Den Ouden, 1997b). In the 2nd phase of the project the economic effects (concerning e.g. investments, labour and energy) of the several implemented (housing) measures will be assessed, at first using literature and practical experience. When necessary the opinion of experts in this field can also be obtained. This phase will be worked out in close co-operation with the LEI-DLO (Agricultural Economics Research Institute). Within this analysis the method of partial budgeting will be applied (Dijkhuizen & Morris, 1997). In the 3rd and last phase the welfare perceptions and economic affects of several measures will be brought together into an optimization model (see also Den Ouden, 1997b). By means of a linear programming model calculations can be performed to assess what measures should be taken to improve welfare at minimum costs. This can be done separately for each stage of the chain and for the chain as a whole. In this way a flexible tool becomes available to support policy making in a quantitative way.

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RISK FACTORS FOR COUGHING IN THOROUGHBRED RACEHORSES

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Coughing, an important problem in the racing industry (Bailey, 1998; Bailey et al., 1997; Jeffcott et al., 1982; Rossdale et al., 1985), is a relatively specific indicator of lower airway disease (Burrell et al., 1996). Potential causes of coughing include inflammatory airway disease (Moore et al., 1995), pneumonia and pleuritis, viral and bacterial respiratory infections, exercise induced pulmonary haemorrhage, chronic obstructive pulmonary disease and pharyngeal lymphoid hyperplasia (Rose and Hodgson, 1993).

Risk factors for pleuropneumonia have been investigated (Austin et al., 1995). However, risk factors for coughing in racehorses are unknown. Racehorses are a unique population, and differ from the general horse population in several regards that may be relevant to the epidemiology of coughing. For example, racehorses tend to be young, involved in strenuous activity, are regularly transported and mixed with other horses, and are generally housed in stables (Moore, 1996).

The multiplicity of potential causes of coughing makes management difficult. Understanding the factors that alter the risk of disease may help determine the likely aetiology. Also, by understanding the risk factors for coughing, control interventions may be targeted to specific high-risk areas. Therefore, the aim of this study was to evaluate a number of potential risk factors for coughing in Thoroughbred racehorses in Sydney, Australia. The variables investigated included management and horse levels factors. Some of these may have been surrogate measures of other variables, or risk factors for other variables on the causal pathway.

MATERIALS AND METHODS

Horses were identified from participating stables at 5 racetracks in and around Sydney by daily contact with the trainers and their consulting veterinarians. The study was conducted between February 1997 and February 1998. A matched case control study design was used. Attempts were made to collect data from two controls for each case.

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Case selection

Cases were defined as Thoroughbred racehorses in training that coughed at least 4 times in a 10-minute period during exercise. Horses may also have coughed at rest, but this was not necessary for case selection. Horses could be identified as cases more than once during the study period. However, a period of at least 2 weeks without the horse fulfilling the selection criteria had to elapse between episodes of coughing in order for the second episode to be classified as a new case. The case definition was deliberately kept simple because case identification relied on the observations of the trainer and the staff of the stable.

Control selection

Controls were randomly selected from among non-cases in the stable from which the case was identified. A horse was defined as a non-case if it had not had a recognized episode fulfilling the selection criteria within the past two weeks. Controls were randomly selected using published random number tables (Armitage and Berry, 1994). If a trainer refused to make a horse available as a control, this horse was excluded from being a case in the future. The controls were sampled at the same time as the case. An attempt was made to collect data and samples from two controls per case. However, this was not always achieved, usually due to time constraints within the stable. Data and samples were always collected from at least one control for each case.

Data collection

Information regarding the signalment and recent history of each case and control was collected using a questionnaire. The questionnaire was completed by the investigator during discussion with the trainer or trainers representative. Details recorded included the name, age and sex of the horse, the number of days since last transportation (1 to 7 days, 8 to 14 days, more than 14 days) and racing (never raced, 1 to7 days, more than 7 days), and the stage of training. Stage of training was categorized as early (mostly trotting and cantering), mid (increasing fast exercise, up to 80% of top speed, usually over short distances), late (training included increasing amounts of exercise top speed gallop) or racing (has raced). Early, mid and late training phases usually lasted 4 to 5 weeks each. Differences in training method between trainers meant that stricter category parameters could defined. Rectal temperature and the presence or absence of inappetence were recorded for cases and controls.

Univariable analyses

An initial univariable matched analysis of each variable was performed to identify factors associated with coughing. As cases and controls were selected concurrently, each case was matched with its control horses on the basis of time and training stable. Therefore, analyses had to take account of this matching. Crude odds ratios were calculated for all variables using conditional univariable logistic regression, with coughing as the dependent variable. Dummy variables were generated for any categorical variable with more than two levels. Conditional logistic regression was performed using Egret (Cytel Statistical Software, Cambridge, Massachusetts, USA) and LogXact version 2.1 (Cytel Statistical Software, Cambridge, Massachusetts, USA).

Multivariable analyses

Logistic regression models were initially constructed using variables considered, *a priori*, to be potential confounders, and those which were significant at $p<0.25$ in the univariable screening (Hosmer and Lemeshow, 1989) and which were considered to be biologically meaningful. The models were refined through backward elimination with variables remaining in the model if removal of that variable resulted in a significant change ($p<0.05$) in the likelihood ratio statistic or if exclusion of the variable altered the estimate of effect of other variables by 5% or more (Hosmer and Lemeshow, 1989; Kleinbaum et al., 1988). Biologically meaningful two-way interaction terms between the independent variables were examined after identification of the reduced set main effects. Each interaction term was introduced into the model and the significance assessed as for the independent variables. The fit of the multivariable model was assessed by examining the delta-betas values (Pregibon, 1981). Horses with the greatest delta beta values for each variable were sequentially excluded from the models. Models were judged as stable and robust when these exclusions did not cause significant effects.

Analysis of correlation coefficients

Correlation coefficients between stage of training and time since last transportation were examined, following stratification by time since last race, in order to examine the relationships between these variables. Correlation coefficients were calculated using Statistica (StatSoft Inc, Tulsa, Oklahoma, USA).

RESULTS

During the period February from 1997 to February 1998, 100 cases of coughing were identified in the participating Thoroughbred racehorse training stables. A total of 148 controls were selected randomly from the same stables as cases. Data were collected from the cases and controls on the day of case identification. Twenty-nine trainers participated in the study and these trainers were located in 23 stable facilities, with some trainers in shared premises.

The overall prevalence of vaccination against EHV-1 and -4 was low. Overall, 12% of horses were vaccinated. The prevalence varied with age, with young racehorses having the highest rate of vaccination (Table1).

Univariable analysis

The Box-Tidwell method (Hosmer and Lemeshow, 1989) provided evidence that age was not linear in the logit. Therefore, age was categorized into four levels that reflected the age groupings of racing Thoroughbreds. Results of the univariable screening are summarized in Table 2. The risk of coughing decreased with increasing age. There was also a significant effect of sex, with male coughing horses being more than four times more likely to be colts than geldings.

Recently transported horses were at lower risk of coughing compared to those transported more than 14 days previously. However, the risk of coughing decreased as training proceeded. Also, horses that had raced were less likely to cough than those that had never raced were. Vaccination

using a combined EHV-1 and -4 vaccine was positively associated with coughing in univariable analysis (Table 2).

Multivariable analysis

For horses that raced recently, the measure of time since last transportation was often the same as the time since last race. However, these variables were different for many horses (Table 3) and were not correlated ($r=0.07$, $p=0.2$). This may have occurred because horses raced at their home tracks, or because horses were transported for reasons other than racing. When developing multivariable conditional logistic regression models, the effect of including time since transportation and recent racing was considered individually and together. Addition of both terms improved the fit of the final model. Also, inclusion of these terms did not greatly alter the magnitude of effect of other variables or each other (<10% change), further suggesting the terms were not highly correlated. Therefore, both variables were considered for inclusion in the final model.

Variables identified by multivariable logistic regression as being significantly associated with coughing included age, stage of training, time since last race and time since last transportation (Table 4). The odds ratio of racing within the last 7 days were 11.1 (1.6-76.5) compared to never having raced, and the odds of having last been transported more than 14 days ago were 5.5 (1.7-17.8). Addition of interaction terms did not improve the model. Following adjustment for the effects of other variables, the use of a combined EHV-1 and -4 vaccine was not associated coughing and was excluded from the model.

Examination of delta-beta values indicated the models presented were stable and robust, as determined by the lack of significant effect of sequential exclusion from the model of data sets with the largest leverage for each variable.

Analysis of correlation coefficients

Amongst horses which had never raced ($n=98$) time since last transportation was correlated with stage of training ($r=0.4$, $p<0.001$). This correlation took the form of increasing time since transportation as stage of training increased. This group of horses included 57% of cases. When considering horses that had never raced, the majority that were in mid or late stage training had been transported more than 14 days previously (Table 5). In contrast, the distribution of time since last transportation was more even amongst those horses in early stage training.

Table 1. Age related prevalence of vaccination against EHV-1 and -4 amongst coughing and healthy horses in training

Age (years)	Vaccinated	Not vaccinated	Number in each age group	Prevalence of vaccination (%)
1-2	18	93	111	16.2
3	6	49	55	10.9
4	2	48	50	4.0
>4	4	28	32	12.5
Total	30	218	248	12.1

Table 2. Univariable analysis of categorical variables investigated for association with coughing in Thoroughbred racehorses in training

	Cases	Controls	unadjusted Odds Ratio	unadjusted 95% CI		p value
Age (years)						**<0.001**
1 or 2	62	49	**1**[a]			
3	21	34	**0.3**	0.1	0.7	
4	10	40	**0.1**	0.04	0.3	
>4	7	25	**0.1**	0.05	0.4	
Sex						
Gelding	36	77	**1**[a]			**0.003**
Colt	22	13	**4.5**	1.8	11.3	
Female	42	58	**1.9**	1.0	3.7	
Recent transport (days)						**0.003**
1-7	19	50	**1**[a]			
8-14	18	33	**1.9**	0.8	4.7	
>14	63	65	**3.6**	1.6	8.0	
Stage of training						**<0.001**
Early	49	30	**1**[a]			
Mid	18	20	**0.8**	0.3	1.9	
Late	11	16	**0.3**	0.1	0.9	
Trialed or racing	22	82	**0.1**	0.05	0.3	
Previous racing						**<0.001**
Never	57	41	**1**[a]			
1-7days	13	32	**0.3**	0.1	0.7	
>7days	30	75	**0.2**	0.1	0.5	
EHV 1 & 4 vaccination						**0.02**
No	82	136	**1**[a]			
Yes	18	12	**5.5**	1.1	26.1	

[a] reference category

Table 3. Association between the time since last race and time since last transportation

	Time since last race			
Transportation (days)	Never	1-7 d	>7d	Total
1-7	25	31	13	69
8-14	18	0	33	51
>14	55	14	59	128
Total	98	45	105	148

Table 4. Factors identified by conditional logistic regression model as being associated with the risk of coughing in Thoroughbred racehorses

	unadjusted OR	**OR**[a]	95% CI[a]	
Age (years)				
1-2	1[b]	**1**[b]		
3	0.3	**0.5**	0.2	1.7
4	0.1	**0.2**	0.04	0.7
>4	0.1	**0.2**	0.04	0.9
Stage of training				
Early	1[b]	**1**[b]		
Mid	0.7	**0.6**	0.2	1.6
Late	0.3	**0.1**	0.04	0.6
Trialed or racing	0.1	**0.05**	0.009	0.2
Previous racing				
Never	1[b]	**1**[b]		
1-7days	0.3	**11.1**	1.6	76.5
>7days	0.2	**1.2**	0.4	3.9
Recent transport (days)				
1-7	1[b]	**1**[b]		
8-14	1.9	**3.5**	0.9	13.6
>14	3.6	**5.5**	1.7	17.8

[a] Adjusted for age, stage of training and previous racing, [b] Reference category.

Table 5. Association between time since transportation and stage of training in horses that have never raced

	Time since transportation (days)		
Stage of training	1-7	8-14	>14
Early	22	14	23
Mid	3	3	21
Late	0	1	11

DISCUSSION

This is the first study to identify risk factors for coughing in Thoroughbred racehorses in training. In the multivariable model age, time since last race, stage of training and time since last transportation were significantly associated with coughing.

By selecting controls from the same training stable and at the same time as the cases were identified, the cases and controls were matched for a range of variables including the sensitivity of observation by trainers, climatic conditions and some management practices. Some of these variables would have been difficult to measure and therefore difficult to allow for during analysis. An advantage of the matched design was that the presence of cases provided incentive for the trainers to permit access to healthy horses for use as controls. As the trainers recruited for this investigation had not previously been involved in this type of study, the likely level of cooperation was impossible to determine prior to commencement of the study. The use of a matched design ensured that cases and controls arose from the same population of horses. This may not have been the case using an unmatched design if some trainers had ceased to cooperate. In fact, trainer cooperation was excellent and no trainers left the study.

Unfortunately, matched studies have disadvantages such as the inability to assess the effect of the matched variables (Schlesselman, 1982). Also, as a case control study was used, it was not possible to calculate incidence of coughing, or to determine sensitivity of coughing as a diagnostic test. Despite these limitations, the association between coughing and horse and management level variables may improve our understanding of risk factors for lower respiratory tract disease in racehorses. However, given the observational nature of the study, the possible role of chance, bias and confounding by unmeasured variables should be considered when the results are evaluated.

Several factors were identified by conditional logistic regression to alter the risk of coughing, after allowing for the other variables. The horses at greatest risk of coughing were young horses and those in early stage training. Horses were also at increased risk in the week after a race and when they had not been transported in the past two weeks

The decrease in the odds of coughing with increasing age in the current study is in agreement with previous studies that younger horses are more at risk of respiratory tract inflammation (Burrell et al., 1996), and bacterial (Wood et al., 1993) and viral (Matsumura et al., 1992; Powell et al., 1974; Sugiura et al., 1987) infections. The decreased risk with increasing age suggests horses develop immunity or tolerance to the aetiological agents of coughing, or that some component causes are more prevalent in younger horses.

The significant decrease in risk of coughing as training progresses, after controlling for age, suggests that early training is a period of higher risk even in horses that have previously been exposed to the training environment. This may reflect the relative short duration of immunity following exposure to etiological agents, loss of tolerance to irritants following lack of exposure, or may suggest that unidentified features, peculiar to early training, contribute to the risk of coughing. Alternatively, exercise training may be associated with enhanced immunity, as has been suggested to occur in humans (Nieman, 1993) and has been demonstrated in mice (Good and Fernandes, 1981; Liu and Wang, 1987). In humans, psychological stress is also associated with an increased risk of acute respiratory illness, both in terms of rates of serological evidence of infection and rates of clinical signs of infection, following challenge with one of a number of

viruses (Cohen et al., 1991). There is some evidence that stress levels in racehorses decrease as training progresses (Nogueira and Barnabe, 1997). Therefore, stress associated immunosuppression may be greatest at the commencement of training and decrease as the horses become accustomed to training (Nogueira and Barnabe, 1997). However, the decline in cortisol concentrations with training may, at least in part, be due to increased utilization of cortisol with increased work demands rather than alterations in rate of cortisol production (Wilson et al., 1991).

Increasing time since transportation was associated with an increased risk of coughing in the current study. This finding may initially appear anomalous as prolonged transportation has been associated with an increased risk of bacterial pneumonia (Austin et al., 1995; Raidal et al., 1997; Raphel and Beech, 1982). Transport is thought to increase the risk of respiratory disease via a number of mechanisms. Prolonged head elevation causes inflammatory changes and bacterial colonisation of the healthy equine airway (Raidal et al., 1995). Bacterial and inflammatory cell products may impede mucociliary function (Denny, 1974; Johnson and Inzana, 1986; Sykes et al., 1987; Wilson and Cole, 1988; Wilson et al., 1986) and interfere with phagocyte function (Caruso and Ross, 1990; Dom et al., 1992). Transport stress, mediated by cortisol and other hormones, further reduces phagocytic activity by the pulmonary macrophages (Huston et al., 1987). Finally, dehydration, due to decreased water consumption prior to, and during, transport may reduce mucociliary clearance (Dixon, 1992).

However, there are a number of possible explanations for the effects of transportation identified in the current study. The physiological effects of transportation are altered by its duration. Road transport for 24 hours, with rest stops every 4 hours, does not alter pulmonary aerosol clearance rates (Smith et al., 1996), and transport for 90km does not affect haematological variables, serum electrolyte concentrations or subsequent performance during submaximal exercise (Beaunoyer and Chapman, 1987). Although the duration of transportation was not considered in the current study, it was likely to have often been of short duration. This may explain the lack of increased risk of coughing immediately following transportation, but does not explain the increased risk with increasing time since transportation.

The increased risk of coughing with increasing time since transportation may be due to correlation between time since transportation and stage of training amongst horses that had never raced. In this group of horses, the most likely reason for transportation was entry into the training stables to commence training. Therefore, the majority of horses in mid or late stage training had not been transported within the previous two weeks. However, time since transportation was more evenly distributed in the group of horses in early stage training. In this group of horses, time since transportation would be related to duration of exposure to the stable environment. Hence, the greater risk in horses transported more than 14 days previously may represent a combination of latent period and increased odds of exposure to agents within the stable. As 57% of cases were in the never raced group, this effect was likely to have influenced the role of transportation evident in the final model.

The stable environment of racehorses has been implicated as a risk factor for respiratory disease. Stable air hygiene may affect the incidence and severity of respiratory disease by increasing the magnitude of challenges from micro-organisms or allergens, changing the rate at which pathogens once deposited are cleared from the respiratory tract, and altering the horse's local or systemic resistance (Clarke, 1987). Acute episodes of chronic obstructive pulmonary disease (COPD) can be induced in susceptible horses by introducing them into a poor quality stable environment, with

poor quality straw and hay (Derksen et al., 1985; McGorum et al., 1993). Even in horses without COPD there is evidence that exposure to stable dusts induces airway inflammation (Tremblay et al., 1993). Also, poor stable air hygiene has been implicated in delayed recovery from EHV infection (Clarke et al., 1988). However, there is a lack of conclusive scientific evidence that poor air quality is a risk factor for respiratory disease in young racehorses. As the risk of coughing decreased as training progressed in the current study, it seems unlikely that simple cumulative exposure to the stable environment was a major risk factor.

The role of endotoxin in dust as a cause of respiratory disease has recently been recognized in mice (Jagielo et al., 1996). Endotoxin has been measured in similar concentrations to that which causes disease in mice in vegetable dusts, including corn, wheat, oats and barley (Olenchock et al., 1986). Also, some horse stables contain concentrations of endotoxin which are capable of inducing pulmonary inflammation and hyper-responsiveness in humans (McGorum et al., 1998). While inhalation of as little as 1 mg of endotoxin can induce a pulmonary neutrophilic response in horses (Pirie et al., 1998), the concentration of endotoxin in stables in Sydney, and the effect of this on the respiratory tract of young racehorses is unknown. However, it is interesting to note that tolerance to endotoxin (Coffee et al., 1992) develops in mice following repeated exposure (Schwartz et al., 1994). Therefore, should inhaled endotoxin be found to be an important cause of coughing in racehorses, tolerance may, in part, explain the lower risk of coughing observed as training progressed in the current study.

In the current study, recent racing was associated with an increased risk of coughing. Racing may increase the risk of coughing by exposing the respiratory tract to pathogens, or by altering immune function. Congregation of horses at race meetings may facilitate spread of communicable agents. Racing, particularly on dry, dusty surfaces, may aerosolize potential pathogens (Robinson and Slocombe, 1986). Also, straightening of the head and neck during fast exercise reduces airway impedance [Petsche, 1995 #423], potentially reducing the rate of deposition of particulate matter in the upper airways due to inertial impaction (Salvaggio, 1994). Combined with large increases in respiratory rate and tidal volume during strenuous exercise (Art and Lekeux, 1989), these factors increase the risk of particulate matter reaching the lower airways. Therefore, it is not surprising that racetrack debris has been observed in the tracheae of horses post-race (Arthur, 1983; Sweeney et al., 1991).

Exercise induced pulmonary hemorrhage (EIPH) is common is racehorses (McKane et al., 1993) and the presence of blood in the airway may induce coughing (Pascoe, 1991). Several authors have suggested a link between EIPH and lower respiratory tract infection in racehorses (Hoffman, 1995; Raphel and Beech, 1982; Robinson, 1997). Also, instillation of autologous blood into the airway induces inflammatory changes (Tyler et al., 1991). Hence, there is the suggestion that EIPH can directly result in coughing, predispose to infection, and induce inflammatory airway disease.

Racing and strenuous training may also affect the susceptibility of racehorses to respiratory disease. A previous study (Austin et al., 1995) showed that Thoroughbreds that have raced within 48 hours were 6.1 (95% CI: 1.3 to 38.7) times more likely to develop pleuropneumonia than Thoroughbreds that have not raced. The factors associated with racing that predispose to pleuropneumonia may also increase the risk of coughing. In horses, strenuous exercise causes reduced macrophage function (Wong et al., 1990) and elevations in serum cortisol for up to 24 hours (Church et al., 1986; Huston et al., 1987; McCarthy et al., 1991; Thornton, 1985) and bronchial lavage fluid cortisol for at least 30 minutes (Huston et al., 1987).

The effect of exercise on respiratory immunity has also been reported in humans. Extremely strenuous physical exercise, in contrast to moderate exercise, has been associated with immunosuppression (Budgett, 1990; Sharp and Koutendakis, 1992; Weidemann et al., 1992) and increased rates of respiratory disease in humans (Heath et al., 1991; Peters and Bateman, 1983). This may be due to impairment of host resistance following extreme stress and fatigue, (Smith and Weidemann, 1990; Weidemann et al., 1992), and to the physical effects of cold and dry air on local mucosal defenses (Peters and Bateman, 1983). Increased rates of respiratory disease have been reported in competitors in a marathon compared to sedentary people, with faster runners and those that trained over greater distances prior to the race, at highest risk (Peters and Bateman, 1983). In the current study, the effect of recent racing was only apparent after adjusting for the effects of age, stage of training and recent transportation.

After controlling for other variables, sex was not a risk factor for coughing in the current study. In contrast, human male runners have an increased risk of symptoms of respiratory tract disease, compared to females (Heath et al., 1991). However, in humans, the effect of gender is modified by alcohol consumption (Heath et al., 1991). Hence, the apparent difference between male and female humans may be due largely to lifestyle differences, factors that are less likely to vary between fillies, colts and geldings.

Once other variables were controlled for, there was no effect of vaccination using an EHV-1 and -4 vaccine. The lack of apparent effect of vaccination against EHV-1 and -4 is consistent with studies in the United Kingdom that found no association between inflammatory airway disease and infection (Burrell et al., 1996). However, it should be noted that EHV-1 and -4 vaccines were only released in Australia in April 1997, two months after the commencement of the study. Therefore many horses identified as having been vaccinated may not have received the complete vaccination schedule.

In conclusion, conditional multivariable logistic regression identified age, stage of training, time since last race and time since last transportation as being significant risk factors for coughing in Thoroughbred racehorses in training at racetracks in and around Sydney. The results identify young horses in early training as being most at risk of coughing. Racing was identified as an activity that increases the risk of coughing. The effect of transportation was difficult to interpret. The results for the effect of transportation may indicate that time since transportation was a surrogate measure for duration of time in the stable, particularly for horses in early training or it may reflect a delayed effect of transportation. Further investigation of the causes of respiratory disease affecting racehorses in early training and following racing may help in the development of control measures to reduce the risk of disease in these horses.

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THE EPIDEMIOLOGY OF HEMORRHAGIC KIDNEY SYNDROME - INFECTIOUS SALMON ANEMIA IN ATLANTIC SALMON IN ATLANTIC CANADA

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In late 1996, salmon producers in the Bay of Fundy first reported unexplained high levels of mortality among 1996 year class Atlantic salmon in seawater sites. Some of the fish demonstrated histological lesions in the kidney which were considered unique to this problem, resulting in the descriptive term of Hemorrhagic Kidney Syndrome (HKS). Although initial testing for known fish viruses was negative, later samples resulted in positive virus isolation for the orthomyxovirus responsible for Infectious Salmon Anemia (ISA) experienced by Norway and also a new virus tentatively classified in the *Togaviridae* family. The number of sites involved in the outbreak increased to more than twenty by late 1997 and cost the industry more than $10 million (Cdn).

In the summer of 1997, producers in the region initiated an epidemiologic study of the disease with two main objectives. The first was to describe mortality patterns in affected cages from which they hoped to be able to better predict the impacts of the condition being identified in a cage. The second was to identify risk factors which contribute to increased mortality among farms (site level risk factors) and among cages within an affected site (cage level risk factors).

MATERIALS AND METHODS

Mortality data collection

Starting in August 1997, all sites in the affected area were visited and their mortality records for the 1996 year class were examined. Mortality data was collected (retrospectively) for the period starting with the time fish were transferred to cages at each site until the time of data collection. A prospective data collection system was then established to monitor mortalities through until November 1997.

An estimate of the initial number of fish in each cage was obtained from production records on the farm. All movements into and out of each cage were recorded and the "population at risk" was adjusted accordingly in the data. If the number of mortalities were recorded by the producer as a total per week, or in some cases as total per month, then the daily mortality rate was computed by dividing the total number of mortalities by the number of days in the interval. Since the farm records lacked sufficient detail to be more precise, it was necessary to assume that the mortality rate was constant over the entire period. All records were kept confidential and all site and cage identifiers used in the report are randomly generated numbers.

Outbreak definitions

At the time this investigation was launched, no etiologic agents had been identified as responsible for the outbreaks. While characteristic pathologic lesions had been defined, they were very

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inconsistently observed and some cages which experienced very high mortality did not have the characteristic lesions in any of the fish examined. Consequently, the "case" definition was based solely on increased levels of mortality in a cage. A cage was considered a case when it experienced an outbreak of unexplained mortalities as defined by one of the following criteria:

- seven or more days with daily mortality rates greater than one per 1000 fish (i.e. 0.1% per day), or
- a cumulative mortality of greater than 5% of the initial number of fish in that cage.

The duration of the outbreak was computed as the number of days between the first "high mortality day" (daily mortality > 1 per 1000 fish) and the last "high mortality day". If there were more than 30 days which were not considered "high mortality days" recorded during the outbreak, the duration of that outbreak was not computed.

Sites were classified as problem sites if greater than 50% of cages at the site with 1996 year class fish were classified as cases.

Cages were excluded from all analyses if they met any of the following criteria:

- mortality data for less than 3 months,
- a total population of less than 1,000, or
- no fish present on April 1st, 1997

Mortality data obtained from the first 30 days after the fish were transferred to salt water were also not included in the calculations.

Risk factor data collection

All sites were visited in late 1997 and a detailed personal interview questionnaire was administered to obtain information about potential risk factors. Data on cage size and construction, source and method of handling smolts, cage level management practices, disease occurrences and treatments used over 1996/97, and site level management variables were collected. A copy of the questionnaire is available on request from the investigators.

Statistical analysis

Mortality patterns were described by developing a data set with 1 record per cage per day for the period from spring 1996 (usual time that fish were put in salt water) to November 1997. Spike plots and descriptive statistics were generated to describe mortality patterns. The case definition was evaluated by providing each of three veterinarians who worked with the industry a complete set of spike plots showing the mortality patterns and asking them to classify each cage as to whether or not they felt it had experienced an outbreak. A kappa statistic was used to compare our case definition with the results from the veterinarian who worked most closely with the outbreak from its start. We also compared his classification with those from the other two veterinarians.

Cage level risk factors were analysed using a dataset with 1 record per cage with the outcome variable being a case as defined above. Descriptive statistics, unconditional associations (between risk factors and the dependent variable), correspondence analyses, and logistic regression analyses were used to evaluate cage level risk factors. Both fixed and random effects logistic models which tried to account for clustering of cages within site were attempted.

A site level dataset was created with 1 record per site with the outcome variable being problem site as defined above. Descriptive statistics and unconditional associations between risk factors and the dependent variable were evaluated.

A combined dataset (1 record per cage) containing both cage and site level risk factors was created for use in survival analyses. In addition to risk factors identified as potentially significant, a time dependent covariate which represented whether or not any other "case" cages had been identified at

the site was created. Cox proportional hazards models were fit to identify important risk factors.

Unconditional associations were evaluated at P=0.1 while the logistic regression and proportional hazards models used a significance threshold of P=0.05. All statistical analyses were carried out using STATA, Version 5 (Stata Corp., College Stn. Texas) except for the random effects logistic regression which was attempted using MlwiN (Multilevel Models Project, Inst. of Education, Univ. of London).

RESULTS

Outbreak definitions

Mortality data were available from 218 cages at 16 sites. These cages contained an estimated 2.2 million fish. By November 1997, 106 cages had experienced an outbreak (ie. classified as a case) and 112 were unaffected. Of the 106 case cages, 97 met the criteria of ≥ 7 high mortality days while 9 were classified as a case based on cumulative mortality only. There was considerable variation among the outbreak patterns observed in the cages. Figure 1 presents four selected outbreak patterns which represent:

- a "typical" outbreak pattern with relatively low overall mortality;
- a severe outbreak with early harvesting of the cage (note change in y-axis scale);
- a cage that had two distinct outbreaks, one in 1996 and one in 1997, and
- a cage with several periods of elevated mortality but less than 7 "high mortality days"

The kappa statistic for agreement between our definition and the subjective assessment of the veterinarian who had worked most closely with outbreak sites was 0.81. Of 207 cages classified by both methods, only 7 were identified as cases by our definition but not by the veterinarian. On the other hand, he identified 13 cages as cases that were not considered such by our definition. The kappa values for agreement between that veterinarian and the other two veterinarians were 0.67 and 0.73.

The distribution of dates of onset of cases is shown in Figure 2. Most new cases occurred between June and August 1997. The distribution of proportion of cages at a site classified as cases is shown in Figure 3. Of the 14 sites for which complete data were available, 7 were classified as "problem sites" (ie. > 50% of cages defined as cases).

Mortality rates and duration of outbreaks

All mortality rates will be expressed in terms of deaths per 100,000 fish.

Over the entire study period (spring 96 to Nov. 97) non-affected cages had median daily mortality rates of 2.4 (per 100,000 fish) but this rose to 4.6 after June 1st 1997.

During outbreaks, the median daily mortality rose to 73. The average peak mortality (ie. highest daily mortality observed in each outbreak) was 1507 while the median peak mortality was 492. 25% of cases had peak mortalities over 1400.

The duration of the outbreak could only be computed for 46 cases since many cages were either harvested or split during the outbreak in order to prevent further losses. For these 46 cases, the average duration of the outbreak was 37 days (median = 33) and 25% of outbreaks had a duration over 56 days. The mean proportion of the initial population of fish that died during these outbreaks was 12.2% with the median, 75th and 90th percentiles being 6.6%, 16.7% and 29.3% respectively.

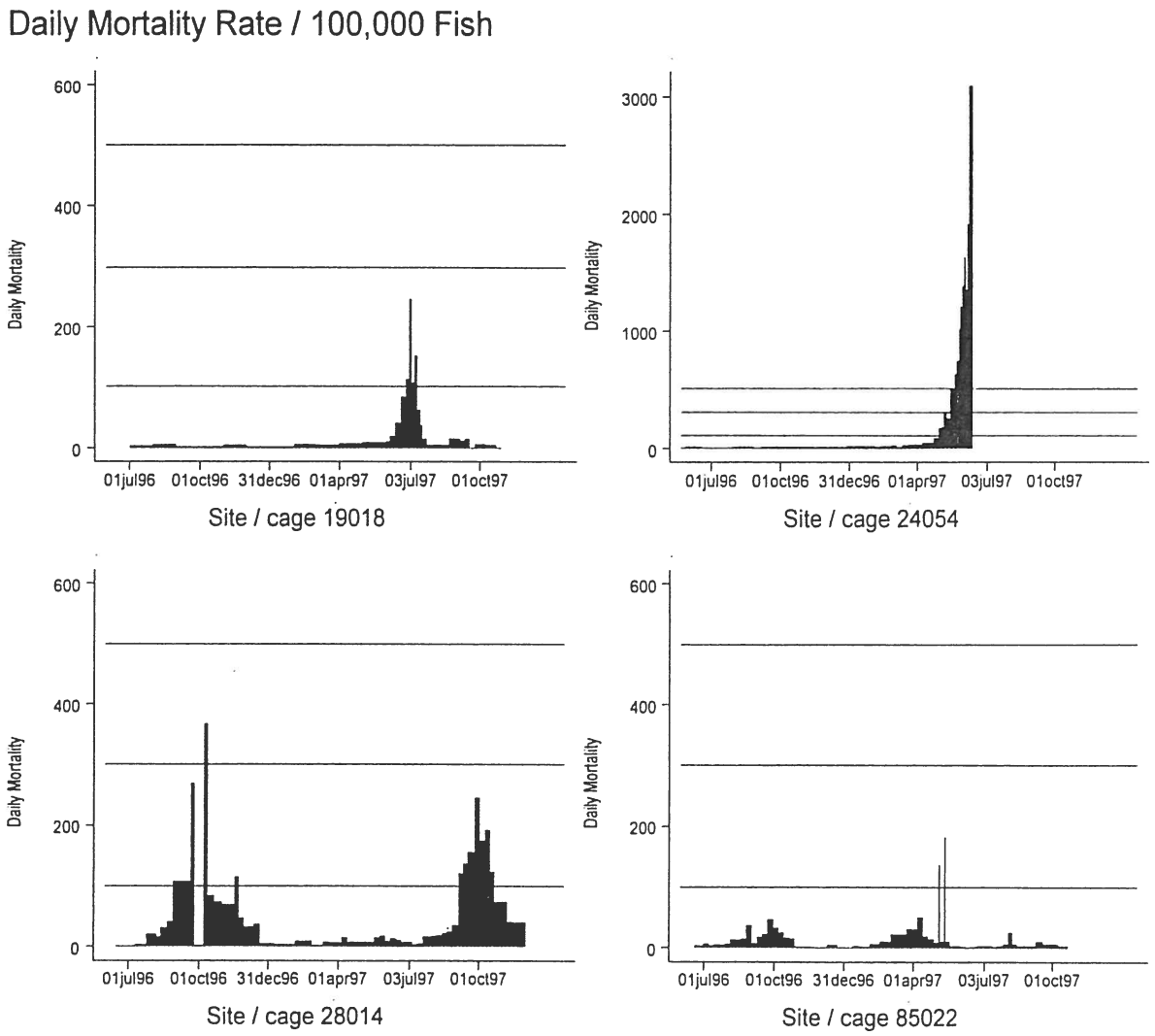

Fig.1 Selected outbreak patterns from HKS outbreaks in Bay of Fundy salmon farms.

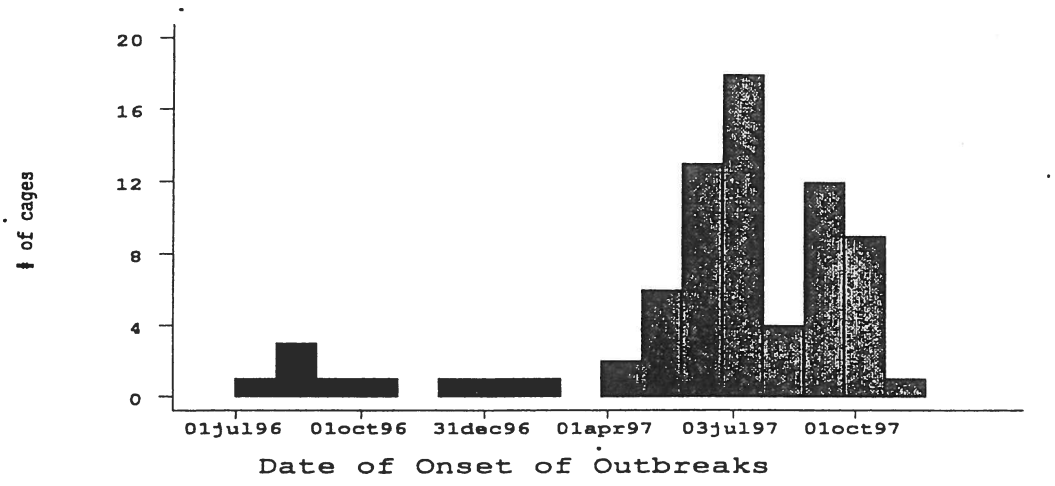

Fig. 2 Distribution of dates of onset of HKS outbreaks in Bay of Fundy salmon cages

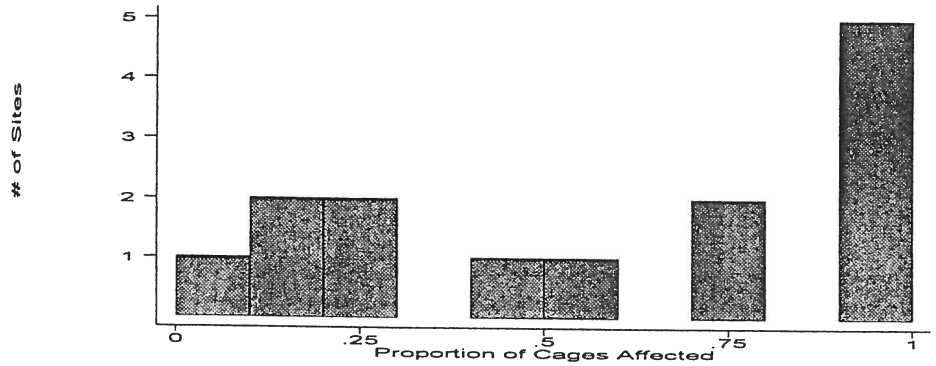

Fig.3 Proportion of cages affected with HKS outbreaks in 1996/97 at each of 14 salmon farm sites in the Bay of Fundy

Cage level risk factors

Table 1 presents the results from several selected cage-level risk factors which had unconditional associations with the cage being classified as a case ($P<0.1$). Not all significant risk factors have been listed since many were highly correlated with factors listed in Table 1. For example, virtually all cages with >12,000 fish were polar circles made of plastic so the variables for cage shape (circular) and material (plastic) were also significant. Investigator judgement and correspondence analyses were used to select variables for further analysis.

Table 1. Unconditional associations between cage level risk factors and outbreaks of HKS (cases) in 178 cages in 14 sites in 1996/97.

Variable	Levels	Cases (cages)	Relative risk	Lower CI	Upper CI	P-value
Initial Population at Risk	< 5,000	15 (72)				0.000
	5,000 - 12,000	28 (57)	2.36	1.40	3.97	
	> 12,000	40 (49)	3.92	2.45	6.27	
Fish Density (Fish/m^3)	< 2.5	11 (55)				0.000
	2.5 - 5.0	43 (60)	3.58	2.06	6.22	
	> 5.0	26 (49)	2.65	1.47	4.81	
Cumulative Mortality during 1996	< 0.003	12 (45)				0.000
	0.003 - 0.007	24 (68)	1.32	0.74	2.37	
	> 0.007 [a]	34 (46)	2.77	1.66	4.63	
Fish were weight sampled during 1997	no	40 (123)				0.000

[a] Data from cages which experienced outbreaks in 1996 excluded since they would contribute to 1996 cumulative mortality

Results from the final logistic regression model of cage level risk factors is presented in Table 2. These results must be interpreted with caution since cages were clustered within site and hence were not independent. The Hosmer-Lemeshow goodness-of-fit test was marginally significant (P=0.06) suggesting that the model did not adequately fit the data. Attempts to control for site using both a fixed effects and random effects logistic regression model were not successful, primarily due to the fact that most sites had either very few cages affected or most/all cages affected.

Table 2. Logistic regression model of cage level risk factors for HKS outbreaks. Data from 152 cages in 14 sites in 1996/97.

Variable	Level	OR	Lower CI	Upper CI	P- Value
Initial Population at Risk	< 5,000				
	5,000 - 12,000	4.42	1.44	13.49	0.009
	> 12,000	15.60	3.86	63.08	0.000
Fish Density (Fish/m^3)	< 2.5				
	2.5 - 5.0	7.27	2.16	24.48	0.001
	> 5.0	1.71	0.48	6.07	0.404
Cumulative Mortality during 1996	< 0.003				
	0.003 - 0.007	1.44	0.49	4.28	0.508
	> 0.007	10.06	2.77	36.61	0.000

Site level risk factors

Unconditional associations(P<0.1) between a number of site level risk factors and whether or not a site had more than 50% of the cages affected by outbreaks are presented in Table 3.

Table 3. Unconditional associations between site level risk factors and the majority of cages at a site being affected by HKS outbreaks in 14 sites in 1996/97. Both Pearson's chi-square P values and Fisher's Exact P values are presented

Variable	Levels	Cases (sites)	Relative risk	Lower CI	Upper CI	P-value (exact[a])
Mortality dives per week	1	4 (11)				0.051
	2	3 (3)	2.75	1.26	6.01	(0.19)
Diver visits multiple sites	no	1 (5)				0.094
	yes	6 (9)	3.33	0.54	20.43	(0.27)
Moist feed fed after transfer	no	6 (8)				0.031
	yes	1 (6)	0.22	0.004	1.39	(0.10)

Variable	Levels	Cases (sites)	Relative risk	Lower CI	Upper CI	P-value (exact[a])
Months moist feed fed between Jan.- July 1997	0	6 (6)				0.004
	1 - 4	1 (4)	0.25	0.05	1.36	(0.03)
	5 - 7	0 (4)	0	-	-	(0.005)
Feed delivered by feed company	no	2 (8)				0.031
	yes	5 (6)	3.33	0.95	11.66	(0.10)
High fat feed fed	no	1 (6)				0.048
	yes	5 (7)	4.29	0.67	27.24	(0.10)
Proportion of cages with 96 year class	<0.9	6 (7)				0.008
	> 0.9	1 (7)	0.17	0.03	1.05	(0.03)
At least one other cage at site had experienced an HKS outbreak	no					
	yes	NA [b]	3.13	1.71	5.72	0.000

[a] Fisher's Exact P-value

[b] Value changed with time so unconditional association (hazard ratio) determined from a survival analysis with a time-dependent covariate

Survival Analysis

The results of the final Cox proportional hazards model are presented in Table 4. It is based on a model in which the start of the time period at risk was defined as the point in time in which the fish were put in the cage (spring 1996) and cages were either classified as cases if they experienced an outbreak or were censored at the end of the data collection period (November 1997). Inclusion of a time dependent covariate representing whether or not another cage at the site had been classified as a case resulted in a very unstable model with unrealistic estimates of coefficients so that variable was not included in the final model.

Table 4. Cox proportional hazards model of cage and site level risk factors for HKS outbreaks. Data from 158 cages at 14 sites in 1996/97.

Variable	Level	Hazard Ratio	Lower CI	Upper CI	P- Value
Proportion of cages with 96 year class	<0.9				
	> 0.9	0.38	0.22	0.67	0.001
Feed delivered by feed company	no				
	yes	1.69	1.00	2.85	0.048
Months moist feed fed between Jan. - July 1997	0				
	1 - 4	0.45	0.23	0.90	0.024
	5 - 7	0.08	0.03	0.19	0.000
Cumulative Mortality during 1996	< 0.003				
	0.003 - 0.007	1.52	0.73	3.19	0.27
	> 0.007	3.61	1.63	8.01	0.002

Having a site that was primarily one year class reduced the hazard of outbreaks, as did feeding moist feed. For the latter variable, the hazard was lowest when moist feed was fed for the longest period of time. Higher levels of mortality during 1996 were associated with an increased hazard of outbreaks. As with the logistic regression analyses, the results from this multivariable model must be interpreted with caution since cages were not independent within site.

DISCUSSION

This study was initiated prior to identification of an etiologic agent for Hemorrhagic Kidney Syndrome (HKS). It is now widely accepted by the industry that the Infectious Salmon Anemia (ISA) virus (orthomyxoviridae) is the cause of these mortalities and the industry refers to this disease as HKS-ISA to reduce confusion over the difference in clinical signs observed with ISA in Norway and HKS observed in Canada. Although the presence of interstitial hemorrhage and nephrosis as first described by Byrne et al. (1997) is still noted, other clinical signs and pathological lesions are similar to the ISAv infections reported in Norway (Evenson, et al., 1991).

This retrospective study identified six cages that appeared to be cases in the summer and autumn of 1996. Although the 1996 year class (ie. fish which were transferred to sea cages in the spring of 1996) was originally believed to be first affected by HKS-ISA, there were cases of HKS-ISA diagnosed by mortality patterns, gross signs, and histopathology in the 1995 year class during the late summer of 1996. It is possible that the 1996 outbreaks included in this study were caused by other infectious agents, but no specific alternative diagnoses had been recorded. Due to the fact that the mortality patterns were very similar to HKS-ISA and these cages were in the immediate vicinity of 1995 year class fish with mortalities attributed to HKS-ISA, these cases were included in the study.

There were a small number of cages which experienced multiple outbreaks (see example site/cage 28014 in Figure 1). If these cages truly were re-infections of HKS-ISA, the protective immune

response following a natural infection may not be as effective under usual farming conditions as laboratory evidence has suggested (Falk and Dannevig, 1995).

Fewer cages lost high numbers of fish to HKS-ISA outbreaks than first suspected by the industry. Only 25% of outbreaks resulted in total mortality over 16.7%. Although this mortality rate may not be excessive in other parts of the world, it was much greater than the expected mortality rate of less than 5% for the entire sea water production phase in Atlantic Canada. The total mortality attributed to HKS-ISA was likely an underestimate due to the fact that many cages were harvested early to reduce losses once HKS-ISA was diagnosed or strongly suspected.

Evaluating risk factors for disease occurrences in Atlantic salmon sea water cage sites of eastern Canada was complicated by the fact that no standardized mortality recording system existed. Obtaining the information about mortality rates required careful scrutiny of the individual records kept by farmers, each utilizing a different recording system. The most difficult aspect was obtaining proper identification of the fish groups within cages. Such potential risk factors as fish characteristics, husbandry methods, environmental characteristics, and physical locations of groups at the site, were reported by the farmer through a combination of recorded information and memory. Cage-level information which was irretrievable on a majority of farms, such as sea lice counts, or was difficult to validate, such as growth rates, was not included in the risk factor analyses. As a result, much of the detailed analysis regarding cage specific factors, such as the sea lice infestation level or prevalence of BKD, and their associations with HKS-ISA outbreaks was not possible. A standardized record of performance system would facilitate such risk factor investigations in the future.

Cages with larger initial population at risk (PAR>12000 fish) were 3.92 times as likely to experience an outbreak compared to cages which had initial populations less than 5000 fish. The greater number of fish within a cage was highly correlated to the cage type (larger PAR tended to be reared in 20(+) meter diameter plastic circle cages, whereas smaller PAR were more often reared in 15 meter steel square cages). Over the past decade, the salmon farming industry has gradually converted to larger cages with greater numbers of fish in each cage as a method of reducing the capital and operating costs. The larger cage populations appear to increase the probability of experiencing elevated mortality rates in the presence of an infectious agent. The ability for fish to contact more naive individuals within a cage group may contribute to the spread which would increase the probability that exposure to a viral agent would result in an increased mortality rate.

Fish density was calculated using number of fish, rather than biomass, per cubic metre of water because fish weight measurements were not comparable between sites. The effect of fish density within cages was difficult to interpret because higher relative risk (3.58) was associated with moderate density compared to high density (2.65). Lower numbers of fish per unit volume of water were the lowest risk group. The fact that moderate density was higher risk than high density cages indicates that low fish densities may reduce the risk, but once a threshold of density is exceeded, there is little additional effect of increasing densities on the risk of experiencing elevated mortality rates attributable to HKS-ISA.

Poor general health of salmon smolts, as measured by cumulative mortality rate, during the first year of seawater growth was associated with a greater risk of experiencing an outbreak of HKS-ISA in the second seawater growth season. Better survival rates in the first warm water season after smolt transfer may be an indirect measure of many health management factors. Cages in which weight samples were taken during 1997 were more than twice as likely to experience an outbreak. Weight samples can induce additional stress on the group of fish being measured and this may account for the increased risk of HKS-ISA. However, it is also possible that farmers who do not sample for weight also tend to manage cages differently in unmeasured ways.

Sea lice have been reported to carry various pathogens, such as *Aeromonas salmonicida*, the bacterial cause of furunculosis (Nese and Enger, 1993), and ISA virus (Nylund et al., 1993; Nylund at al., 1994). The treatment of sea lice has also been observed as more frequent in furunculosis-positive

sites compared to furunculosis-negative sites (Jarp et al., 1994). Farmers in this study suspected sea lice infestation levels as contributing to the risk of HKS-ISA. However, sea lice counts were often unavailable or too variable in frequency and measurement method to be reliable indicators of true sea lice levels. T he number of delousing bath treatments was used as an indirect measure of sea lice control, in which greater bath frequency was considered an indication of a more aggressive sea lice control policy. Since all study sites were located in an area which was assumed to experience similar lice recruitment pressures, cages with greater treatment frequencies were assumed to represent reduced level of sea lice infestations. Greater than two delousing bath treatments was protective against HKS-ISA outbreaks compared to zero or one treatment.

The influence of site on the cage-level factors was difficult to determine in these analyses as the regression model which included site as a random effect would not converge. At the site level, significant (P=0.1) unconditional associations between "problem site" and 8 independent variables were observed (Table 3). The frequency of mortality dives was likely influenced by the presence, or the suspicion, of HKS-ISA at the site and therefore was more likely a result rather than a cause of HKS-ISA outbreaks. Divers in the region which work for more than one company usually disinfect gear between sites, but rarely maintain distinct sets of gear for each site. Consequently, divers moving from site to site may have contributed to the spread of HKS-ISA.

It is common practice for some sites in Atlantic Canada to use moist feed in the first few months following smolt transfer (spring of 1996 for the study population) in the belief that appetite and growth are enhanced with moist feed. Dry, pelletted feed is more economical as the fish become larger and so most farms will convert to dry feed within four months of seawater transfer (i.e. late in the first summer). Unconditional associations suggested that feeding moist feed after transfer to salt water and during 1997 both reduced the risk of HKS-ISA outbreaks. Feeding moist feed during 1997 remained as a strong and significant protective factor in the Cox proportional hazards model. Since moist feed has more crudely prepared components, the risk of introducing a pathogen through the feed was expected to be higher in moist than in dry feeds. Since dry feeds were associated with an increased risk of HKS-ISA, the feed was not suspected of harbouring to the virus. Rather, fish on moist feed may be generally in better health during the first months after smolt transfer and less susceptibility to HKS-ISA outbreaks.

High fat feeds and feed delivery to the site by the feed company are more common with dry feeds than with moist feeds. However, feed delivery was considered a potential method of viral transmission due to the fact that the delivery boats would travel between sites. It was interesting to note that in the final Cox proportional hazards model, the effect of feed delivery remained a significant risk factor for HKS-ISA outbreaks.

Sites which were least exposed to other year classes (i.e. less than 10% of cages were from another year class) had a much reduced risk of being a "problem site". Sites with multiple generations had greater frequency of primary ISA outbreaks in one Norwegian study (Vågsholm et al., 1994). Our study supports this observation and suggests that despite the close proximity of sites to each other (e.g. usually less than 1-2 km from other sites within the study area), year class separation is an important consideration in reducing the risk of HKS-ISA transmission between sites.

Jarp and Karlsen (1997) identified five kilometers as a safe distance between sites to reduce the risk of ISA transmission. The majority of sites in this study were all located within a five kilometer radius and each site was undoubtedly influenced by the close proximity to other infected sites. Despite the close proximity of sites to each other, a previous HKS-ISA outbreak in a cage at a site substantially increased the risk of other cages at the site having an outbreak. This indicates that managing risk factors at the site level may be able to reduce the risk of infectious disease spreading from nearby sites.

In conclusion, management factors appeared to substantially modify the risk of experiencing an outbreak of HKS-ISA. Increasing the health and survival of smolt in the first summer in seawater

and feeding moist feed during the same time period were associated with reduced risk. More aggressive lice control, lower initial stocking numbers, maintaining a single year class at a site and feeding moist feed during the second year in salt water may also help reduce the probability of HKS-ISA outbreaks.

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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 he 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