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**The Gareth Davies Lecture**

# SCIENCE, POLITICS AND ANIMAL HEALTH POLICY: EPIDEMIOLOGY IN ACTION

W. D. HUESTON*

'Animal health policy must be science-based!' This rallying cry is heard from animal health professionals around the world. The second verse of this mantra is often 'science, not politics', as if science is unquestionably 'good' and politics 'evil'. Antipathy toward politics is worn as a badge of honour. The crowning moment frequently comes with the proclamation, 'I am a scientist, I want nothing to do with politics.'

Thank you for the honour of presenting the 4th Gareth Davies lecture. My central premise is that veterinary epidemiologists are uniquely qualified to consider both science and politics in the promulgation of scientifically sound and politically prudent animal health policies. Gareth Davies has spent his career working at the interface of science and politics and I am honoured to have the opportunity to advocate the importance of this critical role. I will begin by dissecting the credo 'science not politics' from the perspective of an epidemiologist. Next, I intend to demonstrate how the epidemiologist can consider both science and politics through the application of risk analysis, and finally I plan to outline the competencies which the epidemiologist must master to be effective in the policy-making arena. By the end of this presentation, I sincerely hope that you will join me in saying 'the best animal health policy decisions result from consideration of both science and politics.' I also hope that you will recognise that effective animal health policy development requires the participation of epidemiologists.

The phrase 'science-based' infers that the underlying justification of animal health policy is derived from knowledge gathered through the systematic observation of, or experimentation with, phenomena (Allen, 1990). Scientific knowledge implies the compilation and analysis of data by individuals with advanced education in specific disciplines. Scientists seek facts, the fundamental truths which explain the world around us. At face value, the 'science, not politics' paradigm has a great deal of appeal. However, the very notion of fundamental truth is illusory, as scientific knowledge changes frequently with new observations and experiments. Furthermore, conjecture and refutation characterise the scientific method, with disagreement and debate the recognised features of scholarly pursuit. Scientists often reach conflicting interpretations of observational and experimental data. Consequently, individual scientists may champion different, even diametrically opposed, sets of ideas and principles, so that any number of alternatives may be justified as 'science-based'. Finally, animal health professionals typically consider only the biological and physical sciences as 'true sciences', dismissing the social sciences.

* University of Maryland campus, Virginia-Maryland Regional College of Veterinary Medicine, 8075 Greenmead Drive, College Park, Maryland, USA 20742

Politics reflect the human need for organisation of authority, whether in public or private life. Politics exist whenever two or more people come together. The terms 'office politics' and 'family politics' are recognised as clearly as the collective activities surrounding local or national governance. Politics exist even in science, affecting scientific organisations, refereed publications and academe. Indeed, politics are inescapable.

All public courses of animal health action adopted by governments emerge from the interplay of science and politics. The policy-making process is governed by rules and regulations, affected by the organisational culture of the government agencies involved, and constrained by legal authorities, political correctness and resource availability. The animal health policy-making process involves consideration of current biological and physical scientific knowledge. Policy decisions also consider social science factors including ideologies, economics and public opinion. Hence the aetiology of animal health policy is multifactorial.

Epidemiologists accept the concept of multifactorial aetiology as a basic tenet. We discuss disease in terms of agent, host and environment interactions. The practice of applied epidemiology demands a breadth of knowledge and the ability to work in interdisciplinary teams. The more complex the problem, the greater the demand for additional knowledge and insights. Veterinary epidemiologists study risk factors for disease in animal populations and develop strategies for health promotion and disease control. However, animal health problems cannot be resolved by consideration of biological and physical factors alone. The veterinary epidemiologist working with field problems soon recognises the critical role played by people in animal health issues. Applying epidemiological principles to animal disease prevention requires consideration of social issues such as attitudes toward animals, cultural and religious mores, and individuals' willingness and capability to implement the prevention strategies. Implementing prevention on a national scale brings additional factors into the equation such as availability of resources, adequacy of veterinary services, and animal health infrastructure, among others. Therefore, animal health policy development and implementation require attention to macroepidemiology, the study of all of the economic, social and political inputs which affect the distribution and impact of animal or human disease at the national level (Hueston & Walker, 1993).

Let me illustrate these concepts with several current animal health issues such as bovine spongiform encephalopathy in Europe, tuberculosis in white-tail deer in Michigan, USA and the global concern over antimicrobial resistance. Bovine spongiform encephalopathy (BSE) has rapidly gained the same level of global recognition as foot and mouth disease or rabies. While the origin of the disease still is debated (scrapie source or spontaneous TSE), the BSE epidemic was propagated by the recycling of infective meat and bone meal in cattle feed. Use of proprietary calf feeds including meat and bone meal was identified as the major risk factor for development of BSE at the farm level (Wilesmith et al., 1992). But why did BSE emerge in the United Kingdom (UK) and not somewhere else in the world? Consideration of the macroepidemiology of BSE identified major differences in available nutrients, feeding systems and animal demographics between the UK and other nations like the USA and Argentina (Walker et al., 1991; Cane et al., 1993). The BSE epidemic emerged in an environment of intensive agricultural production. Consolidation in the animal feed, slaughter and rendering industries had achieved economies of scale and allowed the marketing of commodity-based proprietary feedstuffs throughout the country. Meat and bone meal provided an economical source of high quality protein for cattle rations in a country where plant proteins sources were limited. The resulting animal feeding systems and widespread use of meat and bone meal in

feedstuffs allowed BSE to spread rapidly throughout the country. Therefore, the emergence of BSE can be described in the context of social, economic and political risk factors.

The successes and failures of BSE prevention and control can also be analysed using macroepidemiological principles. Control of the disease has rested on aggressive case finding, destruction of potentially infective tissues, and bans on the incorporation of high risk feed ingredients from the diets of susceptible animals. However, compliance with the control measures has proven to be challenging throughout Europe. The proposed prevention and control measures are scientifically sound prevention strategies, that is, science-based policy. In contrast, compliance involves both science and politics. The most scientifically-sound prevention and control programme is worthless if it cannot be implemented. Ironically, although compliance turns out to be a key factor in the success of BSE prevention and control programmes, little attention has been given to assessing the dynamics of compliance itself. What are the factors influencing voluntary compliance? What are the risk factors for low compliance and consequently, the failure of prevention and control programmes? Consideration of these social science questions is equally as important as the more traditional epidemiological focus on the biological and physical factors of agent, host and environment.

Bovine tuberculosis (TB) in white-tailed deer in Michigan USA presents another excellent example of an animal disease problem that can be tackled only from a macroepidemiological perspective. Bovine TB is close to eradication in cattle in the United States. Sporadic bovine TB infections of deer have been documented in the USA in areas of cattle TB problems, but these were not sustained in the wild populations. However, endemic bovine TB has now been confirmed in white-tail deer in the State of Michigan, a problem which appears to be spreading. The problem emerged in north-eastern Michigan, in an area of marginal farming and poor economic conditions. As cattle farming declined in this region over the last 25 years, establishment of hunting clubs and the feeding of native deer was on the rise. The feeding programmes increased deer populations and density to improve hunting success for the urban sportsmen who flock to the region. Congregations involving over 100 deer at a single feeding station were not unusual. The resulting high concentrations set the stage for the efficient spread of bovine TB infection from deer to deer.

Development of effective control policies in this situation requires a macroepidemiological approach. First, agriculture and wildlife are governed by separate state agencies in the USA, with different mandates and priorities. The agriculture department traditionally has focused on animal health and production, while the wildlife agency encourages wildlife conservation and development of hunting resources. Consequently, a national TB eradication programme exists in the USA for cattle but no such programmes exist for wildlife. From an economic perspective, the wildlife feeding and hunting represent a much more important contributor to the local economy of the endemic area than cattle farming. Finally, the disease control strategies commonly employed in the agricultural sector may not be as palatable for wildlife. Public opinion regarding the destruction of deer is more emotionally charged than equivalent control measures in cattle.

Antimicrobial resistance presents yet another example of the complexities of animal health (and public health) policy-making. Resistant microbes represent an emerging public health threat when alternative treatments are unavailable. Development of resistance occurs in the presence of antimicrobials, so that restriction of antimicrobial use appears a logical strategy for addressing the situation. Some antimicrobials are widely used as animal growth promotants. Therefore, a number of scientists and organisations have called for banning specific antibiotic

uses in food animals. However, antimicrobial resistance must be considered in the context of a dynamic biological system involving animals, plants and people. Antimicrobial use in animal (and plant) production reaches its highest levels in some emerging economies where environmental concerns are secondary to economic growth and the provision of abundant and affordable food supply, that is, food security. Unfortunately however, the global movement of animals, animal products and people means that resistance emerging in one part of the world spreads very quickly to other countries and continents. Consequently, establishment of effective animal health policies with regard to antimicrobial use must emerge in a charged political environment where the developed countries have different needs and priorities than the developing ones.

The public health dangers of resistant organisms appear highest in hospital settings among select populations of the most developed countries. Control of communicable diseases like malaria and sexually transmitted diseases may be much higher priorities for countries with a less developed health care infrastructure and fewer resources. Malnutrition, addressed partly by improving food security, may be a much higher priority for these countries than reducing the risk of antimicrobial resistance development. Furthermore, specific prevention strategies aimed at nosocomial disease protection and additional precautions in care of the elderly and immunocompromised appear of critical importance for minimising the impacts of resistant microbes.

These three examples, BSE, bovine TB in deer, and antimicrobial resistance, serve to illustrate the need for addressing animal health issues from a holistic, macroepidemiological perspective. Both animal and human health issues must be considered, as well as the social science aspects of compliance and public support. Animal health policy decisions cannot be made in a vacuum. Given the complexity of today's animal and public health issues and the necessity of a more integrated and holistic approach to policy-making, the epidemiologist needs to grasp new tools. Risk analysis appears to be a promising prospect.

Risk analysis is a systematic approach to evaluating and responding to potential adverse events. One canon of Murphy's Law states that anything that can go wrong, will go wrong. Therefore, no action is without risk. The concept of risk involves both chance and consequence; that is, the chance or likelihood that something will go wrong and the consequences if this happens. The overarching concept of risk analysis is that risks can be better managed if they are explicitly examined and discussed. The risk analysis process comprises four interrelated elements: hazard identification, risk assessment, risk management and risk communication. Each of these risk analysis terms can be explained most easily by a series of questions. Hazard identification involves 'What can go wrong?' and 'How would it happen?' Risk assessment asks 'How likely is something to go wrong?' and 'What would be the consequences?' Risk management considers 'How can the likelihood of something going wrong be reduced?' and 'How can be consequences of something going wrong be minimised?' Risk communication means 'How can all of the potentially affected public be involved in the risk analysis process?' These four processes can be seen as a cycle; they progress simultaneously in an iterative process. Obviously, we all use risk analysis on a daily basis as we make decisions about our own behaviour. Similarly, risk analysis has been the basis for animal health policy decisions from import permits to disease eradication programmes. However, risk analyses in the past have been largely subjective and implicit. Risk analyses formed part of the decision-making process of the individual assigned the responsibility. These analyses were heavily dependent on the decision-makers previous experience and the prevailing organisational culture.

Completion of the Uruguay round of the General Agreement on Tariffs and Trade and adoption of the Sanitary-Phytosanitary Agreement signalled a major change in policy-making with regard to trade and presaged a broader movement from implicit to explicit risk analysis processes. Risk analysis now has been recognised as the tool for applying scientific knowledge to both domestic and international animal and public health problems. Documentation of the risk analysis and transparency of the risk analysis process are rapidly becoming the global norm, as policy decisions regarding health must be justified by risk analyses. The process of risk analysis has become more formalised, with a series of steps.

Risk analysis begins with the elucidation of the hazard(s) of interest. For instance, the hazard of greatest concern in the BSE example is the human manifestation, variant Creutzfeldt-Jakob Disease (vCJD). Looking at that endpoint, a flow chart or scenario tree is created to illustrate all of the routes by which this hazard might occur. For the vCJD example, the routes would include consumption of infective animal tissues, inoculation with a contaminated product, and tissue transplantation from another individual affected with vCJD. The next step of the process, risk assessment, involves estimation of the likelihood of each scenario and its consequences. The probability of consumption of an infected food product can be estimated by knowing the composition of the product, source of the raw materials, means of fabrication, and consumption patterns. The consequence of vCJD will depend on the age of the patient and whether they serve as an organ donor for others. Consequences must include the social as well as the biological implications. As each potential pathway is outlined and characterised, the options for managing the risk become more apparent. Risk management involves both reduction of the likelihood of the adverse event and minimisation of the implications should the hazard occur. A number of alternative risk management strategies always exist. Deciding the most appropriate strategy involves revisiting the risk scenarios. The success of a particular regulation will depend on the completeness of its implementation and degree of compliance. Sensitivity analysis (or consequence analysis) can be utilised to compare the impacts of different management approaches at various levels of compliance. The fourth component of risk analysis, risk communications, is often undervalued. Risk communications are an ongoing process which begin as the risk analysis is conceptualised. Risk communications involve all sections of society (producers, consumers, industry, regulators, politicians) who are potentially affected by the hazard of interest. Involving these sections of the public early improves the risk analysis by expanding the input into each component.

Various approaches to risk analysis are being developed for consideration of microbial hazards, import risks and toxicities. Disciplines from pharmacology and microbiology to engineering and statistics are making contributions. The approach is very pragmatic: use the existing knowledge to characterise risks and evaluate management options. Interestingly, the risk analysis process is more important than the final risk estimate. The process of hazard identification helps elucidate complex processes. Hazard identification and critical control points (HACCP) represents applied risk analysis used in evaluating production systems. Systems modelling has been borrowed from economics and business to help characterise risks by the steps in the manufacturing or production process: raw material sourcing, harvesting, processing (fabrication) and end product use. The systematic nature of risk analysis also helps to identify data gaps and formulate research priorities. Sensitivity analysis can be utilised to identify the most important data needs in terms of their potential impact on overall risk. Risk analysis, as practised with HACCP and systems modelling, further helps in building redundant systems so that the overall likelihood of system failure, that is, the hazard occurring, is much less than the chance that one control measure will not be totally effective.

The veterinary epidemiologist is well suited to participate in risk analyses involving animal and public health issues. Our training supports the systematic approach embodied in risk analysis. Epidemiologists are excellent at outlining potential pathways associated with the hazard, identifying data sources to support evaluation of the likelihood and consequence and designing alternative management strategies. Furthermore, the statistical training of epidemiologists becomes valuable when risks must be quantified. Epidemiologists also understand the complexity of biological systems and recognise that multiple options exist for handling any particular situation. Since risk analyses, whether implicit or explicit, underlie public policy-making, this is the area through which many veterinarians get their first exposure to the interplay between science and politics.

Unfortunately, public policy-making and the risk analyses underlying it, often occur in an atmosphere of secrecy. All too often, government agencies embark on risk analyses using only internal experts in closed meetings. Discussions of the hazards, their risk, and management options are closed to the public, under the assumption that public forums will create public anxiety. Risk management decisions are made based on these internal risk analyses without the benefit of external review or public comment. The decisions are then announced publicly with the risk analysis document used to defend the decision. This sequence of events is so common that it has been referred to as the DAD fallacy: Decide, Announce, Defend. Risk analyses are improved by public involvement. Risk communication promotes an open and transparent policy-making process. The affected public can contribute immensely to the hazard identification process, as they know the processes at the grass-roots level. In addition, they can help identify potential sources of data and information about critical pathways. The affected public are likely to be involved with the implementation of risk management initiatives, so their input can help in determining which strategies will achieve the greatest compliance. Finally, involvement of the affected public enhances the external credibility of the risk analysis and the likelihood that risk management will be successful. In fact, involving the affected public in the entire risk analysis in itself benefits risk management. Further, risk communication emerges as one of the most critical components determining the success of risk analysis and the subsequent public policy decisions.

Taking risk analysis to public policy involves more interplay between science and politics. While the risk analysis may include economic and social factors in the estimation of risk, the policy-making process has its own politics associated with personalities and differences in priorities. At the highest levels, government risk managers are often political appointees or elected officials. Consequently, they incorporate their own implicit 'political' risk analysis into the decision-making. 'How likely am I to be re-elected or continue my appointment depending on the decision that is reached?' In my experience, communication skills become extremely important at this final phase of policy-making. Understanding the risk analysis is often a prerequisite for the decision-maker to make full use of it. Additionally, the decision-maker must feel comfortable in reviewing the findings and the options with the risk analysts, including the epidemiologist. At the same time, the epidemiologist will play a larger role in policy-making process if they understand the thought processes of the decision-maker and are able to communicate effectively.

Neither communication skills nor an understanding of the public policy-making process are part of most epidemiologists' training. Epidemiology training programmes routinely focus on coursework in statistics and infectious disease prevention and control. These represent critical knowledge for those studying the distribution and determinants of disease. However, to be an effective preventive medicine practitioner and a participant in the animal health policy-making

arena, additional knowledge and competencies are required. Applied epidemiology demands knowledge of the policy-making process and a wide range of social skills, including listening strategies, written and verbal communications, conflict resolution, negotiation abilities and an appreciation of cultural differences and diversity. The veterinary epidemiologist must be able to work as a member of an interdisciplinary team involving individuals with varied education, experience, personal style and personal values. In the absence of these competencies, the scientific knowledge of the epidemiologist may not play its necessary role in the policy-making process. The science may be sound, but if it cannot be articulated in an understandable manner, taking into consideration the unique attributes of the affected public, then politics may weigh disproportionately in the final decision.

I am enjoying my career in veterinary epidemiology. I have applied my epidemiological skills in private veterinary practice, agribusiness, academia and the federal government. I have had the privilege of working in several countries and interacting with other epidemiologists around the world. I also have been in positions of public policy decision-making, and have learned through my experience the importance of the social sciences and the so-called 'soft skills' of communications, negotiation and celebration of diversity. Having returned to veterinary academe, I have accepted a new responsibility to create opportunities for veterinary students and animal health professionals to expand their skill sets so that they can be more effective at the interface of science and politics. Let me share three examples of educational initiatives we have initiated to address these needs, changes in the veterinary curriculum, a residency programme in applied epidemiology and a post-graduate executive fellowship in science, politics and animal health policy.

Veterinary curricula are notoriously rigid. Students are overwhelmed by the volume of information given to them during lectures and practical sessions. The faculty is convinced that students must be exposed to the entire field of veterinary medicine during their veterinary training programme. Class sizes are large enough that evaluation techniques rely on machine-grading of standardised examinations. Every question has one right answer and multiple wrong ones. Veterinary students at my institution joke that they are being trained as 'multiple choice veterinarians in a short answer world'. At the same time, studies of the veterinary profession continue to indicate shortcomings in communication and business skills, not problems with students not knowing enough.

We have a unique advantage at our institution, the Virginia-Maryland Regional College of Veterinary Medicine, in that we instituted a 'tracking' system into our curriculum. Early in their veterinary training, students choose an area for in-depth study. Four clinical tracks exist (companion animal, food animal, equine and mixed) plus a track in government and corporate veterinary medicine, which I prefer to call 'public' veterinary practice. Beginning in the second year of our four year veterinary medicine post-Baccalaureate curriculum, the students take coursework in public policy and related subjects with particular relevance to careers in government, industry or academia. These courses emphasise skill building as much as they provide information about the policy making process at the local, state, national, and international levels. The students write fact sheets, develop issue papers, present issue overviews, learn media skills and practise negotiation tactics. During the senior year of veterinary college, the students spend an intensive 3-week block in the Washington DC area, working on a current animal health issue of their choice. They interact with policy-makers, interview producer organisations and advocacy groups and evaluate risk management alternatives. Our goal is to provide these students with entry-level skills and abilities which will help them to succeed in the public arena. Recognising that not all students will track

government-corporate veterinary medicine and that students from other schools may be interested in aspects of this programme, we have incorporated an intensive experience in science and public policy into a summer fellowship programme. Students spend two weeks at our campus in Washington DC learning about public policy, developing new skills and investigating current issues in veterinary medicine and public health.

Our second initiative is directed at graduate veterinarians desired intensive training in applied epidemiology. We created a residency programme in applied epidemiology through which veterinarians gain practical experience in outbreak investigation, risk analysis supporting public policy, evaluation of surveillance systems, epidemiological studies and risk communication. The residency covers three years, during which a graduate degree (Masters of Science in Veterinary Medical Sciences) is also completed. Our faculty and the residents provide applied epidemiology services to government agencies, agribusiness, international organisations and private producers. Obviously, skill building is an integral part of the residency programme.

We also have developed a programme for mid-career professionals entitled 'Science, Politics and Animal Health Policy'. This fellowship comprises 3 four-day sessions over the course of a year. The course is built around case studies of real animal health issues, such as the deer TB problem in Michigan and trade issues between countries. The case studies offer a concrete example through which we can introduce the importance of leadership skills such as strategic planning, media relations, negotiation skills and conflict resolution. The programme has attracted government officials, university faculty, industry specialists and private veterinary practitioners. Animal health professionals from six countries have participated to date. Evaluations from participants suggest that these new skills have increased their ability to effectively participate in the policy-making process.

All of these programmes are founded on the premise that veterinary epidemiologists can gain additional knowledge and develop further skills to permit them to participate more effectively in animal health policy-making. Some of this knowledge can be provided through didactic settings while other competencies require experiential learning. Through this new knowledge and the accompanying skills, the veterinary epidemiologist gains an understanding of the interplay of science and politics, learns to incorporate social science into risk analysis and disease investigations, and ultimately recognises additional opportunities to help improve animal and human health.

I conclude by reminding you that animal health policy decisions are being made daily. Many of these decisions are being made in the absence of information, information that many of you could provide. In the absence of information, perception becomes reality. Not being involved with animal health policy-making means that the veterinary epidemiologists knowledge and insight are lost, and that more decisions may be made primarily on the basis of perception rather than information. Therefore, I am convinced that we, as veterinary epidemiologists, need to be active participants in the policy arena, and that we can be more effective if we develop additional skills in communications, negotiation, and public policy-making which are not part of our veterinary or epidemiological training. Applied epidemiology means preventive medicine on a population basis and implementing preventive medicine programmes involves both science and politics.

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# DESIGN AND ANALYSIS OF TRANSMISSION EXPERIMENTS

A.H. KROESE[12], M.C.M. DE JONG[2]

## SUMMARY

A vast amount of literature is available about how to estimate quantitatively transmission of an infectious disease. Most of it focuses on observational data, whereas quantitative estimation of transmission from experiments has not been given much attention. Transmission experiments have been carried out with animals and even with human volunteers, but these experiments were used to show qualitatively that certain transmission routes are possible. De Jong and Kimman (1994) have carried out experiments to estimate transmission of Pseudorabies (Aujeszky's disease) virus in pigs. One of the main goals was to see whether vaccination reduces transmission. In this paper a statistical method is set out in more detail for the analysis of data from such transmission experiments. Techniques are described to estimate a measure of transmission, the basic reproduction ratio R, and to test whether R differs between treatments. Some tables are included and several designs are compared. The optimal design is to start with 50 percent infected animals. With this design the test has high power if the R of one treatment is larger than one, and the R of the other treatment is less than one. For infections in animals, a combination of transmission experiments and observational studies is best for the study of transmission. In human medicine transmission experiments with animals can be useful as models.

## INTRODUCTION

A number of studies have been done to quantify transmission of agents of infectious diseases especially among humans. These studies almost exclusively deal with observational, that is non-experimental, data. An explanation for this is that transmission experiments with humans are, in general, not feasible. Moreover, a large amount of observational data is usually available while controlled experiments are limited in size because of costs and ethical reasons.

An obvious disadvantage of observational data, however, is that 'confounding' can occur. For example, in determining whether vaccination reduces transmission, one cannot simply compare vaccinated and non-vaccinated individuals in the population. In the first place, as transmission depends on the vaccination status of the whole population, only independent populations can be compared, and second, even when several independent populations are compared vaccination is often only applied when there is a risk of transmission and not at random. Hence, if the effect to be studied is that a certain intervention will have on transmission in the population, there is an advantage to evaluating it in a controlled experiment. By randomisation of the individuals in the

[1] Centre for Biometry Wageningen, Wageningen, The Netherlands
[2] Institute for Animal Science and Health, ID-Lelystad, Lelystad, P.O. Box 65, 8200AB Lelystad (m.c.m.dejong@id.wag-ur.nl)

experiment and keeping differences between the groups to a minimum, except for the intervention, causal statements can be made about the effect of the intervention.

Transmission experiments have been done, even with human volunteers, e.g., Dick et al. (1987), but these experiments have only been used to investigate qualitative questions, i.e., whether a certain transmission route does or does not occur. More recently an experimental set-up was described and used to study quantitatively the effect of vaccination directed at a virus in pigs (De Jong & Kimman, 1994). Some infective and some susceptible animals were placed together in an isolation unit and the number of the susceptible animals that became infected (the 'final size') was observed. By doing a few such experiments with vaccinated animals and a few such experiments with non-vaccinated animals the effect of vaccination can be estimated.

In De Jong and Kimman (1994) it is suggested that modelling can solve the problem of limited data. By making explicit assumptions about how transmission occurs, statistically significant effects of interventions can be found, even if only a small amount of data is available. No-one, however, has yet developed the methodology to design transmission experiments or analyse the data obtained from them. Although much has been written about analysing field-data (see Becker, 1989), it is not easy to adapt the techniques described because most techniques are designed for large data-sets and are not necessarily valid for the small data-sets obtained in transmission experiments. Furthermore, because field-data are about naturally occurring infections, no-one has investigated how to design transmission experiments. Although De Jong and Kimman (1994) performed such experiments, the statistical analysis was discussed only briefly and no rationale was given for the choices made in the design of the experiment. Moreover, the statistical test used was somewhat liberal, that is, it gave significant results more often than required by the given error rate.

In this paper we have compared various estimation and testing techniques to analyse data from transmission experiments. We have also tried out various experimental designs. Finally, we have presented our results about analysis and designs here in a form that is accessible to a broad epidemiological audience. As a result epidemiologists now have ready access to the methodology needed to perform and analyse these experiments. This is important because transmission experiments are potentially of great value in veterinary control problems and as animal models in human medicine.

## THE MODEL

In the transmission experiments described in De Jong and Kimman (1994) more infections occurred in the non-vaccinated group than in the vaccinated group. This suggests that vaccination leads to a reduction of transmission. It has to be established, though, that the difference observed is statistically significant, that it cannot be attributed to chance alone. A second question is whether the difference is relevant: is the reduction large enough to have the agent disappear from the population?

In this paper we show how assuming a model allows us to make inferences about the population at large on the basis of a small data-set. It is clear, however, that a model is at best only an approximation of the real world and the underlying assumptions can be criticised. Hence, it is important to choose a simple and 'robust' model; that is, the conclusions of the analysis should be relatively stable to changes in the assumptions. Moreover, it should be borne in mind that the data are too limited to be of aid in choosing a model. In the absence of detailed

further information, undesirable subjective choices have to be made in choosing a more complex model (see Becker, 1979a). The model we will use is standard and is called either the 'general epidemic model' (Bailey, 1975), or the 'SIR' (Susceptible-Infective-Removed) model (Dietz, 1982). The assumptions are described below.

When a susceptible animal is infected it enters a latent period. After that it is infectious for a while, before it is recovers. An animal recovered from infection is fully immune, i.e., it will not become infectious again to other animals. The probability that a certain infective infects a certain susceptible in a small interval in time is assumed to be independent of time since onset of infectiousness, is the same for all animals, and is inversely proportional to the number of animals present (n). The latter assumption is appropriate for diseases that are transmitted by close contacts: the more animals in the stable, the smaller the probability that there is a close contact between two animals (De Jong et al., 1995; Bouma et al., 1995). Similarly, we assume that the probability that a certain infective recovers in a small interval in time is also independent of time since onset of infectiousness. This assumption is not appropriate for all agents. As will be discussed below, however, the conclusions based on the model are relatively insensitive to modifications of this assumption. The analysis of a transmission experiment does not depend on assumptions made about the duration of the latent period (see Ludwig, 1975). This is obviously an attractive feature of the model.

The model is used to describe the probability distribution of the outcome of a transmission experiment (the final size) in terms of R. This so-called 'reproduction ratio' is defined as the mean number of new infections that one typical infectious individual causes in a totally susceptible population. The reproduction ratio depends on the agent and population of interest. In Bailey (1975, Sec 6.2 and 6.5) it is shown that R has an important threshold property: loosely stated, if R is smaller than one only minor outbreaks will occur, while if R is larger than one a major outbreak is also possible. The concept is important in eradication programs, where an intervention has to be found that reduces R such that it is smaller than one.

Assuming this model, the probability distribution of the final size of a transmission experiment is completely determined if R is known (Bailey, 1975). Hence, the outcome of such an experiment provides information about this ratio. If experiments are done both with vaccinated and non-vaccinated animals, R can be estimated in both populations, it is possible to test whether vaccination reduces the reproduction ratio, and it is possible to test whether vaccination makes R smaller than one. This will be exemplified in the next sections.

In De Jong and Kimman (1994) an algorithm is described for the calculation of the probability distribution of the final size of a transmission experiment as a function of R. The authors have implemented this algorithm in Pascal and Mathematica. These programs are freely available upon request. No general programs yet been developed which perform estimation and testing as described in the remainder of this paper. For specific situations, though, these techniques are not hard to implement.

## STATISTICAL ANALYSIS FOR ONE POPULATION

Suppose m transmission experiments have been performed, each with s susceptibles and i=n-s infectives at the start. Hence, outcomes $x_1, \ldots, x_m$ are available, where $x_k$ is the final size in the kth experiment. In practice, m, s, and i are typically small. The assumption that all m experiments are identically designed is made for notational convenience only and is not

essential. The 'combined likelihood' $l(R|x_1, \ldots, x_k)$ can be calculated for each value of R. For one transmission experiment the likelihood $l(R|x)$ is the probability of final size x if the reproduction ratio is equal to R. As stated before, this probability can be calculated by the algorithm described in De Jong and Kimman (1994). The combined likelihood is the product of the individual likelihoods, i.e. $l(R|x_1, \ldots, x_k) = l(R|x_1) \ldots (R|x_k)$. It follows that, in theory, the maximum likelihood estimate (MLE) of R can be found, that is the value of R for which the combined likelihood is maximum. In all examples we considered the combined likelihood was 'unimodal', increasing until a certain value of R and decreasing thereafter. This implies that it is straightforward to find the maximum by calculating the likelihood in points chosen by trial and error: there is no risk of ending up in a 'local maximum'.

Another estimate of R is obtained by applying a method of moments for martingales described in Becker (1989, Chapter 7). This leads to the estimate

$$\hat{R} = \frac{\sum_{k=1}^{m} \sum_{j=s-x_k+1}^{s} \frac{n}{j}}{\sum_{k=1}^{m} u_k}$$

where $u_k=i+x_k$ if $x_k<s$ and $u_k=n-z_k$ if $x_k=s$. Here $z_k$ is an 'estimate' of the number of animals in a latent or infectious period, immediately after the last infection occurred. If not all experiments are equally designed, the martingale estimate is also given by the above expression but then s and n depend on k. In the sequel, this estimate is called the 'martingale estimate'.

In Becker (1979b,1981) it is shown that the martingale estimate converges to the true value if more data become available, not only for the model assumed in this paper, but also for more general models. For example, it is not necessary to assume anything about the probability of recovery. In general, this is clearly an advantage of the martingale estimate above the maximum likelihood estimate, for which this does not hold. Transmission experiments, however, only provide a small amount of data and 'large sample' arguments do not apply to such data.

In transmission experiments, calculating the martingale estimate is questionable if, in one of the experiments, all susceptibles get infected such as normally happens in the non-vaccinated 'control' groups. In this case, a choice has to be made for $z_k$, which will be debatable as the moment an animal is infected or stops being infective cannot be determined exactly. In Becker (1981) using the martingale estimator is only recommended if the infectiousness of the disease is such that not all susceptibles get infected. We therefore recommend the use of the MLE instead of the martingale estimate in transmission experiments.

Confidence intervals are important as they provide an indication about the precision of the estimate and they can also be used to perform statistical tests. Confidence intervals are usually given in the form 'estimate $\pm$ 2 * standard error'. For both MLE (based on 'Fisher information', see McCullagh and Nelder (1989, p.470)) and martingale estimate (Becker, 1989, Chapter 7) explicit formulae are available for the calculation of such standard errors. Both formulae, however, are only valid for large data-sets and are not appropriate to analyse the outcome of a transmission experiment.

One-sided and two-sided statistical tests about R can be performed exactly. For example, consider testing the hypothesis $H_0$:R≥1. Rejecting the hypothesis means that it is statistically proven that R is significantly smaller than one and hence only small outbreaks will occur. Let $t=x_1+ \ldots +x_m$ be the total number of infected animals in the m experiments. This hypothesis can be tested by calculating the p-value, that is 'the probability that, in total, t or less infected animals are found if R=1'. As usual the hypothesis is rejected if the p-value is 0.05 or smaller. The calculations are done by writing down all possible final sizes of the m transmission experiments such that the total is t or smaller. The p-value is the sum of all the corresponding probabilities, which can be calculated by the algorithm described in (De Jong & Kimman, 1994) and which was implemented in Pascal and Mathematica by the authors.

Two-sided 95-percent confidence intervals for R can be constructed by finding all r such that the hypothesis $H_0$: R=r is not rejected, that is, the p-value is larger than 0.05. The p-value for such a two-sided hypothesis is given by twice the minimum of (a) the probability that, in total, t or less infected animals are found if R=r and (b) the probability that, in total, t or more infected animals are found if R=r. By calculating these p-values for values of r chosen by trial and error, the lower and upper boundary of the confidence interval can be found. As an example consider the data described in De Jong and Kimman (1994). Two transmission experiments were carried out with Pseudorabies virus using vaccinated pigs. In the first experiment s=i=5 and in the second s=5 and i=4. Note that i=5 was planned for both experiments, but one infectious animal died just before the actual experiment started. The final sizes were two and one, respectively. Based on these data the MLE of R in a vaccinated population is 0.593 and the martingale estimate is 0.525. The p-value corresponding to the hypothesis $H_0$:R≥1 is 0.332 and hence it has not been shown by these two experiments that R<1. An exact two-sided 95-percent confidence interval for R is 0.118-2.332. Note that this interval is not symmetric around the MLE or the martingale estimate. Two transmission experiments were also carried out using non-vaccinated pigs. Here s=i=5 for both experiments and all susceptibles became infected. MLE and martingale estimate ($z_k$=10) are both $+\infty$.

We have included Tables 1 and 2 at the end of this paper. In these tables the MLE is given for all possible outcomes of some plausible designs. Also, for each outcome, the lower and upper boundary of the confidence interval is given and the p-value corresponding to the hypothesis $H_0$:R≥1. The p-value is only given if it is smaller than 0.05. If it is bigger than 0.05 the table states 'NS', i.e. non significant.

In Table 1 the designs with s=i=1 are dealt with for m=2 up to m=10. For example, consider seven experiments carried out with one susceptible and one infective each. Suppose only one of these experiments ended with the susceptible being infected; in the other six experiments the infective recovered before it could infect the susceptible. To find the MLE in the table we look at the row with s=1,i=1,m=7, and C=1. It turns out that the MLE is 0.333 with confidence interval 0.007-2.747. In the column with 'p-value' we find 'NS' and hence it is not statistically proven that R<1.

In Table 2 some designs are included with more than one susceptible and infective. The first three columns describe the design of the experiment, i.e. s, i, and m. The fourth column describes the outcomes, i.e. the final sizes $x_1,\ldots,x_m$ of the m experiments. Here the ordering of the $x_i$'s is not important. For example, suppose that four experiments are carried out with two susceptibles and two infectives each. If, in one of these experiment, no susceptibles were

infected while in the remaining three experiments all susceptibles were infected, we look at the row with s=2,i=2,m=4, and $x_1,\ldots,x_m$=2,2,2,0.

## STATISTICAL ANALYSIS FOR TWO POPULATIONS

Often the interest is in a comparison between two populations, e.g., vaccinated and non-vaccinated individuals. As a model, transmission experiments can be carried out both with vaccinated animals and with non-vaccinated animals. Eventual differences can be interpreted as causal. Hence, suppose for both populations outcomes $x_1^{(j)},\ldots,x_m^{(j)}$ are available from m transmission experiments, each with s susceptibles and i infectives at the start. Here $x_k^{(j)}$ is the final size in the kth experiment for population j. Let t be the difference between the populations in the total number of (contact) infected animals, that is:

$$t = \left| \sum_{k=1}^{m} x_k^{(1)} - \sum_{k=1}^{m} x_k^{(2)} \right|$$

To test whether the reproduction ratio differs between the two populations the p-value has to be calculated, that is 'the probability that a difference in total final size is obtained equal to or larger than t, under the assumption that the reproduction ratios are equal'. The difference found is said to be significant if this p-value is 0.05 or smaller. The calculations are done by writing down all possible final sizes of the 2m transmission experiments such that the difference in total final size is t or larger. The p-value is the sum of all the corresponding probabilities assuming that the reproduction ratios in the two groups are equal. Again, these probabilities can be calculated using the algorithm implemented by the authors.

The only practical problem with this approach is that a value has to be chosen for the common reproduction ratio. In De Jong and Kimman (1994), R is first estimated on the basis of all 2m transmission experiments together. This value is subsequently used in calculating the p-value. It is known, however, that this approach can lead to quite misleading results (Cox & Hinkley 1974). In particular, the test is too liberal, i.e., incorrect significant results are found too often. We also found this in the present case.

The following is a conservative approach: For every common value of the reproduction ratio the probability can be calculated that a difference in total final size is found equal to or larger than t. Now define the p-value as the maximum of all these probabilities. By calculating the probabilities for common values of R chosen by trial and error, this maximum can be found. The test defined thus is conservative in the sense that incorrect significant results are found in less than five percent of the cases. The obvious advantage is that, if a significant result is found, it can be trusted.

One of the main considerations in choosing a test is power, the probability of finding a significant difference if there is one. A disadvantage of a test defined as above is that the test may have insufficient power to be of practical use. The authors have investigated this by calculating the power for many designs and the conclusion is that a reasonable power can be expected if, in one population R is smaller than one, while in the other R is larger than one. Because of the threshold results stated above, this is also the region of the alternative of most

interest. In Figure1 the power function (randomised test, see the next section) is given for m=2 and s=i=5.

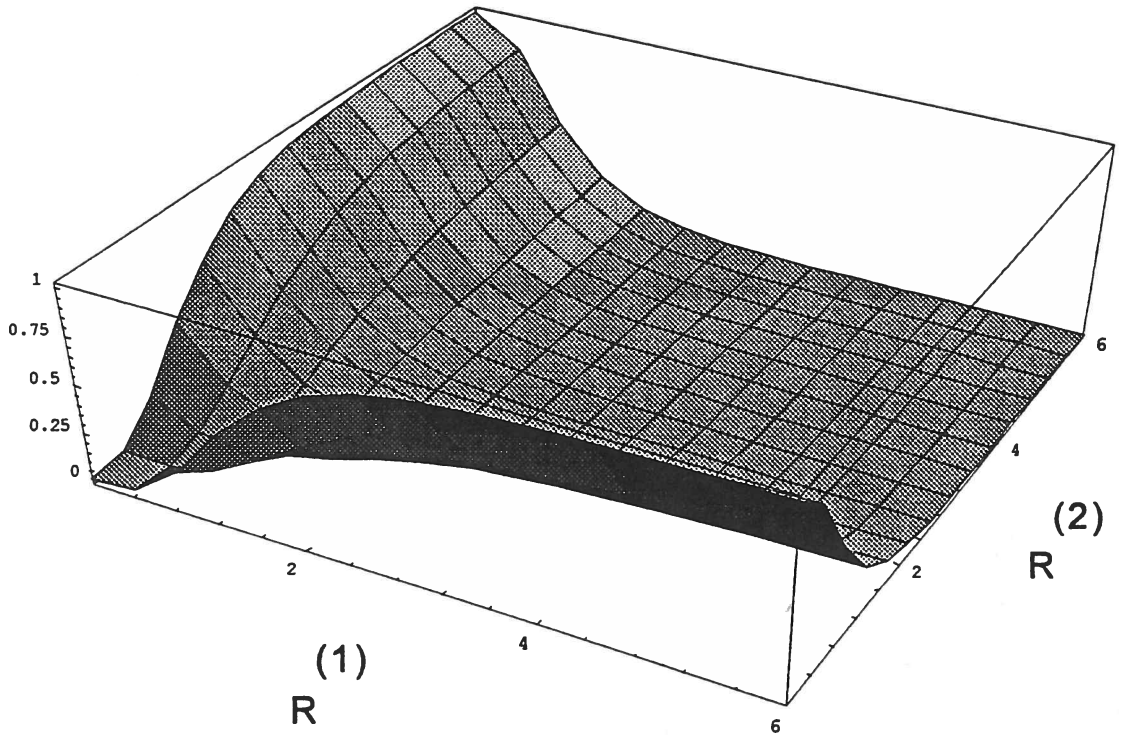


Figure 1 Power of the (randomised) test for H:$R^1$=$R^2$ plotted against possible combinations of $R^1$ and $R^2$. For m=2 and s=i=5.

In field studies, where usually large data-sets are available, one could use a standard likelihood ratio statistic (see McCullagh and Nelder 1989, p.472). However, the authors found that applying the likelihood ratio test to a data-set of the size described in De Jong and Kimman (1994) leads to a test that is too liberal.

As an example, consider the data described in the previous section. Now the interest is in testing whether there is a statistically significant difference between vaccinated and non-vaccinated animals. The p-value obtained by plugging in the MLE of R is 0.024 and the p-value based on the likelihood ratio statistic is 0.004. The maximum probability as defined above is attained for R=1.322 and yields a p-value 0.030. In this specific example a statistical significant difference is found for all three methods. It is not difficult, however, to construct an example where the different tests lead to different decisions regarding the significance of the observed difference, and then the conservative test is the safest to use.

At the end of this paper Tables 3-7 show p-values for the most plausible experimental designs. The p-values presented correspond to a slightly different testing problem than the one treated in this section. In transmission experiments one is often interested in testing H:$R_t$=$R_c$ against the one-sided alternative A:$R_t$<$R_c$. Here $R_t$ denotes the R in a treated (for example, vaccinated) population and $R_c$ denotes the R in the control population. Often it is safe to

presuppose that, in any case, treatment will not result in a larger R and thus a one-sided test is allowed. The test statistic is now the (total) number of contact-infected animals in the control group(s) minus the (total) number of contact-infected animals in the treatment group(s). The hypothesis is rejected for large values of this test statistic. The p-values in the tables are, analogous to the p-values for the two-sided problem, calculated by finding the probability that the test statistic is at least as large as the value actually found if the hypothesis is true. We could also have applied one-sided testing in the example in De Jong and Kimman (1994).

Different tables correspond with different values of s and i. In each tables the values of $n_c$ and $n_t$ are given in the first two columns. Here $n_c$ is the number of times the experiment with untreated animals is performed. Similarly, $n_t$ is the number of times the experiment with treated animals is performed. The columns 3 to 21 give the p-values with, in the top row, the test statistic as calculated from the experimental results. Finally the last column gives the power for the experimental design with $R_t$=0.5 and $R_c$=10.

As an example, suppose that in two experiments with s=i=5 there were zero and four contact infections in the vaccine group, while in the non-vaccinated control groups there were each time five contact infections. The value of the test statistic is then 10-4=6. The p-value can be found in the table with s=i=5 in the row with $n_c$=2 and $n_t$=2. Thus the p-value is 0.034 and hence the vaccine significantly reduces transmission.

## WHICH DESIGN IS OPTIMAL

Given a number of animals available, several designs are possible. For example, one can choose a few large experiments (m small, s and i large) or several small experiments (m large, s and i small). The extreme, on one hand, is a large experiment with all animals in it. The other extreme is as many experiments as possible with s=i=1 each. Furthermore, given the number of animals in an experiment, one can choose s large and i small, s small and i large or s≈i.

It is intuitively clear that it is not efficient to choose s>>i. In this case, minor outbreaks will also occur for R's larger than one and the experiment will not be very informative when a minor outbreak is observed. Intuitively, it is also not efficient to choose s << i. In this case, all susceptibles can be infected, even if R is smaller than one. This suggests that a design with s≈i is a good compromise between different objectives. Furthermore, a large experiment is intuitively more efficient than many small ones if the interest is in predicting the final size in a large population.

The comparison between different designs can also be done more quantitatively. One can pose the question "which design is optimal to estimate a specific function of R?". A sensible choice for this function is 'the expected proportion of infected individuals in a large susceptible population if an infective is introduced'. This proportion is zero if R is smaller than one and goes to one if R increases. An implicit formula can be found in Kermack and McKendrick (1927). If a maximum likelihood estimate is obtained for R the maximum likelihood estimate for a function of R is obtained by substitution. Hence it is straightforward to calculate, for each design of interest, the mean squared error (MSE) of this estimate. Subsequently the design can be chosen for which the MSE, as a function of R is smallest. In this way designs are compared by the information they provide about the size of an eventual epidemic.

Several designs were compared in this way and this confirmed that a design with s≈i is the most sensible choice. As an illustration, some designs with 20 animals were compared. The comparison showed that designs with s>>i have large MSE's for large R's. Designs with s<<i have large MSE's for small R's.

A comparison was made for the MSE's for some designs using 72 animals. The comparison confirmed that one large experiment is better than many small experiments. However, there is not much difference in MSE between one large experiment and a few repetitions of smaller experiments. It is advisable to have at least some repetitions to make the conclusions more robust with respect to the model assumptions.

If the reproduction ratios of two populations are compared, the quantitative criterion in comparing designs is the power of the test described above. For each design the critical region of the test can be obtained and subsequently the probability of rejection under the alternative hypothesis can be calculated. To avoid complications with the discreteness of the probability distribution involved, a mathematical trick is performed: designs are compared by the corresponding randomised tests (see Lehmann (1986). This ensures that the probability of an incorrect significant result is exactly 0.05 for each design. The authors do not propose that these randomised tests are performed in practice, but they can be used for a fair comparison. Many 3-dimensional plots were made of the power for different designs. Uniform dominance is never seen, but it is clear that again, given a number of animals in an experiment, s and i should be chosen approximately equal. Also the power of large and small experiments was compared. It was seen that in the region of most interest, both $R_t$ and $R_c$ approximately equal to one, it is better to have one large experiment. If R≈1, somewhat more infections occur in a large experiment than in the sum of a few small experiments.

## DISCUSSION

In this paper we have compared various estimation and testing techniques to analyse data from transmission experiments. We have also tried out various experimental designs. The purpose of the paper is to provide epidemiologists with ready access to the methodology needed to perform and analyse transmission experiments. As far as we know, this is the first time this has been done. It turns out that, even with relatively few animals, transmission experiments are very informative and are therefore potentially very useful in studying infectious diseases.

As always, it is not obvious how to extrapolate the outcome of an experiment to the situation in the field. Animals used in experiments are often markedly different from animals in practice, and housing conditions differ. Another complication is that the infectivity of experimentally infected animals may not be the same as that of the contact-infected ones. A rough measure of infectivity used in the experiments described in De Jong and Kimman (1994) was virus excretion and no difference was found. In Bouma et al. (1996) a large difference was found and, in Bouma et al. (1997), a similar difference in a different setting, was indicative of differences in infectivity. In spite of these complications, performing transmission experiments is valuable as this leads to information about causal effects of interventions on transmission. Additionally, it remains important to obtain observational data.

As the outcome of a transmission experiment consists of only one observation, strong model assumptions have to be made to derive meaningful conclusions. In this paper a model is described that has been used in practice and leads to acceptable results. However, the

assumptions of the model can be questioned for any particular disease. Therefore we investigated the robustness of the model, that is, how sensitive the conclusions are with respect to changes in the assumptions. More precisely, we studied the mean squared error in the same way as described in the previous section, but with the data drawn from probability distributions corresponding to different models. It turned out that the MSEs were acceptable and, in the case of a constant duration of infectiousness, even smaller than if the observations are sampled from the SIR model. It follows that the model can safely serve as an approximation to reality, even if the assumptions are only approximately and not exactly satisfied.

In the paper it is argued that choosing $s \approx i$ is optimal to study the most relevant characteristics of R. Furthermore, if a certain number of animals is available, it is more efficient to perform one large transmission experiment than many small ones. It is obvious that this conclusion depends on the model. Other models can be constructed for which it is optimal to have more than one repetition. Furthermore, in the model assumed in this paper, there was no big difference between the information provided by one large experiment and that of a few repetitions of smaller ones. Therefore, we recommend at least some replications as this makes the experiment more robust. It is also typically easier and cheaper to perform a few, but not too, large experiments than many small ones.

For experimental data, the maximum likelihood estimator is to be preferred to the martingale estimator. The latter is more robust under changes of the model assumptions, but applying it is questionable if all susceptibles are infected as frequently happens in the control groups. Different populations should be compared on the basis of the test described in this paper. This method is conservative, but has reasonable power in the region of interest.

For experiments of a 'usual' size there are no computational limitations and the techniques can be implemented in many computer programs. If the same methods are used for large observational data-sets the computations take too long. In this case 'asymptotic' methods, for example the likelihood ratio test, are more appropriate.

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Table 1. Maximum likelihood estimates for R[$], lower and upper boundaries for the confidence intervals for R and p-values for the test $H_0$:R≥1. The first three columns give the number of susceptibles (S) the number of infectives (I) and the number of repetitions (m). The fourth column gives the total number of infected animals (C).

| s | i | m | C | MLE | CI-low | CI-up | p-value |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 2 | 0 | 0.000 | 0.000 | 10.649 | NS |
| 1 | 1 | 2 | 1 | 2.000 | 0.025 | 156.993 | NS |
| 1 | 1 | 2 | 2 | ∞ | 0.376 | ∞ | NS |
| 1 | 1 | 3 | 0 | 0.000 | 0.000 | 4.840 | NS |
| 1 | 1 | 3 | 1 | 1.000 | 0.017 | 19.209 | NS |
| 1 | 1 | 3 | 2 | 4.000 | 0.208 | 235.998 | NS |
| 1 | 1 | 3 | 3 | ∞ | 0.826 | ∞ | NS |
| 1 | 1 | 4 | 0 | 0.000 | 0.000 | 3.030 | NS |
| 1 | 1 | 4 | 1 | 0.667 | 0.013 | 8.303 | NS |
| 1 | 1 | 4 | 2 | 2.000 | 0.145 | 27.592 | NS |
| 1 | 1 | 4 | 3 | 6.000 | 0.482 | 314.984 | NS |
| 1 | 1 | 4 | 4 | ∞ | 1.320 | ∞ | NS |
| 1 | 1 | 5 | 0 | 0.000 | 0.000 | 2.183 | NS |
| 1 | 1 | 5 | 1 | 0.500 | 0.010 | 5.053 | NS |
| 1 | 1 | 5 | 2 | 1.333 | 0.111 | 11.640 | NS |
| 1 | 1 | 5 | 3 | 3.000 | 0.344 | 35.918 | NS |
| 1 | 1 | 5 | 4 | 8.000 | 0.792 | 393.980 | NS |
| 1 | 1 | 5 | 5 | ∞ | 1.833 | ∞ | NS |
| 1 | 1 | 6 | 0 | 0.000 | 0.000 | 1.699 | NS |
| 1 | 1 | 6 | 1 | 0.400 | 0.008 | 3.575 | NS |
| 1 | 1 | 6 | 2 | 1.000 | 0.090 | 6.978 | NS |
| 1 | 1 | 6 | 3 | 2.000 | 0.268 | 14.932 | NS |
| 1 | 1 | 6 | 4 | 4.000 | 0.573 | 44.219 | NS |
| 1 | 1 | 6 | 5 | 10.000 | 1.119 | 472.975 | NS |
| 1 | 1 | 6 | 6 | ∞ | 2.355 | ∞ | NS |
| 1 | 1 | 7 | 0 | 0.000 | 0.000 | 1.388 | NS |
| 1 | 1 | 7 | 1 | 0.333 | 0.007 | 2.747 | NS |
| 1 | 1 | 7 | 2 | 0.800 | 0.076 | 4.887 | NS |
| 1 | 1 | 7 | 3 | 1.500 | 0.220 | 8.866 | NS |
| 1 | 1 | 7 | 4 | 2.667 | 0.451 | 18.204 | NS |
| 1 | 1 | 7 | 5 | 5.000 | 0.819 | 52.506 | NS |
| 1 | 1 | 7 | 6 | 12.000 | 1.456 | 551.970 | NS |
| 1 | 1 | 7 | 7 | ∞ | 2.883 | ∞ | NS |
| 1 | 1 | 8 | 0 | 0.000 | 0.000 | 1.172 | 0.039 |
| 1 | 1 | 8 | 1 | 0.286 | 0.006 | 2.224 | NS |
| 1 | 1 | 8 | 2 | 0.667 | 0.066 | 3.728 | NS |
| 1 | 1 | 8 | 3 | 1.200 | 0.186 | 6.168 | NS |

[$] Note that in this particular design the MLE can be derived analytically: $R_{MLE}=2C/(m-C)$

Table 1. continued

| s | i | m | C | MLE | CI-low | CI-up | p-value |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 8 | 4 | 2.000 | 0.373 | 10.738 | NS |
| 1 | 1 | 8 | 5 | 3.333 | 0.649 | 21.465 | NS |
| 1 | 1 | 8 | 6 | 6.000 | 1.073 | 60.786 | NS |
| 1 | 1 | 8 | 7 | 14.000 | 1.799 | 630.962 | NS |
| 1 | 1 | 8 | 8 | ∞ | 3.414 | ∞ | NS |
| 1 | 1 | 9 | 0 | 0.000 | 0.000 | 1.013 | 0.026 |
| 1 | 1 | 9 | 1 | 0.250 | 0.006 | 1.865 | NS |
| 1 | 1 | 9 | 2 | 0.571 | 0.058 | 3.001 | NS |
| 1 | 1 | 9 | 3 | 1.000 | 0.162 | 4.682 | NS |
| 1 | 1 | 9 | 4 | 1.600 | 0.317 | 7.434 | NS |
| 1 | 1 | 9 | 5 | 2.500 | 0.538 | 12.599 | NS |
| 1 | 1 | 9 | 6 | 4.000 | 0.854 | 24.719 | NS |
| 1 | 1 | 9 | 7 | 7.000 | 1.333 | 69.061 | NS |
| 1 | 1 | 9 | 8 | 16.000 | 2.145 | 709.952 | NS |
| 1 | 1 | 9 | 9 | ∞ | 3.948 | ∞ | NS |
| 1 | 1 | 10 | 0 | 0.000 | 0.000 | 0.892 | 0.017 |
| 1 | 1 | 10 | 1 | 0.222 | 0.005 | 1.604 | NS |
| 1 | 1 | 10 | 2 | 0.500 | 0.052 | 2.505 | NS |
| 1 | 1 | 10 | 3 | 0.857 | 0.143 | 3.755 | NS |
| 1 | 1 | 10 | 4 | 1.333 | 0.277 | 5.623 | NS |
| 1 | 1 | 10 | 5 | 2.000 | 0.460 | 8.690 | NS |
| 1 | 1 | 10 | 6 | 3.000 | 0.711 | 14.454 | NS |
| 1 | 1 | 10 | 7 | 4.667 | 1.065 | 27.967 | NS |
| 1 | 1 | 10 | 8 | 8.000 | 1.597 | 77.331 | NS |
| 1 | 1 | 10 | 9 | 18.000 | 2.494 | 788.939 | NS |
| 1 | 1 | 10 | 10 | ∞ | 4.483 | ∞ | NS |

Table 2. Maximum likelihood estimates for R, lower and upper boundaries for the confidence intervals for R and p-values for the test $H_0$:R≥1. The first three columns give the number of susceptibles (S) the number of infectives (I) and the number of repetitions (m). The fourth column gives the numbers of infected animals ($X_1,\ldots,X_m$).

| s | i | m | $X_1,\ldots,X_m$ | MLE | CI-low | CI-up | p-value |
|---|---|---|---|---|---|---|---|
| 2 | 2 | 1 | 0 | 0.000 | 0.000 | 10.649 | NS |
| 2 | 2 | 1 | 1 | 1.008 | 0.025 | 15.489 | NS |
| 2 | 2 | 1 | 2 | ∞ | 0.226 | ∞ | NS |
| 2 | 2 | 2 | 0,0 | 0.000 | 0.000 | 3.030 | NS |
| 2 | 2 | 2 | 1,0 | 0.515 | 0.013 | 4.892 | NS |
| 2 | 2 | 2 | 1,1 | 1.008 | 0.117 | 15.878 | NS |
| 2 | 2 | 2 | 2,0 | 1.684 | 0.117 | 15.878 | NS |
| 2 | 2 | 2 | 2,1 | 2.340 | 0.343 | 21.590 | NS |
| 2 | 2 | 2 | 2,2 | ∞ | 0.719 | ∞ | NS |
| 2 | 2 | 3 | 0,0,0 | 0.000 | 0.000 | 1.699 | NS |
| 2 | 2 | 3 | 1,0,0 | 0.342 | 0.008 | 2.750 | NS |
| 2 | 2 | 3 | 1,1,0 | 0.685 | 0.079 | 4.750 | NS |
| 2 | 2 | 3 | 1,1,1 | 1.008 | 0.213 | 6.884 | NS |
| 2 | 2 | 3 | 2,0,0 | 0.918 | 0.079 | 4.750 | NS |
| 2 | 2 | 3 | 2,1,0 | 1.359 | 0.213 | 6.884 | NS |
| 2 | 2 | 3 | 2,1,1 | 1.743 | 0.408 | 19.874 | NS |
| 2 | 2 | 3 | 2,2,0 | 2.910 | 0.408 | 19.874 | NS |
| 2 | 2 | 3 | 2,2,1 | 3.299 | 0.706 | 26.113 | NS |
| 2 | 2 | 3 | 2,2,2 | ∞ | 1.173 | ∞ | NS |
| 2 | 2 | 4 | 0,0,0,0 | 0.000 | 0.000 | 1.172 | 0.039 |
| 2 | 2 | 4 | 1,0,0,0 | 0.256 | 0.006 | 1.882 | NS |
| 2 | 2 | 4 | 1,1,0,0 | 0.515 | 0.060 | 2.820 | NS |
| 2 | 2 | 4 | 1,1,1,0 | 0.768 | 0.156 | 3.915 | NS |
| 2 | 2 | 4 | 1,1,1,1 | 1.008 | 0.290 | 6.046 | NS |
| 2 | 2 | 4 | 2,0,0,0 | 0.630 | 0.060 | 2.820 | NS |
| 2 | 2 | 4 | 2,1,0,0 | 0.948 | 0.156 | 3.915 | NS |
| 2 | 2 | 4 | 2,1,1,0 | 1.246 | 0.290 | 6.046 | NS |
| 2 | 2 | 4 | 2,1,1,1 | 1.518 | 0.466 | 8.413 | NS |
| 2 | 2 | 4 | 2,2,0,0 | 1.684 | 0.290 | 6.046 | NS |
| 2 | 2 | 4 | 2,2,1,0 | 2.039 | 0.466 | 8.413 | NS |
| 2 | 2 | 4 | 2,2,1,1 | 2.340 | 0.700 | 23.239 | NS |
| 2 | 2 | 4 | 2,2,2,0 | 3.885 | 0.700 | 23.239 | NS |
| 2 | 2 | 4 | 2,2,2,1 | 4.075 | 1.042 | 29.854 | NS |
| 2 | 2 | 4 | 2,2,2,2 | ∞ | 1.576 | ∞ | NS |
| 4 | 4 | 1 | 0 | 0.000 | 0.000 | 3.030 | NS |
| 4 | 4 | 1 | 1 | 0.471 | 0.013 | 4.446 | NS |
| 4 | 4 | 1 | 2 | 0.974 | 0.108 | 5.885 | NS |

Table 2 continued

| s | i | m | $X_1,\ldots,X_m$ | MLE | CI-low | CI-up | p-value |
|---|---|---|---|---|---|---|---|
| 4 | 4 | 1 | 3 | 1.740 | 0.274 | 8.651 | NS |
| 4 | 4 | 1 | 4 | ∞ | 0.548 | ∞ | NS |
| 4 | 4 | 2 | 0,0 | 0.000 | 0.000 | 1.172 | 0.039 |
| 4 | 4 | 2 | 1,0 | 0.244 | 0.006 | 1.786 | NS |
| 4 | 4 | 2 | 1,1 | 0.471 | 0.057 | 2.365 | NS |
| 4 | 4 | 2 | 2,0 | 0.505 | 0.057 | 2.365 | NS |
| 4 | 4 | 2 | 2,1 | 0.722 | 0.142 | 3.048 | NS |
| 4 | 4 | 2 | 2,2 | 0.974 | 0.253 | 4.406 | NS |
| 4 | 4 | 2 | 3,0 | 0.846 | 0.142 | 3.048 | NS |
| 4 | 4 | 2 | 3,1 | 1.061 | 0.253 | 4.406 | NS |
| 4 | 4 | 2 | 3,2 | 1.329 | 0.390 | 5.761 | NS |
| 4 | 4 | 2 | 3,3 | 1.740 | 0.560 | 7.311 | NS |
| 4 | 4 | 2 | 4,0 | 1.515 | 0.253 | 4.406 | NS |
| 4 | 4 | 2 | 4,1 | 1.744 | 0.390 | 5.761 | NS |
| 4 | 4 | 2 | 4,2 | 2.097 | 0.560 | 7.311 | NS |
| 4 | 4 | 2 | 4,3 | 2.778 | 0.784 | 10.448 | NS |
| 4 | 4 | 2 | 4,4 | ∞ | 1.144 | ∞ | NS |
| 5 | 5 | 1 | 0 | 0.000 | 0.000 | 2.182 | NS |
| 5 | 5 | 1 | 1 | 0.378 | 0.010 | 3.223 | NS |
| 5 | 5 | 1 | 2 | 0.755 | 0.087 | 4.175 | NS |
| 5 | 5 | 1 | 3 | 1.210 | 0.216 | 5.393 | NS |
| 5 | 5 | 1 | 4 | 1.949 | 0.393 | 8.023 | NS |
| 5 | 5 | 1 | 5 | ∞ | 0.678 | ∞ | NS |
| 5 | 5 | 2 | 0,0 | 0.000 | 0.000 | 0.892 | 0.017 |
| 5 | 5 | 2 | 1,0 | 0.195 | 0.005 | 1.354 | NS |
| 5 | 5 | 2 | 1,1 | 0.378 | 0.046 | 1.767 | NS |
| 5 | 5 | 2 | 2,0 | 0.394 | 0.046 | 1.767 | NS |
| 5 | 5 | 2 | 2,1 | 0.568 | 0.114 | 2.190 | NS |
| 5 | 5 | 2 | 2,2 | 0.755 | 0.198 | 2.685 | NS |
| 5 | 5 | 2 | 3,0 | 0.622 | 0.114 | 2.190 | NS |
| 5 | 5 | 2 | 3,1 | 0.790 | 0.198 | 2.685 | NS |
| 5 | 5 | 2 | 3,2 | 0.978 | 0.298 | 3.438 | NS |
| 5 | 5 | 2 | 3,3 | 1.211 | 0.414 | 4.276 | NS |
| 5 | 5 | 2 | 4,0 | 0.925 | 0.198 | 2.685 | NS |
| 5 | 5 | 2 | 4,1 | 1.092 | 0.298 | 3.438 | NS |
| 5 | 5 | 2 | 4,2 | 1.289 | 0.414 | 4.276 | NS |
| 5 | 5 | 2 | 4,3 | 1.548 | 0.550 | 5.219 | NS |
| 5 | 5 | 2 | 4,4 | 1.949 | 0.717 | 6.530 | NS |
| 5 | 5 | 2 | 5,0 | 1.480 | 0.298 | 3.438 | NS |
| 5 | 5 | 2 | 5,1 | 1.653 | 0.414 | 4.276 | NS |
| 5 | 5 | 2 | 5,2 | 1.889 | 0.550 | 5.219 | NS |
| 5 | 5 | 2 | 5,3 | 2.246 | 0.717 | 6.530 | NS |
| 5 | 5 | 2 | 5,4 | 2.923 | 0.939 | 9.493 | NS |

Table 2 continued

| s | i | m | $X_1,\ldots,X_m$ | MLE | CI-low | CI-up | p-value |
|---|---|---|---|---|---|---|---|
| 5 | 5 | 2 | 5,5 | ∞ | 1.298 | ∞ | NS |
| 10 | 10 | 1 | 0 | 0.000 | 0.000 | 0.892 | 0.017 |
| 10 | 10 | 1 | 1 | 0.192 | 0.005 | 1.335 | NS |
| 10 | 10 | 1 | 2 | 0.375 | 0.045 | 1.710 | NS |
| 10 | 10 | 1 | 3 | 0.556 | 0.111 | 2.064 | NS |
| 10 | 10 | 1 | 4 | 0.744 | 0.190 | 2.423 | NS |
| 10 | 10 | 1 | 5 | 0.947 | 0.280 | 2.817 | NS |
| 10 | 10 | 1 | 6 | 1.180 | 0.382 | 3.288 | NS |
| 10 | 10 | 1 | 7 | 1.468 | 0.500 | 3.920 | NS |
| 10 | 10 | 1 | 8 | 1.868 | 0.646 | 4.936 | NS |
| 10 | 10 | 1 | 9 | 2.563 | 0.840 | 7.362 | NS |
| 10 | 10 | 1 | 10 | ∞ | 1.158 | ∞ | NS |
| 20 | 20 | 1 | 0 | 0.000 | 0.000 | 0.405 | 0.000 |
| 20 | 20 | 1 | 1 | 0.098 | 0.003 | 0.609 | 0.002 |
| 20 | 20 | 1 | 2 | 0.192 | 0.023 | 0.784 | 0.007 |
| 20 | 20 | 1 | 3 | 0.284 | 0.058 | 0.944 | 0.018 |
| 20 | 20 | 1 | 4 | 0.374 | 0.099 | 1.095 | 0.040 |
| 20 | 20 | 1 | 5 | 0.463 | 0.145 | 1.242 | NS |
| 20 | 20 | 1 | 6 | 0.553 | 0.194 | 1.386 | NS |
| 20 | 20 | 1 | 7 | 0.644 | 0.245 | 1.532 | NS |
| 20 | 20 | 1 | 8 | 0.737 | 0.299 | 1.680 | NS |
| 20 | 20 | 1 | 9 | 0.834 | 0.355 | 1.835 | NS |
| 20 | 20 | 1 | 10 | 0.936 | 0.414 | 1.998 | NS |
| 20 | 20 | 1 | 11 | 1.045 | 0.477 | 2.175 | NS |
| 20 | 20 | 1 | 12 | 1.163 | 0.545 | 2.370 | NS |
| 20 | 20 | 1 | 13 | 1.294 | 0.617 | 2.592 | NS |
| 20 | 20 | 1 | 14 | 1.443 | 0.698 | 2.852 | NS |
| 20 | 20 | 1 | 15 | 1.616 | 0.788 | 3.170 | NS |
| 20 | 20 | 1 | 16 | 1.827 | 0.891 | 3.581 | NS |
| 20 | 20 | 1 | 17 | 2.101 | 1.015 | 4.162 | NS |
| 20 | 20 | 1 | 18 | 2.491 | 1.169 | 5.123 | NS |
| 20 | 20 | 1 | 19 | 3.177 | 1.381 | 7.442 | NS |
| 20 | 20 | 1 | 20 | ∞ | 1.731 | ∞ | NS |

Table 3 – 5. P-values for one sided test $H_0$:$R_t$=$R_c$ against $H_a$:$R_t$<$R_c$ for several designs. Design per replication left top corner of each table. The number repetitions for treatment (t) and control groups are given in the first two columns. Further explanation in the text.

| $(S_0, I_0, N_{tot})$ = (2, 2, 4) | | $R_0c$ = 10 | | $R_0t$ = 0.5 | | $\alpha$ = 0.05 | | | | | | | | | | | | ID-Lelystad |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| repetitions | | critical values | | | | | | | | | | | | | | | | power |
| $n_c$ | $n_t$ | 1-2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19-21 | |
| 1 | 1 | > | | | | | | | | | | | | | | | | | | 0.000 |
| 2 | 2 | > | > | 0.0155 | | | | | | | | | | | | | | | | 0.363 |
| 3 | 3 | > | > | 0.0430 | 0.0115 | 0.0019 | | | | | | | | | | | | | | 0.770 |
| 4 | 4 | > | > | > | 0.0273 | 0.0084 | 0.0018 | 0.0002 | | | | | | | | | | | | 0.819 |
| 5 | 5 | > | > | > | 0.0440 | 0.0176 | 0.0058 | 0.0015 | 0.0003 | 3.0E-05 | | | | | | | | | | 0.940 |
| 6 | 6 | > | > | > | > | 0.0281 | 0.0114 | 0.0039 | 0.0011 | 0.0003 | 4.1E-05 | 3.8E-06 | | | | | | | | 0.950 |
| 7 | 7 | > | > | > | > | 0.0391 | 0.0181 | 0.0074 | 0.0027 | 0.0008 | 0.0002 | 4.1E-5 | 5.8E-06 | 4.7E-07 | | | | | | 0.983 |
| 8 | 8 | > | > | > | > | > | 0.0255 | 0.0118 | 0.0049 | 0.0018 | 0.0006 | 0.0002 | 3.6E-05 | 6.5E-06 | 8.2E-07 | 5.8E-08 | | | | 0.986 |
| 9 | 9 | > | > | > | > | > | 0.0332 | 0.0167 | 0.0077 | 0.0032 | 0.0012 | 0.0004 | 0.0001 | 3.0E-05 | 6.1E-06 | 9.9E-07 | 1.1E-07 | 7.3E-09 | | 0.995 |

| $(S_0, I_0, N_{tot})$ = (5, 5, 10) | | $R_0c$ = 10 | | $R_0t$ = 0.5 | | $\alpha$ = 0.05 | | | | | | | | | | | | | | ID-Lelystad |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| repetitions | | critical values | | | | | | | | | | | | | | | | | | power |
| $n_c$ | $n_t$ | 1-4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | |
| 1 | 1 | > | 0.0133 | | | | | | | | | | | | | | | | | 0.324 |
| 2 | 2 | > | > | 0.0338 | 0.0141 | 0.0048 | 0.0012 | 0.0001 | | | | | | | | | | | | 0.871 |
| 3 | 3 | > | > | > | 0.0395 | 0.0206 | 0.0097 | 0.0041 | 0.0015 | 0.0005 | 0.0001 | 2.3E-05 | 2.3E-06 | | | | | | | 0.981 |
| 4 | 4 | > | > | > | > | 0.0397 | 0.0229 | 0.0124 | 0.0063 | 0.0029 | 0.0013 | 0.0005 | 0.0001 | 5.2E-05 | 1.3E-05 | 2.7E-06 | 4.0E-07 | 3.1E-08 | | 0.997 |

| $(S_0,I_0,N_{tot})$ =(10,10,20) | | $R_0$ c= 10 | | $R_0$ t = 0.5 | | | $\alpha$ = 0.05 | | | | | | | | | | | | ID-Lelystad |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| repetitions | | critical values | | | | | | | | | | | | | | | | | power |
| $n_c$ | $n_t$ | 2-5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | |
| 1 | 1 | > | 0.0420 | 0.0188 | 0.0069 | 0.0019 | 0.0003 | | | | | | | | | | | | 0.849 |
| 2 | 2 | > | > | > | 0.0481 | 0.0290 | 0.0165 | 0.0088 | 0.0044 | 0.0020 | 0.0009 | 0.0003 | 0.0001 | 2.9E-05 | 6.4E-06 | 8.4E-08 | 1.0E-06 | | | 0.996 |

$n_c$ = # of groups with control treatment, $n_t$ = # of groups with experimental treatment, > = p-value larger then 0.05, empty cells; value cannot occur

# CLASSICAL SWINE FEVER

# PREDICTION OF THE LIKELIHOOD OF SPREAD OF CLASSICAL SWINE FEVER VIRUS THROUGH 'NEIGHBOURHOOD INFECTIONS' FOR DIFFERENT REGIONS IN BELGIUM

K. MINTIENS[♣], H. LAEVENS[♦], J. DEWULF[♦] AND F. BOELAERT[♣]

## SUMMARY

This paper describes a study carried out in the frame of the FAIR-project 'Development of Prevention and Control Strategies to Address Animal Health and Related Problems in Densely Populated Livestock Areas of the Community'. One of the objectives of the project was to identify areas in the European Union which contain a higher risk for the spread of emerging diseases such as classical swine fever (CSF) or foot and mouth disease. In an earlier study, the likelihood of neighbourhood infections occurring in the vicinity of a CSF-infected herd was estimated. This was based on a risk analysis of the 1994 Belgian CSF epidemic. In that study, it was found that a bivariate kernel estimate of the intensity of neighbouring pig herds surrounding a CSF-infected herd within a 1-km radius was the best predictor variable.

In this study, the bivariate kernel estimate of intensity of neighbouring pig herds within a 1-km radius was calculated for all Belgian pig herds based on available geographical co-ordinates. In a second step, the likelihood for the occurrence of neighbourhood infections within a 1-km radius was predicted for every Belgian pig herd, given that the herd was infected with CSF-virus. For the prediction of these likelihoods, the model resulting from the risk analysis study mentioned above was used. Finally, the predicted likelihoods were displayed on a national map of Belgium after the application of smoothing techniques. As a result, areas of high or low risk for the spread of CSF-virus spread through neighbourhood infections were identified. The areas in Belgium where CSF-outbreaks occurred in the past decade were all predicted to be of high risk.

## INTRODUCTION

Economic pressures have lead to the development of regions of highly concentrated livestock populations in certain areas of the European Union (EU). These areas also appear to be at an increased risk from epizootic diseases. Therefore, despite the economical advantages, these areas may prove to be unsustainable over the longer term. It was from this framework that

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[♣] Co-ordination Centre for Veterinary Diagnostics, Veterinary and Agrochemical Research Centre, Groeselenberg 99, B-1180 Brussels, Belgium. E-mail: koen.mintiens@var.fgov.be

[♦] Veterinary Epidemiology Unit, Department of Reproduction, Obstetrics and Herd Health, School of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium

the Scientific Veterinary Committee of the European Commission urged for the need to identify and classify the densely populated livestock areas within the EU.

The aim of this study was to classify the different regions within Belgium according to the risk of occurrence of neighbourhood infections from classical swine fever (CSF). This study incorporated data from a CSF epidemic (Westergaard, 1996). The risk was estimated by predicting the probability that pig herds would become infected with CSF when they were located within a 1-km radius of a primary CSF-infected pig herd.

## MATERIALS AND METHODS

### Model

The logistic regression model, which was used for the prediction of the risk for the occurrence of neighbourhood infections, resulted from a risk factor analysis of a data set obtained during the control of the 1994 CSF epidemic that occurred in the East Flanders province of Belgium (Koenen et al., 1996; Mintiens et al., 2000a). Apart from the intercept, the model contained one predictor variable (Table 1) which was a bivariate kernel estimate of intensity of pig herds within a 1-km radius surrounding a primary CSF-infected herd (Bailey & Gatrell, 1995). A higher value for the kernel estimate incorporated a higher risk of the occurrence of neighbourhood infections (Table 1).

Table 1. Parameter estimates for the logistic regression model

| Predictor variable | Parameter estimate |
|---|---|
| Intercept | -2.9580 |
| Bivariate kernel estimate of intensity | 0.2957 |

### Data

The bivariate kernel estimate of intensity was calculated for every pig herd in Belgium where the exact location by geographical co-ordinates was available. The Belgian Veterinary Services obtained these co-ordinates in 1997 through a query in which farmers were asked to indicate their premises on a 1:10,000 scale topographic map.

The distance between the different herds was calculated using the geographical co-ordinates and the Pythagoras theorem. For every pig herd, all other pig herds contained in the neighbourhood (1-km radius) was identified. In a next step, the kernel estimate of intensity was calculated for every pig herd based on the location of the other herds in the neighbourhood. This kernel estimation was used to predict the risk of neighbourhood infections for every pig herd if this herd were primarily infected with CSF-virus.

Graphical presentation of the predicted risk

The predicted risk for the transmission of neighbourhood infection was plotted on a vector map displaying the Belgian municipalities using a geographical information system (ArcView GIS 3.2a, ESRI Inc.). The point estimates of the predicted risk were smoothed out by transfer into a continuous surface variable using an Inverse Distance Weighted (IDW) interpolator (Anon., 1996).

## RESULTS

No geographical co-ordinates were available for 34 (0.003%) out of the 13,115 pig herds located in Belgium during 1997. Of the remaining pig herds, 1,202 (11.9%) did not have other pig herds in their neighbourhood. For the pig herds with neighbouring herds within a 1-km radius, the kernel estimate of intensity varied from $7.7 \times 10^{-8}$ to 13.022 (mean = 2.306; median = 1.817).

For the pig herds with no neighbouring herds within a 1-km radius, the risk for 'neighbourhood infections' was set to zero. For the pig herds with neighbouring herds, the predicted risk varied from 0.05 to 0.71 (mean = 0.11; median = 0.71). The predicted risk is mapped in Fig. 1. Even after smoothing, areas with a potential higher risk for neighbourhood infections can be easily noticed from the map e.g. 'Wingene-Tielt' and 'Tongeren'.

## DISCUSSION

The logistic regression model used for predicting the risk of neighbourhood infections was based on a data set collected during a real CSF outbreak in 1994. In this data set, the kernel estimate of intensity varied from 0.426 to 7.203. For this study, the model was used to predict a risk of neighbourhood infections based on a kernel estimate of intensity, which varied from $7.7 \times 10^{-8}$ to 13.022. This meant that the risk predictions for kernel estimates below 0.426 and above 7.203 were not necessarily correct. Therefore, this map should be seen as an illustration of a possible methodology for predicting the risk for CSF spread, instead of an accurate estimate of the risk. Nevertheless, in a number of the displayed areas of high risk of CSF, outbreaks have occurred in the past e.g. Berlare, Ravels-Merksplas, Wingene-Tielt, Zomergem-Aalter-Nevele and Bocholt-Bree (Lamsens, 1992; Koenen et al., 1996; Mintiens et al., 2000b).

One of the recent developments in the control and eradication of epizootic diseases is the implementation of decision support systems (DSS). The DSS allow the prediction of the evolution of a disease outbreak over variable time periods, based on epidemiological models which contain risk factors for the spread of the disease. This map, along with its underlying methodology, can be used as a DSS since the map can be consulted when a case of CSF infection is diagnosed. Thereafter, specific sanitary measures can be taken depending on the displayed risk of neighbourhood infections. The map can also be used in the situation where concessions are to be assigned to new pig farms. The assignment can then be based on the predicted risk of neighbourhood infections or the kernel estimate of intensity.

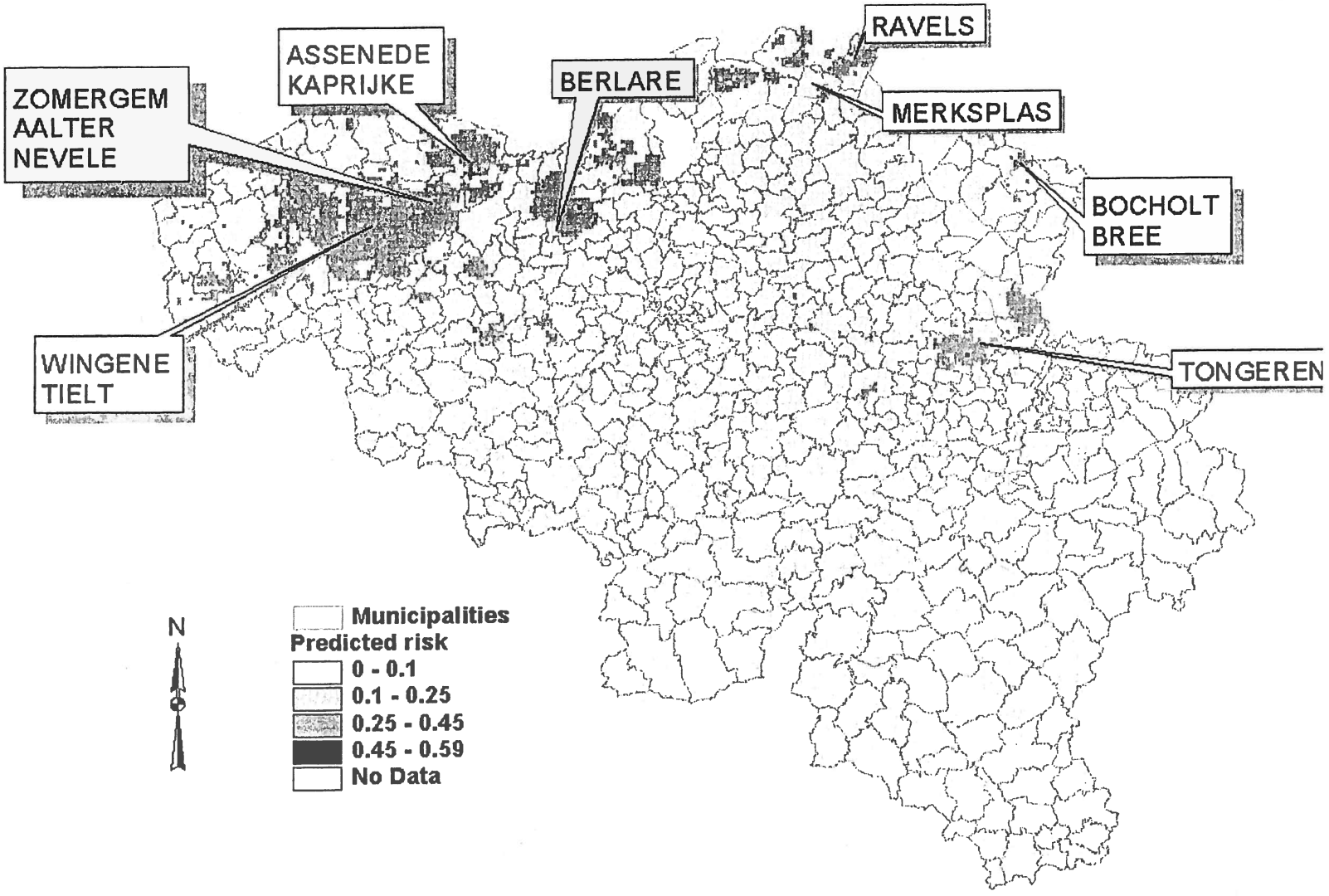


Fig. 1 Predicted risk of the development of CSF based on neighbourhood infections within a 1-km radius

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# FACTORS ASSOCIATED WITH THE INTRODUCTION OF CLASSICAL SWINE FEVER VIRUS INTO PIG HERDS IN THE CENTRAL AREA OF THE 1997-1998 EPIDEMIC IN THE NETHERLANDS

A.R.W. ELBERS [1], J.A. STEGEMAN [2,3] AND M.C.M. DE JONG [2]

## SUMMARY

A matched case-control study of 135 classical swine fever virus (CSFV) infected and 99 non-infected pig herds investigated factors that were associated with the introduction of the disease. The pig herds were selected from farms situated in the central area of the 1997-1998 CSF epidemic in the Netherlands. Five variables were found to be significantly associated with an increased risk of CSFV-infection: 1) The presence of commercial poultry on the premises; 2) Visitors could enter the pig units without wearing overcoat/overalls and boots supplied by the farmer; 3) The driver of the lorry that transported pigs in the framework of welfare slaughter used his own boots instead of boots available on the farm; 4) A moderate herd size (500-1000 animals) and an extreme herd size (> 7000 animals) compared to a small herd size (< 500 animals); 5) Aerosols produced by the high-pressure cleaning of the electrocution equipment used during depopulation of an infected neighbouring herd (< 250 m distance) were carried by the wind onto the farm. Two variables were significantly associated with a decreased risk of CSFV-infection: 1) more than 30 years of experience in pig farming; 2) additional cleaning of the transport lorries used to ship pigs in the framework of the buying-out scheme. This cleaning, which was carried out by the farmer, took place outside the premises before entrance to the farm was allowed.

## INTRODUCTION

After a five year absence of classical swine fever (CSF) from The Netherlands, a pig farm in the southern part of The Netherlands was diagnosed as being infected with CSF on 4 February 1997. This herd was located in an area within Europe which had one of the largest pig populations and highest pig herd densities (Pluimers et al., 1999). It is presumed that the CSF virus (CSFV) was introduced into the area by a transport lorry returning from Germany (Elbers et al., 1999). A total of 429 outbreaks were reported during the epidemic and approximately 625,000 pigs from infected herds were slaughtered. The 1997-1998 CSF epidemic spread extensively around the primary outbreak (Elbers et al., 1999), yet many herds remained uninfected in this central area.

[1] Dept. of Pig Health, Animal Health Service, PO Box 4, 5280 AA Boxtel, The Netherlands Email: a.elbers@gdvdieren.nl
[2] Quantitative Veterinary Epidemiology, Institute for Animal Science and Health, Lelystad.
[3] current address: Dept. of Farm Animal Health, Faculty of Vet. Medicine, University of Utrecht

Tracing of various parameters during the eradication campaign tried to establish how the virus had been introduced into the infected herds. The tracing focused on pigs, visitors, transport vehicles, artificial insemination, manure disposal and the pick-up service for rendering plants as the potential routes of transmission. However, in approximately 50% of the outbreaks none of these possible routes of transmission were found. Indeed, this observation is similar to the findings from other CSF epidemics in Europe (Vannier et al., 1986; Terpstra, 1987; Pittler et al., 1996, Vanthemsche, 1996). Clearly, this is an unsatisfactory situation. Usually, infected herds with an unknown source are subdivided into herds which were within a 1-km distance of an earlier infected herd and herds which were located at a greater distance. The first infected herds were designated as neighbourhood infections and the latter as infections with an unknown origin (Terpstra, 1987). In the 1997-1998 epidemic in The Netherlands, 39% of the outbreaks were classified as neighbourhood infections and 11% as unknown. The phenomenon, that herds at a close distance to a CSFV-infected herd have a higher risk of becoming infected than herds at a larger distance, was observed during the 1930's (Robijns, 1971), and it has continued to be relevant (Wachendörfer et al., 1978; Koenen et al., 1996; Stegeman et al., 2000). These neighbourhood infections may have been caused by contacts with potential sources of CSFV that were not reported to the veterinary authorities. However, they may also have been caused by types of contact that are not normally recognised for their ability to transmit the virus. Apart from an indication that airborne spread may play a role (Laevens, 1998; Mintiens et al., 2000), no other routes for these neighbourhood infections have as yet been identified.

The Dutch CSF epidemic spread extensively in the central area around the primary outbreak, yet there were a considerable number of herds in that area that did not become infected. Whilst unknown routes of infection or unreported contacts may have caused this difference, it is also possible that differences in hygienic measures may also have been important. The purpose of this study was to find out whether alternative types of inter-herd contacts and herd hygiene factors were associated with the introduction of CSFV. Such findings may lead to the generation of new hypotheses concerning the transmission of CSFV and assist in the subsequent prevention of infection.

## MATERIALS AND METHODS

Infected herds (cases) were matched with non-infected herds (controls) into clusters on the basis of herd type (with or without sows) and shortest geographical distance (within a radius of 2 km). A total of 60 clusters (a combination of cases and controls) were established with an average of 4 herds per cluster (minimum was 2 herds; maximum was 10 herds).

The study population consisted of herds located in the central area around the primary outbreak. In this area, 233 herds became infected. Herds infected by the following routes were excluded from the investigation: 1) infection via animal contact; 2) possible infection from the use of contaminated semen from an infected boar station; 3) estimated infection date was before the detection date of the primary outbreak; 4) two infected boar stations.

A total of 196 infected pig herds met the inclusion criteria, of which 61 refused to participate in the study (69% response). Of the remaining 135 pig herds, 29 herds had no sows (21.5%). This distribution was not significantly different from the herds that met the inclusion criteria. Of a total of 131 non-infected herds that were asked to participate in the investigation, 32 herds refused to participate in the study

Information on farm management and hygiene control was collected by means of a questionnaire. Furthermore, the opinions of farmers with respect to possible routes of infection or perceived reasons for non-infection of their herd and of their neighbours, were recorded by personal interview. The questionnaire was based on expert opinions. The layout and the way in which questions were asked was supported by a professional market research agency (MarketResponse, Amersfoort, The Netherlands). The questionnaire was pre-tested on four pig farmers and their families. Their responses were evaluated and the questionnaire was then revised accordingly. The farmers were interviewed by qualified staff, who had received training in interview techniques and questionnaire completion. The questionnaire interview took 1-1½ hours to complete and farmers were compensated for their time by an allowance (US$ 35).

The questionnaire consisted of questions on general demography (e.g. age, family, experience in pig farming) and on the farming enterprise (e.g. herd size, other species, entrance to the premises, use of a hygiene lock). Furthermore, the contact frequency of persons/agencies that contacted the herd for professional reasons was requested. This included visits to the premises (but not the pig-units) and to the pig units (with or without contact with the pigs) in the period before and after the detection of the primary outbreak.

The contact frequency of the following persons/agencies was requested: colleagues with pigs; neighbours, family and friends; playmates of children; pig, sheep, cattle and poultry traders; forage trader; milkman; customers buying fruit or vegetables at the farm; greengrocer; baker's man; oil and/or liquid gas supplier; lorry bringing frozen snacks; representatives of farmers' associations; dairy herd improvement agency service; breeding company consultant; agricultural extension service; representative of the slaughterhouse organisation; veterinary practitioner; veterinary consultant of the national animal health service; climate-control technician; feed-mill consultant; vermin control consultant; gas and electricity fitter; contractor; manure/slurry transporter; pig and cattle artificial insemination technician; sow scanner; broiler catchers; poultry, cattle, sheep or dairy transporters; rendering pick-up service; journalists; excursions; agricultural trainee; temporary farm-help; Agricultural Inspection Service; National Inspection Service for Livestock and Meat; screening and tracing teams of the CSF eradication campaign.

Questions were asked about hygiene attitudes and measures taken after the detection of the primary outbreak in the area, and procedures regarding the pick-up of cadavers by the rendering service. Information concerning contacts with the veterinary practitioner, the artificial insemination service, the pest control service, the slurry transport service, the sow-scanning service and mechanics was also sought. Additional questions were asked about the presence and behaviour of dogs, cats, birds and rodents.

The total contact frequency of different professional persons and agencies per year was not normally distributed, so differences in median total contact frequency between infected and non-infected herds were tested by the non-parametric Kruskal-Wallis test.

The questionnaire data were analysed using a generalised linear model in SAS (SAS, 1993). To account for the 'matched design', clusters of matched pig herds were included in the model as a fixed effect. The response (infected or non-infected) was modelled using a binomial probability distribution and the fixed and random components of the model were linked by a logit-link function. Furthermore, the response was also modelled as an infection rate, in which the response was scaled by the 'population of pigs at risk per unit time'. Using this scaling

process, herds could be compared more accurately as the level and duration of exposure was quantified. The scaling factor was computed by accumulating the average number of pigs present in a herd per week between 1 January 1997, (the presumed date of introduction of CSFV into The Netherlands) and the estimated date of infection of a herd. For the non-infected herds, this period ended on the date of pre-emptive slaughter or complete buy-out of the herd (finishing-pig herds) in the framework of welfare slaughter. For herds with sows that continued to produce piglets and were able to prevent infection, this period ended in the summer of 1997 when the last infected herd in this data set was detected. The infection rate was modelled using a Poisson probability distribution and a log-link function. As modelling the infection rate produced virtually the same model as modelling the response, the results of modelling the infection rate are not presented separately.

The first step in the statistical analysis involved screening of all single explanatory variables in a bivariable regression model. Variables where $P < 0.20$ were considered for further analysis if there was a biologically plausible association between the variable and the response. In the second step, a backward stepwise selection of variables in a cluster of biologically related variables was performed in a multivariate model. In the final step, variables found to be significantly related during the second step were integrated into a multivariable model by a backward selection process. Variables were retained in the final model if $P \leq 0.05$.

## RESULTS

### Self-reported contact frequency

There were no significant differences between infected and non-infected herds in the median total number of contacts by different professional persons and agencies per year for contacting the premises (not in the pig-units), and the pig units (with or without contact with the pigs), in the period before and after the detection of the primary outbreak. The median total number of contacts by different professional persons and agencies per year for contacting the premises and for contacting the pig-unit without having contacted the pigs was significantly higher (Kruskal-Wallis Test: $P < 0.001$) in the period before detection of the primary CSF outbreak. In contrast, there was a slight tendency for the median total number of contacts by different professional persons and agencies per year for contacting the pig-unit (and the pigs inside) to be larger in the period after the primary detection. In general, there was no positive relationship between herd size and median total number of contacts by different professional persons and agencies per year. The exception to this was pig-finishing farms where an increase in the mean herd size was related to an increase in median total number of contacts with the premises.

### Factors associated with an increased risk of infection

In the final model, a total of seven factors remained ($P \leq 0.05$), five of these were associated with an increased risk of infection with CSFV:

1) The presence of commercial poultry on the premises;
2) Visitors could enter the pig units without wearing overcoat/boots or overalls/boots supplied by the farmer;

3) The driver of the lorry that transported the pigs in the framework of welfare slaughter used his own boots instead of boots available on the farm;
4) A moderate herd size (500-1000 animals) and an extreme herd size (> 7000 animals) compared to a small herd size (< 500 animals);
5) Aerosols produced by the high-pressure cleaning of the electrocution equipment used during depopulation of an infected neighbouring herd (< 250 m distance) were carried by the wind onto the farm.

Two factors were associated with a decreased risk of infection with CSFV:

6) More than 30 years of experience in pig farming;
7) Additional cleaning of the transport lorry (used to ship pigs in the framework of welfare slaughter) by the farmer outside the premises before entrance to the farm was allowed.

Three factors, associated with an increased risk of infection with CSFV, could not be included into the model because of the complete separation of the infected and non-infected groups by the covariate:

1) The lorry carrying portable electrocution equipment, which was used for depopulation of an infected neighbour, had turned in the farmyard of the farmer. This was necessary due to the difficulties encountered in manoeuvring of lorries on small country roads;
2) The container in which cadavers of small to medium size pigs were disposed, was transported by the farmer onto the farmyard after the pick-up service of the rendering plant had emptied the container;
3) The household helper/cleaner has pigs at home.

There were no associations found between the presence or increased presence (after depopulation of an infected nearby neighbour herd) of wild birds, cats, rats or mice around the premises with an increased risk of infection with CSFV.

Perception of farmers concerning routes of transmission

In the farmers' opinion, the three most important reasons for infection of their herd were visits by governmental employees' involved with the eradication campaign, airborne transmission of the virus and transmission of infectious material to the herd during the depopulation of an infected neighbouring herd. However, whilst the majority of farmers could not indicate a specific route of infection, airborne transmission and depopulation of an infected neighbour were suggested as important routes of infection.

The majority of farmers did not express an opinion on how they could prevent infection in their own or neighbouring herds However, of those who did express an opinion, additional rigorous hygienic practices and minimizing contacts with personnel from outside the farm were considered the most important measures for preventing the introduction of infection.

## DISCUSSION

The main purpose of this study was to investigate both alternative routes of transmission and hygienic measures to prevent the introduction of CSFV into pig herds.

The number of contacts between infected herds and non-infected herds, the infectivity of these contacts, and the susceptibility of the target herds determine whether transmission will occur from a primary outbreak to other herds. The current policies of the European Union (EU) discourages vaccination (apart from emergency vaccination) against CSFV (EU Directive 80/217) therefore, there is no possibility of minimizing the susceptibility of pigs to CSFV. The effect of a total restriction of animal movements and the general zoo-sanitary measures taken during a CSFV eradication campaign are aimed at minimising both the number of potentially infectious contacts and the infectivity of the contacts. It is clear from this study that there is a decrease (but not very impressive) in the median number of contacts with the premises and with the pig unit (but without contacting the pigs) after the detection of the primary outbreak.

However, these findings indicate that there is no difference (even a tendency for slight increase) in the median number of contacts with pigs in the pig units after the detection of the primary outbreak. This slight increase was thought to be due to the increased number of visits by screening and tracing teams during the eradication campaign. In general, the self-reported contact frequency with pigs in the pig-units (high-risk) during the study period was comparable to another study in high density pig areas in the southern part of The Netherlands (Van der Gaag et al., 1998). There was no difference in the frequency of contacts between the infected and non-infected herds in our study population. This may indicate that differences in infectivity of the contacts, influenced by the hygienic measures taken, play a major role in explaining why certain herds did not become infected in an area where the epidemic spread extensively.

A number of factors, associated with the introduction of CSFV into pig herds, point towards lapses in hygiene standards by the eradication organisation e.g. aspects of welfare slaughter and the additional cleaning of lorries before entering premises. Furthermore, driving electrocution equipment onto the premises of neighbouring herds and the possible transmission of aerosols produced by the high-pressure cleaning of the electrocution equipment, hint at further lapses in hygiene standards during the eradication campaign. Other factors point to flaws in the farmers' awareness of hygiene control e.g. equipping visitors with clothes and boots by the farm; attitude towards the cadaver container. A striking factor was the association between a large number of years of experience in pig farming with increased 'protective value' against CSFV. Pig farmers with more than 30 years experience have encountered situations in the 1960s and 1970s in which hundreds to thousands of CSF outbreaks were common in The Netherlands (Robijns, 1971). It is possible that those experiences caused a higher awareness of how to prevent introduction of CSFV by infectious contacts.

Preventing the introduction of porcine pathogens into a pig herd is a continual challenge for pig producers and veterinarians. The installation of biosecurity protocols and compliance to them will systematically reduce the risk of introducing contagious agents into the pig herd. This will have to become part of a basic package utilised by pig producers for maintaining the health of their pig herds (Amas and Clark, 1999). A higher standard of hygienic awareness and compliance to a high level of biosecurity by the eradication organisation was recommended by

the evaluation report of the Ministry of Agriculture, Nature Management and Fisheries (1998) and these have been incorporated into the newly updated CSF contingency plans.

In this study, no indications of any new important routes of infection were found. There were no associations found between the presence (or increased presence after depopulation of an infected nearby neighbouring herd) of wild birds, cats, dogs, rats or mice around the premises and an increased risk of infection with CSFV. These routes are mentioned as possible sources of infection in the literature (Westergaard, 1996), but there is very little evidence to support this within this study.

In the opinion of the farmers, airborne transmission was among the most important modes of transmission. Again, there is very sparse data supporting this hypothesis. Airborne transmission of CSFV has been experimentally demonstrated over a short distance and with a forced air stream (Hughes and Gustafson, 1960; Terpstra, 1987) but it is conceived to play only a minor role in the transmission of CSFV and only where mechanically ventilated pig barns are in very close proximity (< 10 metres).

Indications for airborne transmission were found in the 1994 Belgium CSF epidemic in East Flanders (Laevens, 1998; Mintiens et al., 2000). It was demonstrated that, the more frequently the neighbouring herd was down wind of the infected herd, the higher the likelihood of infection. As the present study matched farms by distance, it was not possible to investigate this hypothesis. However, the data of the 1997-98 CSF epidemic in The Netherlands provide excellent opportunities to address this issue and it will certainly be a subject of further research (Crauwels et al., 2000).

The results of this epidemiological study should be interpreted with care. It is a retrospective study, in which reliance on the memory of specific situations and circumstances played an important role. The recollection of these situations may differ between farmers of infected and non-infected herds. As all the respondents were deeply involved and affected by the epidemic, recall bias was not considered a serious problem in this study. However, it is possible that bias could have been introduced by unreported personal and material contacts of the infected herds This could have been either deliberate or accidental. Furthermore, it is possible that 'socially accepted' answers may have been given when asking respondents about their behaviour and attitudes. This would have introduced a further bias resulting in potential risk factors not being identified.

In conclusion, this study indicated the importance of hygiene and hygienic measures in controlling the spread of disease during an epidemic. Aspects of hygiene applicable to an eradication campaign are now incorporated into the recently updated Dutch CSF contingency plans. Pig producers have now to fulfil a basic package of biosecurity in order to maintain the health of pigs in a non-epidemic time. Additional hygienic measures required during any future epidemic and subsequent eradication campaign have to be communicated extensively to farmers.

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# STATISTICAL ANALYSIS OF THE CHARACTERISTICS OF LABORATORY TESTS FOR THE DETECTION OF CLASSICAL SWINE FEVER VIRUS WITHOUT A GOLD STANDARD

A. BOUMA[1], J.A. STEGEMAN, B. ENGEL, E.P. DE KLUIJVER, A.R.W. ELBERS AND M.C.M. DE JONG

## SUMMARY

In this study, the sensitivities of tests for the detection of classical swine fever (CSF) virus under field conditions were estimated without knowledge of the true disease status of the animals tested. Tonsillar samples were collected for the laboratory diagnosis of CSF from pigs of CSF-suspect farms in 1997. These samples were tested using a fluorescence antibody test (FAT1) for the presence of CSF antigen. A total of 225 samples, which included 98 FAT1-negative and 127 FAT1 positive samples, were subsequently tested using a second FAT (FAT2). A suspension of the tissue was also examined using a virus isolation test (VI). A statistical model was formulated and the sensitivities of the tests and the prevalences were estimated by the method of maximum likelihood. The sensitivity of the FAT1 was approximately 78% [confidence interval (CI) 62% – 92%]. The ability of a veterinarian to select a positive animal was 77% [CI 64 - 87%]. The sensitivity of the combination of these two tests (60%) indicated that at least 5 animals should be selected from a CSF-suspect farm to gain a probability of detection of 99%. Knowledge of the sensitivity of these tests under field conditions can be used to design a surveillance programme that increases the effectiveness of the control policy.

## INTRODUCTION

Prevention of infectious diseases is an important part of modern livestock production. Disease prevention can be achieved by either eradication of certain agents or by measures that prevent clinical disease. Eradication is often applied when the disease causes severe economic loss and when it is not endemic in the population. This control strategy is applied to Office Internationale des Epizootie (OIE) List A diseases such as foot-and-mouth disease, classical swine fever (CSF) and swine vesicular disease in Western European countries and the United States of America. The introduction of such pathogens into countries that are free of the disease can have a devastating effect on animal husbandry, as shown by the CSF epidemic in the Netherlands during 1997-1998 (Elbers et al., 1999; Meuwissen et al., 1999). In these situations, eradication of the pathogen is preferable, and efforts to control epidemics should focus on controlling the spread of infection. The strategy for OIE List A diseases consists of the depopulation of infected herds, supported by other veterinary legislative and zoo-sanitary

[1] A. Bouma, ID-Lelystad, PO Box 65, 8200 AB Lelystad, The Netherlands.
Tel: +31 320 238238; Fax: +31 320 238050; Email: a.bouma@id.wag-ur.nl

measures (OIE manual). The success of this strategy depends highly on a rapid detection of infected herds. Consequently, the quality of the diagnostic procedure is critical to achieving eradication.

The quality of a test is usually characterised by the parameters, sensitivity (fraction of infected individuals that show up as positive in the test) and specificity (fraction of non-infected individuals that show up as negative in the test) (Kraemer, 1992). For tests used to detect infectious animal diseases, these two parameters are generally determined by use of test panels including specimens from individuals with a well known history of infection, such as experimentally infected animals. Such information is very useful for indicating test possibilities and for detecting systematic test errors.

However, a test panel cannot be used to determine the sensitivity and specificity of a test under field conditions. This is because the choice of the specimens in the panel is subjective and it is not necessarily representative of the situation found in the field with respect to the history and frequency of infection (Stegeman et al., 1996). The field population is heterogeneous, which means that the selected animals differ in disease status or may be infected by different field strains. Moreover, the distribution of different specimens within the test panel may affect the test parameters. If the frequency distribution in the panel is changed, the sensitivity and specificity may change.

Consequently, for estimation of the sensitivity and specificity of a test in the target population it is essential that specimens are taken from animals in the field. Since the true disease status of the animals in the field is unknown, it is necessary to develop a statistical method to estimate the sensitivity of a laboratory test in the absence of a gold standard.

In this paper, tissue specimens were analysed using three different tests for the detection of CSF antigen (CSFV). Additionally, a statistical model was constructed to estimate the sensitivity and specificity of these tests and to determine the true prevalence in the sample. Although the true disease status of the animals in the field was unknown, the sensitivities of CSFV tests can be estimated using the Dutch CSF data from the 1997 epidemic. Knowledge of the sensitivity of CSF tests under field conditions can then be used to design a CSFV surveillance programme that may increase the effectiveness of the control policy.

## MATERIALS AND METHODS

### Diagnostic procedure for CSF

CSF is a viral infection of swine and is one of the list A diseases of the OIE. This epizootic virus is a continuous threat to the swine industry in many European countries. A rapid diagnosis and detection of an infected herd is essential for successful eradication.

The diagnosis of CSF involves three steps: (1) clinical inspection of pigs on a farm, (2) pathological examination and (3) laboratory testing on blood and tissue specimens. The clinical signs and pathological lesions, however, can vary considerably and are not always specific (Moennig, 1990; Terpstra, 1991). A final diagnosis is therefore impossible without the identification of viral antigens or specific antibodies against CSFV.

Data from the 1997-1998 CSF epidemic in the Netherlands were used for the analysis of the diagnostic tests described in this paper. The standard procedure used during the epidemic was as follows. Clinically affected pigs (mainly pigs with fever) were selected by the official veterinarian from farms suspected of being infected with CSFV. These pigs were slaughtered on-farm for post-mortem examination and transported to the diagnostic laboratory of the Animal Health Service. Subsequently, tissue specimens were collected for laboratory testing. The selection of the animals was mainly based on rectal temperature measurements and experience in recognizing diseased animals. This implied that the clinical inspection by the veterinarian was very important for the eventual diagnosis of CSF. The official diagnosis of CSF was based on all available test information including clinical history, clinical inspection, pathological examination and laboratory test results.

Samples

During the 1997-1998 CSF epidemic in The Netherlands, specimens (tonsil, spleen, kidney and ileum) were collected at the diagnostic laboratory from pigs killed on suspect farms and sent to the Reference Laboratory for diagnosis of CSF.

For the purposes of this paper, a specimen was defined as one piece of tissue from one animal (tonsillar tissue was the only specimen used for the analyses described herein). A sample was defined as a series of specimens from different animals in a single herd that were sent to the laboratory on the same day. A case was defined as a set of specimens (tonsils) from a suspect farm at one sampling point. Each case consisted of a series of one to eight specimens of each tissue. After arrival in the laboratory, a unique test number was allocated to each sample. All samples were prepared for the diagnostic tests as described below. Analyses presented within this paper was restricted to the tonsillar specimens since most of the stored samples were tonsillar tissues and also because the tonsil is the first tissue in which the CSFV replicates. After testing in the laboratory during the epidemic, the tonsil specimens were stored at –70°C. These specimens were either positive or negative in the first test, but all samples originated from CSFV-positive farms. These specimens were used for further analysis of test sensitivities.

Laboratory tests

The first antigen detection test that was performed with material from a CSF-suspected pig was a fluorescence antibody test (FAT1) in combination with a confirmation test, the immuno-peroxidase test (IPT). Duplicate cryostat sections (4 μm thick) from the tissue samples were fixated in acetone for 10 minutes, and incubated for 30 minutes with a polyclonal swine anti-pestivirus FITC-conjugated globulin. After washing, the sections were interpreted under a fluorescence microscope (Ressang and Den Boer, 1968). Intracellular, brilliant-green fluorescence seen intracellularly was indicative for an infection with a pestivirus. FAT1-positive samples were confirmed in an IPT. The slides for the IPT were prepared for staining by the same method as was used for the FAT1. The slides were incubated at 37°C for 45 minutes with CSFV-specific horseradish peroxidase conjugated monoclonal antibodies (Mabs) against CSFV. The slides were rinsed, and then incubated 20 minutes at room temperature with chromogen substrate. After washing, the slides were examined under a light microscope. A red intracellular staining of cells was considered evidence of a recent infection with CSFV. The test results were either positive (+) or negative (–) (Wensvoort et al., 1986).

Another test used for the detection of CSFV was virus isolation (VI). A 10% tonsil suspension was made from the remainder of all tonsillar samples included in this study. Sample suspensions were tested in duplicate M-24 well plates. Approximately 300 µl of the suspension was added to a well with a monolayer of SK6 cells. After incubation for 1 hour at 37°C in an atmosphere of 5% $CO_2$ in a humid chamber, wells were washed with Eagle BS solution. Subsequently, 800 µl growing medium was added to each well, and the plates were cultured at 37°C in an atmosphere of 5% $CO_2$ in a humid chamber. After 4 days, an immunoperoxidase monolayer assay (IPMA) was carried out. The monolayers were washed in a 0.15% NaCl solution, dried for 1 hour at 80°C and washed again. Subsequently, the monolayers were incubated for 1 hour with PBS containing 4% horse serum, and CSFV-specific horseradish peroxidase-conjugated monoclonal antibodies, washed and stained with AEC and 0.2% $H_2O_2$. The monolayers were read microscopically for stained cells (Wensvoort et al., 1986, 1989). The test result was recorded as either positive (+) or negative (–).

Testing of samples

After arrival of the samples at the reference laboratory, the first test performed on tissue specimens was an FAT, hereafter referred to as FAT1. Based on this test and the IPT, the particular farm was declared CSF-positive. At the start of the epidemic, after testing in the FAT1, the remainder of all tonsillar specimens (both the FAT1-positive as well as the FAT1-negative specimens) from each farm that was declared CSFV-positive was stored at -70°C. Later on during the epidemic, only FAT1-positive tonsils were stored.

The analysis of the test characteristics was completed after the end of the epidemic (1½ year after the first outbreak) where a second FAT test (FAT2) was performed on the remaining frozen tonsils. FAT1 and FAT2 were carried out on two different parts of the tonsil of the same pig. After the second FAT, a tissue suspension was made of the remaining part of the tonsil for virus isolation (VI). Both FATs and VI were carried out independently, and the tonsil of each pig included in this study was tested three times. FAT1 was carried out on freshly defrosted material, FAT2 on frozen material, while VI was performed on a 10% tissue suspension of the remaining tissue of the frozen tonsil.

The specificity of the FAT1 was determined by testing approximately 6000 tonsils collected during 1999, a CSF-free period.

Statistical analysis

For each diagnostic test used for a series of specimens (one sample from one farm), an apparent prevalence (P) of the sample will be found. This apparent prevalence depends on the true prevalence (p) of positive specimens in the sample, and the sensitivity ($se_1$) and specificity ($sp_1$) of the test used. This implies that for one test, we have one formula with 3 unknown variables p, se, sp. Using a second test for the same sample, there are two more unknown variables. For three tests (FAT 1, 2 and VI), there are 3 formulas with 7 unknown variables. Testing more samples (from different herds), only one unknown parameter, the true prevalence (p) in the sample, is added. This implies that for three or more herds we have 9 formulas with 9 unknown variables, which in principle can be solved.

Test 1 $P = p \cdot ee_1 + (1-p) \cdot sp_1$
Test 2 $P = p \cdot se_2 + (1-p) \cdot sp_2$
Test 3 $P = p \cdot se_3 + (1-p) \cdot sp_3$

For the i-th sample from the i-th farm (i=1,....n), let $p_i$ be the probability that a selected animal is truly positive. It is assumed that the $p_i$ is following a probability distribution with mean $\mu$ and variance $\nu$. The mean $\mu$ is the overall probability for a selected animal being truly positive. It is a measure of the effectiveness of the selection process. Variance $\nu$ offers a measure of the variation between samples from farms, a low value indicating that all $p_i$ are close to $\mu$, a high value indicating that sizeable differences between $p_i$'s may occur.

Let the binary response variable be $y_{ijk}$, $y_{ijk} = 1$, when the j-th animal in the i-th sample was positive according to the k-th test (k = 1, 2, 3 (1 = FAT1, 2 = FAT2, 3 = VI)), and 0 otherwise.

The sensitivity and specificity of the k-th test were denoted by $se_k$ and $sp_k$, respectively:

The $p_i$'s were assumed to follow a beta distribution. The beta distribution is a very flexible family of distributions defined as the interval from 0 to 1 (Johnson and Kotz, 1970) with mean $\mu$ and variance $\nu = \phi\mu\,(1-\mu)$.

Assuming independence between samples and conditional independence within samples, the likelihood is an integral of the form:

$$\prod_i \int_0^1 P(\, Y_j \,;\, \mathbf{y}_i \,;\, \text{conditional upon } p_i \,)\, f(p_i)\, dp_i$$

Here, f is the pdf of the beta distribution, vector $Y_i$ contains the random variables for the test results for the i-th sample, vector $y_i$ contains the corresponding realisations.
Furthermore, P ($Y_i = y_i$; conditional upon $p_i$) =

$$\prod_i \prod_i p_{ik}^{(x_{ik})} (1 - p_{ik})^{(n_i - x_{ik})}$$

where, $p_{ik} = p_i\, se_k + (1-p_i)\,(1-sp_k)$ and $x_{ik}$ is the number of positive animals in sample i according to test k.

The parameters were estimated by the method of maximum likelihood (Cox & Hinkley, 1990). The likelihood was evaluated and optimised numerically employing facilities for numerical integration and optimisation offered in the statistical programming language Genstat 5 (1993). To improve upon the stability of the algorithm and to obtain more accurate confidence intervals, the likelihood was re-parameterized in terms of logit transforms of the parameters. For example, $\mu$ was replaced by $1/(1+\exp(-\eta))$, where $\eta = \text{logit}\,(\mu) = \log\,(\mu/(1-\mu))$.

Confidence intervals were derived for the logits of the parameters, employing a normal approximation with standard errors derived from Fisher's information matrix. Intervals for the original parameters $\mu$, $\phi$, $se_k$, $sp_k$, $k = 1, 2, 3$ were derived by back transformation from the 0.95-interval $(\eta_L ; \eta_H) = (\eta - 1.96\, s ; \eta + 1.96\, s)$ for $\eta$, where $\eta$ is the estimate for $\eta$ and s is the corresponding standard error, the 0.95-interval $(1 / (1 + \exp(-\eta_H)) ; 1 / (1 + \exp(-\eta_L)))$ for $\mu$ was derived.

## RESULTS

### Sensitivity and specificity

In total, 225 tonsils were further analysed (98 FAT1- negative tonsils and 127 FAT1-positive tonsils). These specimens originated from 57 CSF-positive herds. The test results are summarised in Table 1. Two of these samples were positive for fluorescence in the FAT1, but negative in the confirmation test (IPT) and VI test, indicating a recent infection with bovine viral diarrhoea virus or border disease virus. The specificity of the FAT1 was estimated to be approximately 99.97%.

Table 1. The test results of all specimens

| | VI + | VI – | Total |
|---|---|---|---|
| FAT1 negative: | 12 | 4 | 16 |
| FAT2 + | 4 | 67 | 71 |
| FAT2 – | 2 | 9 | 11 |
| Total | 18 | 80 | 98 |
| | | | |
| FAT1-positive: | | | |
| FAT 2 + | 96 | 25 | 121 |
| FAT 2 – | 3 | 3 | 6 |
| Total | 99 | 28 | 127 |

### Statistical analysis

The estimated value for the specificity of VI ($sp_3$) approaches the value 1. This implies that the algorithm is forced to use larger and larger values for the logit of $sp_3$. Because a specificity of (nearly) 1 is highly probable for this test (Weesvoort et al., 1989), specificity $sp_3$ was fixed at value 1. Thus, 7 unknown parameters remain to be estimated: $\mu$, $\phi$, $se_1$, $se_2$, $se_3$, $sp_1$, $sp_2$. Their estimates and 95%-confidence intervals (CI) with all specificities fixed at value 1 are presented in Table 2.

Table 2. Estimates for $\mu$, $\phi$, $se_1$, $se_2$, $se_3$ and the accessory 95% confidence intervals with all specificities fixed at value 1

| Parameter | Estimate | 95% CI interval |
|---|---|---|
| Overall probability $\mu$ | 0.77 | (0.64 ; 0.87) |
| Variance parameter $\phi$ | 0.17 | (0.08 ; 0.31) |
| Sensitivity of FAT1 ($se_1$) | 0.78 | (0.62 ; 0.88) |
| Sensitivity of FAT2 ($se_2$) | 0.82 | (0.65 ; 0.92) |
| Sensitivity of VI ($se_3$) | 0.71 | (0.56 ; 0.83) |

Calculation of the sample size

Using the sensitivity of the FAT1, the effectiveness of the selection process ($\mu$) and the given sample size, the probability of detecting a CSFV-infected herd was calculated (Table 3). The probability of detecting an infected herd is $1-(1-se_{combi})^n$ where $se_{combi} = \mu \times se_1$ (i.e.$0.77 \times 0.78 = 0.60$) and n = number of specimens in one sample (series of specimens from one farm). From this table, it can be derived that a high probability (99%) can be obtained when a sample of 5 specimens from clinically affected animals is sent for laboratory testing by FAT1 when a farm is suspected of CSF.

Table 3. Probability of detecting a CSF-infected herd with the combined test

| Number of specimens | Probability (%) |
|---|---|
| 1 | 0.598 |
| 2 | 0.838 |
| 3 | 0.935 |
| 4 | 0.974 |
| 5 | 0.989 |

## DISCUSSION

The sensitivity of FAT1, which was used for the rapid diagnosis of CSFV infections, was estimated to be 75% [95% CI: 0.59 - 0.87]. The sensitivity of the FAT1 was estimated using samples from the CSF epidemic in the Netherlands during 1997-1998. The effectiveness of the selection of proper specimens for laboratory diagnosis of CSF was estimated to be 72% [95% CI: 0.52 - 0.86]. In other words, 72% of the specimens selected by the veterinarian on a CSF-suspect farm, were positive for CSF virus. In combination with the sensitivity of FAT1, this implied that when a farm is suspected of CSF, approximately 4-5 specimens from sick pigs should be send to the laboratory. In this situation, the probability of detecting a CSFV-infected herd is almost 99%. A higher number of specimens does not contribute to a higher probability.

An epidemic of CSF in countries in which the disease is not endemic is normally controlled by a stamping-out policy of infected herds. It is essential for the effectiveness of this policy that a CSF-infected herd is depopulated before it has, on average, infected more than one other herd (Stegeman et al., 1999a). In other words, the reproduction ratio between herds ($R_h$) should be reduced to a value of below one. The value of $R_h$ is approximated by the product of the number of new infections caused by one infectious herd per unit of time and the infectious period. So, obviously the speed and quality of the diagnostic procedure in detecting infected herds are crucial for the successful control of a CSF epidemic. The rapid detection of an infection will decrease the infectious period and consequently, will reduce $R_h$. Accordingly, the quality of the diagnostic tests and procedures is critical. Knowledge of the sensitivity of CSFV diagnostic tests under field conditions can be used to design a CSF surveillance programme that may increase the effectiveness of the stamping-out policy. The adoption of the above mentioned sampling protocol will maximise the detection of infection and consequently will reduce $R_h$. Increased sampling will not significantly improve detection rates.

The sensitivity of the FAT1 in the target population (75%) was lower than the sensitivity of the same test in a selected panel of samples originating from animal experiments (estimated to be 99%). This may have been caused by the fact that the time between infection and the taking of the tonsillar samples differed between the epidemic and experimentally infected animals. Moreover, an experimental infection is more standardised and the pigs are often infected with a highly virulent strain, which is usually detectable in the tonsils for a longer time than strains of a lower virulence (Terpstra, unpublished). The CSFV strain that caused the 1997-1998 epidemic in The Netherlands was a strain of lower virulence.

In the statistical analysis, the tests that were evaluated were assumed to be conditionally independent with respect to their test errors. Test dependence can change the theoretical values of sensitivity and specificity of combined tests (Gardner et al., 2000). With respect to the random test errors, this seemed a reasonable assumption as the tests were done on randomly selected parts of the tissue sample, and all tests were performed on various days by various persons. With respect to possible systematic biological errors, FAT1 and FAT2 are not independent. The FAT and VI are probably to a large extent independent, because they are based on two different biological principles. However, it can also be argued that there might be some dependence as both tests will follow a similar time dependent pattern (Gardner et al., 2000). The presence of a systematic biological error in FAT as well as in VI seems unlikely, because they have never been found in large panels of samples originating from animals with a known history of infection.

Although CSF-infected herds was determined by a positive result in one of the laboratory tests, it was clinical inspection by the veterinarian that played an essential role in the detection procedure. This was because the veterinarian determined which pigs were to be tested for CSF. If pigs that were not infected by CSFV were selected, an infected herd will remain undetected despite the high quality of laboratory tests. The overall probability for a selected animal being truly positive ($\mu$) is a measure for the effectiveness of the selection process by the veterinarian. In this study, this parameter $\mu$ was estimated to be 72 % [95% CI: 0.52 - 0.86]. Although this estimate seems rather high, the quality of the clinical inspections during the 1997-1998 CSF epidemic in the Netherlands cannot be determined. For this, $\mu$ has to be linked to the average prevalence of CSFV-infected pigs in these herds. However, earlier clinical inspections of the

herds in this study, at times when no diagnosis was made but when they were infected as determined by back calculation (Stegeman et al., 1999b), have to be included in that analysis.

The sensitivity for the VI was estimated to be 77% [95% CI: 0.53 - 0.91]. In experimental infections, the probability of a positive VI is higher than the probability of a positive FAT (Kaden et al., 1999). However, based on the comparable estimates of the sensitivity of the VI and FAT tests described in this paper, FAT is to be preferred to VI for the diagnosis of CSFV infected herds. This is because the VI is a very time consuming test. Of course, the VI test is still valuable for confirmation of a positive FAT, when the IPT is inconclusive, because of the high specificity. In addition, VI may also be valuable as an additional tool for testing blood samples. The advantages of using VI on blood samples are that, more samples can be taken, the animals do not have to be killed and the specificity of the test is high. It would be worthwhile comparing FAT with VI on blood by use of paired samples from the same animal. These samples, however, are not available.

The specificity of the FAT1 was estimated using data collected in 1999. In that year, no outbreaks of CSF were recorded in The Netherlands. Based on these data, the specificity of the FAT1 was estimated to be 99.97%. The question is whether the characteristics of the FAT1 during an epidemic are the same as during times when no outbreaks are recorded. For example, it can be expected that the specificity decreases during testing of a series of samples from one herd with at least one positive sample, because the tester may assume that the next sample will also be positive. Conversely, in disease free periods, the specificity might increase because one does not expect to find a case of CSF. In addition, the sensitivity might also be influenced by several factors. For example, the sensitivity may decrease during testing a positive series of samples, because finding the last positive sample in a series of positive samples is less important. However, the sensitivity may also increase during testing a positive series of samples, due to the assumption that a sample is likely to be positive when it is part of a series of positive samples. The position of the FAT1-negative samples in each series of samples was determined and there were no indications that the FAT1-negative samples were mainly found at the beginning or at the end of a series of samples (data not shown).

It is not yet clear whether the available data set is large enough to fit more complicated models. Work is still in progress with models allowing for within sample correlation and a mixture of beta distributions to model between sample variation. Also the variables of time and veterinarian have yet to be included in the model. To determine the sensitivity of clinical inspection, more data will have to be collected concerning the course of infection in each herd, dates of clinical inspections in the herds, samples collected during these visits, the veterinarian responsible for collection of the samples and the prevalence of infection in each herd at the moment of clinical diagnosis. Therefore, this information, in combination with the information on the sensitivity and specificity of CSF tests under field conditions and quantitative knowledge of the transmission of CSFV between herds, can help in the design of a surveillance programme that increases the effectiveness of the eradication policy. The focus must remain on quicker detection of an infected herd that will reduce the probability of contact herds becoming infected which in turn, will minimise the destruction of many, often healthy, pigs.

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# EPIDEMIOLOGICAL TOOLS AND TECHNIQUES / DIAGNOSTICS

# DESIGN OF SURVEILLANCE PROGRAMMES:

# AN EXAMPLE WITH MATHEMATICAL MODELLING

E.A.M. GRAAT[1], M.A.A.J. VAN OIJEN[1], K. FRANKENA[1], G.J. BOENDER[2], P. FRANKEN[3], M.C.M. DE JONG[1,2]

## SUMMARY

Previous studies have demonstrated that a surveillance strategy of IBR free herds can be used to keep the reproduction ratio between herds ($R_h$) below 1. $R_h$ was derived using mathematical models for individual or bulk milk sampling. Both models consist of 1) within-herd infection dynamics, and 2) a probability of detection of an infected herd. These parts combined with 3) contact rate, showed that surveillance programmes exist to keep $R_h$ below 1. To improve the model, both the actual number of contacts and contact structure between different herd types, as occur in The Netherlands, were added. Information about animal contacts was collected from an Identification and Registration database. Both models and contacts were combined into a next-generation matrix. $R_h$ is equal to the dominant eigenvalue of this matrix. Preliminary results show that spread of infection between herds cannot be prevented ($R_h$>1) if there is even a small proportion of contacts between "trading herds".

## INTRODUCTION

In relation to disease eradication programmes, surveillance programmes need to detect infection at an early stage in herds free of a specified disease. That is, an infected herd should be detected before it infects, on average, more than one other herd. In other words, the reproduction ratio between herds ($R_h$) should be smaller than 1 (De Jong, 1995). In general, $R_h$ might depend on within-herd dynamics of infection, sample size, sampling frequency, test sensitivity, herd size, herd type and contact rate and structure. The following example of modelling surveillance was completed for Infectious Bovine Rhinotracheitis (IBR), but the model could be generalised for other diseases.

In May 1998, an eradication campaign for IBR was started in The Netherlands. In an earlier study (Graat et al., 2001) it was shown how surveillance of certified herds could be carried out, in order to detect BHV-1 infection on a positive, but certified free, herd timeously That is, in such a way that R between herds is smaller than 1. The estimation of R was done with

[1] Wageningen University, Department of Animal Sciences, Wageningen Institute of Animal Sciences, Quantitative Veterinary Epidemiology Group, P.O. Box 338, 6700 AH Wageningen, The Netherlands. Email: Lisette.Graat@genr.vh.wag-ur.nl

[2] Institute of Animal Science and Health (ID-Lelystad), Quantitative Veterinary Epidemiology Group, P.O. Box 65, 8200 AB Lelystad, The Netherlands

[3]Animal Health Service, P.O. Box 9, 7400 AA Deventer, The Netherlands

knowledge and assumptions regarding the dynamics of a BHV-1 infection in cattle herds. In both simulation models, based on individual sampling and bulk milk sampling, the sampling frequency and proportion of positives that introduce infection into other herds were the most important factors that influenced spread of infection between herds. The proportion of positives that introduces infection into other herds is more or less directly related to the number of animals sold to other herds. For dairy herds, a monthly bulk milk sample was sufficient and this criterion can easily be met in existing bulk milk sampling programmes. For non-dairy herds (veal calves, fattening bulls, "trading herds", suckling cows) individual sampling needs to be carried out. Sampling should be done at least twice a year, provided that not more than 8% of the animals is sold to other herds.

Besides the number of animals leaving the farm alive, the number of contacts, and structure of these contacts between different herd types, might also influence spread of infection. In the previous models, however, contact structure between different herd types was not taken into account. Therefore, to improve the surveillance programmes, both the actual number of contacts and contact structure between different types of herds, as occur in The Netherlands, were taken into account in this study.

## MATERIALS AND METHODS

Information regarding purchase and replacement of animals (either leaving to go to other herds or to go to the slaughterhouse) was collected for 1999 from the Identification and Registration (I&R) database. Herds were categorised into the following types:

1. dairy herds
2. fattening bulls
3. veal calves
4. "trading herds"
5. mixed herds: non-dairy + other type
6. mixed herds: dairy + other type (surveillance through bulk milk sampling)
7. other herds: all other herds (suckling cows, hobby farms, etc)

For each herd type, the following information was collected:

- number of herds
- average number of animals present on July 1st 1999 (including variance, minimum and maximum) per age category (0-3 months, 3-6 months, 6-12 months, 12-24 months, >24 months)
- average number of removed animals (including variance, minimum and maximum), animals sold to other herds as well as to slaughterhouses.

The following information, at herd and animal level, appeared in the database:

| | |
|---|---|
| At herd level : | Unique Herd Number (UBN) |
| | Herd type |
| | Postal code |
| | Number of animals present (herd size) |
| | Number of replaced/purchased animals |
| At animal level: | Animal identification number. |
| | UBN herd |

Date of birth
Replacement date
Age at replacement (in months)
UBN of herd of destiny
Sold for "dead", i.e., slaughterhouse or "life", i.e., other farms

For both models, for individual sampling as well as for bulk milk sampling, the formulae of Graat et al. (2001) were used with a minor adaptation. In short, both models consist of three parts. Part (1): A formula for the dynamics of infection within a herd, given a certain reproduction ratio between animals within a herd, number of positive animals immediately after an outbreak (based on Kermack & McKendrick, 1991a,b,c), and the proportion of animals that is replaced by non-selective culling. This latter proportion, in this paper, is divided into those animals leaving to go to the slaughterhouse and those animals leaving to go to other farms. Part (2): A formula for the probability of detection, which is based on the sampling of a number of animals per herd with a certain sampling frequency, given the dynamics of infection within a herd. Part (3): An expression for the basic reproduction ratio between herds. In this expression, a measure for "contact rate" is included. The division of culling into true culling and selling rate (see Part 1) consequently implies the parameter "contact rate" also consists of two parts. The first part is a multiplication factor for animals leaving the farm alive that can spread infection to other farms, and the second is a multiplication factor for contacts that arise other than through selling positive animals (e.g., contact over fence, neighbourhood, vermin, persons (veterinarian, dealer, inseminator), materials, etc.). The assumption is that culled animals immediately go to the slaughterhouse and do not spread the infection.

Both simulation models and contacts between herds were combined in a so-called next-generation matrix. This next-generation matrix ($M_r$) is an *nxn*-matrix, in which *n* is the number of herd types that is distinguished in this study (n=7). In the matrix, the element $a_{ij}$ is the number of herds of type *j* being infected by herds of type *i*. In other words, this element is the value of the reproduction ratio resulting from one of the two simulation models, multiplied by the proportion of contacts. With this next-generation matrix, the reproduction ratio between herds, $R_h$, can be calculated. This $R_h$ is equal to the dominant eigenvalue of the next-generation matrix, provided that there is a constant contact rate over *r* "generations", that is, $M_1 = M_2 = M_3 .... = M_r$ (Diekmann et al., 1990).

Table 1 shows the fraction of herd types delivering animals to other herd types, and shows from which simulation model the reproduction ratio was used in the calculation of the next-generation matrix. The presented fraction was derived from the I&R database. The fractions are based on 1,033,914 animal movements in 1999.

By using the seven specified herd types, a next-generation matrix with 49 elements was produced. Default values for a number of parameters in both models can be found in Table 2. Parameters in both simulation models can be varied, as well as the value of the fraction of animals sold to other herd types. A sensitivity analysis was performed in which parameters were varied one by one, while the others were kept constant. In this sensitivity analysis, only parameters which were assumed to play an important role in spread of infection were systematically increased or decreased to examine the influence on the outcome of the model, the R between herds. As in the models without contact structure (Graat et al., 2001), sampling frequency as well as number of positive animals that introduce infection in other herds greatly affected the R between herds. These were studied in detail.

Table 1. Fraction of animals sold by herd type "x" delivering (columns) to other herd types (rows). The simulation model that is used for the next-generation matrix is shown between brackets (B=bulk milk sampling, I=individual sampling)

| FROM<br>TO | 1 (B) | 2 (I) | 3 (I) | 4 (I) | 5 (I) | 6 (B) | 7 (I) |
|---|---|---|---|---|---|---|---|
| 1. dairy herds | 0.12 | 0.09 | 0.02 | 0.05 | 0.09 | 0.10 | 0.23 |
| 2. fattening bulls | 0.01 | 0.14 | 0.04 | 0.05 | 0.04 | 0.04 | 0.04 |
| 3. veal calves | 0.43 | 0.10 | 0.51 | 0.43 | 0.42 | 042 | 0.10 |
| 4. 'trading herds' | 0.06 | 0.16 | 0.02 | 0.11 | 0.06 | 0.06 | 0.10 |
| 5. mix/non dairy | 0.22 | 0.38 | 0.34 | 0.25 | 0.29 | 0.23 | 0.03 |
| 6. mix/dairy | 0.02 | 0.01 | 0.01 | 0.01 | 0.02 | 0.02 | 0.24 |
| 7. other | 0.14 | 0.12 | 0.06 | 0.10 | 0.08 | 0.13 | 0.26 |

Table 2. Default values as used in the simulation models

| Parameter \ Herd type | dairy herds | fattening bulls | veal calves | "trading herds" | mixed non dairy | mixed dairy | other herds |
|---|---|---|---|---|---|---|---|
| Herd size | 50 | 100 | 300 | 100 | 50 | 50 | 50 |
| Sensitivity | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 |
| Sample size[1] | bulk milk | 3 | 3 | 3 | 3 | bulk milk | 3 |
| Sampling freq. (per year)[1] | 9 | 2 | 2 | 2 | 2 | 9 | 2 |
| Threshold[3] | 0.15 | NA[2] | NA | NA | NA | 0.15 | NA |
| R within herds[4] | 5.6 | 5.6 | 5.6 | 5.6 | 5.6 | 5.6 | 5.6 |
| Fraction of animals sold for "life" | 0.04 | 0.001 | 0.001 | 0.5 | 0.04 | 0.04 | 0.04 |

[1] Minimum number as required in the current IBR eradication campaign in The Netherlands
[2] NA = Not Applicable
[3] 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)
[4] R within herds is equal to 5.6 in herds that became free without vaccination (Bosch, 1997)

## RESULTS

With the use of default parameter values, the outcomes of each model, bulk milk sampling or individual sampling, produce an R between herds, i.e., the number of herds infected by one infected herd. For example, for dairy herds, the number of infected herds (determined by surveillance with bulk milk sampling) is 0.26. By multiplying this by the matrix of contact rates between herd types, this results in the number of infected herds per herd type. This is the next-generation matrix, which is shown in Table 3. From Table 3, it is clear that with only the default surveillance of "trading herds" (type 4) some reproduction ratios are higher than 1.

The resulting $R_h$, calculated as the dominant eigenvalue of this next-generation matrix (see Table 3), is equal to 1.58, implying that a surveillance programme, according to the default parameter values, cannot prevent major outbreaks of BHV-1 infection in certified-free Dutch dairy herds.

Table 3. The next-generation matrix, from which the reproduction ratio between herds, $R_h$, can be calculated. The numbers in the table represent the number of herds infected by herds from a certain type

| FROM / TO | 1 (B) | 2 (I) | 3 (I) | 4 (I) | 5 (I) | 6 (B) | 7 (I) |
|---|---|---|---|---|---|---|---|
| 1. dairy herds | 0.031 | 0.006 | 0.007 | 0.645 | 0.052 | 0.026 | 0.053 |
| 2. fattening bulls | 0.003 | 0.010 | 0.013 | 0.645 | 0.023 | 0.010 | 0.009 |
| 3. veal calves | 0.112 | 0.007 | 0.169 | 5.548 | 0.243 | 0.109 | 0.023 |
| 4. 'trading herds' | 0.016 | 0.011 | 0.007 | 1.419 | 0.035 | 0.016 | 0.023 |
| 5. mix/non dairy | 0.057 | 0.026 | 0.113 | 3.226 | 0.168 | 0.060 | 0.007 |
| 6. mix/dairy | 0.005 | 0.000 | 0.003 | 0.129 | 0.012 | 0.005 | 0.055 |
| 7. other | 0.036 | 0.008 | 0.020 | 1.290 | 0.046 | 0.034 | 0.060 |

Figure 1 shows the results of the sensitivity analysis. From Figure 1, it can be seen that the fraction of animals that are sold by "trading herds" is a crucial factor. Only when these farms sell less than 25% of their animals per year can spread of infection be prevented (see Fig. 1a). The destination of animals leaving "trading herds" is an especially important aspect (see Fig. 1d). By lowering the fraction of animals that are sold to other "trading herds", $R_h$ becomes lower than 1. In 1999, this fraction was 0.11 (see Table 1); by lowering it to less than 0.05 (see Fig. 1b), spread of BHV-1 infection might be prevented. Another way for $R_h$ to be made lower than 1 is to increase the sampling frequency on "trading herds" to at least every 3 months (see Fig. 1c). The fraction of animals sold for "life" by dairy herds, does not influence the outcome of the model very much (see Fig. 1b). Related to this, is the fact that the $R_h$ is not affected by the fraction of animals sold by dairy herds to "trading herds" (see Fig. 1e).

## DISCUSSION

In this paper, modelling of surveillance was performed for Infectious Bovine Rhinotracheitis (IBR), but the model could be generalised for other diseases. The model of Graat et al. (2001) was improved. Besides the number of animals leaving the farm alive, the number of contacts and structure of these contacts between different herd types also might influence spread of infection. However, in the models described previously, contact structure between different herd types was not taken into account. Therefore, to improve the surveillance programmes, both the actual number of contacts and contact structure between different types of herds, as occurs in The Netherlands, was taken into account in this study.

A surveillance programme is only successful when positive animals in certified herds are sufficiently quickly detected, i.e., the R between herds ($R_h$) should be below 1. As in Graat et al. (2001), it should be emphasised, that the results presented deal with the aspect of staying free of BHV-1 once the population is negative. Another assumption made was that all animals within

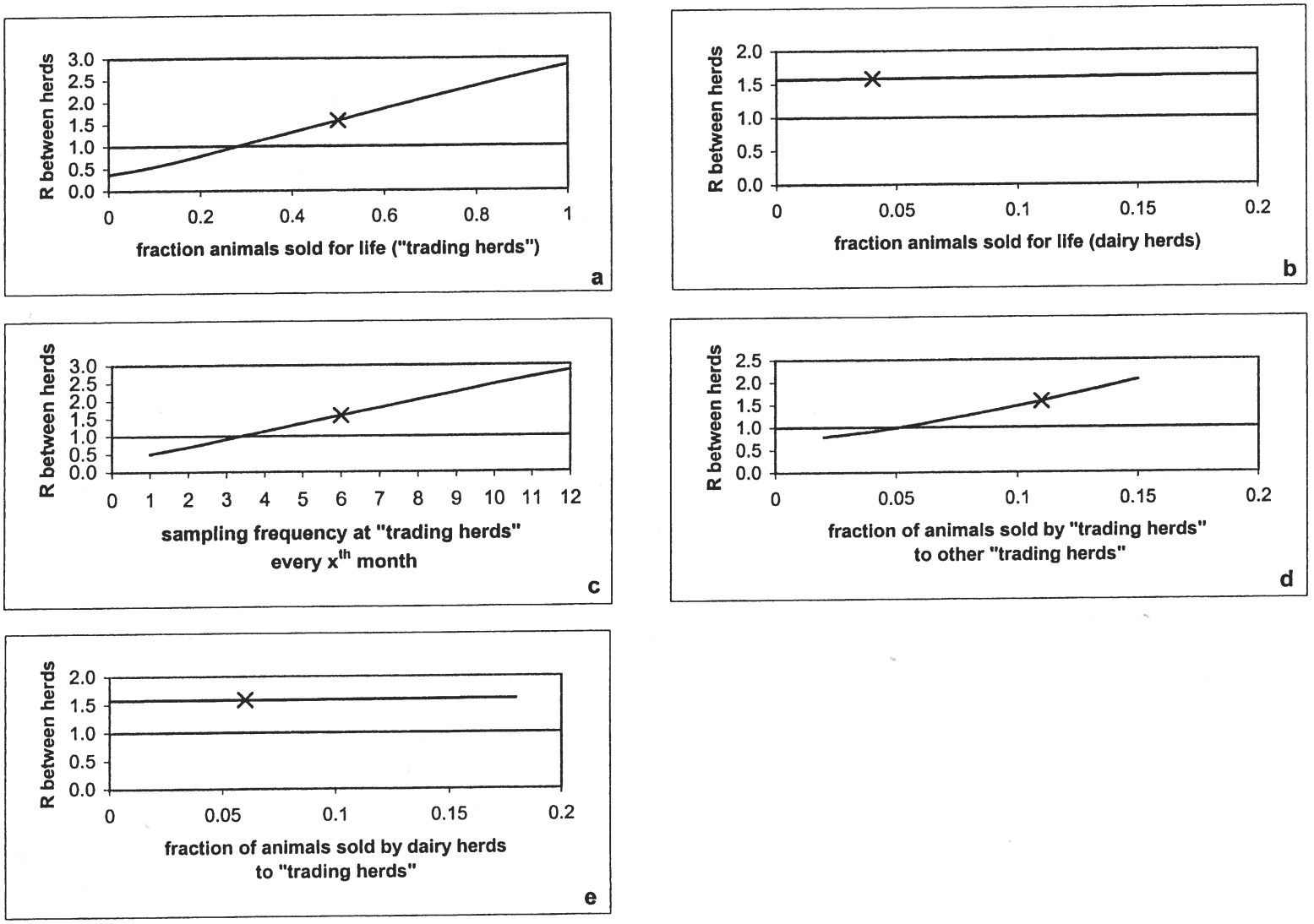


Fig. 1 R between herds ($R_h$) with varying the fraction sold for life by "trading herds" (a), and dairy herds (b), sampling frequency at "trading herds" (c), fraction of animals sold by "trading herds" to other "trading herds" (d) and fraction of animals sold by dairy herds to "trading herds"

Note: the marker (x) in the figure denotes the outcome with all default values ($R_h = 1.58$).

herds are fully susceptible (no vaccinated animals are present), implying a rigorous surveillance programme (Graat et al., 2001; Vonk Noordegraaf et al., 1998). In addition, the assumption that the outbreak in a herd occurs instantaneously could be debated. Furthermore, the calculated $R_h$ is equal to the dominant eigenvalue of the next-generation matrix, provided that there is a constant contact rate over *r* "generations" (Diekmann et al., 1990). This assumption is still to be verified with data from the I&R database.

From the results of this study, it can be concluded that spread of BHV-1 between herds can only be prevented if special actions are taken on so-called "trading herds". One action might be to restrict contact with other herds, and especially with other "trading herds". Another measure could be to increase the sampling frequency of these herd types to at least four times per year. This sampling frequency or the time between sampling occasions, should be regarded as the time since last sampling, and the time of the implementation of control measures until the next sampling. This becomes especially important when there is a short sampling interval.

As in Graat et al. (2001), the sensitivity of the test and sample size is of lesser importance (data not shown). With the default sample size, the existing diagnostic tests (Wellenberg et al., 1997) are sufficiently good for surveillance purposes.

This simulation model, which describes surveillance programmes and accompanying transmission of BHV-1 between herds, might be used as a framework for modelling surveillance and transmission of other diseases, e.g. (para)tuberculosis. Of course, economic analysis is needed to determine the optimal surveillance programme in terms of costs and benefits. For this cost-benefit analysis the work of Metz (1978) is noteworthy. Decreasing the $R_h$ from 0.999 to, e.g., 0.8, considerably reduces the number of herds that become infected in a minor outbreak. A further reduction in $R_h$ does not reduce this number of herds very much. Furthermore, prior to economic analysis, I&R data could be used to examine whether surveillance of non-dairy farms is feasible by taking samples at slaughterhouses instead of sampling animals at the farm. If this is possible, then the costs of sampling might be lowered considerably.

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# "TAGS", A PROGRAM FOR THE EVALUATION OF THE DIAGNOSTIC VALUES OF TESTS IN THE ABSENCE OF A GOLD STANDARD

R. POUILLOT*, G. GERBIER*

## SUMMARY

Performances of new diagnostic tests are frequently evaluated by comparison with a reference test, considered to be a "gold standard". These classical methods generally work on the assumption that this latter test is perfect, i.e., its sensitivity and specificity are equal to one. Most of the time, no real "gold standard" is available. This can seriously bias the estimation of the diagnostic values of the challenged tests. In the absence of a "gold standard", statistical methods are available for the estimation of the accuracy of diagnostic tests. These approaches assume that the infectious status of the individuals is unknown, but may be estimated from their result pattern; these models are referred to as "latent class models". Maximum likelihood methods may then be used for the estimation of the diagnostic values and the disease prevalence within the population(s). This paper describes the development of functions for this purpose, implemented in S-Plus and R software: these functions may incorporate data obtained from several populations, using several tests, and may take into account data obtained from reference population(s), i.e. population(s) with a known infectious status (infected or disease-free), if available. Two estimation methods are used: the classical Newton-Raphson procedure, and an Expectation-Maximisation (EM) procedure. Test independence is assumed conditionally on the infectious status of the individual, as is constant test performance within each population. Use of a goodness-of-fit statistic and the residuals of pairwise correlation coefficients are proposed as checks for validity of these assumptions. These functions, available on request from the author, can help epidemiologists in this important field of test validation. Two examples are used to illustrate the use and the limitations of the tool.

## INTRODUCTION

Test validation is a topic of primary interest in the veterinary field. Reliable estimates of the sensitivity (i.e., the probability that a truly infected animal reacts positively in the test) and the specificity (i.e., the probability that a non-infected animal reacts negatively in the test) are needed for any evaluation of disease surveillance and monitoring. For example, a good knowledge of the performances of diagnostic tests is essential for a decision-maker to declare an area (or even a herd) as "disease free". Reliable estimates of test performances are also needed for any evaluation of aggregate test performances based on probabilistic theory.

Tests are generally evaluated in comparison with another test, referred to as a "gold standard", whose sensitivity and specificity are both assumed to be unity. A "gold standard" is

* French Agency for Food Safety, BP 19, F-94701 Maisons-Alfort cedex. r.pouillot@afssa.fr
* g.gerbier@afssa.fr

perfect, i.e., is positive in all animals with the disease and negative in all animals which do not have the disease. In this case, the true disease state of the animals is known according to the result of the gold standard test, and the validation of the new test is direct, using a 2×2 table. The use of a reference test with known sensitivity and specificity less than one would also lead to easy estimation of the challenged test performances (Staquet et al., 1981, cited by Enoe et al., 2000).

If the reference test is not perfect or if the performances of the reference test are not known with accuracy, a misclassification of the animals according to the infectious status occurs, and the associated evaluation of any new diagnostic test is biased. In practice, there are very few real "gold standards" available. It has been shown that one third of medical articles dealing with diagnostic test evaluation used no well-defined "gold standard" (Sheps & Schechter, 1984). For example, in animal brucellosis, the bacterial isolation is frequently used as a reference test and whilst one might assume that its specificity is one, it has been shown that its sensitivity may be far from unity (Hornitzky & Searson, 1986). The use of this test as a "gold standard" will mathematically bias the estimates of the specificity and the sensitivity of a challenged test.

For some 20 years, in statistical research and medical science (Hui & Walter, 1980), original methods have been used to deal with the estimation of new test performances in the absence of a gold standard. One approach is to use Maximum Likelihood methods. The simplest model estimates parameters without any additional assumptions other than the conditional independence between tests, as long as the number of parameters to be evaluated is lower or equal to the degree of freedom permitted by the data. Hui and Walter's original article (1980) developed a model using two tests applied to two populations with different prevalences, with the additional assumption of constant diagnostic values within each population. Various other models have been developed since that date (for a review up to 1988, see Walter & Irving, 1988; in veterinary science, see Enoe et al., 2000).

These methods have been recently applied in the veterinary field (e.g., Spangler et al., 1992; Enoe et al., 1997; Singer et al., 1998), but are still not widely used, probably due to the absence of accessible software. In this paper, "TAGS" ("Test in the Absence of a Gold Standard"), is presented as a set of R (© The R Development Core Team) and S-PLUS (© MathSoft, Seatle, WA) functions developed for this purpose. R (Ihaka & Gentleman, 1996) is freeware which shares a lot of common functions with S-Plus; it is available at http://cran.R-project.org/.

## MATERIALS AND METHODS

### Experimental design and data

The model is restricted to binary criteria: the tests should take only two values, one signifying the assumed presence of the infection, the other signifying the absence of the infection.

The study designer applies dichotomous tests independently to the same animals. In order to increase the degree of freedom of the model (see below), these animals should be from several populations with unknown, but varying, prevalence of the disease. For example, the investigator could use the tests in an area where the presence of infection is almost certain (e.g., ascertained by clinical observation) and in another area where the infection is less likely. It is important here to note that the infected animals of all sub-populations should be comparable with regard to the

infection. Bias may occur if the animals of one population are selected on the basis of a previous test (e.g., previous slaughter of the most heavily infected).

It is sometimes possible to get data from individuals whose infectious status is known with certainty. Disease-free animals may be obtained from areas where the disease has not been observed for several years, and has not been observed since the experiment was performed. Animals known to be infected may also be obtained in some cases: For example, when a "gold standard" exists but cannot be applied to all of the animals in the experiment. If these animals react in exactly the same manner as those of unknown infectious status, the information from these groups may be used in TAGS. It must be emphasised that obtaining animals from these reference groups, which are comparable to those in the experiment, can be very difficult. For example, infected animal data could be obtained from experimentally infected animals, but it might be difficult to affirm, without major assumptions, that these animals are comparable to naturally infected animals.

In fact, the TAGS algorithm may be used on any set of data, as long as the model is identifiable, i.e. the degree of freedom given by the data allows the calculation of all the parameters. Given $T$, the number of evaluated tests, $P_u$ the number of population(s) with unknown prevalence, $P_r = (0, 1, 2)$ the number of reference populations (0: no reference population, 1: one infected reference population or one disease-free population, 2: one infected and one disease-free reference population), the number of estimated parameters will be: $par = 2T + P_u$, i.e., the sensitivity and the specificity of each test, and the prevalence of each population with unknown prevalence. Regarding the sample sizes as fixed, the degree of freedom will be: $df = (P_u + P_r)(2^T - 1)$, i.e., the number of combinations of results obtained with $T$ dichotomous tests minus one, multiplied by the total number of populations.

The design should, *a minima*, use one test on a population plus two reference populations ($par = 3$, $df = 3$), 2 tests on a population plus one reference population ($par = 5$, $df = 6$), 2 tests on two populations with different prevalences (the classical Hui and Walter's model, $par = 6$, $df = 6$), or 3 tests ($par \geq 7$, $df \geq 7$, $par \geq df$).

Algorithm and assumptions

The real infectious status of the animals from the $P_u$ populations is unknown, and makes the classical evaluation of test values by a 2×2 table impossible. It is nevertheless possible to make inference on these parameters using the Maximum Likelihood (ML) method. This method evaluates the most probable values of the parameters which would have lead to the observed data. A generalisation of the Hui and Walter's (1980) likelihood function to all the possible study designs outlined above has been implemented (see Appendix A). The higher the function of the parameter, the higher the likelihood of the observation given these parameters; the estimates are then obtained by maximizing this likelihood function. Estimates are asymptotically unbiased and efficient. The Maximum Likelihood estimates for all parameters are obtained using two iterative methods: the Newton-Raphson (NR) algorithm, and the Expectation-Maximisation (EM) algorithm (see Appendix B). Both algorithms should lead to the same estimates.

Through the NR algorithm, the standard error of the estimates may be calculated as the square root of the diagonal of the variance-covariance matrix, this latter being estimated by the Fisher Observed Information Matrix. A confidence interval can then be derived, assuming that

the estimators follow a normal distribution. This assumption is asymptotically correct; users should be careful if the number of individuals is low.

This likelihood function is nevertheless obtained with two major assumptions: *i*) test results are independent conditionally on the infectious status of the animals, and *ii*) the accuracy of tests is the same in all sub-populations.

Check of fit and assumptions

The likelihood function used in this program is based on several assumptions that, if not valid, could lead to bias estimation (Vacek, 1985).

Tests results are assumed independent conditionally on the infectious status on the animals. In this case, this not only means that tests are performed independently (i.e., blindly), but that the probability that a test result is positive is the same whatever the result of the other tests, for a given infectious status. In practice, this could be the case when the tests are based on different biological properties (Vacek, 1985; Gardner et al., 2000). For example, in brucellosis, two indirect ELISA will probably not be considered as independent, whilst a serological test and an allergic test could *a priori* be considered as independent.

The second assumption is that the accuracy of all tests is the same if applied to different sub-populations. This old paradigm of the constant sensitivity and specificity of tests is now revisited (Chriel & Willeberg, 1997; Greiner & Gardner, 2000).

In order to check for the validity of these assumptions, TAGS compares the observed results to the theoretical ones obtained from the NR estimates. The user should carefully check these results, especially if $df > p$. If some combinations of results are under- or over-estimated, one of these two assumptions may be incorrect. A likelihood ratio chi-square deviance test is also provided as a goodness-of-fit test; this test is based on asymptotic theory and so may be unreliable. The power may be low if some expected cell values are small.

Moreover, TAGS provides the correlation residuals between tests, for each sub-population. These residuals of $T(T-1)/2$ pairwise correlation coefficients are defined as the difference between the observed and the expected correlation (Qu et al., 1996) (see Appendix C). If tests are independent conditionally on the infectious status, the residuals should be distributed randomly around 0. If the dependence between tests is the same in each sub-population, these correlation residuals should be the same in all sub-populations. A 95% bootstrap confidence band could be added, but because the utility of these values is in modelling and to check for model validity, this option has not been included in TAGS.

## ILLUSTRATION

Two functions have been implemented in R and S-Plus: one function allows the loading of the data (`load.data()`), while the second one (`evaluate()`) allows the calculation of test diagnostic values. Two examples from the literature are provided: `evaluate(Hui)` or `evaluate(Sae)`. These examples are discussed below.

The program prompts the user for *i*) the number of tests to evaluate, *ii*) the number of sub-populations, *iii*) the number (and the group, i.e., infected, disease-free or both) of reference

population(s). For each population, the number of observations according to the possible combinations of test results is requested. Last, "best guesses" are provided for each parameter (i.e. sensitivity of each test = 0.8, specificity of each test = 0.9, prevalence in each population = 0.2), although the user may load different values. These "best guesses" will be the initial values used by the iterative procedures (NR and EM).

Example 1 (Hui and Walter, 1980):

The classical Hui and Walter (1980) data set concerns two tests for tuberculosis (the Mantoux and Tine tests), given to individuals in two different populations. The results given by the program are presented in Frame 1. The goodness-of-fit test is not considered because the degree of freedom *df* is equal to the number of parameters *par*.

The estimates seem to fit the observed data. Nevertheless, since *par* = *df*, it is not really possible to check the adequacy of the model. Vacek (1985) showed with these data that an underlying conditional dependence could bias the estimation. Caution should be exercised in drawing conclusions about the accuracy of models that do not leave a *df* for test validation.

Example 2 (Saegerman et al.,1999):

Saegerman et al. (1999) applied three brucellosis tests (test 1: a combination of classical serological tests, test 2: brucellin skin test and test 3: an indirect ELISA test) to one population with an unknown prevalence, and to a disease-free population (no brucellosis in the area for more than 10 years and 3 years following the experiment). TAGS output is presented in Frame 2.

Since *df* > *par*, a goodness-of-fit test is available: the model does not fit. One can see that the expected number of animals (0,1,0), i.e., positive in the test 2 only, is lower than the observed data (20.16 *vs* 33). The correlation residuals between tests show that test 1 and test 3 cannot be considered as independent ($corr_{1-3} = 0.21$). Estimates are probably biased. Note that this result is unexpected since serological and brucellin skin tests are based on two different physiologic phenomena (humoral and allergic response), while serological and ELISA are based on the same underlying physiology (humoral response).

## DISCUSSION

Test validation is an essential, but difficult job.

It is important to emphasise that the classical "rules" for the selection of animals in order to evaluate diagnostic tests should be applied. Several guidelines exist in the medical and veterinary literature (Greiner & Gardner, 2000) for experimental design in validation studies. No accurate estimates can be inferred from an inappropriate study design.

Many biologists have to deal with the evaluation of a test or tests in the absence of a "gold standard": the functions described should help to assess the diagnostic values of challenged tests in such cases. The objective of this project was to provide a free "black-box", but with the tools to diagnose whether or not the model was valid for available data. In most cases, as soon as the

degree of freedom availed by the data is large enough, these functions will provide enough information to check for estimate validity.

The functions also allow the user to incorporate data from a reference group or groups. While these data may be difficult to obtain, they are of great importance in refining estimates. Usually, estimates from these reference populations are considered as perfectly accurate, even though the small number of individuals in these categories does not always allow such a conclusion.

The assumptions used in this model have been discussed over recent years. The assumption of constant diagnostic values over different populations may be considered correct if all populations are comparable. Moreover, as soon as $T \geq 3$, the user may check the validity of this assumption by using TAGS independently on each of the sub-populations: estimates should be equivalent if the assumption is valid.

As seen in the second example, a lack of conditional independence between tests may invalidate the model. This has been noticed since the inception of this model, and various other models have been developed to deal with the issue, e.g., random effect models (Qu et al., 1996) or latent class models using more than two groups (Formann et al., 1994). Since this latter assumption is often invalid, another set of functions should be provided. First assay using log-linear modelling through an EM algorithm have been implemented and will soon be available in R.

```
SUMMARY
2 Population(s);  2 Tests;  0 Reference Population(s)
df: 6 ; parameters: 6
  test1 test2 pop1 pop2 RefInd RefInf
1     0     0  528  367      0      0
2     1     0    4   31      0      0
3     0     1    9   37      0      0
4     1     1   14  887      0      0

           p(inf) 1 p(inf) 2 Sp 1 Sp 2 Se 1 Se 2
Best Guess      0.2      0.2 0.95 0.95  0.8  0.8

 EXPECTATION MAXIMISATION
$Iterations
[1] 27
$Likelihood
[1] -1207.617
$Estimations
    p(inf) 1 p(inf) 2   Sp 1   Sp 2   Se 1   Se 2
Est   0.0268   0.7168 0.9933 0.9841 0.9661 0.9688

 NEWTON-RAPHSON
$Iterations
[1] 29
$Likelihood
[1] -1207.617
$Estimations
      p(inf) 1 p(inf) 2   Sp 1   Sp 2   Se 1   Se 2
Est     0.0268   0.7168 0.9933 0.9841 0.9661 0.9688
CIinf   0.0159   0.6911 0.9797 0.9684 0.9495 0.9540
CIsup   0.0450   0.7412 0.9978 0.9921 0.9774 0.9790

WARNING 1: test results are supposed independent conditionally to the
infectious status
WARNING 2: tests are supposed to have same diagnostic values in all
populations

Expected Results (NR) and Goodness-of-fit test
$Expected
  test1 test2 pop1 pop2 RefInd RefInf ExpectedPop1 ExpectedPop2 ExpectedRefInd
1     0     0  528  367      0      0          528          367              0
2     1     0    4   31      0      0            4           31              0
3     0     1    9   37      0      0            9           37              0
4     1     1   14  887      0      0           14          887              0

$Test
[1] "NA"
$Commentary
[1] "The number of parameters = df: no goodness-of-fit test available"

Correlation residuals between test
$ResCor
              Corr1-2
pop 1 : -7.888958e-06
pop 2 :  1.867175e-06
The residuals should be randomly distributed around 0
```

Frame 1: The Hui and Walter example

```
SUMMARY
1 Population(s);  3 Tests;  1 Reference Population(s)
df: 14 ; parameters: 7
  test1 test2 test3 pop1 RefInd RefInf
1     0     0     0  275   1142      0
2     1     0     0    6      2      0
3     0     1     0   33     25      0
4     1     1     0   11      0      0
5     0     0     1    0      3      0
6     1     0     1    0      0      0
7     0     1     1    6      0      0
8     1     1     1   32      0      0
           p(inf) 1 Sp 1 Sp 2 Sp 3 Se 1 Se 2 Se 3
Best Guess      0.2 0.95 0.95 0.95  0.8  0.8  0.8

 EXPECTATION MAXIMISATION
$Iterations
[1] 63
$Likelihood
[1] -494.4022
$Estimations
    p(inf) 1   Sp 1   Sp 2   Sp 3   Se 1   Se 2   Se 3
Est   0.2064 0.9972 0.9748 0.9979 0.6261 0.9363 0.5072

 NEWTON-RAPHSON
$Iterations
[1] 20
$Likelihood
[1] -494.4022
$Estimations
      p(inf) 1   Sp 1   Sp 2   Sp 3   Se 1   Se 2   Se 3
Est     0.2064 0.9972 0.9748 0.9979 0.6261 0.9363 0.5072
CIinf   0.1590 0.9883 0.9626 0.9936 0.4848 0.7633 0.3730
CIsup   0.2634 0.9993 0.9830 0.9993 0.7487 0.9853 0.6403
WARNING 1: test results are supposed independent conditionally to the
infectious status
WARNING 2: tests are supposed to have same diagnostic values in all
populations

Expected Results (NR) and Goodness-of-fit test
$Expected
  test1 test2 test3 pop1 RefInd RefInf ExpectedPop1 ExpectedRefInd   ExpRefInf
1     0     0     0  275   1142      0       280.33        1136.86           0
2     1     0     0    6      2      0         2.26           3.20           0
3     0     1     0   33     25      0        20.16          29.45           0
4     1     1     0   11      0      0        21.66           0.08           0
5     0     0     1    0      3      0         1.48           2.35           0
6     1     0     1    0      0      0         1.52           0.01           0
7     0     1     1    6      0      0        13.32           0.06           0
8     1     1     1   32      0      0        22.27           0.00           0
$Test
 Max Likelihood: Achievable  Obtained Deviance d.f.      p value
                  -477.3426 -494.4022 34.11911    7 1.636273e-05
$Commentary
[1] "The model does not fit: Assumptions may not be justified (see below)"

Correlation residuals between test
$ResCor
           Corr1-2   Corr1-3    Corr2-3
pop 1 : -0.0563845 0.2124539 0.03600714
The residuals should be randomly distributed around 0
```

Frame 2: The Saegerman et al. (1999) example

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## APPENDIX

### A : the Likelihood Function

It is assumed that $T$ tests have been applied to $N$ animals grouped in $P$ sub-populations of size $N_p$. We note $y_n = (y_{1n},\ldots y_{Tn})$ the vectors of length $T$ of results for animal $n$. If animal $n$ is positive in test $t$, $y_{tn} = 1$; otherwise $y_{tn} = 0$. For example, with $T = 3$, $y_n = (0,0,1)$ will correspond to an animal that yields a positive result in the third test only. Only $2^T$ patterns of results $y_n$ exist. $Y_t = 1$ represents that the subject is diagnosed as having the disease under study while $Y_t = 0$ represents that the subject is diagnosed as not having the disease. Each animal has one true infectious status, $D$: 1 means that the animal has the disease, 0 means that it does not have disease.

The objective is to estimate for each test $t$ the following conditional probability: $\theta_{1t} = \Pr\{Y_t = 1 | D = 1\}$, the sensitivity, and $\Pr\{Y_t = 0 | D = 0\} = 1 - \Pr\{Y_t = 1 | D = 0\} = 1 - \theta_{0t}$, the specificity. In the absence of a "gold standard", the real infectious status $D$ of the animals is unknown.

$\pi_{pd} = \Pr\{D = d | p\}$ is the probability that $D = d$ for an animal belonging to the sub-population $p$. $\pi_{p1}$ is the prevalence of the infection in the population $p$ and $\pi_{p0} = 1 - \pi_{p1}$.

If animals belong to a reference group, this latter parameter is assumed to be known. Therefore, $\pi_{p1} = 1$ for an infected reference population and $\pi_{p1} = 0$ for a reference disease-free population. No inference is made from these parameters for these reference populations.

Assuming conditional independence between tests, the likelihood of the combination $y_n$ for an animal $n$ from population $p$ is:

$$V_{pn} = \sum_{d=0}^{1} \pi_{pd} \prod_{t=1}^{T} (\theta_{dt})^{y_{tn}} (1-\theta_{dt})^{1-y_{tn}}$$

Assuming the same test parameters $\theta_{\bullet\bullet}$ for each sub-population, the log-likelihood for the global population is:

$$LnV = \sum_{p=1}^{P} \sum_{n=1}^{N_p} \log(V_{pn})$$

The likelihood function is the same for any animals of the sub-population $p$ that yields a common result pattern $y_k$. The calculation is much more computationally efficient using the formula:

$$LnV = \sum_{p}^{P} \sum_{k=1}^{2^T} X_{pk} \log(V_{pk})$$

where $X_{pk}$ is the number of animals in population $p$ that yields a $y_k$ combination of results.

Parameter estimation is obtained by maximisation of this function. The function uses a Newton-Raphson algorithm. In fact, all parameters $\theta_{\bullet\bullet}$, $\pi_{\bullet\bullet}$ are bounded by [0 – 1] and the algorithm uses a logit transformation for each ($\Theta = \text{Logit}(\theta) = \log(\theta(1-\theta)^{-1})$. Estimates are then provided using a back transformation. The standard error of the logit of the estimates may be estimated as the diagonal element of the Variance–Covariance matrix, through Fisher's information matrix. Using the normal asymptotic distribution of the ML estimator, a confidence interval for all parameters can then calculated.

B: The EM algorithm.

This algorithm is reported in Qu et al. (1996). A less general, but more intuitive, version of this algorithm may be found in Singer et al. (1998).

In the E-step, given a current set of parameter estimates, the posterior probability $h_{pdn}$ of $D = d$ for an individual showing the results pattern $y_n$ in population $p$, is evaluated, using Bayes theorem, as following:

$$h_{pdn} = \Pr\{D = d | y_n, p\} = \frac{\Pr\{y_n | D = d, p\}\Pr\{D = d, p\}}{\Pr\{y_n, p\}} = \frac{\pi_{pd} \prod_{t=1}^{T} (\theta_{dt})^{y_{tn}} (1-\theta_{dt})^{1-y_{tn}}}{\sum_{d=0}^{1} \pi_{pd} \prod_{t=1}^{T} (\theta_{dt})^{y_{tn}} (1-\theta_{dt})^{1-y_{tn}}}$$

In the M-step, the disease prevalence in each sub-populations of unknown prevalence $p$ are updated as:

$$\pi_{p1} = \Pr\{D = 1 | p\} = \frac{\sum_{n=1}^{N_p} \Pr\{D = 1 | y_n, p\}}{\sum_{d=0}^{1} \sum_{n=1}^{N_p} \Pr\{D = d | y_n, p\}} = \frac{\sum_{n=1}^{N_p} h_{p1n}}{N_p}$$

and $\pi_{p0} = 1 - \pi_{p1}$. For the disease-free reference population, $\pi_{p1}$ is set to 0; for the infected reference population, $\pi_{p1}$ is set to 1.

For each test $t$, the parameters $\theta_{dt}$ are updated as:

$$\theta_{dt} = \Pr\{y_t = 1 | D = d\} = \frac{\Pr\{D = d, y_t = 1\}}{\Pr\{D = d\}} = \frac{\sum_{p=1}^{P} \sum_{n=1}^{N_p} h_{pdn} y_{tn}}{\sum_{p=1}^{P} \sum_{n=1}^{N_p} h_{pdn}}$$

The E-step and the M-step are performed iteratively until convergence of the estimates. The EM algorithm converges to the ML estimator.

C The pairwise coefficient correlation

The correlation between test $i$ and $j$ in each sub-population is given by:

$$\text{corr}_{ij} = \frac{\Pr\{y_i = 1, y_j = 1\} - \Pr\{y_j = 1\}\Pr\{y_j = 1\}}{\sqrt{\Pr\{y_i = 1\}\Pr\{y_i = 0\}\Pr\{y_j = 1\}\Pr\{y_j = 0\}}}$$

For the observed correlation, the estimation for $\Pr\{y_i = 1|p\}$ is

$$\Pr_{obs}\{y_i = 1|p\} = \frac{\sum_{n=1}^{N_p} y_{in}}{N_p}$$

The estimation for $\Pr\{y_i = 1, y_j = 1\}$ is

$$\Pr_{obs}\{y_i = 1, y_j = 1|p\} = \frac{\sum_{n=1}^{N_p} y_{in} y_{jn}}{N_p}$$

For the expected correlation, $\Pr_{exp}\{y_i = 1\}$ is $\pi_{p0}\theta_{0i} + \pi_{p1}\theta_{1i}$, and the estimation for $\Pr_{exp}\{y_i = 1, y_j = 1\}$ is $\pi_{p0}\theta_{0i}\theta_{0j} + \pi_{p1}\theta_{1i}\theta_{1j}$.

The residuals of pairwise correlation coefficients are then $\text{corr}_{obsij} - \text{corr}_{expij}$.

# SENSITIVITY ANALYSIS BY EXPERIMENTAL DESIGN AND METAMODELLING FOR INTERIBR-ENDEMIC

A. VONK NOORDEGRAAF*, M. NIELEN* J.P.C. KLEIJNEN **

## SUMMARY

In many scientific studies, sensitivity analysis of simulation models is performed by changing only one factor at a time. Such an approach results in less accurate estimates of factor effects, and does not allow for estimation of interaction between factors. Experimental design and metamodelling (Kleijnen & Sargent, 2000) supports a structural approach to sensitivity analysis, and is more effective and efficient in estimating factor effects, including interactions. This paper applies these techniques to the simulation model InterIBR-endemic, which simulates the spread and control of BHV1 within and between cattle farms (Vonk Noordegraaf et al., 2000). Linear (OLS) and non-linear (logistic and tobit regression) regression metamodels were fitted to the input-output data of the simulation experiments. When dealing with a censored outcome variable, tobit regression is considered more appropriate than OLS. Future field studies should focus on getting better estimates of factors to which the simulation model is most sensitive.

## INTRODUCTION

To support decision makers in the national BHV1-eradication program in The Netherlands, the spatial, dynamic and stochastic simulation model InterIBR-endemic was developed (Vonk Noordegraaf et al., 2000). InterIBR-endemic simulates the spread and control of BHV1 within and between cattle farms in The Netherlands. This model contains many uncertain input factors and as part of verification and validation, it is important to evaluate the sensitivity of model-outcome to these factors. Sensitivity analysis allows for identification of parameters that have most impact on model outcome. In many scientific studies, sensitivity analysis is performed by changing only one factor at a time (OAT designs). This results in less accurate estimates of factor effects, and does not allow for estimation of interaction between factors (Kleijnen, 1998). The techniques of Design of Experiments (DOE) and metamodelling (Kleijnen and Sargent, 2000) support a structural approach to sensitivity analysis, and are more effective and efficient in estimating factor effects, including interactions.

In a simulation context, DOE can be defined as selecting, from the great number of possible combinations of factor levels, the set that actually needs to be simulated in an experiment with the simulation model, in order to quantify factor effects (Hunter & Naylor, 1970). The

* Department of Social Sciences, Farm Management Group, Wageningen University, Hollandseweg 1, 6706 KN Wageningen, The Netherlands

** Department of Information Systems, Center for Economic Research, Tilburg University, 5000 LE Tilburg, The Netherlands

simulation model is run for this set of combinations, and resulting input-output data are analysed by regression analysis to derive conclusions about the importance (sensitivity) of the factors. This analysis is based on a metamodel, which is defined as a model of the simulation model. Figure 1 shows the relationships among metamodel, simulation model and problem entity.

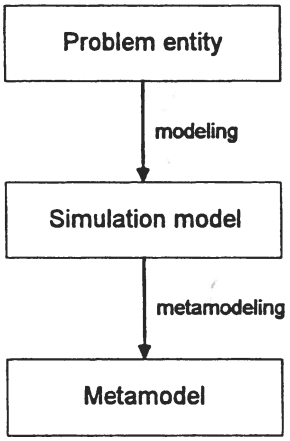


Fig 1. Metamodel, simulation model and problem entity .

The concept of metamodels can be explained by viewing the simulation model as a function that turns input factors into output performance measures. The explicit form of this function is unknown, but by experimentation with the model this function is approximated with a metamodel. The metamodel then treats the simulation model as a black box (Bettonvil & Kleijnen, 1996). The purpose of a metamodel is to estimate the response surface; the metamodel can then be used, instead of the actual simulation program, to learn about how the response surface would behave over various regions of the input-factor space (Law & Kelton, 2000).

The main goal of this paper is to show how the techniques of experimental design and metamodelling can be applied in the sensitivity analysis of complex simulation models. Furthermore, the application of three regression techniques fitting the simulation input–output transformation is demonstrated: Ordinary Least Squares (OLS), logistic and tobit regression. A theoretical discussion on the application of these regression techniques in veterinary epidemiology has been presented at the SVEPM in 1998 (Carpenter, 1998).

## MATERIALS AND METHODS

The ten steps suggested by Kleijnen and Sargent (2000) were adopted for development of a metamodel: (1) Determine the goal of the metamodel; (2) Identify the inputs and their characteristics; (3) Specify the domain of applicability; (4) Identify the output variables and their characteristics; (5) Specify the accuracy required of the metamodel; (6) Specify the metamodel's validity measures and their required values; (7) Specify the metamodel, and review this specification; (8) Specify a design including tactical issues, and review the DOE; (9) Fit the metamodel and (10) Determine the validity of the fitted metamodel. In this paper only a few of

these steps are highlighted, with the main emphasis on step 9, regression techniques to fit the metamodel.

Metamodel variables

In steps 2 and 3, factors and their levels were identified. Just as in OAT designs, there was no general prescription for which factors to select and what levels to assign to each factor; this depends on the goal of the study. A total of 31 factors used in the simulation model InterIBR-endemic was selected. These factors were all related to disease spread, and had in common that they were uncontrollable by decision makers and their estimation contained uncertainty. The control program was considered fixed. Sensitivity analysis requires that each factor has at least two levels, and therefore a lower and upper level were determined for each factor. Values assigned to each level reflected uncertainty of factor values in real life, based on data if available, or expert opinion otherwise. These levels also determined the experimental frame for which the metamodel was valid. Factor levels were standardised to 0 and 1, to enable comparison of factor effects by relative importance.

In step 4, simulation outputs of interest in the sensitivity analysis were selected. These were used as dependent variables in regression analysis, and for each of these outputs a metamodel was specified. In this paper the focus is on only one simulation outcome; mean number of weeks necessary to reduce the prevalence level to 5% in the dairy cattle population, applying the national control programme. Because simulation stopped when this prevalence level was not reached within 1000 simulated weeks, data were considered to be censored.

Metamodel definition and analysis

Specification of the form of the metamodel was required in step 7 of the metamodelling process. Initially, the metamodel was specified as a simple first-order polynomial, in which the independent variables (X) were standardised at either 0 or 1:

$$y_i = \beta_0 + \sum_{h=1}^{k} \beta_h x_{i,h} + e_i$$

In this additive metamodel, $y_i$ denoted the simulation response of factor combination i, $\beta_0$ the overall mean, $\beta_h$ the main effect of factor h, $x_{i,h}$ the value of the standardised factor h in combination i, and $e_i$ represented approximation error. Later, this metamodel was extended with effect modifiers (interactions).

To allow efficient estimation of the coefficients (factor effects) in this metamodel, an experimental design was constructed (step 8). In this design, each scenario represented a combination of factor levels. Dealing with 31 factors and two levels for each factor, a total of $2^{31}$ scenarios could be constructed. However, to give unique estimates of the 31 main effects and overall mean of the metamodel, a minimum of 32 factor scenarios would suffice. Because estimation of certain two-factor interactions requires more scenarios, a design with 64 scenarios was constructed, by applying the Foldover principle to a $2^{31-26}$ fractional factorial design (Kleijnen, 1998). The resulting design matrix was orthogonal, thereby minimising the variance of the estimated factor effects. In total, 64 simulation experiments were performed with the simulation model, each experiment replicated twice. Using 5 computers, (Pentium III, 600 Mhz), total calculation time was about 2 weeks.

Fitting the specified metamodel to the resulting input-output data (step 9), classic DOE uses Ordinary Least Squares (OLS). When the 5% prevalence level was not reached within 1000 weeks (threshold), simulation output was set to 1000 weeks, although the true value could have been much higher. This is called upper censoring. With OLS regression, censored observations will result in underestimation of the factor effects, and therefore produce inconsistent estimates. Dealing with censored data, a censored regression model or tobit model may be more appropriate (Long, 1997; Greene, 1997; Carpenter, 1998). The tobit model includes information about the censoring, and thereby provides consistent estimates of factor effects (Long, 1997). The form of the underlying tobit metamodel was similar to the OLS metamodel, with the difference that the dependent variable y was now a latent variable. Observations were never seen above the threshold value of 1000 weeks. Tobit regression is based on maximum likelihood estimation, where the log likelihood of the censored regression model consists of two parts; one corresponding to the classical regression for the non-limit observations and one corresponding to the probabilities for the limit observations (Greene, 1997). Using tobit regression, the expected value of an upper censored variable equals (Long, 1997):

$$E(y \mid x_i) = [\Pr(\textit{uncensored} \mid x_i) \times E(y \mid y < \tau) + [\Pr(\textit{censored} \mid x_i) \times E(y \mid y = \tau)]$$

where $\Pr(\text{censored}|x_i)$ is the probability of a scenario with factor combination $x_i$ being censored and $\tau$ the threshold value. Long (1997) shows that $E(y|x_i)$ is non-linear in x. To identify which factors significantly contributed to the event that the simulation outcome censored at 1000 weeks, logistic regression was performed. For the logistic model, the dependent variable was made dichotomous by transforming simulation output to 1 if censored (y=1000), and to 0 if not censored (y<1000). Logistic regression uses a log linear model in which the probability of the simulation outcome being censored (y=1) is modelled as (Hosmer & Lemeshow, 1989):

$$E(y \mid x_i) = \pi(x_i) = \frac{e^{\beta_0 + \beta_1 x_1 + .... + \beta_k x_k}}{1 + e^{\beta_0 + \beta_1 x_1 + .... + \beta_k x_k}}$$

Estimation of factor effects by logistic regression is based on a maximum likelihood procedure, using the logit transformation of $\pi(x)$ (Hosmer & Lemeshow, 1989). For all regression models, factors were excluded from the model with a backward elimination procedure. Possible interaction terms were investigated and added to the regression model with a forward conditional selection procedure.

Metamodel validation

To validate the metamodel with respect to the simulation model (step 10), new scenarios could be run, and simulation output compared with metamodel output. Because the simulation model required a lot of computer time, the technique of cross-validation, which requires no new simulation runs, was applied. Cross-validation means that factor input combinations (scenarios) are eliminated one by one, the regression model re-estimated, and the resulting metamodel used to predict simulation realisation for the combination eliminated. These predictions are then compared with the corresponding simulation responses (Van Groenendaal & Kleijnen, 1997; Kleijnen & Sargent, 2000). Cross-validation was applied to the metamodel estimated by OLS, deleting all 64 scenarios one by one, re-estimating coefficients on 63 scenarios, and predicting simulation realisation for the deleted scenario.

## RESULTS

From the 64 scenarios simulated, 23 did not reach a 5% prevalence level in the dairy cattle population within 1000 weeks. Table 1 shows the fitted metamodel applying OLS and tobit regression of y on x for all observations, with the censored observations included as y=1000.

Table 1. Factor estimates and individual p-values of fitted metamodel for simulation outcome 'weeks to 5% prevalence', using OLS and tobit regression.

| | OLS regression | | Tobit regression | |
|---|---|---|---|---|
| Factor | Estimate | P-value | Estimate | P-value |
| Intercept | 217.6 | 0.002 | -18.0 | 0.840 |
| Local spread | 175.9 | 0.005 | 251.5 | 0.004 |
| Reactivation rate transport | 158.5 | 0.000 | 220.8 | 0.000 |
| Yearly reactivation rate | 226.1 | 0.000 | 297.1 | 0.000 |
| Professional contact | 92.4 | 0.011 | 139.7 | 0.005 |
| $R_0$ non-vaccinated | 91.4 | 0.070 | 109.6 | 0.093 |
| $R_0$ killed vaccine | 125.5 | 0.014 | 200.0 | 0.004 |
| Weeks young stock infected | -89.6[a] | 0.013 | n.s.[b] | |
| Hygiene certified farm | -52.4 | 0.294 | -64.0 | 0.325 |
| Bulk threshold prevalence | -98.9[a] | 0.007 | n.s.[b] | |
| Sero sensitivity | -118.7 | 0.001 | -117.2 | 0.020 |
| Vaccine type used | 103.7 | 0.041 | 173.1 | 0.011 |
| *Interactions* | | | | |
| Vaccine type x $R_0$ killed | 188.0 | 0.010 | 212.6 | 0.042 |
| Local x Hygiene | -201.2 | 0.006 | -297.1 | 0.004 |
| Local x $R_0$ non | 280.8 | 0.000 | 445.3 | 0.000 |

[a] Sign of factor estimate opposite to expectation

[b] Main effect of factor not significant ($p<0.05$) in backward elimination procedure and therefore not included in final metamodel

The adjusted $R^2$ of the linear regression model using OLS was 0.82. Estimates for each factor in Table 1 reflect the expected change of the outcome variable when changing a factor from its low (0) to high (1) level. For example, changing the yearly reactivation rate in the simulation model from its low to high value increased the number of weeks required to reach the 5% prevalence level in dairy cattle to 226 weeks according to the metamodel fitted with OLS. In general, using upper censoring, tobit regression resulted in increased estimates compared to OLS regression. Most factors had a positive estimate due to increased risk of virus transmission. However, increasing hygiene on certified farms and sensitivity of serological tests, reduced the value of the outcome variable both in the OLS and tobit model, reflecting preventive effects. In the OLS metamodel, two factors had negative signs not in keeping with prior expectation, but these factors were not significant using tobit regression. In both models, three interactions had a significant effect on simulation outcome.

Table 2 shows the metamodel based on logistic regression, where the event of interest was the simulation not reaching a 5% prevalence level within 1000 weeks. Factors in this model also appeared significant in the OLS and tobit regression model.

Table 2. Factor estimates and individual p-values of fitted metamodel using logistic regression where event was the simulation outcome being censored at 1000 weeks.

| Factor | Estimate | St. error | P-value |
|---|---|---|---|
| Constant | -10.6 | 3.2 | 0.001 |
| Local spread | 4.2 | 1.5 | 0.013 |
| Yearly reactivation rate | 3.4 | 1.4 | 0.004 |
| $R_0$ non-vaccinated | 4.1 | 1.4 | 0.004 |
| $R_0$ killed vaccine | 4.1 | 1.2 | 0.013 |
| Hygiene certified farm | -2.9 | 1.4 | 0.004 |
| Vaccine type used | 4.1 | 3.2 | 0.001 |

From this metamodel, the probability of the outcome value being censored was calculated for each scenario, and compared to the simulation outcome. Using a cut-off value of 0.5, the overall fraction of correctly classified scenarios by the metamodel was 92.2%. From the scenarios being censored, 21 out of 23 were classified correctly by the logistic metamodel, and from the uncensored scenarios, 38 out of 41 were classified correctly.

Figure 2 shows a scatter plot of the results from cross-validating the metamodel based on OLS regression. The correlation coefficient between predicted and true outcome was 0.97.

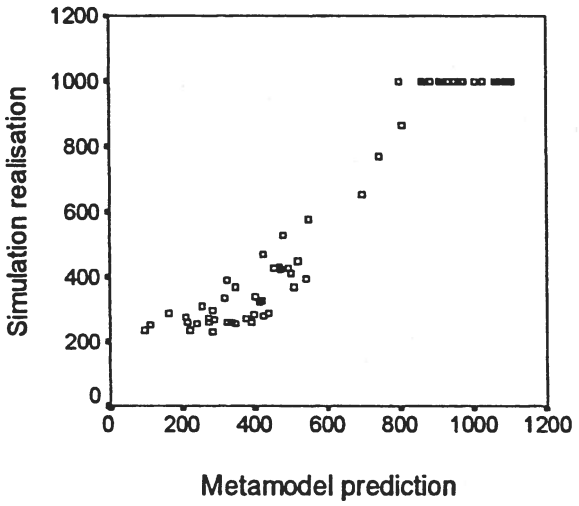


Fig 2. Scatter plot of OLS regression prediction and simulation realisation, where metamodel prediction was based on cross-validation procedure.

## DISCUSSION

The main goal of this paper was to present the application of experimental design and metamodelling as part of verification and validation of a complex simulation model. Whereas changing one factor at a time is common practice for sensitivity analysis of simulation models in the field of veterinary epidemiology and economics, it does not meet the statistical requirements that are obtained by experimental design and metamodelling in estimating and testing for significance of factor effects and interactions between factors. An exception is the study of Stärk and Pfeiffer (1999), although their focus was on five factors only.

Another goal of this paper was to show the application of three different regression techniques to the fitting of simulation input-output data obtained from this experiment. Whereas OLS and logistic regression are well known in veterinary epidemiology, tobit regression has only been applied recently (Ekstrand & Carpenter, 1998). Based on the adjusted $R^2$ and cross-validation results, model fit using OLS was quite good and the metamodel appeared to be valid with respect to the simulation model. Two factors that entered significantly into the metamodel, however, had an estimated sign opposite to prior belief. Programming code for these factors was verified and tested, but no errors were found. These factors did not appear significant in the metamodel using tobit regression. In general, dealing with censored data, OLS will produce inconsistent estimates of factor effects, whereas tobit regression takes into account information obtained from censored data (Greene, 1997). Logistic regression uses the information less efficiently than tobit regression, because continuous output is transformed into binary data. In this study it did provide additional information on factors for which the simulation model was most sensitive.

This paper only showed the metamodel for one output of the simulation model. Other outcome variables were investigated, such as the total disease control costs and number of outbreaks on certified farms. For each output a separate metamodel was developed. Because response variables were correlated, multivariate regression was also applied.

The goal of this study was to identify which uncertain factors had greatest impact on model outcomes of interest. Factors included in the final metamodel had most impact on outcome of the simulation model, changing factor level from low to high. It is essential to realize that the importance is based on the low and high level assigned to each factor (i.e., experimental frame). Low and high values chosen in this study, were supposed to reflect uncertainty of these factors in the real world. If the model is a good representation of the real system, a sensitive region established in the model can, by association, be considered to be so in the real system. With this assumption, it can be concluded that field studies must focus on getting better estimates of factors included in the metamodels. These may include; local spread, reactivation rate at transport and on farm, professional contact, $R_0$ for non-vaccinated herds and for herds vaccinated with killed vaccine. Also, some factors found to be important can be used to support advice given to farmers in the current eradication programme, such as the importance of hygiene on certified farms and preference for live vaccine. Three interactions between factors were found to be significant. If factor 'vaccine type used' was at its high level (all farmers use killed vaccine), the level of $R_0$ for killed vaccine was found to be very important on model outcome. Also, interactions between 'local spread' and 'hygiene certified farms' and between 'local spread' and '$R_0$ non-vaccinated herds' were found to be significant. Most interactions were related to the risk of introduction of virus on a farm (local spread), and the consequent virus circulation (hygiene, $R_0$ killed vaccine and $R_0$ non-vaccinated herd). If a sensitivity analysis had

been performed with one factor at a time, these interaction effects could not have been estimated.

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# APPLICATION OF PRINCIPAL COMPONENT ANALYSIS FOR CLASSIFYING EAST COAST FEVER REACTIONS IN CATTLE CHALLENGED WITH *THEILERIA PARVA*.

G. J. ROWLANDS, A.J. MUSOKE, V.A. NENE, R.P. BISHOP, S.M. NAGDA, AND S.P. MORZARIA,*

## SUMMARY

A statistically derived disease reaction index, based on daily parasitological, clinical and haematological measurements observed in 440 5-9 month old Boran cattle following laboratory challenge with *Theileria parva* in clinical trials of a sub-unit vaccine against East Coast fever, is described. Principal component analysis was applied to 13 variables including first appearance of schizonts, first appearance of piroplasms and first occurrence of pyrexia, together with duration and severity of these symptoms, and white blood cell count. The first principal component, which accounted for over 80% of the total variation expressed by the 13 variables, provided the definition for the disease reaction index, defined on a scale of 0-10. The extension of the method to 133 cattle exposed to natural tick challenge, for which incubation periods are unknown and white blood cell count is impracticable to measure, is also described. A correlation of 0.98 was found between the laboratory and field reaction indices.

## INTRODUCTION

Clinical scoring methods have in recent years been applied in human medicine to provide generic methods for assessing health status in clinical trials. However, until recently (Rowlands et al., 2000) there appears to have been no published application of such methods in veterinary medicine. The publication by Rowlands et al. (2000) refers to experimental work undertaken to evaluate a *Theileria parva* sporozoite surface antigen (p67) as a potential sub-unit vaccine against the parasite in cattle (Musoke et al., 1992). *Theileria parva*, a tick-borne parasite, causes a disease known as East Coast fever (ECF). The disease is of immense economic importance in eastern, central and southern Africa and inhibits the development of livestock production (Mukhebi et al., 1992). In comparing responses between immunised and non-immunised animals, disease reactions have been principally based on a subjective interpretation by clinicians of the severity of observations on pyrexia, parasitosis and haematology and other clinical symptoms. Responses to infection have then been categorised into those for which there was either no reaction (NR) or only a mild reaction (MR) and those for which the reaction was moderate (MODR) or severe (SR) (Anonymous, 1989). Animals that died or were euthanased (humanely killed) were among those categorised as severe reactors.

* International Livestock Research Institute, P.O. Box 30709, Nairobi, Kenya

The reliance on a clinician's judgement of what constitutes a mild, moderate or severe reaction has led to uncertainty in the reliability of the final outcome. A more objective method was considered necessary to reduce the levels of subjectivity in defining the severity of reactions of cattle to ECF in vaccine trials. The method chosen was that of principal components. This is a statistical method that finds linear combinations of a set of measurements (in this case describing the extent and severity of parasitological and clinical symptoms) that maximise the variation contained within them, thereby displaying most of the original variability in fewer dimensions. The first principal component, which is the new variable that accounts for the highest proportion of the variation expressed by the original variables, often provides a general average representation of the contribution of each of the individual measurements. As will be shown later, this is what tended to happen here. The method has already been applied to 13 variables derived from daily parasitological, clinical and haematological measurements observed in 309 5-8 month old Boran cattle following various laboratory challenge experiments with *Theileria parva* (Rowlands et al., 2000). The first principal component, which was based on approximately equal contributions of the 13 variables, provided the definition of the disease reaction index, defined on a scale of 0-10.

Following several years of experimentation with needle challenge under laboratory conditions it was decided to evaluate the sub-unit vaccine in the field to assess its efficacy under natural tick challenge. The ECF reaction index in its existing form (Rowlands et al., 2000) was not directly applicable for field use since the individual incubation periods when cattle are exposed to natural tick challenge are unknown. Thus, the time to first detection of schizonts, one of the 13 variables used for the index, cannot be derived. Furthermore, determination of white blood cell count, another variable, is often impracticable under field conditions. The purpose of this paper is to describe the statistical approach taken in extending the method of Rowlands et al. (2000) to deriving a disease reaction index suitable for both laboratory and field use.

## EXPERIMENTAL METHODS

### Cattle

Boran cattle (*Bos indicus*), 5-9 months old, free of antibodies to *Theileria parva* by ELISA (Katende et al., 1998), were used. A total of 440 cattle challenged in 20 laboratory experiments and 133 cattle challenged in three field trials were available for calculating the ECF reaction index. The numbers of records available had thus increased from those used by Rowlands et al. (2000) in the initial development of the index.

### Infection of cattle

Cattle were infected in laboratory experiments with Muguga stocks of *T. parva* stabilates. Each stabilate was inoculated subcutaneously over one of the parotid lymph nodes (local drainage lymph node) as described by Rowlands et al. (2000). Various immunisation regimens were used in these experiments, which led to the p67 formulation and treatment regimen evaluated in the field. Three field trials were undertaken in Kenya. Five laboratory experiments that used the same p67 formulation and treatment regimen as applied in the field are used to demonstrate the application of the reaction index in assessing efficacy of immunisation.

Monitoring of cattle

Rectal temperatures of cattle in both field and laboratory experiments were recorded daily after challenge. On day 5 after inoculation of cattle in laboratory experiments, and at daily intervals thereafter, needle biopsy smears were made from the local drainage lymph node, stained with Giemsa's stain and examined for the presence of schizonts. The degree of parasitaemia detected was scored on a scale from 1 to 3. Biopsy smears were similarly taken daily from the contralateral, pre-scapular lymph node from the day after the draining lymph node was found to be positive with schizonts. Both parotid lymph nodes were examined in cattle exposed to field challenge from day 5 after exposure. As soon as schizonts were detected in one of the lymph nodes this was designated as the local drainage node. Thereafter, biopsy smears were taken from both lymph nodes

Blood smears, prepared from the ear vein, were collected daily from the day after an animal first became positive with schizonts. These smears were stained with Giemsa's stain and piroplasm parasitaemia was determined as the number of infected erythrocytes per 1000. For cattle receiving a laboratory challenge blood for determining total white blood cell count (WBC) was taken from the jugular vein in EDTA before infection and three times per week thereafter.

Clinically severe cases of ECF

Cattle which became severely affected with theileriosis, the predominant signs of which included long durations and high levels of pyrexia and parasitosis, low white blood cell counts and poor general body condition, appetite and respiration rate, were humanely killed. Animals less severely affected were allowed to recover or, in one of the field trials, treated with an anti-theilerial drug.

## PRINCIPAL COMPONENT ANALYSIS

Laboratory experiments

A number of variables were derived from the parasitological, haematological and rectal temperature measurements taken (Table 1). One hundred and twenty six animals inoculated with a laboratory challenge were either killed on the basis of parasitological, clinical and haematological signs, or died, between day 14 and 22 post challenge. Thus, the lengths of the periods over which measurements were taken were shorter for these animals than for animals that recovered. In order to allow all animals to be compared on an equal basis, the period of measurement was fixed to 23 days, both for animals that died or were killed, and for animals that recovered. Thus, records were extended to day 23-post inoculation for any animal that did not survive that long. In doing so, the assumption was made that symptoms apparent at the time of death would have persisted at the same average intensity had animals survived until day 23. When an animal's temperature had returned to normal by the time of death, however, duration of pyrexia was not extended.

Table 1. Variables defined from measurements made on 440 5 to 9-month-old Boran cattle challenged in laboratory experiments and 133 challenged in field experiments for defining an ECF reaction index.

| Abbreviation | Description | No. of records available | |
|---|---|---|---|
| | | Laboratory experiments | Field experiments |
| SC1-FST | First day post inoculation that schizonts were detected in the local drainage lymph node. | 358 | 0 |
| SC1-LEN | Length of period (days) over which schizonts were observed. | 232 | 113 |
| SC1-INT | Average score intensity over the period that schizonts were observed. | 358 | 129 |
| SC2-FST [a] | First day post SC1-FST that schizonts were detected in contralateral, pre-scapular lymph node. | 311 | 73 |
| SC2-LEN | As for SC1-LEN | 185 | 57 |
| SC2-INT | As for SC1-INT | 311 | 73 |
| TEMP-FST [a] | First day relative to SC1-FST that a temperature > 39.4°C was recorded. | 354 | 109 |
| TEMP-LEN | Length of period (days) over which pyrexia was observed. | 228 | 93 |
| TEMP-INT | Average intensity of pyrexia, expressed as deviation from 39.4°, over the period that pyrexia was observed. | 354 | 109 |
| PIRO-FST [a] | First day post SC1-FST that piroplasms were detected. | 324 | 88 |
| PIRO-LEN | Length of period (days) over which piroplasms were observed. | 198 | 74 |
| PIRO-MAX | Maximum value recorded. | 324 | 88 |
| WBC | Mean white blood cell count between day 13 and day 19-post inoculation. | 440 | 0 |

[a] The definitions of SC2-FST, TEMP-FST and PIRO-FST are given as deviations from SC1-FST rather than as days post inoculation, which are slightly different from those of Rowlands et al. (2000).

In cases where either schizonts, piroplasms or pyrexia were not observed (for example, 73 animals that demonstrated no parasitosis or pyrexia, and 47 animals that were detected with schizonts in the local drainage and not the contralateral, pre-scapular lymph node) values were substituted for missing days of first occurrence. Thus, a value of 14 for days post challenge was substituted for 'first detection' of schizonts in the local drainage lymph node (SCI-FST). Similarly, values of 8, 6 and 10 were substituted for the subsequent number of days to 'first detection' of schizonts in the contralateral, pre-scapular lymph node, for pyrexia and for piroplasms, respectively, following SC1-FST. Frequency distributions showing the variations in the first day of occurrence of schizonts, piroplasms and pyrexia are shown in Fig. 1. The above definitions were based on the observation that the majority of observed reactions to infection had taken place by the respective time point. In each case when symptoms were not recorded their duration was defined to be zero.

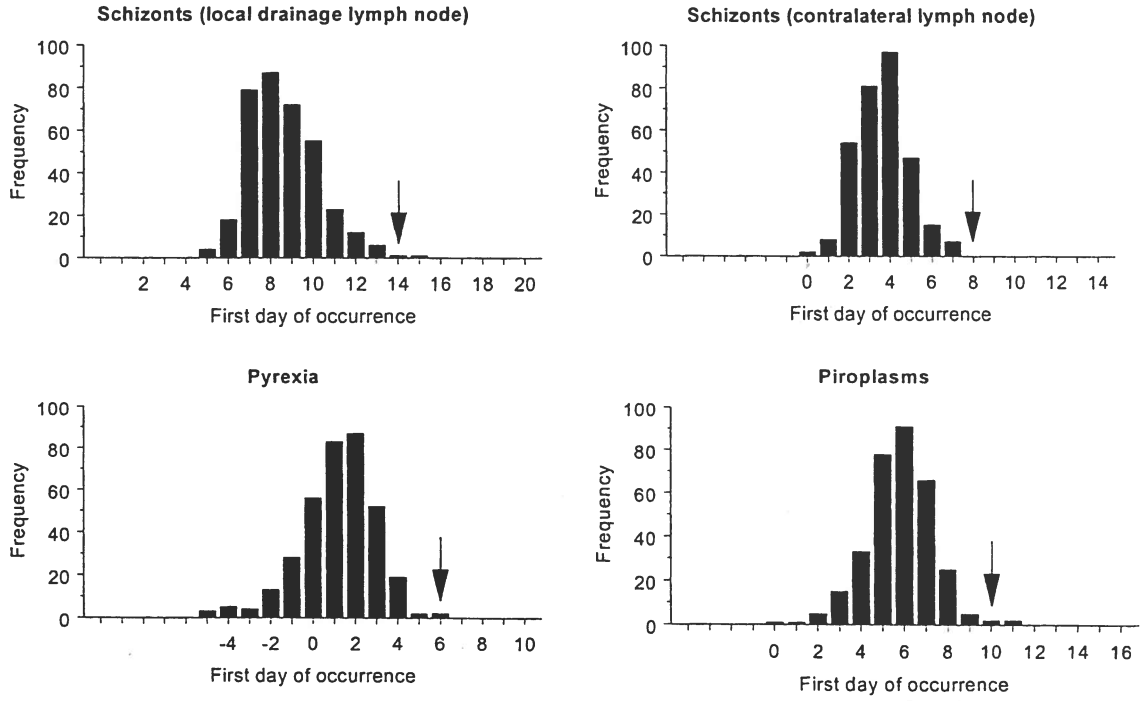


Fig. 1. Histograms of the first days of detection of schizonts in the local drainage node and the number of subsequent days to first detection of schizonts in the pre-scapular, contralateral lymph node, to first detection of pyrexia and to first detection of piroplasms in Boran cattle. The vertical arrows indicate the values substituted for animals that showed no symptoms.

Mean white blood cell count was calculated between day 13 and 19 when minimum counts were generally reached. Mean pyrexia was calculated as the mean deviation (rectal temperature – 39.4°C) over the period for which pyrexia was observed. A rectal temperature of 39.4°C or below during this period contributed a zero value in the calculation of the mean intensity of pyrexia. Similarly, failure to detect schizonts on a particular day during the period that schizonts were recorded counted as zero in the calculation of the average schizont intensity. The maximum piroplasm parasitaemia per 1,000 erythrocytes recorded was transformed to natural logarithms to make the data less skewed.

The 13 variables were then standardised $\left[x' = \left(x - \bar{x}\right)/sd\right]$ before subjecting the data to a principal component analysis using Genstat (Payne et al., 1993). For the purposes of this analysis only 232 animals that recovered and showed some clinical reaction were used, thus excluding observations based on statistically extended periods for animals that were humanely killed or animals that died, and animals that showed no clinical reactions (NR) at all. The method was then repeated on these 232 animals, but without SC1-FST and WBC, variables not possible to determine under field conditions

Field trials

Periods of recording of parasitological and clinical measurements obtained from the field were fixed to 15 days for calculation purposes. This period was based on the assumption that schizonts were first detected in the local drainage lymph node at an average of 8 days post challenge (see Fig. 1). This gives a total period of observation post challenge of 23 days, the same as that used for the analysis of the laboratory data. Other definitions were as described for the laboratory experiments. Ninety animals that recovered from infection under field challenge and showed some clinical reaction were available for verification of the index under field conditions.

## RESULTS

Principal components

Table 2 shows the coefficients of the first principal component when applied to data assembled from both laboratory and field experiments. When all 13 variables were used the component represents an overall 'average' contribution of the six variables representing length and intensity of infection. There are smaller contributions from coefficients representing time to first detection of schizonts and subsequent time to detection of schizonts in the contralateral, pre-scapular node and of piroplasms. The time of onset of pyrexia relative to that of schizonts was of negligible importance. When restricted to the 11 variables that can be determined under field conditions similar coefficients were obtained. Similar values were also obtained when the method was applied to the parasitological and clinical observations determined from 90 cattle under field challenge. This verifies that the principal components derived from disease indicators for cattle under laboratory challenge are applicable to those under natural field challenge. Furthermore, when all animals, including non-reactors and animals that were humanely killed or died, were considered, Rowlands et al. (2000) found that a slightly higher weight was attached to SC1-FST, but that otherwise coefficients were similar. This confirmed that the assumptions made in substituting values for non-reacting animals and animals that were humanely killed or died were reasonable.

The coefficients obtained from the first two columns of Table 2, based on the analyses of 13 and 11 variables derived from the laboratory data, were then applied to all cattle in laboratory and field experiments, respectively. The resulting scores were then standardised to ensure that they lay in a range between 0 and 10. When similar indices for the 440 animals recorded under laboratory challenge were calculated using just 11 variables, their correlation with those derived using all 13 variables was 0.98. This indicates that little information was lost when dropping WBC and SC1_FST from the analysis.

Table 2. Coefficients of the first principal component in a principal component analysis of pyrexia, parasitological and haematological measurements in 5 to 9-month old Boran cattle.

| | Laboratory experiments | | Field experiments |
|---|---|---|---|
| | All 13 variables (232) [a] | Without SC1-FST, WBC (232) | without SC1-FST, WBC (90) |
| SC1-FST [b] | -0.18 | - | - |
| SC1-INT | 0.30 | 0.32 | 0.24 |
| SC1-LEN | 0.32 | 0.33 | 0.31 |
| SC2-FST | -0.25 | -0.28 | -0.32 |
| SC2-INT | 0.31 | 0.32 | 0.34 |
| SC2-LEN | 0.34 | 0.35 | 0.36 |
| TEMP-FST | -0.04 | -0.06 | -0.14 |
| TEMP-INT | 0.31 | 0.32 | 0.28 |
| TEMP-LEN | 0.28 | 0.29 | 0.27 |
| PIRO-FST | -0.20 | -0.23 | -0.30 |
| PIRO-MAX | 0.34 | 0.35 | 0.34 |
| PIRO-LEN | 0.32 | 0.34 | 0.34 |
| WBC | -0.26 | - | - |
| Total variation accounted for (%) | 55 | 61 | 53 |

[a] Numbers of records used in data analysis – these were for animals that demonstrated a clinical reaction to infection and recovered.

[b] For description of variables see Table 1.

Analysis of five laboratory trials

The individual reaction indices for animals under laboratory challenge undergoing the same immunisation regimen as applied in the field trials are illustrated in Fig. 2. These indices are shown as the first principal component along the x-axis, plotted against scores determined for the second principal component along the y-axis. As discussed by Rowlands et al. (2000), this second principal component provided no meaningful biological interpretation; it is useful though for the purposes of displaying the data in the form of a scatter diagram. Inspection of these scatter diagrams shows that those animals that died or were humanely killed tended to have high index scores, whereas those that recovered tended to have low index scores. There was some overlap, however, in the middle. Animals with high index scores that recovered are nevertheless animals that will have developed sufficiently severe clinical symptoms likely to warrant chemotherapy if they were raised on a farm. A score of 6, based on a general assessment of reactions as determined by the clinicians (see Fig. 2), was defined to separate animals showing

signs of severe ECF from those showing mild signs of the disease. An animal with severe ECF was thus considered to be an animal that would die from exposure to the disease or would develop a sufficiently severe reaction (≥ 6) likely to warrant chemotherapy.

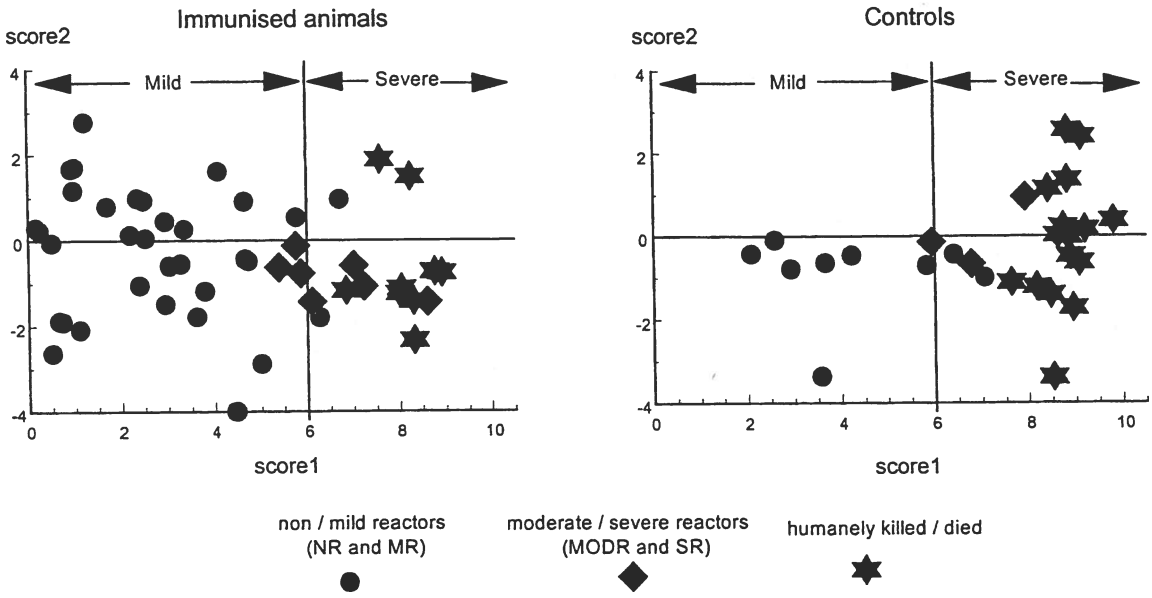


Fig. 2. Scatter diagrams of the first and second principal component scores for Boran cattle, susceptible controls or immunised with a p67 sporozoite antigen in five laboratory trials. The vertical lines separate statistically defined mild and severe cases of ECF. The symbols indicating severity of reaction refer to clinical assessment by the clinicians.

The number of mild and severe reactions defined in this way are summarised in Table 3 and the incidences of severe ECF in controls and immunised animals in each of the experiments are shown in Fig. 3. Application of logistic regression analysis to these data demonstrated reductions in proportional incidence from 0.80 ± 0.07 in susceptible controls to 0.39 ± 0.06 in immunised animals. The corresponding odds ratio, with 95% confidence limits, was 0.13 (0.04 – 0.43).

The data from the field trials have been analysed in the same way and await publication.

Table 3. Numbers of statistically defined mild and severe ECF reactions among controls and animals immunised with the sub-unit p67 vaccine in five laboratory experiments.

| | Mild ECF | Severe ECF | Total |
|---|---|---|---|
| Controls | 6 | 29 (25) [a] | 35 |
| Immunised | 36 | 20 (14) | 56 |

[a] Numbers in parenthesis are animals that died or were humanely killed.

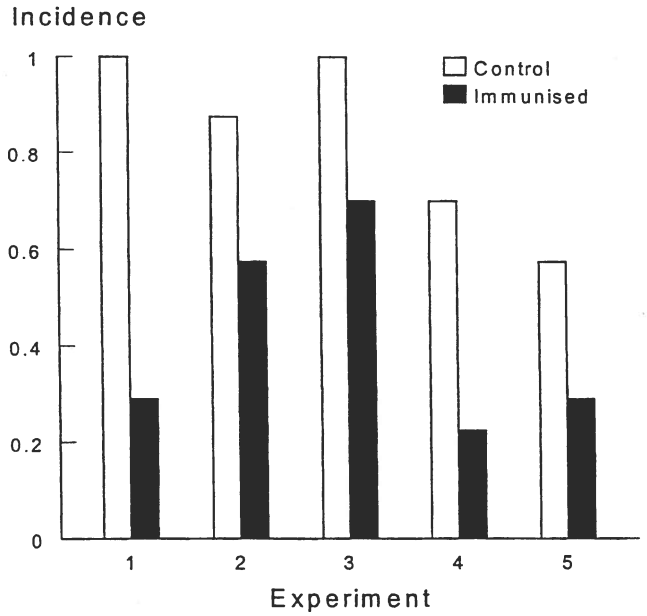


Fig. 3. The incidence of severe ECF in non-immunised and immunised Boran cattle in five laboratory experiments.

## DISCUSSION

Principal component analysis is only one of a number of approaches that have been adopted in human medicine – others include factor analysis, discriminant analysis and other special methods. A brief review is given by Rowlands et al. (2000). The major advantage of any of these indices is that they reduce the element of subjectivity implicit in clinicians trying to formulate a balanced view of the severity of infection, in our case from the various parasitological, clinical and haematological symptoms observed. It is important to emphasise, however, that the index, as developed in our situation, facilitates statistical analysis of trial results once an experiment has been completed, but cannot be used as an aid to clinicians. In our example the data were categorised into cases of severe and mild ECF and analysed by logistic regression. This was done to determine levels of protection provided by the vaccine.

Having developed our index score, however, its use as a continuous variable in an analysis of variance is also possible. In small-scale laboratory experiments, where a qualitative approach to defining severity of disease reaction may yield non-significant results, statistical analysis based on the value of the score itself improves the power of the statistical comparison between immunised and non-immunised groups. This does not mean that sample size can be reduced, since mortality or disease severity will remain an important trait for assessing vaccine efficacy, but rather that the data collected can be more efficiently analysed.

It is reassuring that the statistical method assigned higher index scores to those animals that were considered necessary to kill than to some of the animals that died (Rowlands et al., 2000). This would indicate that in the majority of cases clinical judgements were sound, and that delaying further the decision to sacrifice an animal would have caused animals unnecessary suffering. In contrast, in the few cases where there was overlap with cattle that recovered euthanasia may have been premature. It is furthermore reassuring that good correlation was

found between the statistically derived and clinically defined disease reactions (Rowlands et al. 2000).

Although the first principal component accounted for only 50 to 60% of the total variation expressed by the original variables, this analysis was done on only the subset of those animals that recovered from infection. As shown by Rowlands et al. (2000) the proportions of variation accounted for increased to over 80% when all animals were considered, including those that were humanely killed or died. It is also worthy of note that the indices based on 11 and 13 variables were highly correlated. This suggests that even under laboratory conditions evaluation of WBC may be unnecessary.

In conclusion, the levels of protection of the p67 vaccine determined in the large number of laboratory trials (Musoke et al., 1992) can be concluded to have been based on sound and unbiased clinical judgement. Indeed, use of the statistically derived reaction index has not altered the conclusions that could have been made by clinical judgement alone on the efficacy of immunisation. Rather, the index has provided a degree of objectivity in the reporting of results and a level of credibility in their acceptance that will be crucial for the presentation of the field trial results.

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# EXPERIMENTAL DESIGN TO TEST IF VACCINATION REDUCES TRANSMISSION OF *ACTINOBACILLUS PLEUROPNEUMONIAE*

A.G.J. VELTHUIS[1] M.C.M. DE JONG[1]

## SUMMARY

In this paper the design of an experiment to quantify the transmission of *Actinobacillus pleuropneumoniae* between non-diseased pigs is described. To create pigs that are infectious and non-diseased, an extended transmission experiment was designed in which the transmission was observed between the first group of pigs, that were contact-infected by inoculated pigs, and the second group of pigs. To investigate the effect of vaccination on transmission, only the second group of contact-exposed pigs was vaccinated in one of the experimental groups. Using the experimental data, generalised linear models were used to test the reduction in transmission due to vaccination.

## INTRODUCTION

The effect of vaccination on the interaction between pathogen and host has been studied intensively under experimental and field conditions. However, when vaccination is to be assessed as a tool in an eradication program the effect of vaccination on the transmission of the pathogen should be known. Whether vaccination reduces transmission can be determined in transmission experiments, by comparing the transmission between vaccinated and non-vaccinated animals.

For several viral infections, e.g. Pseudorabies virus (De Jong et al., 1984; Bouma et al., 1996) and Bovine herpesvirus (Mars et al., 2000) transmission experiments have already been designed and used to quantify the effect of vaccination on the transmission. However, this has not yet been done for bacterial infections. The difficulty with bacterial infections is that it is not clear which or when animals should be called infectious. Another problem is that the length of the infectious period is unknown for bacterial infections, whereas for most viral infections the infectious period is known to be short. To analyse data of transmission experiments the above mentioned parameters must be known.

In this paper the design of a transmission experiment of *A. pleuropneumoniae* is described and, secondly, it is shown how the experimental data should be analysed to test whether vaccination reduces the transmission.

[1] Quantitative Veterinary Epidemiology, Institute for Animal Science and Health (ID-Lelystad) & Wageningen Institute of Animals Science (WIAS), Wageningen University, P.O. Box 65, 8200 AB Lelystad, the Netherlands.

## TRANSMISSION EXPERIMENT

### Experimental design

It is believed that carriers, i.e., pigs that are potentially infectious but clinically healthy, play an important role in the transmission of *A. pleuropneumoniae* in the field. To be able to quantify the transmission between non-diseased pigs in this study, a suitable inoculation method to create infectious and non-diseased pigs was developed. A balance between the inoculation dose and route of administration had to be found, because a low dose or incorrect route could lead to no infection or, conversely, a high dose or incorrect route could lead to disease. In the first experiments with *A. pleuropneumoniae* where intranasal inoculation was used, it was shown that the probability of intranasal inoculation resulting in an infectious state was very low: 0.34 (Velthuis et al., submitted). Therefore, endobronchial inoculation was used in the final experimental design. Because pigs become diseased as well as infectious by endobronchial inoculation, an extended transmission experiment was chosen. In an extended transmission experiment the inoculated animals are replaced by new susceptible contact pigs when enough first contact pigs are contact-infected.

The experiment was a modification of that described by Bouma et al. (1997), and a schematic overview is given in Figure 1. In this experiment the transmission is compared between two experimental groups housed in separate isolation units. Each experimental group consists of 15 six-week-old, specific-pathogen-free pigs. One week before the start of the experiment, ten of 15 pigs are housed in an isolation unit; the other five pigs are kept in a separate unit in order to get used to each other and to the environment. On day 0, five of the ten pigs are placed in another unit, and the remaining five pigs are inoculated endobronchially with a 10ml inoculum containing $10^3$ colony forming units of *A. pleuropneumoniae* serotype 9 as described in Velthuis et al. (submitted). On day 1, the five un-inoculated pigs ($C_1$-pigs) are returned and are contact-exposed to the inoculated pigs. When at least four of the five $C_1$-pigs are found positive for *A. pleuropneumoniae*, i.e., when about 40 colonies or more are isolated, the inoculated pigs are removed and replaced by the five second contact pigs ($C_2$-pigs). From this moment on, the infection chain to be observed starts, and is monitored by taking nasal and tonsil swabs.

### Quantitative bacteriology

To determine whether the inoculated pigs are excreting *A. pleuropneumoniae* and whether the $C_1$- or $C_2$-pigs become contact-infected, nasal and tonsil swabs are taken for bacteriological examination. The frequency of swabbing is daily until the second week following replacement and three times a week during the last three weeks of the experiment. The collected swabs are spread directly on to a specific plate, as described by Velthuis et al. (submitted). Subsequently, two solutions of the swabs are made (3ml and a 1:100 dilution) and inoculated on to plates. After 24 hours the number of suspected colonies, i.e., small white/grey colonies surrounded by a large β haemolytic zone, is counted or estimated when there are too many. To confirm that the colonies are *A. pleuropneumoniae*, one typical colony per plate is tested for satellite growth and one for agglutination in a specific anti-serotype-9 serum. The average number of suspected colonies counted on the directly swabbed plate and the plate with the 3ml saline suspension is used for further statistical analysis.

The inoculated pigs (just after having been replaced) and all other pigs (at the end of the experiment) are examined at necropsy. The palatine tonsil, and, if present, pneumonic parts of the lungs are collected for bacteriological examination. For bacteriology of the specimens, the same procedure is followed as for the swabs.

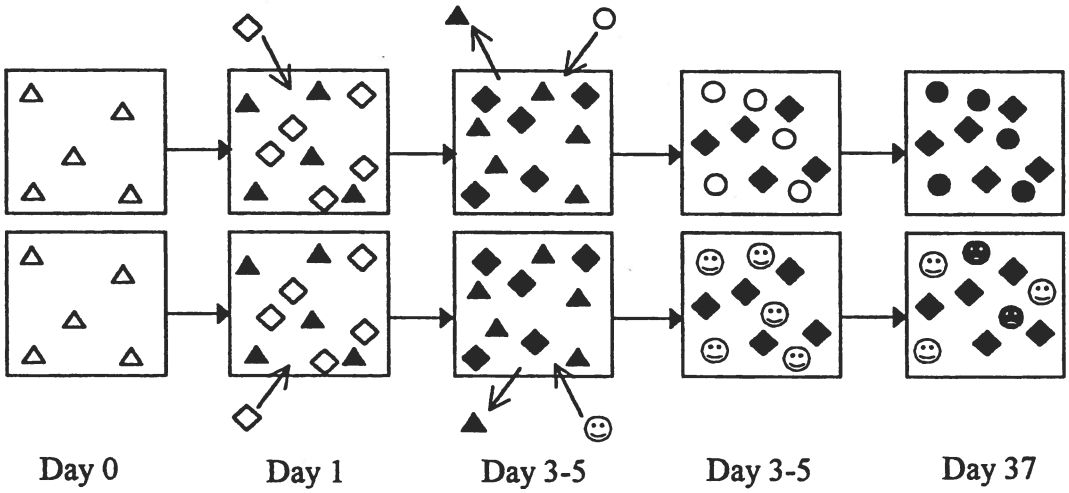


Figure 1. In this schematic overview, the experimental design of an extended transmission experiment to test the effect of vaccination on the transmission of *A. pleuropneumoniae* is shown. The unfilled figures represent non infectious pigs whereas the filled figures represent infectious pigs. The inoculated pigs are indicated with triangles, the $C_1$-pigs with diamonds, the unvaccinated $C_2$-pigs with circles, and the vaccinated $C_2$-pigs with "smileys"

Observing the infection chain

For the analysis, it is essential to know which pigs are susceptible or infectious. The experiment is started with SPF-pigs, so all the pigs are susceptible at the start but it is necessary to know which and when the animals became infectious. In a previous study (Velthuis et al., submitted), it was proven that pigs with an *A. pleuropneumoniae* positive tonsil at necropsy have been infectious during the experiment. It is plausible to assume that an infectious pig became infectious on the first day at which *A. pleuropneumoniae* was isolated from the nasal or tonsil swab. Until that particular day the pig is assumed to be susceptible.

The treatment and control groups

To test the effect of vaccination on the transmission in the extended transmission experiment for *A. pleuropneumoniae*, the $C_2$-pigs in one experimental group are vaccinated, whereas the $C_2$-pigs in the other group remain unvaccinated (Figure 1). The $C_1$-pigs are not vaccinated, since the vaccination probably also has an effect on the excretion pattern of the $C_1$-pigs. Consequently, the moment of replacement, which is dependent on the excretion of *A. pleuropneumoniae* by the $C_1$-pigs, becomes very unsure.

## ANALYSIS OF TRANSMISSION EXPERIMENTS

### Different methods

The following methods can be used to quantify transmission parameters from experimental data: the Final size estimation (De Jong et al., 1984), the Martingale estimation (Laevens et al., 1998), and an estimation based on a Generalised Linear Model (Becker, 1990). The Final size and Martingale estimations are based on the assumption that the final size of the outbreak is reached before the end of the experiment. For bacterial infections like *A. pleuropneumoniae* this assumption is not always valid, because it is possible that there are still infectious pigs present at the end together with (vaccinated) susceptible pigs. The Generalised Linear Model method (GLM) is not based on the final size assumption and is therefore more appropriate to use to analyse the data of transmission experiments with *A. pleuropneumoniae*.

### GLM-method

A stochastic model is used to model the transmission of *A. pleuropneumoniae* in an experimental group, because in small populations chance processes play an important role. The stochastic general susceptible-infectious-recovered model (SIR-model) from Becker (1990) was used as a starting point for this analysis, and was adjusted for variable infectivity for *A. pleuropneumoniae*, which was proven in Velthuis et al. (2000). In this SIR-model, the probability per susceptible individual of becoming infected is defined as the force of infection $\lambda$, which is a function of the infection parameter $\beta$, and the density of infectious individuals: $\lambda = \beta \cdot I/N$ where $I$ is the number of infectious individuals and $N$ the total number of individuals in the population. The relationship between the number of cases in a period (C), the number of infectious animals (*I*) within a period, the number of susceptible animals at the start of a period (*S*) and the infection parameter ($\beta$) is described by the SIR-model. The only unknown parameter is $\beta$, and it can be estimated from the experimental data using a GLM, with a complementary-log-log link function and $\log I/N$ as the offset.

Using data from the extended transmission experiments for *A. pleuropneumoniae* three different infection parameters can be estimated:

- $\beta_{uu}$: the number of contacts between unvaccinated susceptibles and unvaccinated infectious animals per day, multiplied by the probability of transmitting the infection per contact.
- $\beta_{vv}$: the number of contacts between vaccinated susceptibles and vaccinated infectious animals per day, multiplied by the probability of transmitting the infection per contact.
- $\beta_{vu}$: the number of contacts between vaccinated susceptibles and unvaccinated infectious animals per day, multiplied by the probability of transmitting the infection per contact.

In the study of De Jong et al. (1996), different $\beta$s were estimated for the transmission between seropositive and seronegative animals for bovine respiratory syncytial virus. In order to estimate the above mentioned $\beta$s, the following statistics for each period between subsequent samplings are computed: $S_v$ and $S_u$, i.e., the number of vaccinated or non-vaccinated susceptible pigs at the start of each period, $I_v$ and $I_u$, i.e., the average number of vaccinated or non-vaccinated infectious pigs in that period, and N, i.e., the total number of pigs present in that period. The number of cases, Cv and Cu is defined as the average number of pigs that became

infected per day during that period. It is assumed that the cases in a period are caused in the previous period. $C_v$ and $C_u$ have a binomial distribution, thus each $S_v$ or $S_u$-pig has a probability $P_v$ or $P_u$ of becoming infected during a period, where $P_v$ and $P_u$ are functions of $\beta_v$, $\beta_u$, $I_v$, $I_u$ and $N$:

$$P_v = 1 - e^{-\beta_v \frac{I_v + I_u}{N}} \qquad P_u = 1 - e^{-\beta_u \frac{I_v + I_u}{N}}$$

Separate estimates for $\beta_{vu}$ and $\beta_{vv}$ can be obtained by expressing $\beta_v$ as $\beta_v = a + b \cdot Q$ where covariate $Q = I_v/(I_v + I_n)$ is the fraction of the infectious animals that is vaccinated, and $a$ and $b$ the parameters to be estimated using the GLM method. When $Q$ equals zero then $\beta_{vu} = a$, and when $Q$ equals one then $\beta_{vv} = a + b$. The same procedure could be applied to estimate $\beta_{uu}$ and $\beta_{uv}$, but the fraction $Q$ is always zero in these data, so $\beta_u = c = \beta_{uu}$.

Whether vaccination has an effect on the transmission can be tested if, in the model for $C_v$, significantly more variation in $C_v$ is explained when $Q$ is added to the model. When $\beta_{vu} > \beta_{vv}$, it can be concluded that $S_v$-pigs are more easily infected by $I_u$-pigs than by $I_v$-pigs. Thus, vaccination reduces the infectivity of infectious pigs. In addition, whether $\beta_{uu}$ significantly differs from $\beta_{vu}$ can be tested using Student's t-test. When $\beta_{uu} > \beta_{vu}$, it can be concluded that $I_u$-pigs can more easily infect $S_u$-pigs than $S_v$-pigs. Thus, vaccination increases the resistance of susceptible pigs against infection.

## CONCLUDING REMARKS

Until now bacterial transmission experiments have not been developed and the effect of vaccination or other intervention methods on transmission has not been tested under experimental conditions. Data from the experiments and the model frameworks described provide opportunities to investigate the effect of vaccination in bacterial disease.

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# IF SPACE CHANGES ALL –

# THE SMALL-SCALE EPIDEMIOLOGY OF THE FOX TAPEWORM

F. HANSEN[1], K. TACKMANN[2], F. JELTSCH[3], C. STAUBACH[2], H.-H. THULKE[1]

## SUMMARY

Human infections with the larval stage of *Echinococcus multilocularis* are considered to be the most dangerous zoonotic disease in Europe. The parasitic cycle includes foxes as the definitive hosts and small rodents as intermediate hosts. Field studies show that high prevalence in foxes does not lead to accordingly high numbers of infected intermediate hosts. Using the spatially explicit simulation model Echi, this paper describes an investigation into how low prevalence in rodents can be explained. The study demonstrates that an uneven distribution of parasite in the intermediate host populations can cause the observed prevalence ratios. This probable spatial clustering makes control measures more costly. Finally the implications for the planning of control are discussed.

## INTRODUCTION

Recent reports of increasing fox populations, especially the ongoing invasion of urban habitats, have caused a considerable amount of public concern. It is not the fox itself but the fear of disease that comes with it, that raises public awareness. Among the most severe diseases transmitted by the fox is alveolar echinococcosis, a human infection with the larval stage of the fox tapeworm *Echinococcus multilocularis* (Eckert, 1996). Public pressure for information and countermeasures is increasing (Eckert & Deplazes, 1999).

In epidemiology, models can be used to predict the outcome of possible countermeasures (Gemmell & Lawson, 1986; Thulke et al., 2000). Thus, they are useful tools for planning control strategies. However, traditional epidemiological models, such as differential equation models, do not allow for a spatially non-homogeneous distribution of the parasite (Durrett & Levin, 1994). Moreover, they describe a system in equilibrium, whereas control measures are basically directed at disturbing this equilibrium to reduce the parasite's prevalence.

A spatially explicit simulation model is presented: Echi. Echi is used to examine the stability of the parasitic cycle under different assumptions. In endemic areas 60% or more of the fox population is *Echinococcus* infected (Eckert et al., 2000). Individual foxes often carry thousands of adult tapeworms (Hofer et al., 2000). The number of tapeworm eggs shed by these foxes is an

[1]UFZ-Centre for Environmental Research Leipzig/Halle, Dept. of Ecological Modelling, P.O.B. 2, D - 04315 Leipzig, Germany. e-mail: hansen@oesa.ufz.de.
[2]Federal Research Centre for Virus Diseases of Animals, Germany.
[3]University of Potsdam, Inst. for Biochemistry and Biology, Potsdam, Germany

immense infective pressure on the intermediate host population. Surprisingly, only very few (<1%) intermediate hosts carry the larval stage of the tapeworm (Delattre et al., 1988). Eggs of *Echinococcus multilocularis* are known for their sensitivity to elevated temperature and low relative humidity (Veit et al., 1995). In a heterogeneous landscape, abiotic conditions for egg survival most likely change within meters. This paper investigates whether differential mortality of tapeworm eggs in a heterogeneous environment can explain the striking differences in prevalence in the two hosts. If this is the case, differential mortality for tapeworm eggs leads to a spatial clustering of the parasite in the intermediate host. Such local concentrations of infection in the intermediate host have been reported anecdotally (Gottstein et al., 1996) but it remains to be shown that this over dispersed distribution of the parasite in the intermediate host is a general phenomenon. The spatial distribution of a parasite has an influence on the stability of the parasitic cycle (Pacala et al., 1990) and this will affect the success of control measures. Using simple control strategies, the effect of the parasite's spatial distribution on the outcome of control measures is investigated. This may help to identify strategic parameters needed for efficient control programs.

## MATERIALS AND METHODS

### The cycle

The fox tapeworm has a heterogeneous life cycle. Small rodents, predominantly microtine voles, are the most important intermediate hosts (Vogel, 1977). Oral uptake of small numbers of tapeworm eggs leads to infection in rodents (Vogel, 1977). In the liver of the intermediate host, the tapeworm larvae produce protoscolices within three months post infection (Eckert, 1996). Individual voles can harbour many thousands of protoscolices, each developing into an adult tapeworm when the infectious vole is consumed by a definitive host (Frank, 1984). In the mainly sylvatic cycle of the fox tapeworm in Europe, the red fox (*Vulpes vulpes*) is the main definitive host (Eckert et al., 2000). The larvae develop in the fox's intestines into a 4 mm long adults. Approximately four weeks post infection the new generation of tapeworms starts egg production (Eckert, 1996). The eggs are shed in the fox's faeces.

### The simulation model

Echi is formulated as a rule based simulation model, which subsequently is implemented in a computer programme. It combines grid based (Durrett & Levin, 1994) and individual based (DeAngelis et al., 1994) modelling techniques and is best described by a set of rules (see Figure 1). A set of basic rules (B1 to B4) is used, which describe the model's framework, and an additional set of simulation rules (S1 to S5) is used to determine the simulation procedure itself. Simulation rules are executed every simulation step. Finally, a technical rule (T1) is introduced.

Space is included in the model via grid cells, each representing the home range of a family group of voles. Infection of voles is assumed to be a point event in time and space. Infection of foxes is modelled via the predator-prey relationship between foxes and voles. The infection of voles is related to the defaecation behavior of foxes. As fox home ranges are found on a much larger spatial scale than vole home ranges, they are assumed to have no influence on the epidemiology of the smaller scale, and consequently are omitted, for the sake of simplicity. The population of hosts is considered to be homogeneous with respect to age. In special

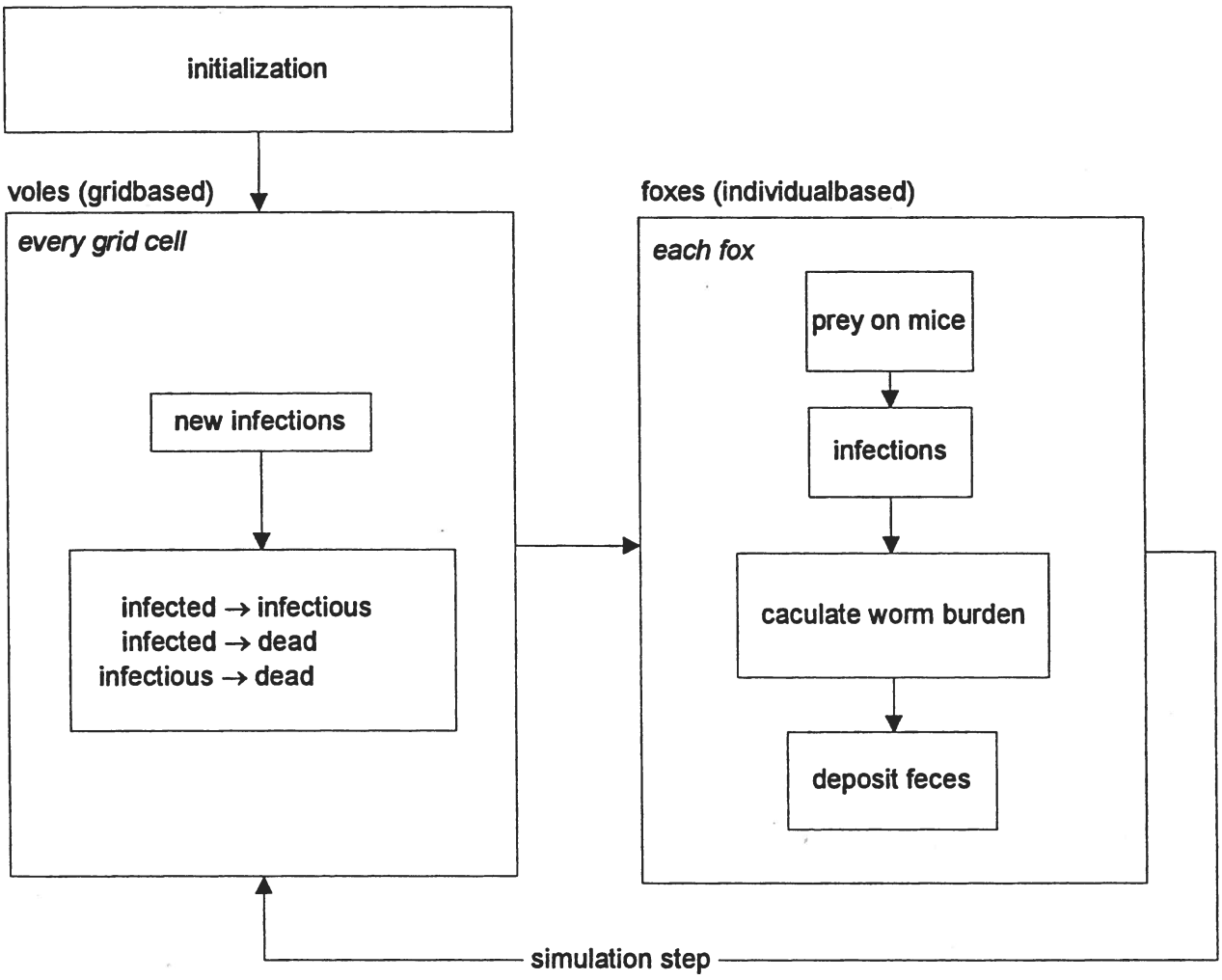


Fig. 1 Flow chart for Echi.
Voles are modelled as subpopulations inhabiting a common home range, a grid cell. Foxes are modelled individually. Infections of foxes and voles take place via the predator-prey interactions between foxes and voles and the defaecation behaviour of foxes, respectively.

circumstances (see below), the simulation model mimics a differential equation model for the population dynamics of the fox tapeworm by Roberts and Aubert (1995). Numerical values for parameters have been extracted from the literature and are given in Table 1. For intermediate hosts, life history parameters of the Common vole (*Microtus arvalis)* have been chosen (Roberts & Aubert, 1995).

Rules

B1: the grid

The space is represented by a set of 500 by 400 grid cells. Each grid cell represents the home range of a subpopulation of voles. Grid cells are defined as measuring 40m by 40m summing to a simulated area of 320 $km^2$.

B2: voles

Voles are represented by infective, but not yet infectious, and infectious proportion of the total subpopulation of a grid cell.

B3: foxes

Foxes are modelled individually. A total of 160 foxes populate the grid of 320 $km^2$.

B4: worms

Tapeworms have three stages; each is represented differently in the model.

- B4.1: adult worms in the definitive host are represented by numbers of individual worms in individual foxes. The time since infection determines the age of the individual worms and the start and duration of egg production.
- B4.2: proportions of the subpopulation of voles in the different infective stages represent larvae in the intermediate hosts. Voles can be either infected but not yet infectious or infected and infectious.
- B4.3: eggs, shed with the fox's faeces, are represented solely by their position in a grid cell.

S1: time step

The model runs in discrete time steps; one simulation step is one week.

S2: foxes

Foxes prey on voles, deposit faeces and suffer mortality.

- S2.1: foxes prey on voles
  Foxes consume a fixed number of voles in each time step. Whether a fox captures an infectious vole or not depends on the prevalence of infectious voles in the chosen grid cell. If a fox consumes an infectious vole, the fox is infected with 1 to 1000 tapeworm larvae. Infected foxes cannot be infected again.
- S2.2: foxes deposit faeces
  Foxes deposit a fixed number of faecal packages in each time step. In general, all faecal packages from an infectious fox contain tapeworm eggs. In the rare case that a fox harbours less adult worms than are defaecated, the number of contaminated faecal packages equals the number of adult worms.
- S2.3: foxes suffer mortality
  Foxes have a fixed probability of dying.

S3: voles

Voles acquire new infections; proportions of different infective stages of larvae in voles change due to maturation of larvae and voles suffer mortality.

- S3.1: voles get infected
  If infective tapeworm eggs are positioned in a grid cell, all susceptible voles become infected.
- S3.2: tapeworm larvae in voles mature
  A fixed proportion of voles that are not infectious, but infected, becomes infectious. This proportion equals 1/(time to maturation of larvae in voles).
- S3.3: voles die
  A fixed proportion of infected, but not yet infectious, and infectious voles die. This proportion equals 1/(mean life expectancy of voles).

S3 in summary: let $S_n$, $V_n$ and $W_n$ be the proportion of susceptible, infected but not yet infectious, and W infectious voles at time n, respectively. Then $S_n+V_n+W_n=1$, the population is constant in time.

If infective eggs are positioned in the grid cell, then $V_n = V_n + S_n$;

Proportions change in such a way that:

$$W_{n+1} = W_n + V_n/d - W_n/L \text{ and } V_{n+1} = V_n - V_n/d - V_n/l,$$

where d denotes the mean duration of the larval development until the first protoscolices appear and l denotes the mean life expectancy of voles.

S4: worms
Worms in foxes age, produce eggs and die, tapeworm larvae in voles mature and eggs lose their infectivity.

- S4.1: worms in foxes age, produce eggs and die
  Worms in foxes age. After "time to maturation of larvae in foxes" time-steps they are considered to be adult and begin egg production. After "mean life expectancy of worms in foxes" time-steps, the worms die.
- S4.2: tapeworm larvae in voles mature
  See S3
  If the prevalence drops below a numerical threshold (0.001), it is set to zero.
- S4.3: eggs lose their infectivity
  After one time-step, eggs lose their infectivity.

T1: Grid cells in which foxes prey or deposit faeces are drawn randomly from all grid cells.

In a model where, exceptionally, homogeneous mixing of vole population takes place, the following technical rule is added:

T2: in each time step, the averages of proportions of infected voles, both infectious and not infectious, are calculated. This average is assigned to every grid cell.

The homogeneous mixing model mimics classical differential equation models.

Robustness

A model's robustness can be understood as its ability to reproduce observed prevalences unaffected by variation in crucial parameters (Jeltsch et al., 1997; May & Anderson, 1983). Here the focus is on the infection probabilities of voles and foxes. Both are suspected of having the biggest influence on resulting prevalences. Under natural conditions, these parameters will vary to a certain degree. The order of magnitude to which they will vary can be estimated because, in the process-oriented model, they represent ecological relations. The infection probabilities for foxes are proportional to the number of voles each fox consumes in a given time. From the literature (e.g., Ansorge, 1991) it can be concluded that foxes rarely consume more than 60 voles per week, so the realistic range for the infection probability of foxes lies between 1 and 60. Voles are infected only when an infectious fox deposits tapeworm eggs, in faeces, in the voles' home range. The infection probability for voles thus is proportional to the number of different sites on which foxes deposit their faeces. In the absence of available data, informed estimates suggest a fox chooses not more than 35 different sites per week to deposit faeces. For 100 random combinations of these infection probabilities, the model's output prevalence in voles and foxes can be classified according to the match with field data. A realistic prevalence in voles lies between 0 and 2% while 7.5 to 60 % of infected foxes are assumed to represent field conditions.

Two scenarios are compared; a homogeneous scenario, in which all tapeworm eggs survive long enough to cause infections in intermediate hosts, and a heterogeneous scenario where eggs survive in only 1/16 of all grid cells. The heterogeneous scenario takes account of the known sensitivity of the eggs to low relative humidity and high temperature (Veit et al., 1995) that might lead to the loss of infectivity before coming into contact with susceptible voles.

Control

A simple control strategy is simulated by randomly curing 70% of foxes every four weeks. This mimics baiting campaigns with Praziquantel-enriched fox baits, which are used in field trials (Romig et al., 1999, Tackmann pers. Comm.) and a bait-uptake rate of 70% (Trewhella & Harris, 1991). Different control intervals are used later in the study.

Parameters

Parameters not defined elsewhere are displayed in Table 1.

Table 1. Parameters

| Parameter | Value | Reference |
|---|---|---|
| Time to maturation of larvae in voles | 12 weeks | (Roberts & Aubert, 1995) |
| Time to maturation of worm in foxes | 4 weeks | (Eckert, 1996) |
| Time interval for to worms produce eggs | 1 week | (Heath & Osborn, 1991) |
| Fox density | 0.5 $km^{-2}$ | (Roberts & Aubert, 1995) |
| Life expectancy of voles | 26 weeks | (Roberts & Aubert, 1995) |
| Life expectancy of worm in foxes | 13 weeks | (Eckert, 1996) |
| Death rate of foxes | 0.011923 $week^{-1}$ | (Roberts & Aubert, 1995) |

## RESULTS

Figure 2 shows the robustness of the homogeneous and the heterogeneous scenarios.

Only 2% of parameter combinations of infection probabilities lead to prevalences in accordance with observed patterns, if all tapeworm eggs cause infections in intermediate hosts. If most eggs are removed from the parasitic cycle, the model is 10 times more robust. Thus, the homogeneous scenario is only realistic in special cases while the heterogeneous scenario exhibits a wider generality (May & Anderson, 1983). As interest is in a general insight into the ecology of the fox tapeworm population dynamics, only the heterogeneous scenario is used for further analysis.

In the natural system, differential survival of tapeworm eggs due to different abiotic conditions will lead to a clustered occurrence of the tapeworm in the intermediate host. In classical epidemiological models of the Anderson-May type (Anderson & May, 1979) the assumption of homogeneous mixing of host populations will blur this spatial aggregation. To investigate how the spatial distribution of the parasite in the intermediate host population affects control measures, the Echi model is compared in its general form with a special-case model where the vole population is homogeneously mixed, by including rule T2. Control starts at week

300 and continues for various times. Figure 3 shows the fox prevalence at the end of the 10-year simulation period, i.e., the running mean of the last 150 weeks.

Less than two years of control have no persistent effect on fox prevalence. With and without spatial clustering, eradication can be achieved but the spatial aggregation of the fox tapeworm in the intermediate host requires a much higher control effort for the same goal. In the model, after four years of baiting, the fox tapeworm could be eradicated if no spatial aggregation occurs. In the more realistic case, that infections of the intermediate host are not evenly distributed in space, seven years control are necessary.

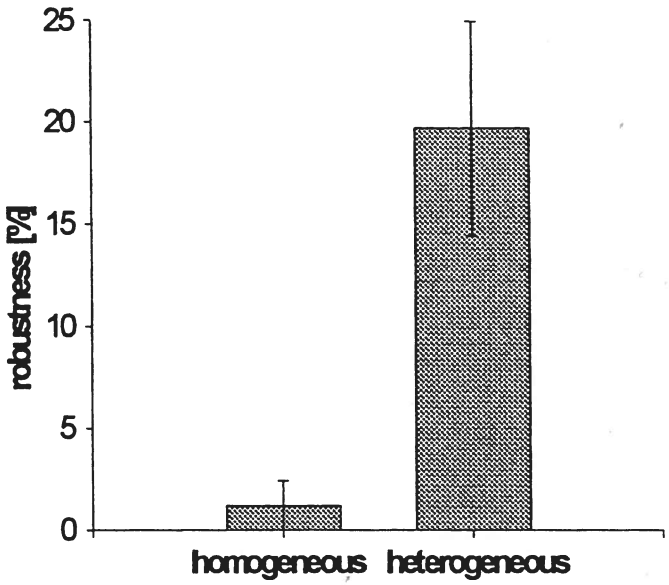


Fig. 2 Robustness of Echi on random combinations of infection probabilities.
The heterogeneous scenario, in which most tapeworm eggs die before they cause infections in voles, is more robust than the homogeneous scenario in which all eggs cause infections. Higher robustness reflects greater generality. Mean and standard deviation are from 30 repetitions of 100 random combinations each.

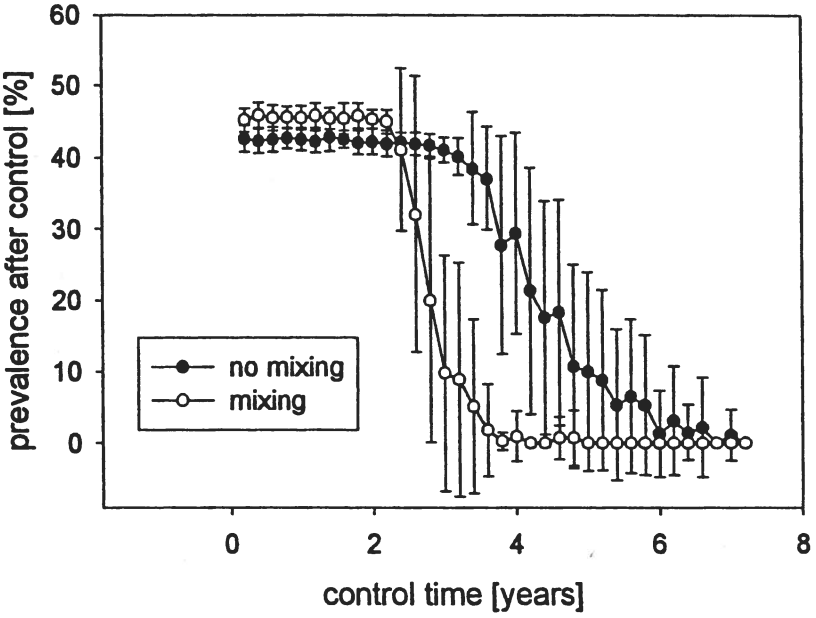


Fig. 3 Prevalence in foxes after varying control time with (open dots) and without (filled dots) homogeneous mixing. Without homogeneous mixing the parasite is clustered in space, which makes a longer control time necessary to achieve eradication. Infection probabilities 12 each, 10% of grid cells support egg survival, mean and standard deviation of 30 simulation runs.

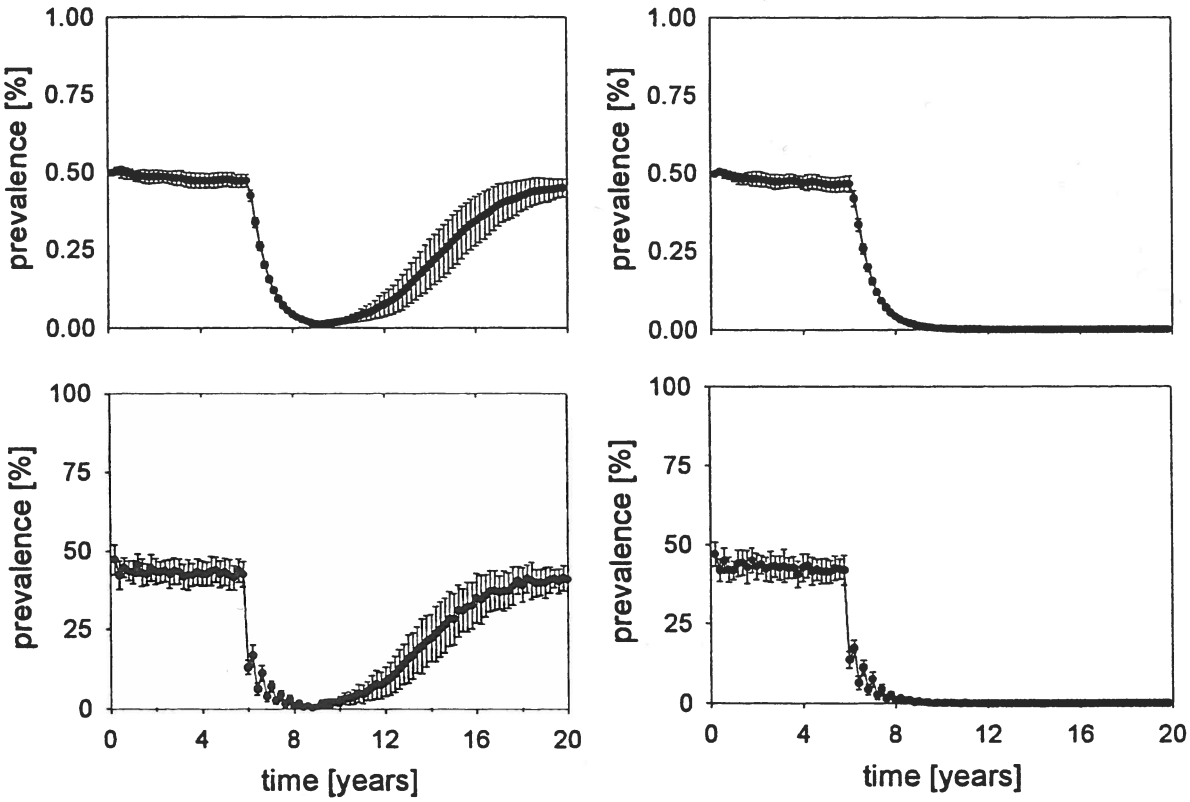


Fig. 4 Temporal development of prevalence in voles (top) and foxes (bottom) under control for 3 years (left) and 8 years (right). After 3 years of control, prevalence recovers quickly. After 8 years of control, eradication is achieved. Infection probabilities for both hosts: 12, 10% of grid cells support egg survival, mean and standard deviation of 30 simulation runs.

The effect of different control duration is shown in Fig. 4. Baiting quickly reduces prevalence in foxes. At the end of control, if eradication is not achieved (left), prevalence recovers quickly and reaches the pre-control level. Only when prevalence drops to zero is the effect persistent.

In Fig. 5 three different control strategies are compared. Triangles mark the control success when the original strategy from field trials (1 year every 6 weeks, then 12 weeks) is used. Open dots show control success for 6-week baiting intervals and filled dots for 4-week baiting intervals. The original strategy fails to show any persistent decline in prevalence. Both 4- and 6-week intervals reduce prevalence until the end of the 10-year simulation time. The 4-week intervals achieve a faster reduction than 6-week intervals. However, for the same cost (i.e., number of baiting campaigns, arrows), the 6-week intervals are judged to perform better.

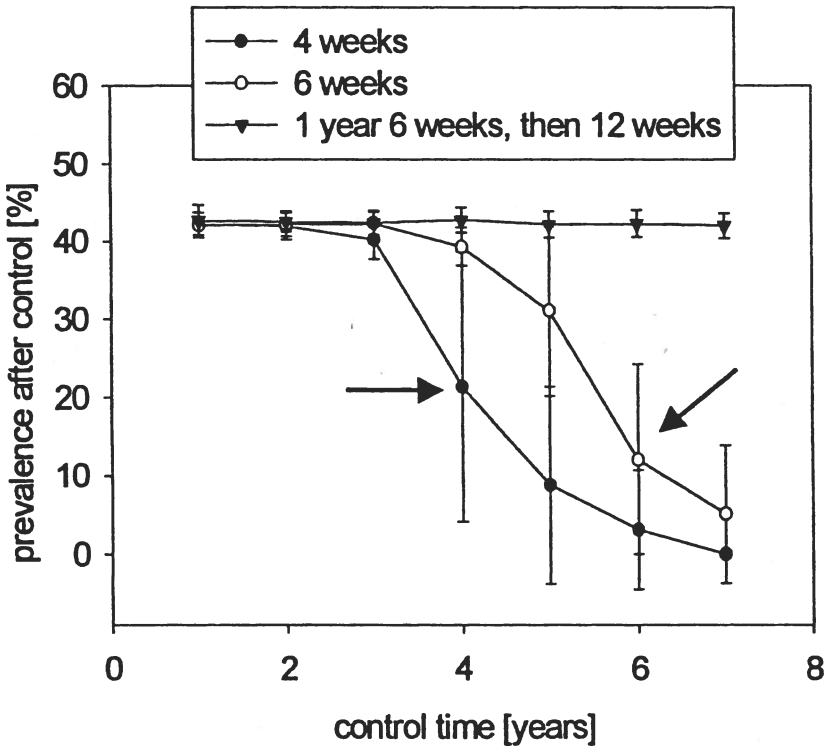


Fig. 5 Prevalence in foxes after varying control times and different intervals between consecutive baiting campaigns. The strategy used in field experiments (triangles) shows no persistent reduction of prevalence. Four-week intervals (filled dots) between campaigns shows a faster reduction of prevalence than six- week intervals (open dots). For equal control effort, however, (arrows), six-week intervals are more effective than four-week intervals. Parameters as in Fig. 3.

## DISCUSSION

The model produces realistic prevalences over a wider range of parameters when most tapeworm eggs are removed from the parasitic cycle (Fig. 2). A high number of infectious tapeworm eggs otherwise leads to unrealistically high prevalence in the intermediate host. The comparatively low numbers of infected voles can only be explained by the assumption that most eggs do not cause infections in voles. At present, it is unclear which mechanism is responsible

for this removal of eggs under natural conditions. Nevertheless, the reported sensitivity of the tapeworm eggs to elevated temperatures and low relative humidity offers an intuitive explanation. In a natural landscape, temperature and humidity most likely differ between sites, providing different conditions for the survival of *Echinococcus* eggs. Thus, from the point of view of the parasite, the landscape appears like a mosaic where only a limited area is suitable for the completion of the parasitic cycle. Empirical findings suggest that foxes more often are infected with *Echinococcus* if they live close to open water (Staubach et al., 1998) where conditions can be assumed to guarantee egg survival. Consequently, differential egg survival leads to a spatial clustering of infectious voles, which concentrates the infection risk for foxes in a few epidemiological hot spots. Since control measures are exclusively directed towards foxes, the source for reinfection of recently cured foxes remains unaffected. The reduction of this potential for infection is a simple die-out phenomenon (rule S3.3). Because a large population of infected voles in a local hot spot needs longer to die out than many small populations the infection risk for foxes lasts longer, even when no new voles are infected. Thus, it is not surprising that a clustered parasite is harder to eradicate (Fig. 3).

Classical epidemiological models most often use the assumption of homogeneous mixing between hosts to model new infections. This approach is inappropriate if the parasite is clustered in space. To investigate the consequences of the spatial distribution of the parasite, a spatially explicit model is needed. Another drawback of conventional differential equation models of the Anderson-May type is that they assume continuous time. Control effort is defined as a variable that indicates how much parasite reproduction needs to be hampered to reduce the basic reproduction rate, $R_0$, below unity (Mollison, 1984). How this reduction can be achieved cannot be investigated with this class of model. The simulation model Echi works in discrete time steps and enables this specific question to be addressed. While Fig. 3 shows how the outcome of control depends on the control effort (i.e., control time) Fig. 4 shows the effect of control on the endemic equilibrium. Although the prevalence in both hosts is reduced almost to zero, at the end of control the prevalence reaches the pre-control level. Apparently, the equilibrium value of the prevalence is determined by epidemiological and ecological factors. The system, after destruction of the equilibrium by control methods, will return to the same values. Thus, only eradication has a persistent effect.

One way to achieve eradication is simply to increase control effort by prolonging control time. Another control parameter is the control strategy. The control strategy used in two field trials in Germany fails to reduce *Echinococcus* prevalence in foxes persistently (Fig. 5). Shorter intervals between consecutive baiting campaigns and longer overall control duration have the potential to eradicate the parasite in a given area. However, the optimal control strategy must be a compromise between cost efficiency and control success. Without considering the economics in too much detail, it can be assumed that a constant baiting in order to reduce infection risk for humans is not feasible. Therefore, only local eradication, possibly in urban areas or under moderate endemic conditions, can be a realistic desired outcome of control. This can be achieved with different control strategies. The term "control strategy" here is used to describe the intervals between consecutive baiting campaigns. However, these intervals are not the only adjustable parameters that compose a "strategy". Other factors include the season in which to begin the baiting campaign, the number of baits per $km^2$ etc. (Tackmann & Conraths, 2000). Finding the optimal strategy is a time consuming task that requires a considerable amount of economic and epidemiological knowledge. The presented model will prove to be a valuable tool in this process.

The extent to which local eradication can be a feasible goal for urban areas or local endemic foci has to be a subject for further research. In the presented form, the simulation model Echi is not capable of addressing this question. Whether or not local eradication can be achieved obviously depends, among other factors, on the movement of foxes. For the sake of simplicity, the fox population in Echi for now is homogeneously mixed. This clearly limits the observed mechanisms in their generality. However, foxes do move around considerably (Trewhella et al., 1988) and so, on a smaller spatial scale of a few square kilometres, homogeneous mixing of foxes might be a reasonable assumption. Spatial segregation of foxes also leads to a clustering of the parasite in the definitive host. As foxes are the targets of control measures, unlike voles which cannot be accessed directly, spatial clustering will make it easier to achieve eradication. Although the assumption of a homogeneously mixed fox population is artificial, it is suggested that the predictions of Echi are conservative in such a way that it investigates the worst-case scenario. A spatially structured fox population will require more elaborate modelling of the baiting itself.

Recent reviews (Eckert et al., 2000; Tackmann & Conraths, 2000) have pointed out the need for further investigations on the epidemiology of the fox tapeworm. This paper is a first attempt at combining empirical knowledge with theoretical considerations. However, theory can only draw a crude image of natural processes. It can, however, identify likely hypotheses that can be discussed and refined. In this respect, the presented work is a snapshot of a "work in progress."

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# ASSESSING DYNAMICS OF ANTIBIOTIC RESISTANCE IN FAECAL *ESCHERICHIA COLI* AND IN YOUNG CALVES USING CLUSTER ANALYSIS TECHNIQUES.

A.C.B. BERGE[1], E.R. ATWILL[1], W.M. SISCHO[1]

## SUMMARY

The current use of antibiotics in food animal production may contribute to high levels of antibiotic resistance in commensal and pathogenic bacteria. This study describes the dynamic nature of antibiotic susceptibility patterns seen in bovine enteric *Escherichia coli* using cluster analysis techniques. Ten cohorts of 30 calves from six farms were sampled at two-week intervals during the preweaning period and thereafter at intervals during the feedlot period. At each sampling occasion five faecal *E. coli* isolates per calf were analyzed for antibiotic susceptibility to 12 antibiotics using the disk diffusion method, according to the NCCLS guidelines. All isolates had a profile consisting of the measured inhibition zone size for each of the evaluated antibiotics. Using cluster analysis methods, *E. coli* isolates with similar inhibition zone patterns were formed into clusters. The clusters were determined using Ward's minimum variance method. Fourteen clusters described the observed resistance patterns of the *E. coli* from the six farms, and provided balanced numbers within clusters to allow for future statistical analysis of the predictive factors associated with cluster affiliation. Across the six farms, there were consistent dynamic temporal patterns. Newborn calves had a relatively diverse population of *E. coli* with clusters representing susceptible and variable resistant clones. There was a rapid transition to a farm-specific faecal flora with the dominant clusters reflecting *E. coli* clones with high levels of resistance to several antibiotics. As the calves aged, the *E. coli* population tended to form clusters of less resistant strains. There were farm-specific clusters that persisted over time within a calf cohort and there were clusters that were ubiquitous and found on all farms. Variability was observed in the bacterial susceptibility within the calf, between calves and over time under different management practices. For example, a population of *E. coli* with resistance to a large number of antibiotics was observed in a calf environment that was considered relatively antibiotic-free. The resistance to certain drugs could be correlated to farm use, while other drug resistances, such as gentamicin and chloramphenicol, were not directly linked to farm use.

## INTRODUCTION

The emergence of antibiotic resistance in zoonotic bacteria has raised concern that the use of antibiotics in food producing animals may contribute to increasing levels of resistance (Bates, 1997; Levy, 1997; Tollefson et al., 1997; Fedorka-Cray et al., 1998; Witte, 1998; Aarestrup et al., 2000). Although the genetic mechanisms of antimicrobial resistance have been investigated extensively, relatively little is known about the emergence and dynamics of antimicrobial

[1] Veterinary Medicine Teaching and Research Center, University of California, Davis, CA. e-mail: caberge@ucdavis.edu

resistance in farm animal populations. Specifically, information is lacking describing the influence of sub-therapeutic and therapeutic use of antibiotics on the antimicrobial resistance in the enteric flora of calves. Many calf-rearing facilities receive multiple-source, colostrum-deprived, day-old calves. The use of prophylactic antibiotics is considered essential in these management systems to suppress growth of enteric and respiratory pathogens (Quigley et al., 1998). Dairy heifers and calves raised on the dairy of origin are usually not administered routine in-feed antibiotics and are therefore exposed to less antibiotic pressure than the calves in the calf ranches.

Studies have shown that several factors influence the prevalence and persistence of bacterial resistance on farms. Factors such as age of animal, the animal's housing conditions and the use of therapeutic antibiotics to treat diseases have been identified as predictors for resistance traits (Smith, 1975; Wierup et al., 1975; Dunlop et al., 1998a,b; Langlois et al., 1988a,b). There is evidence that the age of the animal influences the level of resistance in the enteric commensal bacteria. Calves have been found to harbour more resistant bacterial strains than older animals (Smith, 1966; Wierup, 1975; Hinton et al., 1984; Martel et al., 1993). Resistance genes can also persist in herds long after removal of sub-therapeutic antibiotics. This may be due to an increased fitness of the resistant strains of bacteria or to linkage of resistance genes to some other genetic trait important for survival and reproduction (Sogaard, 1973; Gellin et al., 1989). Antibiotic resistance has also been detected in commensal bacteria from wild animals. In these cases, the resistance occurs despite the apparent lack of antibiotic pressure for selection (Gilliver et al., 1999).

This prospective cohort study describes the nature of antimicrobial resistance patterns in faecal *E. coli* over the first six weeks of life of the calf in different calf-rearing facilities. The null hypothesis was that there was no temporal or farm-specific variation in the resistance phenotypes of faecal *E. coli* in young calves.

## MATERIALS AND METHODS

A convenience sample of six farms was selected for participation in the study. On each farm, a cohort of 30 calves was enrolled on the day they arrived or were born on the farm. These calves were monitored for six weeks. Faecal samples from calves were collected using cotton-tipped swabs. The calves were sampled at two-week intervals.

*E. coli* was cultured from the swabs by direct plating onto McConkey agar according to standard microbiological procedures (Quinn, 1994). From each sample, five lactose-positive colonies were isolated. At least one isolate per sample was biochemically confirmed to be *E. coli*. Antibiomicrobial disk susceptibility tests were performed as recommended by the National Committee Clinical Laboratory Standards (NCCLS) (Bauer et al., 1966; Anon., 1999). The antibiotic panel consisted of 12 antibiotics: amikacin, amoxicillin/clavulanic acid, ampicillin, cephalotin, ceftiofur, chloramphenicol, gentamicin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, sulfamethoxazole/trimethoprim.

The data from the *E. coli* antibiograms were treated as a series of continuous measurements representing the size of the inhibition zone to the 12 antibiotics. Each isolate had a profile consisting of the measured inhibition zone size for each of the evaluated antibiotics. Using Ward's minimum variance method, these profiles were used to develop clusters of relatedness (Ward, 1963).

The final number of antibiotic resistance clusters that described the population was determined using several approaches. The first, and most important, criterion was to evaluate individually and subjectively the clusters to determine the within- and between-cluster variability. To support this decision, the Calinski-&-Harabasz index, the Je(2)/Je(1) rule and the Cubic Clustering Criterion were also used. (Duda et al. 1973; Calinski et al., 1974; Sarle, 1983a,b).

Canonical projections of the cluster distribution from calves of different ages were made to visualize the temporal changes in the antibiotic resistance patterns across the farms. The 12-dimensional characteristics of the strains (i.e., the inhibition zone diameters to 12 antibiotics) were projected on to a 3-dimensional space. This was performed through principal component analysis where the original variables were replaced by the eigen vectors of their covariance matrix. The first, second and third canonical vectors were used as the axes for the graphs (Everitt et al., 1997).

## RESULTS

Six farms were enrolled in the study. Three cohorts of calves were enrolled from a large beef calf ranch during three different seasons. Two cohorts were monitored on a 4000 cow dairy during two seasons. Single cohorts were enrolled on two other large dairies. Cohorts were also enrolled from two veal calf ranches. From each cohort, approximately 500 isolates were obtained during the six week sampling period. Biochemical tests indicated that more than 95% of the isolates were *E. coli*.

Antibiograms were performed on more than 4500 isolates. As defined by the NCCLS, isolates were usually either susceptible or completely resistant to an antibiotic. For most antibiotics there was thus a bimodal distribution of the inhibition zones. The cluster distribution of the non-*E. coli* isolates did not differ significantly from the *E. coli* cluster distribution.

Using Ward's minimum variance method, 14 to 15 clusters provided reasonable partitioning of the isolates and described the variability in the resistance phenotypes. In the largest cluster, which contained 25% of the isolates, the *E. coli* were susceptible to all 12 antibiotics. These isolates were mainly recovered in the day-old calves and then virtually disappeared from the older calves. Some completely susceptible strains reappeared in the 4 and 6 week old calves in the dairies. There were relatively few strains resistant to only one antibiotic and the most common single-antibiotic resistance was towards tetracycline. All the other clusters contained multiple-resistant isolates (resistance to between three and 10 antibiotics). Eight clusters covering roughly 60% of the strains had a common pattern of penta-resistance to ampicillin, streptomycin, sulfisoxazole, tetracycline and sulfisoxazole/trimethoprim, which was embellished with other resistance. In addition to the five common resistances, approximately thirty percent of these isolates, in four clusters, were also resistant to chloramphenicol and gentamicin. There were age-specific trends across the farms. There was a high variability in resistance patterns in day-old calves. A hutch-calf-specific flora rapidly replaced these strains. On the beef calf ranch and the dairy where several cohorts were monitored, there was similar cluster affiliation over time in the cohorts on the same premise. When the calves were six weeks old, an increased recovery of isolates belonging to the more sensitive clones was noted in the dairies, but not in the calf ranches. At 2 and 4 weeks of age the phenotypic diversity of antibiotic resistance patterns is less than at 0 and 6 weeks. Certain resistance patterns were predominating in the 2 to

4 week old calves on some premises but rarely recovered from the other farms. The resistance patterns of these farm specific strains could not be clearly correlated to antibiotic use.

## DISCUSSION

The present study has shown that there is a high degree of variability in antibiotic resistance phenotypes in faecal *E. coli* from calves of different ages and from different farms. The multivariable characteristics of an isolate, such as antibiotic resistance patterns, have been captured by the use of cluster analysis techniques.

Several different clustering methods were evaluated and the Ward's method was found to produce good discrimination and grouping of the strains. The assessment used to optimize the number of clusters was based on visual examination of the clusters and their member isolates and several diagnostic statistics. The goal was to produce clusters that captured the variability of the population but avoided forming clusters containing small numbers of isolates. The clusters developed will be used in further statistical models as outcomes to assess predictive factors for cluster membership.

This study has shown that the presence of antibiotic resistance is governed not solely by antibiotic use on the farm. Although higher levels of resistance were noted in calf ranches where antibiotics were continuously used compared to dairies, there was an unexpected occurrence of highly resistant strains in the dairies with no routine antibiotic treatments. This demonstrates that on-farm xenobiotic pressures are not the sole selection process for resistance genes.

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# USE OF SPATIAL STATISTICS AND GIS TO STUDY THE DISTRIBUTION OF SEROPOSITIVITY FOR *RICKETTSIA CONORII* IN DOGS IN PIEMONTE (ITALY)

MANNELLI A.,[1] MANDOLA M.L.,[2] PEDRI P.,[3] TRIPOLI M.,[1] NEBBIA P.[1]

## SUMMARY

A Geographic Information System and second order neighbourhood analysis were used to evaluate the spatial association of seropositivity for *Rickettsia conorii*, the causative agent of Mediterranean spotted fever (MSF), in dogs, and human cases of the infection in Piemonte, northwestern Italy. Eighteen out of 116 examined dogs were IFA+ (titre ≥ 1:160). Residence sites of dogs were clustered within distances < 1 km from residences of human cases of MSF. Monte Carlo simulations demonstrated that there was a statistically significant departure from a random spatial distribution, and clustering of IFA+ dogs was significantly higher than that for all dogs, at distances of between 0.36 and 0.59 km.

## INTRODUCTION

Tick-borne zoonoses are emerging diseases of increasing frequency and expanding geographic ranges. Surveillance plays a major role in the recognition and control of emerging infections, and relies on well-directed data collection, and on analysis by appropriate epidemiological tools and techniques (Lederberg et al., 1992).

The detection of antibodies against pathogens in sentinel animals can be used in surveillance of tick-borne zoonoses and geographic risk assessment (Keysary et al., 1988; Segura-Porta et al., 1998). Geographic Information Systems (GIS) and spatial statistics can improve the usefulness and interpretation of these types of studies (Kitron & Mannelli, 1994; Kitron, 1998). In this study, basic GIS capabilities are exploited to carry out point pattern analysis of the distribution of dog seropositivity against *Rickettsia conorii*, the causative agent of Mediterranean Spotted Fever (MSF), in Piemonte, northwestern Italy.

Mediterranean Spotted Fever is endemic in southern Italy and, in recent years, it has been increasingly reported from central and northern regions. In Piemonte, the first cases of MSF are from the mid 80's. *Rickettsia conorii* is transmitted by the ixodid tick *Rhipicephalus sanguineus*, which commonly feeds on dogs (Maroli et al., 1996). In this host species, the infection elicits an intense antibody response, with no clinical signs.

[1]Dipartimento di Produzioni Animali, Epidemiologia ed Ecologia, Università di Torino, Via Nizza, 52, 10126 Torino, Italy; Email: alessandro.mannelli@unito.it ; [2]Istituto Zooprofilattico Sperimentale, 10152 Torino, Italy. [3]AUSL 15, Cuneo, Italy.

By studying clustering of dogs with high antibody titres against *R. conorii*, in the vicinities of residences of people that had MSF, an attempt was made to optimise information from canine serology in the surveillance and risk assessment of this emerging tick-borne zoonosis.

## MATERIALS AND METHODS

Serum samples were collected from dogs during mandatory tattooing carried out by public veterinarians, during four sessions from October 1997 through September 1999, in four municipalities of the province of Cuneo, Piemonte (Morozzo, Margarita, Castelletto Stura, Montanera, overall study area 61.1 $km^2$). Other sera were collected from dogs during routine veterinary activities at animal farms. At the time of blood collection, a questionnaire was filled out based on information provided by the dog owners. In addition to dogs' breed, size, sex, age, colour and hair length, the following information on potential tick exposure were collected: Type of residence area (urban, suburban, rural); land use (type of agriculture, woods, pasture); use of dogs, confinement (in house, yard – chain, free); presence of other animals; detection of ticks at the time of blood collection or previously detected by owners; previous treatment against ectoparasites or recent administration of drug for other reasons; clinical signs of disease. The sites of residence of dogs that were bled during tattooing were recorded on a map, whereas farm co-ordinates were obtained by a GPS (Garmin, GPS 38). Dog sera were tested for antibodies against *R. conorii*, at the Istituto Zooprofilattico Sperimentale of Piemonte, Liguria, e Valle d'Aosta, using indirect immunufluorescence assay (IFA, Biomerieux®). In order to reduce the probability of false positive results, that can be due to cross-reactions with antigens of bacteria other than *R. conorii* including non-pathogenic rickettsiae, dogs with antibody titre ≥ 1:160 were considered as positive (La Scola & Raoult, 1997).

Dog residences were digitised as points in a map of Piemonte (UTM coordinate system, zone 32), in a Geographic Information System (Atlas-GIS, Strategic Mapping Inc., Santa Clara, CA). Questionnaire data and serology results were entered in GIS as attributes. In order to study the spatial association between seropositivity in dogs and risk of MSF in people, a modified second order neighbourhood analysis was applied to the data-points of locations of all dogs and of seropositive dogs, relative to residence sites of two human cases of MSF that were reported in the villages of Morozzo and Margarita in 1997 and 1998. In general, second order analysis tests the randomness of spatial patterns by comparing the observed number of the total possible pairs of points that are within a specified distance of each other, with the number that would be expected in a random spatial distribution. Distances where clustering occurs and where departure from randomness is strongest can be identified (Getis & Franklin, 1987; Boot & Getis, 1988; O'Brien et al., 1999). Second order neighbourhood analysis was modified by Kitron et al. (1992) to verify the higher degree of aggregation of tick-infested white-tailed deer, as compared to all examined deer, around a known focus of Lyme borreliosis in northwestern Illinois. In the present study, the same technique was applied to study clustering of IFA+ dogs at different distances from two points; human cases of MSF, as compared to clustering of all examined dogs:

$$L(d) = \sqrt{A \sum_{i=1}^{n} \sum_{j=1}^{2} k_{ij} / \pi 2n} \qquad (1)$$

where $k_{ij}$ is the sum of points (dogs' residence sites) that are within distance $d$ from residences of the two human cases of MSF ($j$=1, 2), $A$ is the study area, $n$ is the number of dogs. The square root makes $L(d)$ a linear function of $d$. If points are randomly distributed, in the absence of clustering, $L(d)=d$.

GIS was used to obtain distances of dogs from human cases of MSF, and to calculate correction factors for irregular boundaries of the study area. In fact, second order neighbourhood analysis is based on the assumption that a point process is continuous beyond the boundaries of the study area. Such assumption can be satisfied by weighting points whose distance from another point is greater than the distance to the border of the study area. The weight will increase $k_{ij}$ to take into account the "missing" points outside the study area. In this case, it was accomplished using, as $k_{ij}$, the ratio between the area of the circle of radius $d$, to the fraction of the same area falling within the study area (Boots & Getis, 1988). Areas were measured using the functions OPERATE, BUFFER, SPLIT, in Atlas-GIS software (Strategic Mapping, 1990), for ten distance bands, one km wide, around each human cases of MSF (Fig. 1).

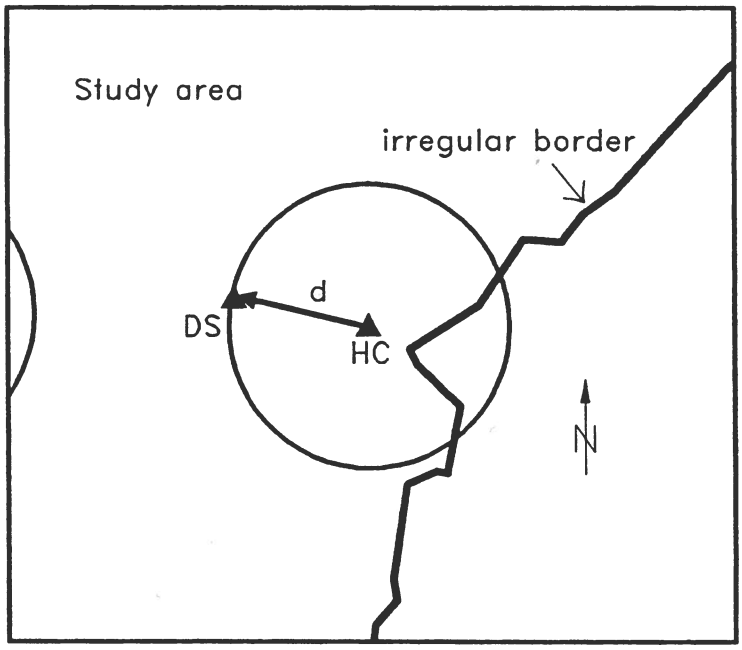


Fig. 1 Use of GIS to calculate correction factors for irregular borders in second order neighbourhood analysis. When the distance, $d$, between the residence site of a human case of MSF (HC), and a dog's residence site (DS) was greater than the distance between HC and the study area's boundaries, the correction factor for $k_{ij}$ was calculated as the ratio of the area of the circle of radius $d$, to the portion of the same circle falling within the study area.

Equation (1) was applied to the distribution of all dogs, and subsequently to IFA+ dogs, after exporting distance data from GIS to the SAS System, Version 8. The observed distance matrix was used to fix increments to calculate $L(d)$ (Boot & Getis, 1988). In order to test the null hypothesis of a random distribution of dogs with regard to human cases of MSF, Monte Carlo simulations were implemented (Manly, 1991). The SAS System macro facility was used to generate statements and commands for multiple simulated data sets (SAS, 1990a, b). Ninety-

nine sets of random points, comprised within the range of coordinates of the observed dog residences, were generated using the RANUNI function. Points falling outside the boundaries of the study area were discarded by fixing limits for X coordinate values at different Y value steps. A sample of points, corresponding to the number of examined dogs or, alternatively, to IFA+ dogs, was drawn from each data set. Distances of selected points from human cases of MSF were obtained using the Pythagorean theorem, and *L(d)* was calculated for each distance step, taking into account GIS-generated border corrections. Clustering of dogs around human cases of MSF was considered to be statistically significant, at the 1% level, for those distances where observed values of *L(d)* were greater than the maximum value obtained in the 99 simulations.

Two different approaches to Monte Carlo simulation were used to compare clustering of IFA+ and all dogs. In the first approach, the coordinates of residences of the dogs, that were examined, were kept as fixed. During each of 99 simulations, a random sub-sample of point locations was drawn and considered as corresponding to IFA+ dogs. The maximum, simulated differences between *L(d)* for IFA+ dogs and for all dogs were then compared with the observed differences. In a second approach, 99 random point patterns were generated, for IFA– and for IFA+ dogs, within the study area. Unlike the previous simulation, dogs' locations were allowed to vary randomly. *L(d)* values were obtained separately for the IFA+ dogs, and for the total points resulting from the combination of the IFA+ and IFA– dog-data points. Differences between *L(d)* for IFA+ and all dogs were again compared to the observed data and were considered as significant if greater than those obtained by simulation.

In order to evaluate the potential confounding effects of other factors on the spatial clustering of IFA+ dogs, the association of each questionnaire factor with the dogs' IFA status was tested using two by two tables (PROC FREQ, SAS, 1989). To evaluate the effect of distance of dogs' residence from human cases of MSF, a coding variable was created, taking value = 1 if distance < 1 km, and zero otherwise. Questionnaire factors that were significant at the 10% level were then included in a logistic regression model to obtain the adjusted effect of distance from human cases of MSF, on dogs' IFA status (PROC LOGIST, SAS, 1989).

## RESULTS

Eighteen out of 116 examined dogs were IFA+ (titre ≥ 1:160) for antibodies against *R. conorii*. As evaluated by second order neighbourhood analysis, examined dogs were clustered within distances < 1 km from residence sites of human cases of MSF. Monte Carlo simulations demonstrated that there was a statistically significant departure from randomness for all dogs. Moreover, clustering of IFA+ dogs was significantly higher than that for all dogs at distances between 0.36 and 0.59 km (Fig. 2, 3; Table 1), and simulation results were the same regardless of whether dog residence sites were kept as fixed, or allowed to vary randomly. Based on two by two table analysis, distance < 1 km from human cases of MSF was significantly associated with IFA+ (OR=4.1, 95 % CI, 1.4-11.5). There was a tendency toward associations between seropositivity and breed - mixed breed versus all breed, OR=2.6 (0.7-7.4) - and treatments against ectoparasites (OR=3.1, 0.96-10.3). All other examined factors, including season of blood collection (spring versus autumn), were not associated with IFA status of dogs. Logistic regression, including breed and treatments as covariates, yielded an adjusted, significant effect of distance < 1 km from human cases of MSF: OR=5.2 (1.7-16.4).

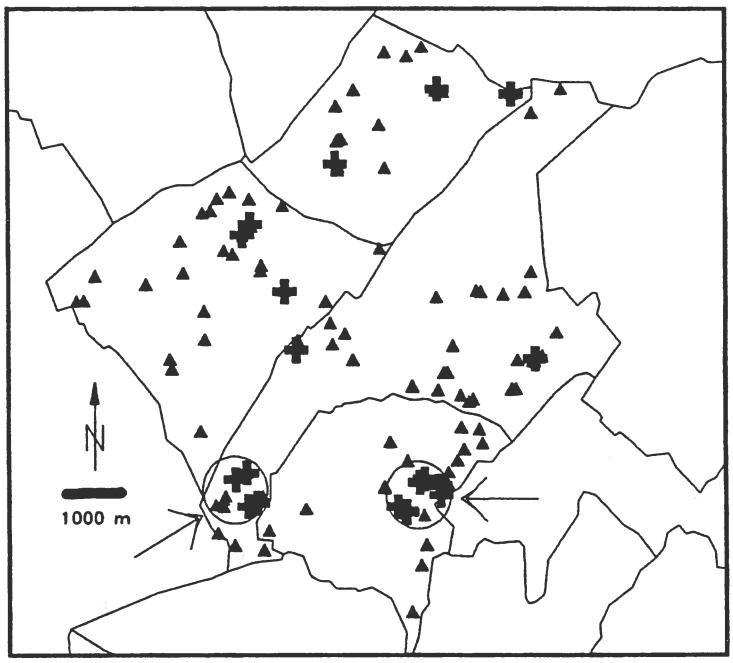


Fig. 2 Spatial distribution of dogs that were tested for antibodies against *Rickettsia conorii* in four municipalities in Piemonte, Italy, in 1998 and 1999. Triangles represent dogs that were IFA–, crosses mark IFA+ dogs. Arrows indicate circles, of 600 m radius, around residences of two human cases of Mediterranean spotted fever.

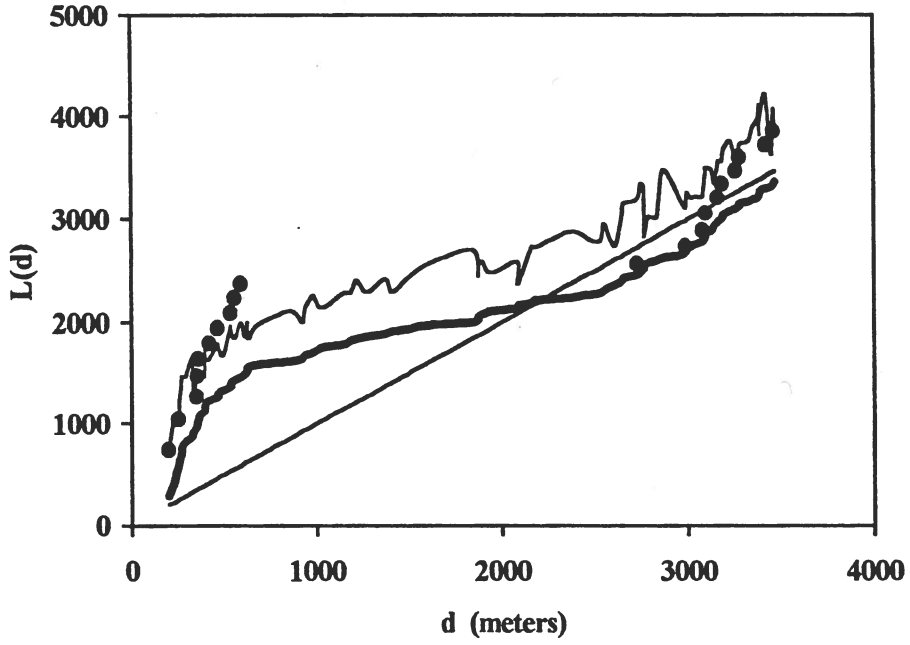


Fig. 3 Graphical output of second order neighbourhood analysis of the distribution of dogs with regard to residences of human cases of MSF, in Piemonte. The bold line is relative to all dogs, whereas filled circles are relative to IFA+ dogs. The thin line represents the upper limit of the 99% confidence interval of $L(d)$ for IFA+ dogs, under the null hypothesis of IFA+ dogs being a random sample of all dogs, obtained by Monte Carlo simulation (dogs' residence sites kept fixed). The straight line, ($L(d)=d$), corresponds to a random distribution of dogs.

Table 1. Second order neighbourhood analysis (first 25 distance steps)

| Dog ID | IFA | $d$ (meters) | $\Sigma k_{ij}$ | $L(d)$ | $L(d)$ IFA+ |
|---|---|---|---|---|---|
| M128 | + | 200.2 | 1 | 289.5 | 734.9 |
| M96 | − | 225.9 | 2 | 409.9 | |
| M97 | − | 236.2 | 3 | 501.4 | |
| M30 | + | 252.4 | 4 | 579.0 | 1039.3 |
| M74 | − | 260.3 | 5 | 647.3 | |
| M129 | − | 267.6 | 6 | 709.1 | |
| M67 | − | 269.2 | 7 | 765.9 | |
| M98 | − | 291.3 | 8 | 818.8 | |
| M68 | − | 326.5 | 9 | 868.5 | |
| M72 | − | 332.8 | 10 | 915.4 | |
| M71 | + | 350.8 | 11 | 960.1 | 1272.9 |
| M37 | + | 352.0 | 12 | 1002.8 | 1469.8 |
| M26 | + | 361.7 | 13 | 1043.7 | 1643.2* |
| M94 | − | 367.5 | 14 | 1083.1 | |
| M95 | − | 391.2 | 15 | 1121.2 | |
| M93 | − | 394.6 | 16 | 1157.9 | |
| M22 | − | 399.7 | 17 | 1193.6 | |
| M126 | + | 418.3 | 18 | 1228.2 | 1800.1* |
| M103 | + | 463.9 | 19 | 1261.8 | 1944.3* |
| M60 | − | 464.1 | 20 | 1294.6 | |
| M27 | − | 491.7 | 21.14 | 1331.0 | |
| M66 | + | 534.2 | 22.28 | 1366.4 | 2096.7* |
| M20 | − | 534.3 | 23.28 | 1396.7 | |
| M64 | + | 556.8 | 24.42 | 1430.5 | 2238.7* |
| M65 | + | 590.1 | 25.56 | 1463.5 | 2372.2* |

In Table 1, the first five columns are relative to all dogs that were tested for antibodies against *Rickettsia conorii* by IFA in 1998 and 1999. The last column contains $L(d)$ values that were obtained for IFA+ dogs. Asterisks mark values indicating that clustering of IFA+ dogs around human cases of MSF was significantly higher ($P<0.01$) than clustering of all dogs, at the corresponding distance ($d$).

## DISCUSSION

Through the examination of 116 dogs in a 61 km$^2$ area, it was found that seropositivity for *R. conorii* was spatially associated with residences of previously recorded human cases of MFS. Using second order neighbourhood analysis, it was possible to detect the scale of clustering of dogs (< one km), and to make comparisons between IFA+ and all subjects. GIS was most useful for generating data for spatial analysis. In particular, the capability of GIS to measure areas allowed the application of boundary corrections in order to implement spatial analysis in an irregular region (i.e., not a rectangle). Monte Carlo simulations were used to assess the statistical significance of the observed clustering, as opposed to the null hypothesis of random spatial distribution. Comparing the degree of aggregation of IFA+ and all dogs, concordant results were obtained using two different simulation approaches.

This type of study should be repeated at other MSF foci in Piemonte. Should the results be confirmed, canine serology could be profitably used as a sensitive indicator of the risk of MSF at the municipality level. Resulting risk maps would be most useful from a public health perspective. Habitat characterisation, and the evaluation of natural and human-related factors should be carried out to explain spatial variations in the risk of MSF. In the study area, which is mostly comprises arable flatland and therefore seems environmentally homogeneous, population density of dogs near villages could affect the abundance of *R. sanguineus* and pathogen transmission. In fact, the density of host for adults is the most important determinant of ixodid tick abundance in condition of environmental homogeneity. Moreover, further studies should be directed to the distribution and abundance of populations of potential hosts for immature stages of *R. sanguineus*, and competent reservoirs for *R. conorii*, such as wild rodents, that may be critical for the intensity of transmission of the infection (Mumcuoglu et al., 1993).

## ACKNOWLEDGEMENTS

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# EQUINE EPIDEMIOLOGY

# SURVIVAL ANALYSIS OF POST-OPERATIVE EQUINE SURGICAL COLIC CASES.

C.J.PROUDMAN, N.P.FRENCH, J.SMITH G.B.EDWARDS[1]

## SUMMARY

A long-term survival analysis was conducted on horses recovering from colic surgery. The study followed the post-operative progress of 341 horses, recording 321 horse years of survival. Cumulative probability of survival for the cohort excluding grass sickness cases was triphasic. There was marked mortality in the first 10 days post-operatively. The probability of survival was decreased to 0.87 by 10 days; at 100 days post-operatively it was 0.82. Thereafter, the probability declined slowly and was 0.75 at 600 days. Determinants of mortality were established using Cox proportional-hazards modelling and generalised additive models. The final model included PCV on admission, time to surgery, duration of surgery and resection length. The dichotomous variable epiploic foramen entrapment also had a significant influence on post-operative survival (RR= 2.2, P=0.046) and was included in the final model. Contrary to prior beliefs, age and heart rate on admission were not significantly associated with survival. The inclusion of referring veterinary surgeon, surgeon and anaesthetist as random effect terms, explained little of the residual variation. Model diagnostics indicated that the assumption of proportionality was valid.

## INTRODUCTION

Colic is a common disease of horses. It is a major cause of mortality in managed horse populations (Tinker et al., 1997). Many colic episodes resolve with medical treatment but approximately 7% require surgical management (Tinker et al., 1997). A number of studies have reported short-term survival following colic surgery, and the prevalence of post-operative complications (Hunt et al., 1986; Kobluk et al., 1989; Phillips & Walsmley, 1993, Edwards & Proudman, 1994; Freeman et al., 2000). These studies have concentrated on the immediate post-operative period, typically seven to ten days, prior to discharge from the hospital. Events occurring after discharge are not reported. Only the studies of MacDonald et al. (1989), and Freeman et al. (2000) used survival analysis methods to study post-operative survival beyond discharge from the hospital.

There are increasing pressures on veterinary clinicians to practice evidence-based medicine. Recent high profile litigation cases in the medical field illustrate the demands that the public make on their medical services. These demands are already being made of veterinary services and clinicians must have access to reliable data in order to advise their clients of prognosis and to guide their own clinical practice. Epidemiology offers the analytical tools required to make scientifically justifiable conclusions about survival data from clinical cases.

[1] Faculty of Veterinary Science, University of Liverpool, Leahurst, Neston, CH64 7TE, United Kingdom.

This study was designed to collect event time data about post-operative progress of equine surgical colic cases. The long-term prognosis of different colic types is an outcome of interest to clinicians and horse owners. The study also seeks to elucidate the determinants of survival time to allow appropriate intervention in the peri-operative period. Information from this study will allow the development of simple models of long term survival following colic surgery.

## MATERIALS AND METHODS

### Case definition

The study was conducted in the Philip Leverhulme Large Animal Hospital, University of Liverpool, between March 1998 and August 2000. All horses undergoing abdominal surgery for the investigation or treatment of abdominal pain (colic) were eligible for inclusion. Only those horses that survived surgery and that walked out of the anaesthetic recovery box were included. All horses euthanised during surgery or that failed to recover from anaesthesia were excluded.

### Data recording

For each horse in the study, details of pre-operative management, examination on admission to the hospital, surgical findings and post-operative treatment were extracted from custom-designed data recording forms used in the hospital.

### Event time reporting

Events of interest included: Death (all causes), death (colic related), colic, diarrhoea, weight loss, wound suppuration, incisional hernia formation, return to ridden exercise and change of ownership. Events occurring during hospitalisation were recorded on case records. On discharge from the hospital, each owner was informed about the study, given literature explaining the aims of the study and invited to participate. The importance of the exact date of the event of interest was emphasised. A dedicated telephone/fax line with a message recording facility was used for the study. The referring veterinary surgeon was sent a letter explaining the study and requesting notification of any events of interest.

In addition to owner or veterinary surgeon initiated reporting of events, periodic telephone and postal questionnaires were conducted. These were brief, and concentrated on the dates of events of interest. During the first post-operative year, the owner received a telephone questionnaire at three and nine months, and a postal questionnaire at six and 12 months. Thereafter, postal and telephone questionnaires were alternated at six-monthly intervals.

### Censoring

Observations were censored if the owner could not be traced, if the horse changed ownership and the new owner was unwilling to participate in the study, or if information gained by questionnaire was considered unreliable. All remaining observations were censored in November 2000, when the final questionnaire returns were received.

Data analysis

Survival: Univariable and multivariable relationships were explored with Kaplan-Meier plots and Cox proportional-hazards models using Splus2000 (Mathsoft). Following preliminary analysis of the data set, grass sickness cases were excluded. Surgery is performed on these cases for confirmation of diagnosis rather than surgical correction of a problem. Confirmation of diagnosis occurs within 48 hours of taking an ileal biopsy at laparotomy and, if positive, horses are euthanised.

Assessing functional form of the relationship between continuous variables and mortality: The shape of the relationship between continuous variables (eg. heart rate at admission, age, length of resection) and mortality was explored using generalised additive models (GAM).These are extensions of generalised linear models (in this case the logistic model) that fit nonparametric functions (e.g. spline smoothers) to estimate the relationships between outcome and explanatory variables. The results can be displayed graphically to illustrate the multivariable functional form of these relationships (e.g. linear, quadratic or cubic).

Statistical modelling: Survival data were modelled using a Cox proportional-hazards model. Final models were constructed using backwards elimination procedures and an assessment of the effect of variable inclusion on parameter estimates. Residual variability that could be attributed to random effects was tested by including variables such as referring vet, surgeon and anaesthetist as frailty (gamma) terms in the model. The assumption of proportionality was tested by examining the Schoenfeld residuals (Therneau, 1994).

RESULTS

Study population

A total of 341 horses were recruited to the study generating 321 horse-years of survival time. Forty-one horses failed to recover from colic surgery and were therefore excluded from the study. Eight different breed categories were recorded, the largest being Thoroughbreds and Thoroughbred crosses (including hunters) which comprised 45% of the population. Ponies represented 19% of the study population. Mean and median age for the population was 10.7 and 9 years old respectively. The oldest horse recovering from surgery was 33 years old.

Proportion of colic types

A total of 66 different causes of colic were documented. Of the 341 cases, small intestinal cases accounted for 216 (63%); large colon disease accounted for 97(28%) cases; caecum and small colon for 13 (4%) and 10 (3%), respectively. Seven cases had a diagnosis that was not attributable to a particular part of the intestinal tract e.g. peritonitis, multifocal lymphosarcoma. The prevalence of major diagnoses (more than ten cases recorded) is given in Table 1.

Response to questionnaires

A total of 769 telephone questionnaires and 213 postal questionnaires were administered during the study. Response rates of 98% and 96%, respectively, were achieved. Of the eight non-

responders to postal questionnaires, seven responded to telephone administration of the same questionnaire. Five cases were censored prior to termination of data collection, one due to unreliability of data provided, the other four due to change of ownership.

Table 1. Proportion of common (>10 cases) diagnoses for each section of intestine in horses with colic.

| Diagnosis | Number | % |
|---|---|---|
| **Small intestinal** (216 cases) | | |
| Pedunculated lipoma strangulation | 57 | 26 |
| Grass sickness | 30 | 14 |
| Epiploic foramen entrapment (L-R) | 20 | 9 |
| Jejunal strangulation | 20 | 9 |
| Volvulus | 15 | 7 |
| Ileal impaction | 13 | 6 |
| Anterior enteritis | 11 | 5 |
| **Large intestinal** 97 cases | | |
| Colon torsion <360° | 27 | 28 |
| Colon torsion >360° | 20 | 21 |
| Nephrosplenic entrapment | 13 | 13 |

Patterns of survival

Survival of the whole cohort: This is presented in Figure 1 as a Kaplan-Meier plot. Also presented on this graph is survival of the cohort excluding grass sickness cases. The difference between these two curves can be explained by the 96% mortality (29/30) of the grass sickness cases diagnosed, all within the first few days of surgery (Figure 2). Subsequent analyses were performed on a subset of the whole data set excluding grass sickness cases. The survival curve (excluding grass sickness cases) shows marked mortality occurring within the first few days of surgery. Probability of survival to 10 days post-operatively is reduced to 0.87. This is followed by a further decrease in survival probability up to 100 days. Beyond 100 days post-operatively, the gradient of the curve is very shallow reflecting a low mortality rate. However, horses were dying of colic-related problems up to 652 days post-operatively.

Survival of small intestinal cases: Figure 3 shows Kaplan-Meier survival plots for the most commonly recorded small intestinal problems. Ileal impaction, a non-strangulating obstruction of small intestine, has a probability of survival of 0.97, which does not change with time. Pedunculated lipoma strangulation and jejunal strangulations have a probability of survival in the region of 0.8 by 100 days post-operatively. Again, the survival curve is observed to plateau after 100 days. In contrast, epiploic foramen entrapment cases, another strangulating obstruction, have a probability of survival of 0.7 by 100 days but this continues to decline with time. Median survival time for epiploic foramen entrapment cases is 390 days. Probability of survival of epiploic foramen entrapment cases is significantly different to that of ileal impaction and pedunculated lipoma cases in a Cox proportional-hazards model (RR=2.2, P = 0.046).

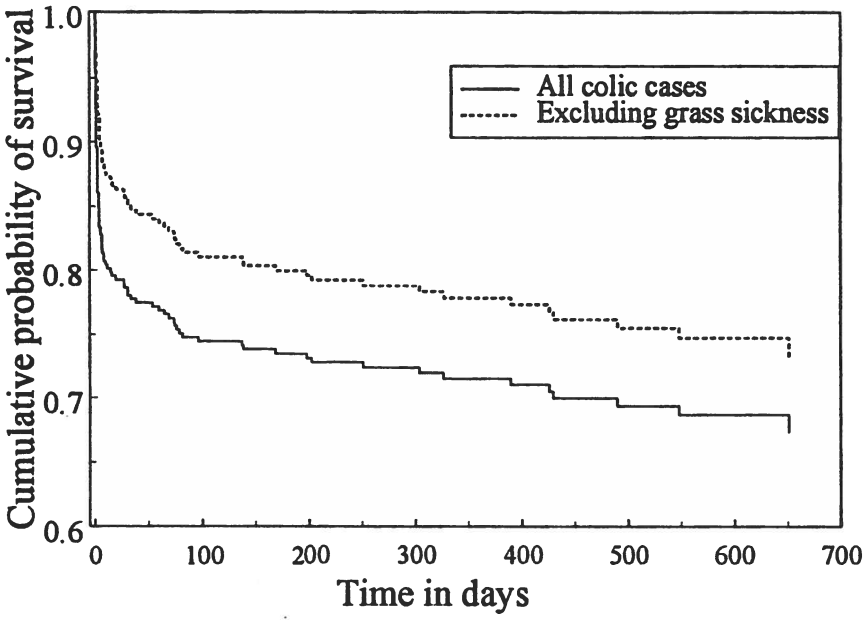


Fig. 1 Kaplan-Meier plot of survival of all horses, and for the study population excluding grass sickness cases.

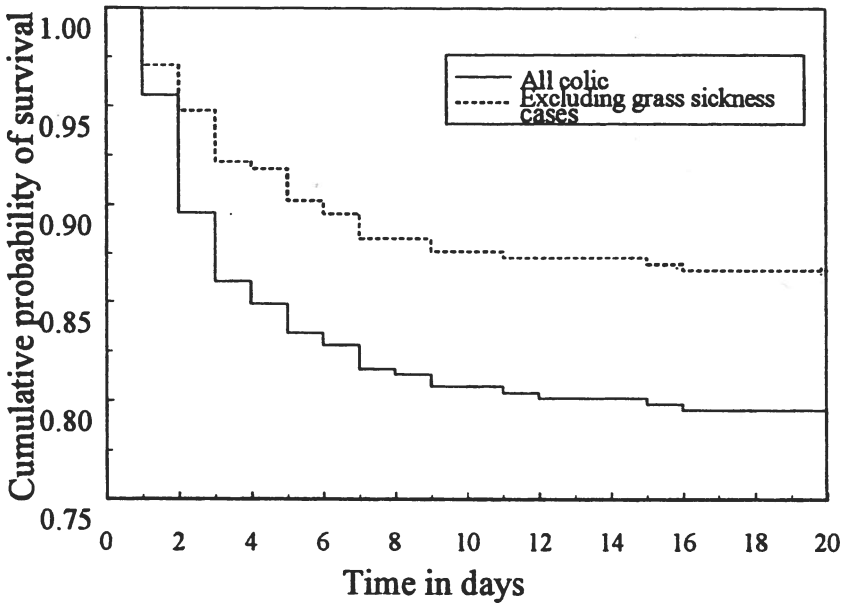


Figure 2. Kaplan-Meier plot of survival probability for first twenty days post-operatively.

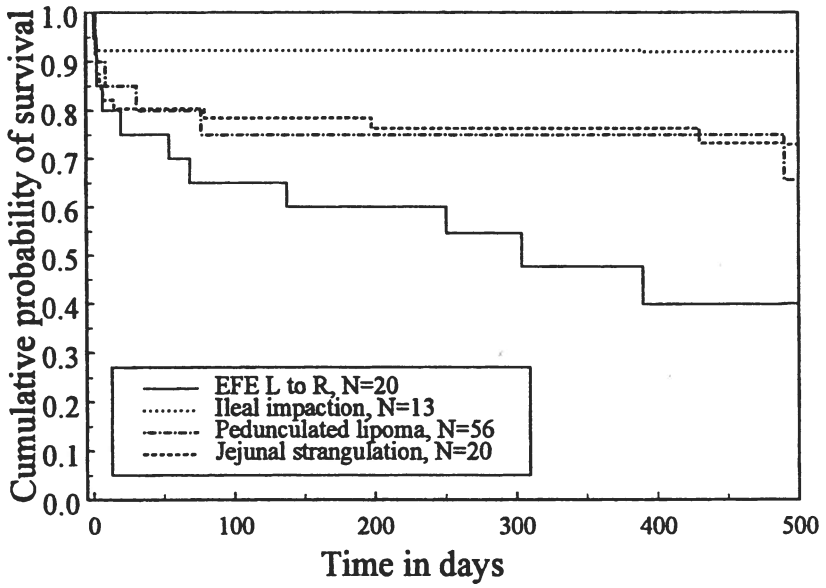


Figure 3. Kaplan-Meier plots of survival probability for the four most commonly recorded small intestinal lesions. EFE = epiploic foramen entrapment.

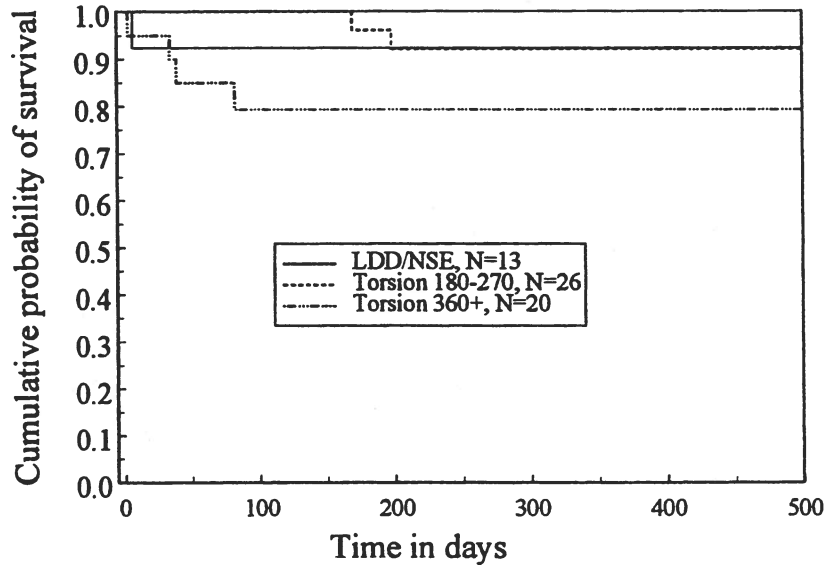


Figure 4. Kaplan-Meier plots of survival probability for the three most commonly recorded large intestinal lesions. LDD/NSE = left dorsal displacement/nephrosplenic entrapment.

Survival of large intestinal cases: Survival of the three most commonly diagnosed large intestinal problems is illustrated in Figure 4. Nephrosplenic entrapment of the colon and torsions of 180° to 270° are non-stangulating obstructions. These cases have a probability of long term survival in excess of 0.90, and this changes little with time. Probability of survival following 360° torsion, a strangulating lesion, declines steadily to 0.8 by 100 days and then remains at this level.

Risk factors for survival

Continuous variables: Figure 5 illustrates the multivariable smoothed relationships between continuous variables, thought *a priori* to influence post-operative survival, and mortality (i.e., GAM fits from a six variable model). It is apparent that age, heart rate at admission and time to surgery (interval between the onset of colic and surgery) show no marked or consistent association with mortality. Packed cell volume at admission (PCV), resection length and duration of surgery all show reasonably linear, monotonic increases in mortality with increasing values.

Categorical variables: Survival was categorised by breed and by laparotomy diagnosis and the influence of each examined using Cox proportional-hazards model. Only epiploic foramen entrapment emerged as a significant variable.

Model construction

Based on the results described above, a Cox proportional-hazards model for post-operative survival was constructed including PCV, resection length, time to surgery, duration of surgery and epiploic foramen entrapment. Table 2 gives details of parameter values for the model.

Table 2. Parameter values for fixed effects model of post-operative survival.

| Variable | Coefficient (β) | Standard error | P value |
|---|---|---|---|
| **Fixed effects model** | | | |
| Resection length (increment per foot) | 0.032 | 0.014 | 0.0190 |
| Time to surgery (increment per hour) | 0.007 | 0.004 | 0.0940 |
| Duration of surgery (increment per min.) | 0.013 | 0.004 | 0.0005 |
| PCV (increment per 1%) | 0.053 | 0.017 | 0.0016 |
| Epiploic foramen entrapment (y/n) | 0.82 (Relative rate 2.26) | 0.35 | 0.0200 |

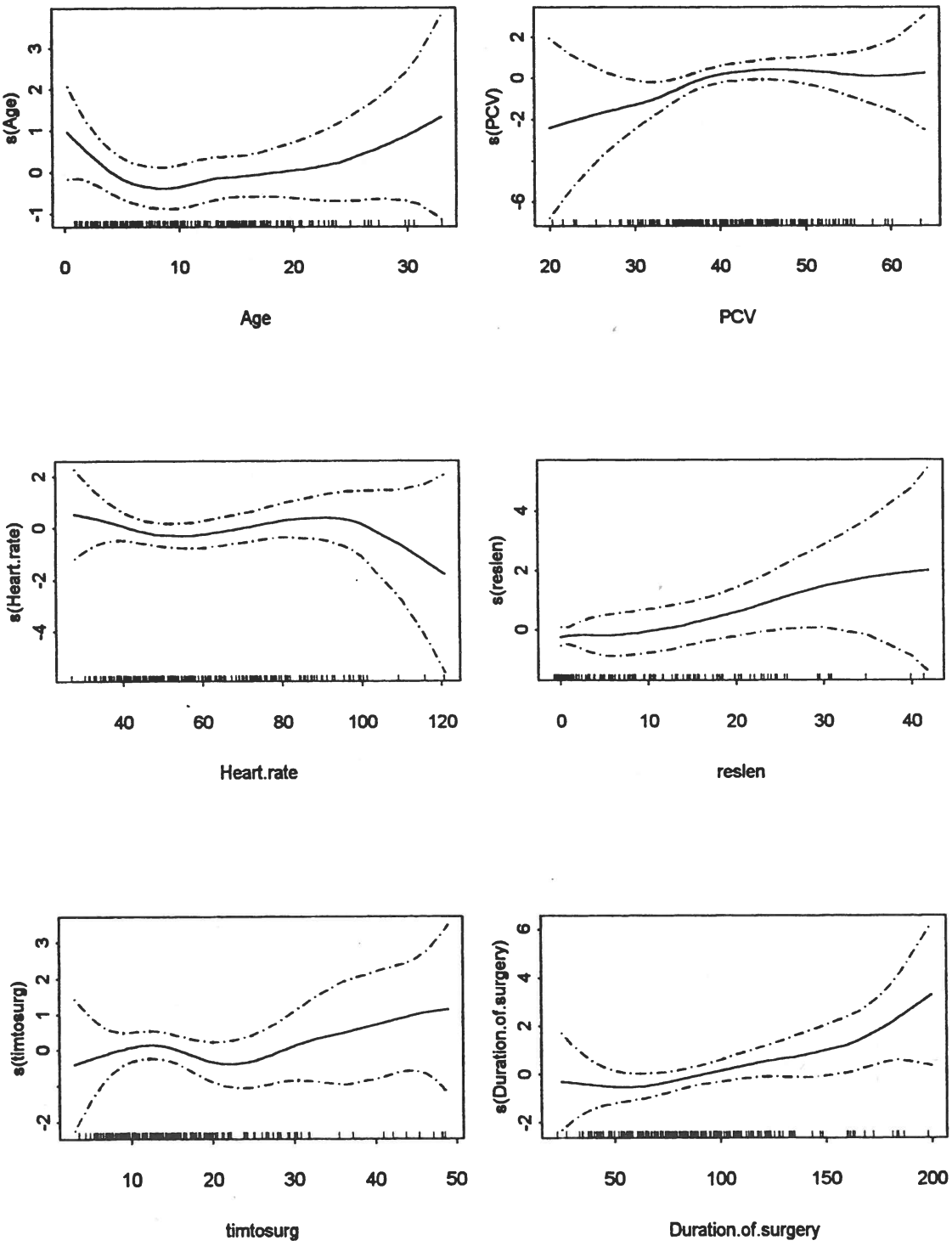


Figure 5. GAM plots of the major continuous variables considered for inclusion in the fixed effects model. Rug plot on x-axis indicates number of data points.

Random effects: The residual variation due to referring veterinary surgeon, surgeon and anaesthetist were explored by inclusion of each as a random effect (frailty) term in the fixed effects model. The *a priori* hypothesis being tested was that some referring veterinary surgeons, surgeons and anaesthetists were associated with better long term survival. The variance estimates for these effects are given in Table 3.

Table 3. Variance estimates for random effects terms in the fixed effects model.

| Variable | Variance estimate | P value |
|---|---|---|
| **Random effects** | | |
| Referring veterinary surgeon | 0.00 | 0.93 |
| Surgeon | 0.06 | 0.15 |
| Anaesthetist | 0.14 | 0.25 |

It is apparent that little of the residual variation can be attributed to either anaesthetist, surgeon or referring veterinary surgeon.

Model diagnostics

The assumption of proportionality for each fixed effect variable included in the final model was tested both graphically and statistically by plotting the rescaled Schoenfeld residuals versus time. The smoothed plots give a direct estimate of each regression coefficient (β) over time and Table 4 gives the global and individual variable tests for a significant slope (if the proportionality assumption holds then the slope would not be significantly different from zero). Although there is no significant deviation from the assumption, the variable PCV shows some indication of a decline in β over time. The final plot shows the deviance residuals from the final model and shows little evidence of poorly predicted subjects.

Table 4. Statistical tests for significant slope in the Schoenfeld residual plots

| Variable | Rho | Chi-squared | P-value |
|---|---|---|---|
| Resection length | 0.014 | 0.015 | 0.902 |
| Time to surgery | 0.009 | 0.006 | 0.937 |
| Duration of surgery | 0.023 | 0.041 | 0.839 |
| PCV | -0.282 | 3.800 | 0.051 |
| Epiploic foramen entrapment | 0.212 | 2.950 | 0.086 |
| **Global** | - | **8.664** | **0.123** |

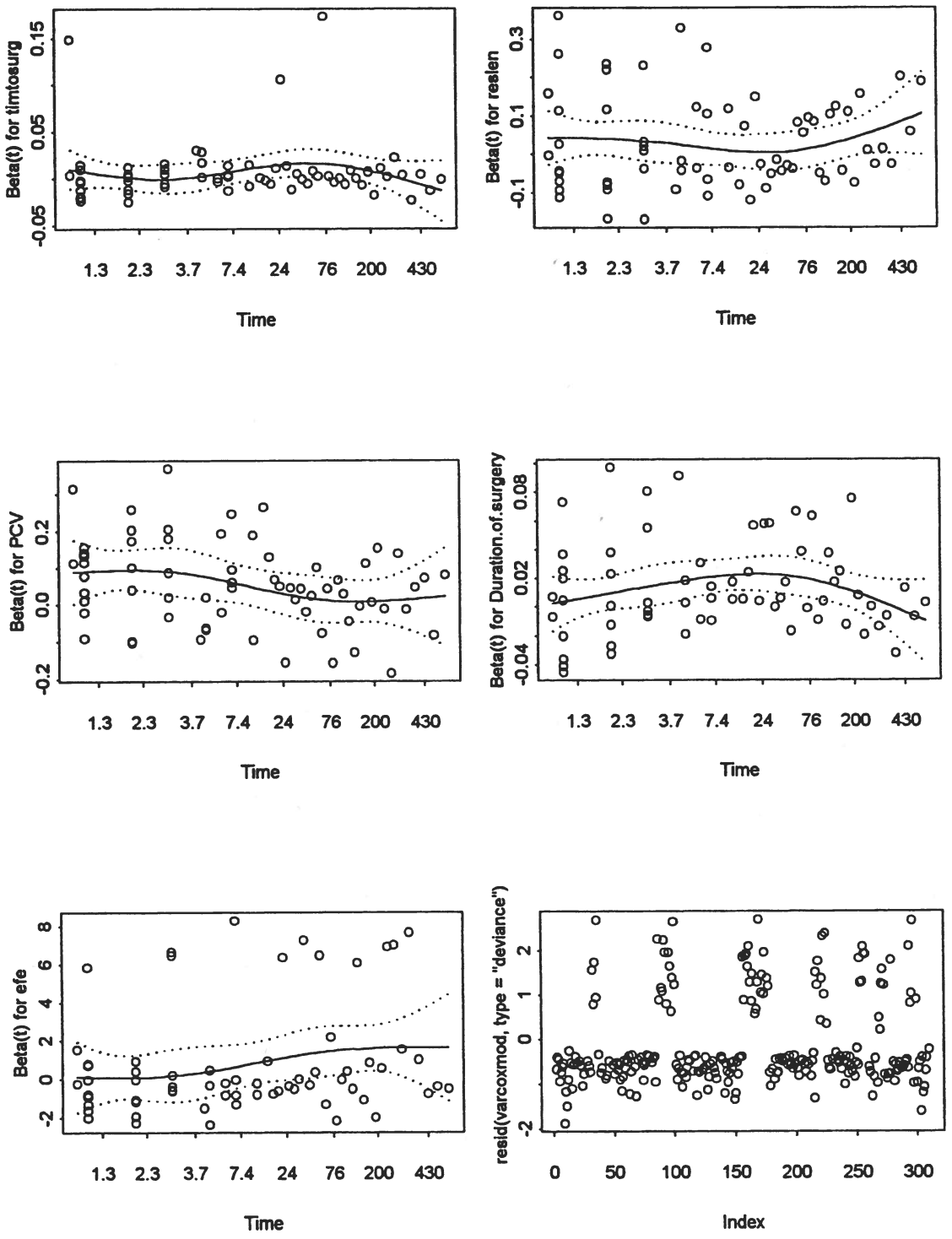


Figure 6. Schoenfeld residual plots with spline curves for the five fixed effect variables included in the final model, and for deviance residuals from this model.

## DISCUSSION

Based on the survival probabilities reported above, approximately 75% of surgical colic cases that recover from surgery are likely to survive to 600 days post-operatively. Survival in the whole surgical colic cohort appears to be triphasic. There is marked mortality in the first ten days post-operatively. The survival curve then has a less steep gradient to 100 days. Most of these cases will have died after discharge from the hospital. Without rigorous reporting of such deaths, clinicians could be unaware of them, thereby allowing an incomplete and inaccurate impression of post-operative survival to be gained. Beyond 100 days post-operatively, the survival curve has a shallow gradient, possibly representing baseline mortality within the referral population. Horses surviving to 100 days could perhaps be considered "cured" and thereafter be subject to normal baseline mortality.

The reported relationships between continuous variables and long term survival are contrary to previous studies. Reeves et al. (1990) described the development of a prognostic model for colic using multivariable logistic regression methods. They concluded that the best prognostic variables were those describing cardiovascular status (e.g., heart rate, PCV). The results presented above suggest that PCV is related to post operative survival but that heart rate is not. However, the present study was considering only survival of horses recovering from surgery. Other studies considered survival of all colic cases. Freeman et al. (2000), in a smaller study of exclusively small intestinal colics, also reported no effect of heart rate.

Many clinicians perceive the time interval between onset of colic and surgery as an important prognostic variable. This may hold true for survival for all colics to discharge from the hospital. This was not investigated in the present study as we did not consider horses that died or were euthanised prior to recovery from surgery. We demonstrate that long-term survival of horses recovering from colic surgery is not influenced by time to surgery. A further perception held by veterinary surgeons is that older horses have a reduced prognosis for survival. This study refutes this perception. It offers no evidence that older horses that recover from surgery have any worse a prognosis than younger ones.

The relatively poor survival of horses suffering from epiploic foramen entrapment is of interest. Engelbert et al. (1993) reported 75% (12/16) survivors to at least three months, Vachon and Fischer (1995) reported 70% (31/44) survival to at least one year post-operatively. This is better survival than in the present study where cumulative probability of survival is 0.70 at 100 days and has fallen to 0.55 by one year. However, neither previous study had a rigorous protocol for obtaining information on post-operative progress. Clinically, this condition is characterised by severe pain, which often leads to rapid referral and early surgery. What may be important is the difficulty often encountered when trying to reduce such entrapments. This will lead to prolonged duration of surgery which, according to the analysis presented here, is an important determinant of long-term survival. The condition often results in resection of long lengths of small intestine which in our study was a significant risk factor.

The final model developed with the survival data from this study illustrates factors that are important in long term survival following colic surgery. Recognition of these factors will assist veterinary surgeons when advising an owner of the likely outcome of colic surgery. In particular, the recognition of a lack of age-effect will allow more accurate advice to be given. The model also focuses attention on the duration of both surgical procedure and general anaesthesia as important determinants of post-operative survival. Further analysis of the data set will concentrate on these aspects in order to determine why duration of surgery is so critical.

The lack of significant contribution to survival variation by referring veterinary surgeon, surgeon and anaesthetist is reassuring. This finding differs from that of Freeman et al. (2000) who reported reduced probability of survival in horses operated on by surgeons with experience of less than nine colic operations. The population of surgeons in the present study included three with this level of experience but failed to demonstrate an effect. The use of random effects modelling demonstrates how non-divisive surgical audit can be conducted. Variability between surgeons or anaesthetists is measured without attributing results to individuals. Clearly, if marked variability is detected then further investigation is warranted. Benchmarking of post-operative survival and audit of clinical skills are two areas currently highly topical in the medical world. Veterinary clinicians should not be slow to learn from the medical experience and to implement appropriate monitoring strategies. Epidemiology can play an important role in designing and analysing such studies.

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# MODELLING A RETROSPECTIVE STUDY OF DEATH ON RACECOURSES

J.L.N.WOOD[1], J.EASTMENT[1], K.H.LAKHANI[1], L.HARKINS[1] K.ROGERS[1]

## SUMMARY

Horses die and are seriously injured during racing in every racing country around the world. Various epidemiological studies have identified factors associated with death during racing, but other than in Japan where a racecourse was modified to reduce the death rate, their results have not usually been translated into action to reduce the frequency of the problem. Sometimes action has been difficult due to the comparison used in a case control approach (i.e., dead horses being compared to seriously injured ones) and sometimes studies have failed to identify easily modifiable factors.

A dataset of every death and every start during racing on the 59 courses in Britain from 1st January 1990 to 31st December 1999 has been analysed to determine factors associated with risk, as well as levels at which variation was occurring. There were around 719,000 starts made by 63,754 horses in 71,739 races, resulting in 2,015 deaths. Ordinary logistic regression models of the likelihood of dying in a race have been developed and validated against external, independent datasets. Hierarchical, multilevel models were used to estimate the proportion of variance residing at different hierarchical levels of racing (Dohoo et al., 2001). Race starts were nested within race, which was nested within race meeting, or date of race, which was nested within racecourse.

A relatively small proportion of the variance was associated with race, course, race day, trainer or jockey levels (<c.14%) in both hurdle and flat race types. Risk did vary significantly between racecourses, although much of this variation was associated with firmness of racing surface. There was significant variation at the level of trainer in both flat and hurdle races, as well as significant variation at the level of race in hurdle races (c.12%), but not in flat races (<0.1%). The results suggest that while greater understanding of start level variables is most important, trainer and course level variation should not be overlooked.

## INTRODUCTION

Serious injury to racehorses, associated with exercise, is very important in the international Thoroughbred racing industry. There is considerable public disquiet about catastrophic injuries on the racecourse as these are so highly visible and greater understanding of the likely causes of these accidents would produce benefits for equine welfare and for the racing industry.

The rates of fatal injury per race start in races that do not involve jumping over obstacles vary from 0.04% in Australia (Bailey et al., 1997), to 0.08% in the UK (McKee, 1995), 0.13% in California (Wilson et al., 1994) to 0.14% on 4 tracks in Kentucky (Peloso et al., 1994). Fatality rates in jump races per start are much higher, being from 0.49 to 0.7% in the UK (McKee,

[1] Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, CB8 7UU

1995). Limb (Vaughan & Mason, 1976) or musculoskeletal (Johnson et al., 1994) injuries account for 70 to 80% of all deaths on racecourses, although the nature of the injuries varied between the different racetypes (Vaughan & Mason, 1976).

Epidemiological studies in the USA have highlighted risk factors for both fatal and non-fatal musculoskeletal injury (Robinson et al., 1988; Mohammed et al., 1991, 1992; Peloso et al., 1994; Estberg et al., 1995) and these reports identify factors that would be expected to confound direct comparison of crude, overall death rates. Several studies have found that risk of severe injury increases with age (e.g. Robinson et al., 1988; Mohammed et al., 1991). "Going" (firmness of racing surface) and track surface has also been reported to be an important predisposing factor. Death rates were lower in the USA in races on turf compared with races on dirt (Mohammed et al., 1991) and this is consistent with the finding that the overall mortality figures are lower in the UK than in the USA (0.08% v. 0.14-0.19%) where most racing tends to be on dirt rather than on grass. Hardness of ground on racetracks has also been linearly correlated with rates of lameness in the horses running on them (Cheney et al., 1973). Other effects that have also been associated with the probability of musculoskeletal injury include horse shoe characteristics (Kane et al., 1996) and the number of seasons that the horse has raced (Mohammed et al., 1991). Although greater race-speed exercise distances covered in both the two or six month periods prior to injury were associated with catastrophic injury in one study (e.g. Estberg et al., 1995), such an effect was not detected in another (Cohen et al., 2000) and this apparent increased risk may have resulted from confounding by the effects of racing (Cohen et al., 2000).

Death rates have been reported to vary widely between racecourses in both the USA and the UK (Wilson et al., 1994; McKee, 1995). However, direct comparisons, such as of crude death rate between locations, without simultaneous adjustment for other factors can produce highly misleading results (Bailey et al., 1997).

In the United Kingdom, studies of death on racecourses will only include a relatively small proportion of horses that suffer a fracture when in training (Bathe, 1994) as little training occurs on racecourses. However, they will include a substantial proportion of the animals that suffer fatal injury (Verheyen et al., unpublished). Studies of fatalities on the 59 British racecourses are thus highly worthwhile. The data are likely to be complete, reliable and not subject to selection bias due to high notification rates and their analysis may shed light on risk factors that are particular to British racing.

Some workers have taken a horse level approach (Estberg et al., 1995; Cohen et al., 2000), but the majority of researchers have used ordinary logistic regression methods to identify factors associated with fatal or severe injury in racehorses. These models assume that all observations (starts) are independent. Clearly starts on any particular racecourse (e.g., due to course characteristics), or any particular day (e.g. due to weather conditions), or in any particular race (e.g., due to accidental pile-ups) are unlikely to be independent. In addition, horses sent out by any trainer may be similar (e.g. because of training techniques, degree of fitness or intensity of veterinary treatment) and thus not independent. As well as the clustering of data at any particular level, there is a clear hierarchy of data structure, in particular considering the races on any particular day, or any particular racecourse. Modelling this hierarchy explicitly can be very informative, both to determine the level of the hierarchy which explains most of the variance and to improve the validity of the estimates of the regression coefficients (e.g., Dohoo et al., 2001).

The main objective of this study was to estimate the incidence of fatal accidents per start in different types of races in Britain and to identify predisposing factors. There was particular interest in determining whether horses that raced when 2 years of age were at increased risk compared to older animals, considering both the age at start of the race and at start of racing career. Data included all races held in Britain from 1990 to 1999 inclusive. Multilevel models were used to check the validity of ordinary logistic regression models and to estimate the contribution that the different organisational level of the data made to the variability in the odds of the horse dying on the racecourse. In particular, the estimation of variance components was considered, using the general approach of Dohoo et al. (2001). The results for flat and hurdle races are presented, although hurdle races on turf only have been considered. Hurdle races on "all weather" (sand based) surfaces were excluded due to their discontinuation in 1994.

## MATERIALS AND METHODS

### Data and case definition

The study utilised data from all UK race starts from 1st January 1990 to 31st December 1999. Weatherbys Ltd provided these data in electronic format. For each start, the information that was provided is shown in Table 1. Horses were only included if they started the race in question and so "late withdrawals", even if they were just prior to the start, were excluded.

Data from flat races (races with no obstacles), hurdle races (races which involved jumping a flexible hurdle) and chases (including both hunter chases and steeplechases, both of which were races involving races over solid "brush" fences) were dealt with separately. There were insufficient data from "National Hunt Flat races" to justify separate analyses (68 deaths from 17,829 starts over the period), but these data were used in the validation of the ordinary logistic regression models of the flat data.

Data were imported into SAS and new variables were derived; these included age in days since birth date, official age (taking January 1st as birth date) and speed of winning horse in race. In addition, variables that should have replicated information provided by Weatherbys Ltd were derived from the primary data (e.g. number of runners in each race, date of previous race (if after 1st January 1990) and number of runs in the last 3 months after 1st March 1990 and compared with those provided. The data were also cross-checked for validity and internal consistency. For instance, flat races were not allowed on racecourses restricted to National Hunt jumping.

Initially, numerous discrepancies were detected and in all cases the correct answer was obtained from checking independent data sources (e.g. Racing Post online (www.racingpost.co.uk) and Raceform/Chaseform publications). All analyses were performed after the data were internally consistent.

A horse was classified as a case if it died or was killed on the racecourse after starting. Cases that died on the racecourse prior to the start were excluded from the dataset, again even if they were withdrawn late, e.g., "under starters orders". The majority of horses were killed by a member of the veterinary team after severe injury had been sustained, but a few horses died as a direct result of the injury (e.g. from a broken neck or severe pulmonary haemorrhage).

Table 1. Descriptions of variables received electronically from Weatherbys Ltd

| Variable type | Descriptions |
|---|---|
| Horse identity | Name and internal numerical code |
| Date of birth | Date recorded in general Stud Book |
| Gender | Colt, gelding, filly or rig at time of start |
| Date of first race | Date of first race under orders<br>a) in same race type<br>b) in any race type |
| Date of last race | Date last ran under orders<br>a) in same race type<br>b) in any race type |
| Racing intensity | Number of runs in<br>a) last three months<br>b) last 12 months<br>c) season to date |
| Race date & identity | Date and various different ID codes |
| Racecourse | Name of course |
| Type of race | Flat, hurdle, national hunt flat, steeplechase, hunter chase |
| Prize money available in race | In pennies |
| Race class | Official class of race (A-G, incl. listing of Group A races) |
| Race classification | Handicap, wt. for age, novice, maiden, maiden before close |
| Race and jockey restriction | - |
| Prize money won by horse | In pennies |
| Finishing position | Numerical, 99=did not finish |
| Time recorded by winner | In seconds |
| Number of runners | Number of horses in race |
| Distance of race | In yards |
| Racing surface | Whether turf or "all weather" |
| Official "firmness of going" | Hard through to soft, recorded by the steward of the course |
| Trainer ID & classification | Name and whether fully licensed |
| Jockey ID & classification | Name and whether fully licensed |
| Weight carried by horse | In pounds |
| Official rating | Official rating of horse at time of race |

Weatherbys Ltd recorded data about the races and starts as part of their normal business. At each race meeting during the period, there was a veterinary team consisting of official Jockey Club Veterinary Officers and Racecourse Veterinary Surgeons. This team recorded all deaths and serious injuries likely to result in long term incapacity on standard forms. These were submitted after each race meeting to the Jockey Club racecourse department. For the years 1990-1994 inclusive, forms were retained and summarised on annual forms. For years after 1995, they were forwarded to Weatherbys Ltd, where they were entered on a customised computer database. Returns where no horse died were also required and each one was checked to ensure that a report had been made for every race. The data from 1990-1994, held on paper forms, were entered into a customised Access database, before extracting for analysis in SAS.

Statistical analyses

Ordinary Logistic Regression. Data were analysed initially and separately for each racetype (flat, hurdle and chase races) using a standard ordinary logistic regression (OLR) approach (Hosmer & Lemeshow, 1992). Univariable screening of all variables was performed using contingency tables for categorical variables and t-tests or univariable logistic regression models for continuous variables. All variables of any likely significance in univariable analyses ($p<0.4$) were tested for inclusion in logistic regression models, which were built using forward build-up methods. Variables were included in models if they significantly improved the fit of the model (likelihood ratio $\chi^2$ statistic $p<0.05$), or if they were significantly associated with the outcome (Wald $p<0.05$). Biologically meaningful two way interaction terms were tested between all main effect variables. The quality of model fit was assessed by way of the Hosmer-Lemeshow statistic. Where possible, models developed from one source of data were validated against an independent data source. All these data analyses were performed using SAS/STAT.

Multilevel Modelling. For the multilevel modelling, analyses were performed using MLWin 1.10 (Rasbash et al., 2000) and Egret (Cytel, 1999). In MLWin, 1st order marginal quasi-likelihood (MQL) and 2nd order penalised quasi-likelihood (PQL) estimates were derived, where possible, using restricted generalised iterative least squares (RIGLS). Initially, simple two level random intercept models were fitted using both MLWin and Egret, looking at each level of clustering separately. The logistic binomial model for distinguishable data in Egret (version 2.0.3), using 7 prior points was used to fit two-level random intercept only models.

Subsequently, models with more than two levels were fitted in MLWin, representing the detailed hierarchy in the data. The significance of the variance estimates was tested using the Wald option in MLWin and by using the likelihood ratio statistic associated with the addition of the random effects term in Egret. All levels were retained in the models, whatever their statistical significance. The contribution of each level of the hierarchy was then estimated.

After fitting random intercept only models, the models were extended to include all fixed effects terms identified as being significant in the OLR analyses and the contribution of the different levels of the hierarchy to the variance was again estimated.

Variance Component Assessment. The relative contribution of each random term to the total variance was then assessed. In all these (binary) models, no extrabinomial variation was allowed and the start level (level 1 in multilevel model terminology) was constrained to be equal to 1 on the binomial scale. The total level 1 variance on the logit scale was taken to be fixed and equal to $\pi^2/3 \approx 3.29$ (Searle et al., 1992; Snijders et al., 1999). This calculation was based on the assumption that any horse's death (died/survived) was the result of an underlying latent process, with a continuous, logistic distribution (Snijders et al., 1999). When estimating the proportions of variance attributable to different levels, the variance at that level was divided by the total of ($\pi^2/3$ + the total variance estimates from the higher level, random terms).

Due to limitations of software and computing facilities, all multilevel modelling was done using reduced datasets using all cases and a lower number of controls. In the hurdle data set, a case control ratio of around 1:50 was used resulting in a dataset containing 42,837 starts and in the flat data a ratio of around 1:100 was used, resulting in a dataset of 38,938 starts. Controls were randomly selected using the pseudo-random number generator in the analysis module of Epi-info 6.04c (Dean et al., 1994).

## RESULTS

The numbers of starts made by horses in the period is shown in Table 2.

Table 2. Descriptive statistics for race starts in GB from 1990 to 1999

| Racetype | No. Starts | No horses | No fatalities |
|---|---|---|---|
| Flat | 420,607 | 40,065 | 378 |
| Hurdle* | 170,514 | 28,251 | 838 |
| Chase | 103,712 | 14,218 | 695 |
| National Hunt Flat | 17,829 | 9,581 | 68 |

* Hurdle starts on all weather surfaces not included

The hierarchical structures of the data in the complete dataset and in the cut-down data analysed in the multilevel models are shown in Table 3. Most levels had a reasonable number of observations per individual, apart from races, where the average cluster level was small.

Table 3. Hierarchical structure of data, showing numbers at each level.

| | | Data Analysed | |
|---|---|---|---|
| Hierarchical level | Complete Data | Numbers | Mean starts per level |
| **Flat races** | | | |
| Racecourses | 43 | 43 | 905.5 |
| Race day | 2,697 | 2,685 | 14.5 |
| Races | 39,813 | 24,204 | 1.6 |
| Starts | 420,607 | 38,938 | - |
| Trainers | 814 | 730 | 53.3 |
| Jockeys | 1,835 | 935 | 41.6 |
| *Deaths* | *378* | *378* | - |
| **Hurdle races** | | | |
| Racecourses | 45 | 45 | 952.0 |
| Race day | 2,356 | 2,355 | 18.2 |
| Races | 16,377 | 14,847 | 2.9 |
| Starts | 170,514 | 42,837 | - |
| Trainers | 1,557 | 1,345 | 31.8 |
| Jockeys | 1,420 | 1,067 | 40.1 |
| *Deaths* | *838* | *838* | - |

Ordinary logistic regression analyses. In the multivariable model of the odds of a horse dying in a flat race, risk increased with age at first race (i.e., age at start of racing career), as well as with the firmness of going and the distance of the race. Better horses (those with a higher rating) were at lower risk of death. A statistical interaction between distance of race and racing intensity was found so that horses racing for the first time ever and in long races were at particularly increased risk. The fit of these models was assessed using the Hosmer-Lemeshow

statistic and was also validated using an independent source of data (initially from the 17,829 National Hunt flat race starts from the same period). The model fit the data well (Hosmer-Lemeshow statistic P=0.76) and predicted the fatality rate per start in National Hunt flat races reasonably accurately (predicted 3.5 deaths /1000 starts, whereas 3.8 deaths /100 starts were observed).

In hurdle races, the risk varied significantly with firmness of going, winning speed, distance of race, month and year of race, gender, racing intensity (number of runs in the last 12 months), age at the time of start and age at the start of racing career. There was evidence of statistical interaction between the firmness of the going and the racing speed.

Multilevel modelling. Two level (race start and one other level) random intercept models were fitted in both MLwiN and Egret. The results are shown in Table 4, along with the level of statistical significance of the random term.

Table 4. Results from 2 level, intercept only models from hurdle and flat races.

| Level 2 Variable | Variance estimate (s.e.) MlwiN* | Egret** | Egret S.E. (s.e.) | Egret Significance†† |
|---|---|---|---|---|
| **Flat Racing** | | | | |
| Racecourse | 0.054 (0.037) | 0.048 | 0.220 (0.078) | 0.022 |
| Race day | 0.124 (0.172)† | 0.140 | 0.375 (0.242) | 0.213 |
| Race | 0.000 (0.000) | 0.187 | 0.433 (0.894) | 0.403 |
| Trainer | 0.178 (0.083) | 0.138 | 0.372 (0.110) | 0.023 |
| Jockey | 0.000 (0.000) | 0.000 | 0.000 (0.161) | 1.000 |
| **Hurdle Racing** | | | | |
| Racecourse | 0.041 (0.021) | 0.038 | 0.196 (0.053) | 0.003 |
| Race day | 0.135 (0.076)‡ | 0.134 | 0.366 (0.110) | 0.039 |
| Race | 0.469 (0.190)‡ | 0.566 | 0.753 (0.144) | 0.003 |
| Trainer | 0.266 (0.064) | 0.178 | 0.422 (0.064) | <0.001 |
| Jockey | 0.001 (0.021) | *** | *** | 1.000 |

*2nd order penalised quasi likelihood models fitted using RIGLS unless †where 1st order PQL or‡ where 1st order MQL models fitted with RIGLS.
** derived by squaring standard error estimate in Egret
*** no convergence.
†† from likelihood ratio statistic.

The results from MLwiN and Egret were generally very similar for the hurdle models (Table 4), both qualitatively and quantitatively, although some non-trivial differences were evident for trainer and race. In flat races, the results were quite different, with the PQL results in MLwiN finding no evidence of variation at the level of race (in terms of variance and statistical significance), whereas the variance estimate from Egret was relatively large, albeit with very large standard error (and thus low statistical significance).

In the hurdle models, the most important random variables were the race itself and the trainer. In flat models, race was not significant. Jockey had no effect in either flat or hurdle models. In flat models, the most significant variables were trainer and racecourse, despite a relatively large (but statistically insignificant) central variance estimate for race day.

Variance Component Assessment. The relative proportion of the total variance at different levels, as estimated in second order PQL models (unless stated), are shown in Table 5. In all cases, the greatest part of the variance was due to the start level, the lowest level of the hierarchy. These results demonstrate that care needs to be taken in model interpretation when some hierarchical levels are excluded. This was evident when models B1 and B3 were compared (race level not included in model B3). The components were vastly different. The results were not the same between types, as an insignificant amount of variance was due to the flat race level, in comparison with hurdles (see also Table 4). Racing hierarchy models alone are presented for flat racing as the fitted models containing trainer and jockey terms were unstable.

Table 5. Results from multilevel, intercept only models in MLwiN.

| Model | Level | Variable | Variance (s.e.)** | Variance % |
|---|---|---|---|---|
| | | **Flat Racing** | | |
| A1 | 4 | Racecourse | 0.050 (0.035)† | 1.40% |
| | 3 | Race day | 0.164 (0.270)† | 4.50% |
| | 2 | Race | 0.000 (0.000)† | 0.00% |
| A2 | 3 | Racecourse | 0.050 (0.035)† | 1.50% |
| | 2 | Race | 0.062 (0.516)† | 1.80% |
| A3 | 3 | Racecourse | 0.050 (0.035)† | 1.40% |
| | 2 | Race day | 0.164 (0.270)† | 4.50% |
| | | **Hurdle Racing** | | |
| B1 | 4 | Racecourse | 0.038 (0.020)‡ | 1.00% |
| | 3 | Race day | 0.008 (0.140)‡ | 0.20% |
| | 2 | Race | 0.468 (0.236)‡ | 12.30% |
| B2 | 3 | Racecourse | 0.038 (0.020)† | 1.00% |
| | 2 | Race | 0.318 (0.185)† | 8.70% |
| B3 | 3 | Racecourse | 0.038 (0.020)‡ | 1.10% |
| | 2 | Race day | 0.179 (0.114)‡ | 5.10% |
| B4 | 3 | Racecourse | 0.039 (0.020)† | 1.10% |
| | 2 | Trainer | 0.136 (0.113)† | 3.90% |
| B5 | 3 | Racecourse | 0.039 (0.020)‡ | 1.10% |
| | 2 | Jockey | 0.101 (0.104)‡ | 2.90% |
| B6 | 3 | Trainer | 0.210 (0.057) | 6.00% |
| | 2 | Jockey | 0.017 (0.083) | 0.50% |

**2nd order penalised quasi likelihood models fitted using RIGLS unless †where 1st order PQL or ‡ where 1st order MQL models fitted with RIGLS.

Models were also fitted which included fixed effects identified as being significant in ordinary logistic regression analyses (not shown) and the impact of the fixed effects on the random effect variance estimates evaluated. The most notable effect of this in the hurdle model was inclusion of the term for firmness of going, which resulted in a 50% reduction in the course level variance, although the residual rankings of the different courses were largely unchanged.

## DISCUSSION

The results of multilevel, random intercept (only) modelling from these studies of the risk of horses dying during racing in Britain in the 1990s have been presented. Results were similar whether derived from PQL/MQL techniques in MLwiN or by maximum likelihood estimation techniques in Egret. Although several starts were made by many horses during the study (average=*c*10 starts per horse), as only one fatal outcome per horse could occur, lack of independence of starts was unlikely to be important. It was thus not explicitly modelled.

Care needs to be taken with the results from the first order MQL estimates (Tables 4 and 5), as these results may be biased in binary response models (Rodríguez & Goldman, 1995), particularly where the number of level 1 units per level 2 unit was low (see Table 3, e.g., races). First order PQL models tend to be less biased than first order MQL models, but second order PQL models are preferred in these situations (Goldstein & Rasbash, 1996). It is for this reason that second order PQL estimates were presented wherever possible. Monte Carlo Markov Chain (MCMC) techniques may produce improved confidence estimates in these situations (Goldstein et al., 1998), but these models took a unrealistic time to fit (≤2 days using a high powered PC).

A more full discussion of the techniques used in this study has been made by Dohoo et al. (2001). The same approach as Dohoo was adopted to estimate total variance in the binary outcome models, as has been reported elsewhere (e.g., Atwill et al., 1995). As in Dohoo et al. (2001), central estimates of variance in estimating contributions were used. While this technique appears satisfactory for hurdle races, the wide confidence intervals around non-zero estimates in the flat races (e.g., race date in A1) could be producing biased estimates, particularly given the differences between Tables 4 and 5. Further work will make use of MCMC techniques to improve central estimates and credibility intervals, enabling firmer conclusions to be drawn. It is quite possible that the outcome (died/survived) is not the result of an underlying latent process with a continuous logistic distribution, as might be expected from pass/fail scores in examinations, and this theoretical assumption was not tested. A recently web published paper (https://multilevel.ioe.ac.uk/team) by Goldstein et al. suggests that four methods of estimating variance components be used and the results compared as all currently available methods are no more than approximations. Whether or not the assumption is correct in this situation, the comparison of the higher level (level ≥2) variances can still provide useful insight into the problem.

These results suggest that none of the higher level variables tested provided the greatest contribution to the total variance in the risk of a horse dying during racing for both race types (all <15%). Thus, efforts to modify appropriately any lower level (i.e. start level) variables that are significantly associated with the risk of a horse dying in any race are appropriate and more likely to have an impact than interventions at other levels. However, despite this, risk of death did vary at the level of the racecourse in both flat and hurdle models. The variance was not great and a large part of it was attributable to the effects of firmness of going. Although around 12% of the variance was due to the race level in hurdle racing, this figure was negligible in flat races (<0.1%). Such figures are entirely plausible, as the injuries associated with dying vary between race types (e.g. Vaughan & Mason, 1976). Moreover, injuries associated with falling or failing to clear a jump, which would be expected to cluster in hurdle races, are numerically important. In contrast, many serious injuries in flat races appear to be related to underlying, pre-existent injury in particular animals (Stover et al., 1992).

It was interesting that trainer was significant in both flat and hurdle races, particularly the latter. This is consistent with the large degree of variation in incidence rates for fractures that observed between different trainers in studies in flat training yards (Verheyenet al., unpublished). Trainer could have a significant effect on the risk of a horse dying in a flat or a hurdle race in several different ways. Individual trainers might tend to have a particular type of horse that is predisposed to dying, perhaps because of genetic reasons. Also, some trainers and their veterinary surgeons might be particularly good at detecting stress fractures prior to more catastrophic breakdowns occurring. In the OLR models for both race types, horses racing for the first time were the group at highest risk of dying. Preparation for racing, particularly for jumping, is likely to vary between trainers and this may be an important factor.

To summarise, the results from this modelling should be interpreted with some caution, particularly where first order MQL estimates were presented. The results do suggest that efforts to reduce the rates of fatal injury during racing should continue to be concentrated at the start level variables. Changing racecourse factors, particularly going, may also be beneficial. Efforts to identify factors associated with trainer level variation should be continued. Further work should include improvement of estimates of risk, perhaps by using MCMC techniques. Results will also be confirmed using another, independent random sample of controls.

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# A CASE CONTROL STUDY OF FACTORS AND INFECTIONS ASSOCIATED WITH CLINICALLY APPARENT RESPIRATORY DISEASE IN UK RACEHORSES

J.R. NEWTON*, J.L.N. WOOD*, N. CHANTER*

## SUMMARY

A matched case-control study was used to determine infections and other factors associated with clinically apparent respiratory disease in young racehorses in training in the UK. A total of 170 cases, defined as horses with sudden onset coughing, nasal discharge or pyrexia, were identified and matched to 632 non-affected controls, by trainer and time period. Factors examined included age, sex, time since entry into the training yard, time since last race and different infections including tracheal and nasopharyngeal bacteria and viruses. Multivariable conditional logistic regression modelling was used to evaluate the risk of being a case for variables after adjustment for other factors. Three analyses were conducted using clinical cases, which were compared with i) controls without evidence of subclinical inflammatory airway disease (IAD) ii) controls with evidence of subclinical IAD and iii) all controls irrespective of IAD status. A fourth analysis was conducted comparing the two groups of controls i.e. those with and without IAD.

Results indicated that younger horses and those that had entered training more recently were at increased risk of suffering episodes of clinically apparent respiratory disease. Among the infections, increasing numbers of *Pasteurella* spp. in tracheal washes were associated with increasing risk of clinical disease. Tracheal infection with *Streptococcus zooepidemicus* was associated with both clinical respiratory disease and subclinical IAD when compared with controls with no evidence of IAD. This explained the lack of association between clinical cases and *S. zooepidemicus* when all controls were used. Tracheal isolation of *Mycoplasma felis* was also associated with clinical disease after controlling for other factors. An inverse association was also identified between risk of clinically apparent disease and isolation from tracheal washes of the transient, non-pathogenic bacteria *Staphylococcus* and *Acinetobacter* spp. There was no significant association identified between clinical disease and infection with equine herpesviruses-1 and -4, rhinoviruses-1 and -2 or adenovirus. Although equine influenza was significantly associated with clinical respiratory disease, it was a very rare infection in this well vaccinated population and in order to maximise available data all multivariable analyses were conducted after exclusion of the only two matched sets in which influenza was diagnosed.

## INTRODUCTION

Respiratory disease is a significant cause of days lost during training in Thoroughbred racehorses (Rossdale et al., 1985; Bailey et al., 1999). Clinically apparent respiratory disease can be readily recognised by both veterinarians and trainers by signs of coughing during exercise or at rest, abnormal nasal discharge and raised rectal temperature. Other signs include reduced

* Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, CB8 7UU

athletic performance or so-called "poor performance" (Mumford and Rossdale, 1980), swelling of the distal limbs or abnormal haematological parameters. The equine industry has unwittingly perpetuated the theory that the cause of clinically apparent respiratory disease and associated poor performance is exclusively viral in origin by repeatedly referring to the disease as "the virus", with diagnosis often based on non-specific and poorly standardised abnormal haematological parameters. However, detailed investigations of several outbreaks of respiratory disease in racehorses have failed to identify a definitive viral aetiology. Fibreoptic endoscopy and lavage of the distal trachea is now widely used for the diagnosis of lower respiratory tract (LRT) disease, which may or may not be clinically apparent (Whitwell and Greet, 1984). Subclinical LRT disease characterised by endoscopically visible mucus and cytologically evident neutrophilic inflammation has been shown to be associated with lower airway infection by several bacterial species including *Streptococcus zooepidemicus*, *Streptococcus pneumoniae* and *Pasteurella* spp. (Wood et al., 1993) and is commonly referred to as inflammatory airway disease (IAD). A matched case-control study of clinically apparent respiratory disease in young racehorses in the UK was conducted with care taken to use different case and control definitions to account for possible confounding from IAD among controls.

## MATERIALS AND METHODS

### Study design

A matched case-control study nested within a longitudinal study of respiratory disease (Wood, 1999) and with concurrent selection of controls was used. The longitudinal study was conducted in seven flat training yards in the UK between 1993 and 1996 with a random sample of 10 to 15 horses monitored in five yards at any one time. Horses were monitored daily by stable staff for signs of clinically apparent respiratory disease and were examined and sampled on a monthly basis, irrespective of their respiratory health status at the time of examination. As well as horses routinely monitored, cases of clinical respiratory disease amongst other horses in the study yards were investigated during the study if requested by the trainer. Controls were selected from horses that were routinely monitored in the study yards close to the time of the case and which did not have clinical signs of respiratory disease.

### Definition and selection of cases

Cases of clinical respiratory disease were horses which demonstrated at least one of the following signs: sudden onset coughing at exercise or rest, abnormal and obvious serous or mucopurulent nasal discharge or rectal temperature ≥38.6°C (101.5°F). Cases were identified irrespective of endoscopic findings or cytological interpretation of tracheal washes.

### Definition and selection of controls

Where possible and subject to restrictions required for matching, up to four matched controls were initially selected at random for each case from eligible horses routinely sampled during the longitudinal study. Horses were eligible as controls if they did not have clinical signs of respiratory disease at the time of sampling, were under the same trainer and had been sampled within six weeks of examination of the case. This selection was made irrespective of endoscopic findings or cytological interpretation of tracheal washes.

Subdivision of controls according to airway inflammation: A summary measure of airway inflammation was made using an aggregated scoring system comprising three equally weighted parameters (Whitwell & Greet, 1984). A score of one was given for each of i) moderate or severe amounts of endoscopically visible tracheal mucopus, ii) ≥1000 nucleated cells/ml of tracheal wash and iii) moderate or greater proportions of neutrophils in the tracheal wash. Under this scoring system the degree of inflammation ranged from zero to a maximum score of three, with a score of zero indicating no evidence of airway inflammation. Control horses were defined as "healthy" if endoscopic findings and tracheal wash cytological parameters demonstrated no evidence of lower respiratory tract disease, i.e. an inflammation score of zero. Control horses were defined as "subclinical" if there was evidence of airway inflammation, i.e. an inflammation score of one or more.

Clinical examination and sample collection

Presence or absence of abnormal and obvious serous or mucopurulent nasal discharge and coughing during the examination period was noted. The amount of mucopus and/or haemorrhage visible on endoscopic examination of the trachea was recorded as either absent, slight, moderate or severe. Clotted blood samples were collected from the jugular vein and unguarded gauze swabs were used to sample the nasopharynx. Tracheal wash samples were collected transendoscopically from the distal trachea by the instillation and aspiration of 30ml of sterile phosphate buffered saline using a sterile polythene catheter in the biopsy channel. Following collection and thorough mixing, tracheal wash samples were immediately divided into three aliquots: one into EDTA for nucleated and red blood cell counts, another fixed with an equal volume of 10 percent (v/v) saline buffered formalin for cytological examination and another for quantitative bacteriological examination. After each examination the endoscope was disinfected by immersion and thoroughly rinsed with sterile saline.

Laboratory methods

Tracheal wash samples were examined bacteriologically by standard qualitative and quantitative methods, as described by Wood et al. (1993). Nasopharyngeal (NP) swabs were examined qualitatively using the same methods. Tracheal wash samples were qualitatively and quantitatively examined for *Mycoplasma* spp. using the culture and identification techniques described by Wood et al. (1997). Tracheal washes were examined cytologically for presence of inflammatory changes, using the techniques described by Whitwell and Greet (1984), with airway inflammation classified according to inflammation score as already described. Sequential clotted blood samples were quantitatively tested for serum antibody titres to equine herpesviruses (EHV-1 and -4) and equine rhinoviruses (ERV-1 and -2) using the complement fixation test. Haemagglutination inhibition was used to measure antibody titres to equine influenza (A/equine-1 [H7N7] and A/equine-2 [H3N8]) and equine adenovirus. Seroconversion was defined as a four-fold or greater increase in specific antibody titres between paired samples and was taken to indicate viral infection around the time of collection of the first blood sample.

Statistical analyses

Classification of controls into two subgroups on the basis of tracheal inflammation scores meant that factors associated with different case and control definitions (datasets) could be examined. Comparison of the factors associated with each dataset would establish whether

different factors were likely to be associated with clinically apparent and subclinical presentation of respiratory disease in this population of racehorses. Three analyses were conducted using clinical cases, comparing them with: i) "healthy" controls (inflammation score = 0/3), ii) "subclinical" controls (inflammation score ≥ 1/3) and iii) all controls irrespective of IAD status. A fourth analysis was conducted comparing the two groups of controls. Statistical analyses were conducted using Egret software (Cytel Software Corporation).

Univariable analyses: All explanatory variables were examined for their univariable (crude) association with the probability of being a clinical and subclinical case by inclusion in a simple conditional logistic regression (CLR) model in which cases and controls were matched by trainer and time period. The association between outcome and each explanatory variable was expressed as an estimated crude odds ratio (OR). Risk of disease and $\log_{10}$ cfu/ml numbers of individual bacterial species were examined graphically to assess the most suitable way to express variable levels for the purposes of logistic modelling.

Multivariable analyses: Variables believed *a priori* to be associated with disease and others that were associated on univariable analyses at a significance level $P<0.25$ were entered in conditional regression models during the model building process. A forward stepwise approach was adopted for multivariable analyses with systematic addition of *a priori* and significant main effects variables in descending order of their strength of association with the outcome. Variables were only retained if they were associated with disease (Wald $\chi^2$: $P \leq 0.05$) or if their inclusion resulted in a significant improvement in model fit as measured by the likelihood ratio statistic (LRS $\chi^2$: $P \leq 0.05$) (Hosmer and Lemeshow, 1989). Final multivariable models were examined for goodness-of-fit using the Hosmer-Lemeshow technique (Hosmer and Lemeshow, 1989). Statistical interaction was examined by the addition of biologically meaningful, two-way interaction terms between main effect variables. Interaction terms that provided a significant improvement to model fit as measured by likelihood ratio statistic (LRS $\chi^2$: $P \leq 0.05$) would be retained.

Examining data prior to conducting multivariable logistic regression indicated that infection with influenza virus was extremely rare in this well vaccinated population. Influenza virus infection was not detected among "healthy" controls and this presented particular problems with its inclusion in multivariable analyses involving this control group. In order to maximise available sample size and minimise the confounding effects from rare influenza infection, all analyses were conducted after exclusion of the only two matched sets in which influenza seroconversions occurred.

## RESULTS

A total of 170 clinically apparent respiratory disease cases were identified and these were matched to 632 controls. Among controls, 304 were "healthy" because of a zero inflammation score and 328 had "subclinical" respiratory disease with airway inflammation. There was a satisfactory linear relationship between the logit of disease risk and $\log_{10}$ cfu/ml bacteria for *Streptococcus zooepidemicus* and *Pasteurella/Actinobacillus* spp. and the risk of disease was expressed per $\log_{10}$ cfu/ml increase relative to no bacterial culture. The relationships between the logit of disease risk and non-haemolytic *Streptococcus* spp., *Staphylococcus* spp. and *Acinetobacter* spp. were not linear and were best reflected by ordered categories. Due to too few individuals within different categories, the remaining tracheal wash bacterial isolates were classified as binary variables, irrespective of bacterial numbers.

Cases and "healthy" controls

In univariable analyses horses were at significantly increased risk of being a case with younger age, being female, increasing mucopus and inflammation scores and presence of tracheal haemorrhage. Risk was increased in horses that had never raced previously but decreased with the time they had been in the training yard. The risk of being a case increased with greater $\log_{10}$ cfu/ml of tracheal wash for total bacterial count, *Pasteurella/Actinobacillus* spp. and *S. zooepidemicus*. Non-haemolytic *Streptococcus* spp. at $>10^4$ cfu/ml of tracheal wash was significantly associated with clinical disease as was presence of *S. pneumoniae* and *M. felis*. NFGF *Mycoplasma* spp. and *M. equirhinis* were not associated with disease. *Staphylococcus* spp. in tracheal washes at $<10^2$ cfu/ml demonstrated an inverse association with disease. The presence of *S. zooepidemicus* on NP swabs was significantly associated but non-haemolytic *Streptococcus* spp. on swabs was negatively associated with clinical disease. There was no association with viral infections and no diagnoses of influenza were made amongst "healthy" controls. Table 1 summarises the final multivariable CLR model for clinically apparent respiratory disease using only "healthy" controls and excluding the two matched sets in which influenza infections occurred. After controlling for microbiological variables, clinical respiratory disease remained significantly associated with age and time since entry into the training yard; with younger horses and those that had been in training less than one month at increased risk. Risk of being a case was significantly and positively associated with presence of *Pasteurella/Actinobacillus* spp. and *S. zooepidemicus* in tracheal washes, *Pasteurella/Actinobacillus* spp. on NP swabs but was inversely associated with *Staphylococcus* spp. in washes and non-*Streptococcus* spp. on swabs. No statistically significant interactions were identified in this model.

Table 1. Final multivariable CLR model (n=353) for associations between clinically apparent respiratory disease and explanatory variables using "healthy" controls

| Explanatory variable | | Crude OR | Adjusted OR | 95% CI | P-value |
|---|---|---|---|---|---|
| Age group | Yearlings | 13.4 | 14.0 | 1.1 – 179 | 0.042 |
| | 2 year-olds | referent | 1.0 | | |
| | 3 year-olds | 0.4 | 0.5 | 0.2 – 1.0 | 0.057 |
| | ≥4 year-olds | 0.9 | 1.1 | 0.4 – 3.1 | 0.849 |
| Time since entering training yard | <1 month | 4.1 | 4.3 | 1.6 – 11.7 | 0.004 |
| | 1-3 months | 5.9 | 3.4 | 0.9 – 12.6 | 0.065 |
| | >3 months | referent | 1.0 | | |
| TW *Pasteurella/Actinobacillus* spp. | log cfu$^{-1}$ | 1.6 | 1.5 | 1.1 – 1.9 | 0.002 |
| TW *S. zooepidemicus* | log cfu$^{-1}$ | 1.7 | 1.3 | 1.0 – 1.7 | 0.029 |
| TW *Staphylococcus* spp. | not isolated | referent | 1.0 | | |
| | $<10^2$ cfu/ml | 0.3 | 0.4 | 0.2 – 0.9 | 0.032 |
| | $>10^2$ cfu/ml | 0.8 | 0.9 | 0.5 – 1.9 | 0.839 |
| NP non-haemolytic *Streptococcus* spp. | not isolated | referent | 1.0 | | |
| | isolated | 0.5 | 0.4 | 0.2 – 1.0 | 0.039 |
| NP *Pasteurella/Actinobacillus* spp. | not isolated | referent | 1.0 | | |
| | isolated | 1.6 | 2.1 | 1.0 – 4.3 | 0.039 |

TW = tracheal wash isolate NP = nasopharyngeal swab isolate Hosmer-Lemeshow $\chi^2 = 4.32$ ; P = 0.83

Cases and "subclinical" controls

Using "subclinical" controls, horses were at increased risk of being a clinical case with younger age, being female, increasing tracheal mucopus, presence of tracheal haemorrhage and less time since entering the training yard. Previous racing was marginally inversely associated with clinical disease. Cases were significantly associated with tracheal washes containing $>10^4$ cfu/ml total bacteria and non-haemolytic *Streptococcus* spp. and with $\log_{10}$ cfu/ml increases of *Pasteurella/Actinobacillus* spp. and *S. zooepidemicus*. The strengths of these associations were generally lower than for the analyses with "healthy" controls. Presence of *Acinetobacter* spp. and *Staphylococcus* spp. in tracheal washes were inversely associated with clinical disease. The presence of *S. pneumoniae* in tracheal washes was not associated with disease in this analysis. Both *M. felis* and NFGF *Mycoplasma* spp. present in tracheal washes were associated with clinical disease but *M. equirhinis* was not. There was no association between clinically apparent disease and viral infection or presence of any individual bacterial species on NP swabs. Table 2 summarises the final multivariable CLR model for clinically apparent respiratory disease using only "subclinical" controls and excluding the two matched sets in which influenza infections occurred. After controlling for microbiological variables, clinical respiratory disease remained significantly associated with age and time since entry into the training yard; with younger horses and those that had been in training less than three months at increased risk. Risk of being a case was significantly and positively associated with presence of *Pasteurella/Actinobacillus* spp. and *M. felis* in tracheal washes but was inversely associated with *Staphylococcus* and *Acinetobacter* spp. in washes. No statistically significant interactions were identified in this model.

Table 2. Final multivariable CLR model (n=359) for associations between clinically apparent respiratory disease and explanatory variables using "subclinical" controls

| Explanatory variable | | Crude OR | Adjusted OR | 95% CI | P-value |
|---|---|---|---|---|---|
| Age group | Yearlings | 9.1 | 9.5 | 0.9 – 100 | 0.060 |
| | 2 year-olds | referent | 1.0 | | |
| | 3 year-olds | 0.6 | 0.6 | 0.3 – 1.2 | 0.172 |
| | ≥4 year-olds | 0.8 | 0.8 | 0.3 – 2.2 | 0.732 |
| Time since entering training yard | <1 month | 2.8 | 1.9 | 0.8 – 4.2 | 0.134 |
| | 1-3 months | 3.8 | 3.8 | 1.1 – 12.7 | 0.033 |
| | >3 months | referent | 1.0 | | |
| TW *Pasteurella/Actinobacillus* spp. | log cfu$^{-1}$ | 1.3 | 1.2 | 1.0 – 1.4 | 0.050 |
| TW *Staphylococcus* spp. | not isolated | referent | 1.0 | | |
| | <$10^2$ cfu/ml | 0.3 | 0.3 | 0.1 – 0.8 | 0.019 |
| | >$10^2$ cfu/ml | 0.6 | 0.9 | 0.5 – 1.6 | 0.755 |
| TW *Acinetobacter* spp. | not isolated | referent | 1.0 | | |
| | <$10^2$ cfu/ml | 0.3 | 0.3 | 0.1 – 0.8 | 0.022 |
| | >$10^2$ cfu/ml | 0.8 | 0.8 | 0.2 – 3.1 | 0.739 |
| TW *M. felis* | not isolated | referent | 1.0 | | |
| | isolated | 4.0 | 3.7 | 1.0 – 13.8 | 0.056 |

TW = tracheal wash isolate  Hosmer-Lemeshow $\chi^2 = 7.55$; P = 0.48

Cases and all controls

Horses were at significantly increased risk of being a case for the same factors as for "healthy" controls (younger age, female, tracheal haemorrhage, less time in training yard and not raced previously). However the strengths of association were generally smaller than for only "healthy" controls. Similarly, with univariable analysis, clinical cases compared to all controls irrespective of their inflammation score, were significantly associated with the same tracheal wash bacterial variables as "healthy" controls as well as non-felis glucose fermenting (NFGF) *Mycoplasma* spp.. Infections with known equine viruses were not significantly associated with clinical disease other than equine influenza, which had an unadjusted OR of 7.0. The presence of non-haemolytic *Streptococcus* spp in NP swabs was inversely associated with disease. Table 3 summarises the final multivariable CLR model for clinically apparent respiratory disease using all controls and excluding the two matched sets in which influenza infections occurred. After controlling for microbiological variables, clinical respiratory disease remained significantly associated with age and time since entry into the training yard; with younger horses and those that had been in training less than three months at increased risk. Risk of being a case was significantly and positively associated with presence of *Pasteurella/Actinobacillus* spp. and *M. felis* in tracheal washes but was inversely associated with *Staphylococcus* and *Acinetobacter* spp.. No statistically significant interactions were identified in this model.

Table 3. Final multivariable CLR model (n=596) for associations between clinically apparent respiratory disease and explanatory variables using all controls

| Explanatory variable | | Crude OR | Adjusted OR | 95% CI | P-value |
|---|---|---|---|---|---|
| Age group | Yearlings | 11.6 | 12.8 | 1.3 – 128 | 0.029 |
| | 2 year-olds | referent | 1.0 | | |
| | 3 year-olds | 0.5 | 0.6 | 0.3 – 1.1 | 0.098 |
| | ≥4 year-olds | 0.8 | 0.8 | 0.3 – 1.8 | 0.576 |
| Time since entering training yard | <1 month | 3.3 | 2.4 | 1.1 – 5.2 | 0.022 |
| | 1-3 months | 4.5 | 5.5 | 1.7 – 17.6 | 0.004 |
| | >3 months | referent | 1.0 | | |
| TW *Pasteurella/Actinobacillus* spp. | log cfu$^{-1}$ | 1.4 | 1.3 | 1.1 – 1.5 | 0.001 |
| TW *Staphylococcus* spp. | not isolated | referent | 1.0 | | |
| | <$10^2$ cfu/ml | 0.3 | 0.4 | 0.2 – 0.8 | 0.010 |
| | >$10^2$ cfu/ml | 0.7 | 1.0 | 0.6 – 1.7 | 0.981 |
| TW *Acinetobacter* spp. | not isolated | referent | 1.0 | | |
| | <$10^2$ cfu/ml | 0.4 | 0.3 | 0.1 – 0.9 | 0.030 |
| | >$10^2$ cfu/ml | 1.0 | 1.0 | 0.3 – 3.6 | 0.956 |
| TW *M. felis* | not isolated | referent | 1.0 | | |
| | isolated | 5.3 | 4.2 | 1.1 – 15.9 | 0.033 |

TW = tracheal wash isolate Hosmer-Lemeshow $\chi^2 = 6.67$; P = 0.57

Subclinical cases and "healthy" controls

These analyses tested univariable associations between subclinical respiratory disease in controls with airway inflammation (cases in this analysis) and explanatory variables using "healthy" controls that had no evidence of airway inflammation. "Subclinical" respiratory disease was not significantly associated with any of the non-infectious explanatory variables that

were associated with clinically apparent respiratory disease. "Subclinical" disease in controls compared to "healthy" controls was significantly associated with increasing total bacterial count, *Pasteurella/Actinobacillus* spp. and *S. zooepidemicus* and $>10^4$cfu/ml non-haemolytic *Streptococcus* spp. Presence of *S. pneumoniae* and *M. equirhinis* in tracheal washes were also associated with subclinical disease, whereas *M. felis* and NFGF *Mycoplasma* spp. were not. There was no association between subclinical disease and viral infection or presence of any individual bacterial species on NP swabs. Table 4 summarises two final multivariable CLR models for subclinical respiratory disease compared with "healthy" controls and excluding the two matched sets in which influenza infections occurred. After controlling for other variables, subclinical respiratory disease was only significantly associated with increasing *S. zooepidemicus* in tracheal washes. *S. pneumoniae* was forced into model 1 and *Pasteurella/Actinobacillus* spp. forced into model 2 as they each approached statistical significance (Wald P-values = 0.069 and 0.073, respectively). In neither of these models was subclinical inflammatory airway disease associated with age or time since entering training and statistically significant interactions were not identified.

Table 4. Two final multivariable CLR models (n=585) with *S. pneumoniae and Pasteurella/Actinobacuillus* spp. forced in, respectively, for subclinical respiratory disease and explanatory variables using "healthy" controls

| Explanatory variable | | Crude OR | Adjusted OR | 95% CI | P-value |
|---|---|---|---|---|---|
| *Model 1* | | | | | |
| TW *S. zooepidemicus* | log cfu$^{-1}$ | 1.5 | 1.4 | 1.2 – 1.7 | <0.001 |
| TW *S. pneumoniae* | not isolated | referent | 1.0 | | |
| | isolated | 2.2 | 2.0 | 0.9 – 4.1 | 0.069 |
| LRS $\chi^2$ P-value = 0.062 | | | | | |
| Hosmer-Lemeshow $\chi^2$ = 6.45; P = 0.6 | | | | | |
| *Model 2* | | | | | |
| TW *S. zooepidemicus* | log cfu$^{-1}$ | 1.5 | 1.4 | 1.1 – 1.7 | 0.002 |
| TW *Pasteurella/Actinobacillus* spp. | log cfu$^{-1}$ | 1.3 | 1.2 | 1.0 – 1.4 | 0.073 |
| LRS $\chi^2$ P-value = 0.070 | | | | | |
| Hosmer-Lemeshow $\chi^2$ = 6.39; P = 0.6 | | | | | |

## DISCUSSION

Respiratory disease in horses may present in a spectrum of severity and this includes subclinical inflammatory airway disease (IAD) which is not evidenced by any overt clinical signs, being diagnosed with endoscopy and lavage of the trachea after exercise. This condition is being increasingly recognised in athletic young horses (Burrell et al., 1996; Wood et al., 1999, Robinson, 2001). Therefore, an important and unique aspect of this study was the consideration of the occurrence and possible influence of subclinical IAD on the risk factors for clinically apparent respiratory disease. The findings of the multivariable analyses for cases presenting clinically were therefore compared with separate "healthy" and "subclinical" control subgroups

in the first instance and then the factors accounting for differences between "healthy" and "subclinical" controls were considered. Finally, the risk factors for clinically apparent respiratory disease that arose in an overall large model using all controls together, irrespective of IAD status, were considered in light of the findings from the three smaller models.

Cases and "healthy" controls

The "healthy" controls in this analysis did not have signs of IAD as defined by endoscopic and cytological parameters (Whitwell & Greet, 1984; Burrell et al., 1996). However, in contrast to previous studies (Burrell et al., 1996; Wood et al., 1999) the definition of controls here was that horses had inflammation scores of zero and so a very stringent definition of respiratory health was used. Multivariable analysis showed that after controlling for other factors, clinical respiratory disease was significantly associated with younger age and recent entry into the racing yard. This was consistent with, but not necessarily confirmation of, an infectious aetiology for this syndrome as the risk of disease decreased with age (controlling for the effects of time in the yard) and time in the yard (controlling for the effects of age). This would be expected with acquisition of immunity against infection in young horses or recent arrivals experiencing novel infections. In the event that horses entered training at exactly the same time e.g. as yearlings, then these variables would have measured the same thing. However, as there was a wide variation in the time since entry into training within each Thoroughbred age category, it is unlikely that these variables were overall measuring exactly the same phenomenon. A similar association was identified between risk of coughing and stage of training in Australian racehorses in Sydney (Christley et al., 1999). Controlling for these factors, horses were at increased risk of clinical disease with recovery of *Pasteurella/Actinobacillus* spp. and *S. zooepidemicus* from tracheal washes and *Pasteurella/Actinobacillus* spp. from nasopharyngeal swabs. Tracheal infection with these bacterial species has been shown to be associated with pyrexia (Burrell et al., 1994) and coughing (Christley et al., 2001), both signs used in the case definition in this study. There was a significant inverse association found with low numbers ($<10^2$ cfu/ml) of *Staphylococcus* spp. in washes and non-haemolytic *Streptococcus* spp. on swabs. It is not clear whether this is biologically meaningful or represents a differential bias towards detection of small numbers of organisms from "healthy" horses with low overall bacterial counts and despite being present, these bacteria were not detected in the presence of large numbers of pathogenic bacteria in diseased horses.

Cases and "subclinical" controls

Controls in this analysis had signs of IAD based on endoscopic and cytological parameters but unlike previous studies in which horses with inflammation scores of one were defined as non-diseased (Burrell et al., 1996; Wood et al., 1999), "subclinical" controls had inflammation scores of one or greater. Multivariable analysis showed that after controlling for the effects of other variables, clinical disease was still significantly associated with younger age, recent entry into the yard, presence of *Pasteurella/Actinobacillus* spp. in tracheal wash samples and relative absence of small numbers of *Staphylococcus* spp. In addition, horses were at increased risk of clinically apparent disease with the isolation of *M. felis* from tracheal washes, but at decreased risk with isolation of small numbers of *Acinetobacter* spp. in washes. In contrast to the analysis with "healthy" controls, *S. zooepidemicus* in tracheal washes and nasopharyngeal bacterial factors were not significantly associated with clinical respiratory disease.

"Subclinical" cases and "healthy" controls

Multivariable analysis conducted with the same criteria as previous analyses showed that all other variables were confounded by the presence of *S. zooepidemicus* in tracheal washes. There was also an apparently increased risk with *S. pneumoniae* or *Pasteurella/Actinobacillus* spp. isolated from tracheal washes having controlled for *S. zooepidemicus*. These infections which were forced into two final models as their presence after controlling for *S. zooepidemicus* were nearly significant at the 5% level. In contrast to the analyses using clinically apparent respiratory disease cases, "subclinical" disease was not significantly associated with younger age or time since entry into the yard. This suggests that horses may have at least slight airway inflammation, irrespective of their age and time in training and would be consistent with immunity increasing with age and time in training but which is not complete. This finding should be considered in the context that horses with an inflammation score of one out of three (probably indicating airway neutrophilia) were allocated to the "subclinical" case/control group in this study.

Cases and all controls

From looking at each of the separate models, it is clear that the final model using all controls (i.e., "healthy" and "subclinical" controls together), was likely to reflect a composite of factors from these other models. This is most clearly demonstrated by infection with *S. zooepidemicus*, which was shown to be associated with both clinical and "subclinical" disease (and including disease defined by an inflammation score of one), when each was compared with only "healthy" controls but was absent from the all and "subclinical" only control models. This was because *S. zooepidemicus* in tracheal washes was significantly associated with "subclinical" disease, evidenced predominantly by tracheal neutrophilia, among the controls as well as being associated with clinical disease among cases. Overall, there was no apparent significant net effect from *S. zooepidemicus* in favour of clinically apparent respiratory disease when horses with "subclinical" IAD were used as, or included in, the control group. Similarly the effects of nasopharyngeal bacterial isolates of *Pasteurella/Actinobacillus* spp. and non-haemolytic *Streptococcus* spp. that were associated with clinical disease compared with "healthy" controls were not retained in the final model. In contrast, *Pasteurella/Actinobacillus* spp. in tracheal washes, although nearly significantly associated with "subclinical" disease among controls, retained a significant association with clinically apparent respiratory disease when compared with only "subclinical" controls as well as all controls considered together. In multivariable analyses *M. felis* in tracheal washes was associated with clinical disease compared to "subclinical" but not "healthy" controls and this significant association was retained in the overall model with all controls. Age, time since entry into the training yard and *Staphylococcus* spp. in low numbers in tracheal washes were significantly inversely associated with clinical but not "subclinical" disease in these analyses.

Conclusions

Taken together, the analyses conducted in this study using different case and control definitions provide considerable evidence that predominantly bacterial infections of the trachea, sometimes in conjunction with other factors, are associated with a range of presentations of respiratory disease in young horses in training. The milder end of the disease spectrum includes slight inflammation attributed predominantly to tracheal neutrophilia (inflammation score 1/3) in the absence of overt clinical signs and detected only by cytology of tracheal wash samples. The more severe end of the spectrum includes obvious signs of disease such as coughing, nasal

discharge and pyrexia and this is associated with marked airway inflammation (inflammation score ≥2/3) as evidenced by visible tracheal mucopus, neutrophilia and high nucleated cell count. The study identified that tracheal infection with *S. zooepidemicus* was associated with an increased risk of clinically apparent as well as "subclinical" respiratory disease when compared with "healthy" horses that had no clinical, endoscopic or cytological evidence of disease (inflammation score 0/3). This remained true for horses that had a cytologically detectable airway neutrophilia but were otherwise considered to have a healthy respiratory tract. There was evidence that tracheal infections with *Pasteurella/Actinobacillus* spp. and *M. felis* were associated with clinically more apparent disease and that younger horses and those that had arrived more recently in the yards were at increased risk of clinical disease. There was no evidence from this UK based study that after controlling for infections and other non-infectious factors that there was an association between clinical or subclinical respiratory disease and recent racing. This was in contrast to the results of a case-control study of coughing among young Australian Thoroughbreds which found a strong association between this sign and recent racing (Christley et al., 1999).

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# DISEASES RELATED TO INTENSIVE ANIMAL PRODUCTION

# DEVELOPMENT OF THE DUTCH JOHNE'S DISEASE CONTROL PROGRAMME SUPPORTED BY A SIMULATION MODEL

H. GROENENDAAL*[1], M. NIELEN[1] AND J.W. HESSELINK[2]

## SUMMARY

The development of a simulation model, 'JohneSSim', was part of a research effort in preparation for a national Johne's disease control programme. Initially, the focus was mainly directed on different compulsory 'test and cull' strategies. However, the results from the 'JohneSSim' model showed that eradication with only 'test and cull' strategies would not be possible within 20 years. Better calf management seemed more effective in reducing the prevalence. Overall, control was not an economically attractive option. Simulation of a strategy with an 'ideal test' (80% overall-sensitivity) showed that the usage of this test would result in a significant faster decrease of the prevalence but that this strategy was economically not attractive because of the high number of test-positive, young animals that had to be culled. Therefore, a new potential control programme called, Paratuberculosis Programme Netherlands (PPN), was defined which was based on the stepwise improvement of calf hygiene, with little dependency on 'test and culling' at all. The model indicated that if farmers would consistently carry out the necessary management adaptations, this control programme decreases the prevalence considerably, and is economically more attractive (average benefit-costs ratio, excluding extra labour = 1.58) than previous plans. Based on the results of the 'JohneSSim' model, the new national voluntary Johne's disease control programme, PPN, was started in September 2000. The decision making has been greatly supported by the 'JohneSSim' model.

## INTRODUCTION

Paratuberculosis in cattle is an infectious chronic granulomatous enteritis caused by *Mycobacterium avium* subs. *paratuberculosis* (Juste, 1996). In The Netherlands. paratuberculosis has been present for a long time, especially in the low-lying peat moors in the northern part of the country (Benedictus, 1984). Organised disease control started in The Netherlands in the eighteenth century with governmental attempts to eradicate cattle plague (Huygelen, 1997). Since 1919, the Animal Health Service has used faecal cultures from clinical

* Corresponding address: Center of Animal Health and Productivity, University of Pennsylvania, School of Veterinary Medicine, New Bolton Center, 328 W. Street Road, Kennett Square, PA 19348, USA. Email: huybertg@cahp2.nbc.upenn.edu

[1] Farm Management Group, Wageningen University, Hollandseweg 1, 6706 KN, Wageningen, The Netherlands.

[2] Animal Health Service, PO Box 9, 7400 AA, Deventer, The Netherlands

affected and suspected animals as a means of detecting *M.a. paratuberculosis*. The first paratuberculosis control programme started in 1942 (Benedictus et al., 1999). Until recently, all control programmes of paratuberculosis were mainly based on early culling of infected animals and improvement of animal husbandry to prevent further spread of *M.a. paratuberculosis* infections within the herd (Kalis et al., 1999). Results of these programmes have been disappointing because diagnostic procedures have been inadequate and because farmers have not consistently carried out husbandry measures aimed at limiting infection transmission (Benedictus, 1984; Benedictus et al., 1985). The lack of progress with an organised control programme based on test and cull, led to a change of focus towards vaccination with a killed vaccine. This strategy has been successful in reducing clinical paratuberculosis and proved to be much cheaper than the subsidised cull-and-slaughter programme (Benedictus et al., 1985). However, even long-term use of a vaccine does not prevent faecal shedding of the bacteria and thus does not lead to elimination of the infection from the herds (Kalis et al., 1999). In 1997, the leading Dutch institutes working on paratuberculosis developed a plan for eradication of paratuberculosis. The plan was initiated to help alleviate the economic losses caused by the disease and also to address the growing awareness of product quality guarantees. This resulted in the 'First global plan for collective control of paratuberculosis'. In 1998, a new preliminary and voluntary paratuberculosis programme was started which was founded on this plan. This programme was based on management improvement and yearly testing of the animals and consisted of two parts: 1)'Unsuspected herds programme' which has as its objective, identifying unsuspected herds and preventing infection of these herds and 2) 'Assisting infected herds', which has the objective of eliminating the infection from known infected herd. A detailed outline of this voluntary programme is available (Benedictus et al. 1999).

The project, 'Preparation of the collective control of paratuberculosis in The Netherlands', was started on July 1, 1998. The objective of this project was to prepare a national control programme for paratuberculosis with the final aim of eradicating the disease. Scientific foundation of this new programme was deemed essential, because previous programmes had not yielded the desired results. A large research effort was initiated that included studies on test characteristics and improvements, prevalence estimates, monitoring and surveillance programmes as well as on the development of a simulation model, called 'JohneSSim' (Groenendaal et al., 2001). The goal of this model was to evaluate different control strategies on their epidemiological effectiveness and their economical attractiveness.

The aim of this paper is to illustrate the results of the 'JohneSSim' model and the subsequent crucial steps in the decision-making process that led to the implementation of the Dutch voluntary national Johne's disease control programme that started in September 2000.

## MATERIALS AND METHODS

### The 'JohneSSim' model

The simulation model, 'JohneSSim', that was used to evaluate the different Johne's disease control strategies, has been described previously (Groenendaal et al., 2001). This model is a stochastic and dynamic simulation model that simulates the herd dynamics, the disease dynamics, the control of Johne's disease and the economic consequences on a herd level for a default time period of 20 years.

In the herd dynamics, a typical Dutch dairy herd is simulated, including all replacement heifers. Both involuntary and voluntary culling are considered. In the disease dynamics module, five infection routes are considered; (1) fetal infections, (2) infections around birth, (3) infections due to drinking colostrum, (4) infections due to drinking whole milk and (5) infections due to an environment that is contaminated with *M.a. Paratuberculosis*. Control tools that can be simulated with the 'JohneSSim' model can be divided into 'calf hygiene' and 'test and cull' strategies. The benefit-costs ratio (BC-ratio) and the Net Present Value (NPV) are calculated for each control strategy. Both parameters are standard economic measures to value investments that have an extended time component (Dijkhuizen and Morris, 1997) and were calculated for the whole 20 year period, using discounting. However, the NPV should always be the ultimate decision criteria for investments (Brealey and Myers, 2000). The BC-ratio was defined as the total discounted benefits divided by the total discounted costs and the NPV as the total discounted profits minus the total discounted costs. The benefits were calculated as the losses from Johne's disease without control and the losses with the control programme. To discount, the real interest rate (interest rate minus inflation rate) was taken at 5%.

Because of the model's stochastic nature, the model both captures bad and good case scenarios. To represent the difference between dairy farms in the pre-control calf management, eight different herd-profiles were defined. After separately simulating all eight profiles, the model aggregates the different results according to each profile's proportional existence, to determine the results on a national level. A more detailed description of the different herd-profiles is available (Van Roermund et al., 1999).

Input data and control strategies

The strategies that were simulated are shown in Table 1. All strategies were defined by the advisory group 'simulation model Johne's disease control programme', which consisted of eleven experts on Johne's disease. Monthly meetings were held in which the necessary input data and the strategies requiring simulation, were formulated. Input data for the model are extensively described by Groenendaal et al. (2001).

Table 1. Control strategies, simulated with the 'JohneSSim' model

| Stage | | Test-and-cull | Management |
|---|---|---|---|
| First | a-0 | No | No |
| | a-I | ELISA [a], > 3 yr, once a year | No |
| | a-II | ELISA [a], > 3 yr, once a year | Improve for calves ≤ 6 months |
| | a-III | Faecal, > 2 yr, once a year | Improve for calves ≤ 6 months |
| | a-IV | Faecal, > 2 yr, once a year | Improve for calves ≤ 12 months |
| Second | b-0 | No | No |
| | b-I | ELISA, > 3 yr, once in first five years | Step 1 of management |
| | b-II | ELISA, > 3 yr, once in first five years | Step 1 & 2 of management |
| | b-III | ELISA, > 3 yr, once in first five years | Step 1, 2 & 3 of management |

[a] each positive ELISA blood-test was confirmed with a faecal test and, if both tests were positive, the cow was culled

The study was performed in two stages, the first stage was performed from May 1998 to January 1999 and the second stage from January to April 2000.

First stage

The strategies that were simulated in the first stage of the study were mainly focussed on different test strategies for infected herds, combined with a monitoring programme to declare herds as 'unsuspected'. All input data are shown in Groenendaal et al. (2001). However, a few of the differences between stage 1 and 2 are shown in Table 2.

Table 2. The main differences between input parameters in the first and second stage of the 'JohneSSim' simulation study

| Parameter | First stage | Second stage |
|---|---|---|
| Herd size (number of dairy cows) | 50 (constant) | 50, growing with 3.5% per year to 100 after 20 years |
| Introduction of infected animal(s) | Zero or one latently infected replacement heifer per year | Zero to six animals (calves, heifers or cows) per year with variation between farms and years |
| Costs of separate housing (in Dutch guilders) | 2240 per year | 1120 increasing to 2240 per year |

In addition, an ideal test was defined and simulated with the model. The input parameters that were used for this test are shown in Table 3.

Table 3. Characteristics of both the 'default' ELISA test and the 'ideal test' as simulated in the first stage of the study

| | | 'Default test' | 'Ideal test' |
|---|---|---|---|
| Sensitivity | Infection status | | |
| | Latent infected | 1% | 80% |
| | Low infectious | 10% | 80% |
| | High infectious | 60% | 80% |
| | Clinical infected | 80% | 80% |
| Specificity (all uninfected animals) | | 99% | 99% |
| Minimal age of testing | | 3 year | 12 months |
| Frequency | | Once a year | Once a year |
| Costs (in Dutch guilders) | | 10.00 | 4.35 |

Second stage

In the second stage of this study, the focus changed to management strategies (Table 2). A new potential control strategy was defined called, 'Paratuberculosis Programme Netherlands' (PPN), based on stepwise improvement of calf hygiene (Table 4). In this programme, participating dairy farmers will be strongly encouraged to implement an enhanced three-step calf management programme, with advice being given on suitable management practices. The implementation of these measures was arranged in a logical order that followed the course of development of the calf. In the simulations, it was assumed that participating dairy farmers would implement step 1 in year 1, step 2 in year 2 and step 3 in year 3. Furthermore, it was

assumed that all participating farms would test all cows older than 3 years by an ELISA test once in the first five years and cull all cows that were positive by both the ELISA and by a faecal confirmation test.

Table 4. Management adjustments to be made in each of the three PPN steps

| Stages/Steps | Practices |
|---|---|
| Step 1: Calving | • Cleaned cow placed in an individual, clean, calving pen<br>• Separate calf early from dam |
| Step 2: Calving to weaning | • No whole milk, only milk replacer<br>• Housing, separate from cows >2 years<br>• First two weeks in individual calf-box<br>• Clean drinking water<br>• Clean roughage: hay or dried grass<br>• Only given colostrum from own dam |
| Step 3: Weaning to year old | • Housing, separate from cows >2 years<br>• Clean roughage, hay, dried grass or silage from clean pasture that had no fresh manure on it<br>• Only on clean pasture (no fresh manure)<br>• Clean drinking water |

## RESULTS

### Stage 1

Figure 1 shows the mean true prevalence of an average Dutch dairy farm (both infected and uninfected farms included) as simulated in the first stage of the study.

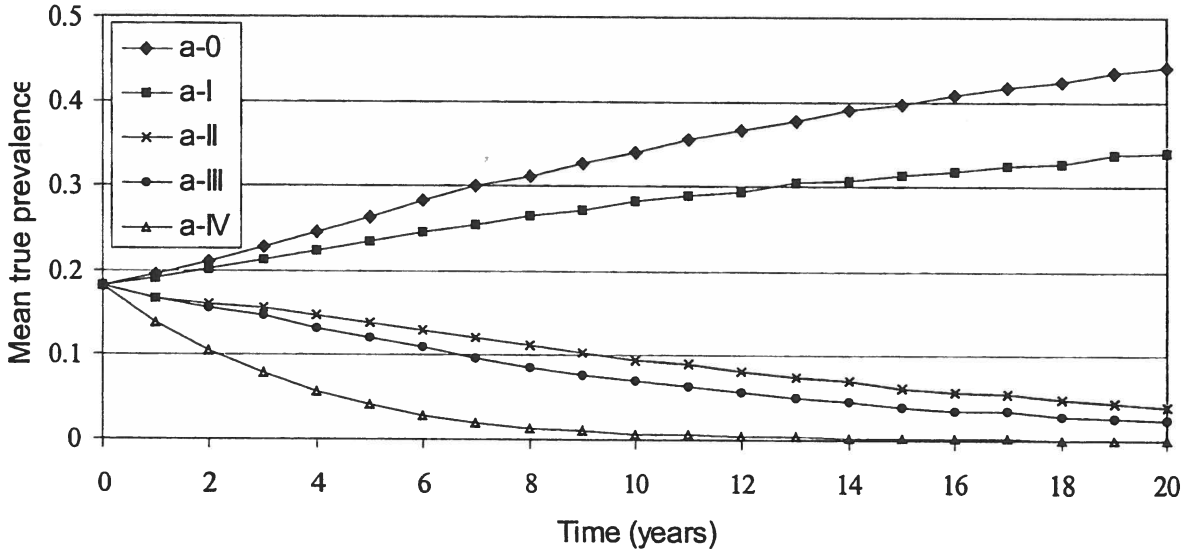


Fig. 1 Mean true animal prevalence over all Dutch dairy farms (infected or uninfected) in the first stage of the study as simulated by the 'JohneSSim' model under no control (a-0) or four different control scenarios (a-I to a-IV)

Without any control (a-0), the prevalence increased gradually. Annual ELISA blood testing with confirmation of positive results by faecal culture with culling if both tests are positive (a-I), resulted in a slower increase, but the prevalence still increased. However, improving the calf hygiene had a much larger impact on the mean prevalence, which can be seen on the difference between strategy a-I and a-II, which is caused only by improved calf rearing management. Furthermore, the difference between strategy a-III and a-IV showed the impact of further improving the calf hygiene, especially using separate housing of 7 – 12 month old calves.

Table 5 shows the economic consequences of the different control strategies, as calculated by the 'JohneSSim' model in the first stage of the study. Without control, the average losses increase considerable because of an increase of the average prevalence in infected herds and an increased of the number of herds infected. For strategy a-II the average BC ratios on farm level were calculated as 0.47 and 0.63 (with or without the costs for extra labour to realise the desired management measures, respectively). There was a large variation around these estimates, the 10% and 90% percentiles of the BC-ratio's were 0.00 and 1.44 for the situation without costs for additional labour. The latter number showed that for 10% of the dairy farms in The Netherlands, control strategy a-II would have a BC-ratio of 1.44 or higher.

Table 5. Losses caused by Johne's disease without control and reduction of the losses (in Dutch guilders), NPV, and BC ratio's of the control strategies as calculated by the 'JohneSSim' model in the first stage of the study

| | Losses without control | Reduction of losses with control | | | |
|---|---|---|---|---|---|
| Year | a-0 | a-I | a-II | a-III | a-IV |
| 1 | 1,541 | 390 | 395 | 391 | 383 |
| 10 | 3,288 | 1,747 | 2,783 | 3,054 | 3,220 |
| 20 | 4,777 | 2,653 | 4,598 | 4,693 | 4,772 |
| Total | 36,871 | 18,691 | 29,070 | 32,064 | 33,218 |
| Costs of control | | 30,054 | 55,196 | 66,426 | 162,610 |
| BC-ratio incl. | | 0.51 | 0.47 | 0.40 | 0.19 |
| BC-ratio excl. | | 0.51 | 0.63 | 0.50 | 0.24 |
| 10%[a] | | 0.00 | 0.00 | 0.00 | 0.00 |
| 90%[a] | | 1.15 | 1.44 | 1.10 | 0.57 |
| NPV incl. | | -11,362 | -26,126 | -34,362 | -129,392 |
| NPV excl. | | -11,362 | -11,234 | -19,466 | -96,664 |
| 10%[a] | | -23,996 | -37,464 | -38,979 | -120,379 |
| 90%[a] | | 5,670 | 22,077 | 6,551 | -62,687 |

[a] 10% and 90% percentiles on farm level

The mean true prevalence under strategy a-II and using the 'ideal test' is shown in Fig. 2. The only differences between the two strategies are the test characteristics as all management measures are the same.

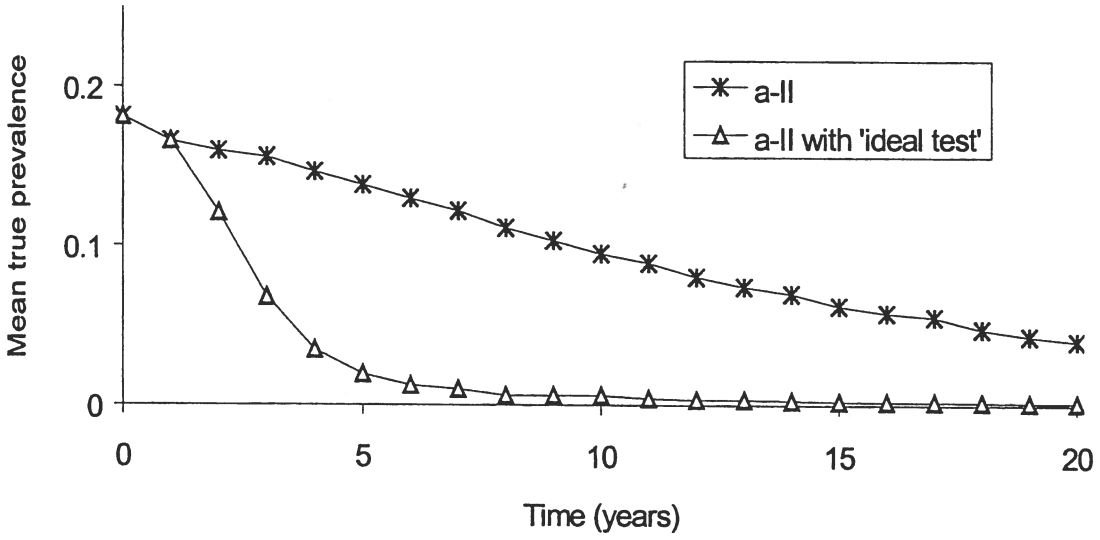


Fig. 2 Mean true animal prevalence over all Dutch dairy farms (infected or uninfected) in the first stage of the study as simulated by the 'JohneSSim' model under strategy a-II with and without an 'ideal test'

It is clear that with an 'ideal test', the prevalence decreased considerably faster but the economic consequences of these two strategies were also quite different (Table 6). A control strategy with an 'ideal test' was economically less attractive because of the much higher costs of culling: the 'ideal test' also found 80% of the latent infected animals (Table 3).

Table 6. Costs of culling test positive animals, total control costs (in Dutch guilders), BC ratio's and NPVs of strategy a-II and the same strategy using an 'ideal test'; calculated by the 'JohneSSim' model in the first stage of the study

| | Strategy a-II | 'Ideal test' |
|---|---|---|
| Costs culling | 6,886 | 34,378 |
| Total costs control | 87,615 | 109,875 |
| Average BC-ratio | 0,63 | 0,44 |
| 10% - 90% [a] | 0 – 1,44 | 0 – 0,94 |
| NPV | -11.234 | -27.224 |
| 10% - 90% [a] | -37,464 – 22.077 | -46.026 - -4.481 |

[a] *the 10% and 90% percentiles on farm level*

Stage 2

Figure 3 shows the mean true prevalence on an average Dutch dairy farm (both infected and uninfected farms included) as simulated in the second stage of the study. It shows a slightly faster increasing average true prevalence without control (Fig. 3, b-0) than in the first stage of the study (Fig. 1, a-I). In addition, fig. 3 shows the additional effect of the three different management steps (see Table 4).

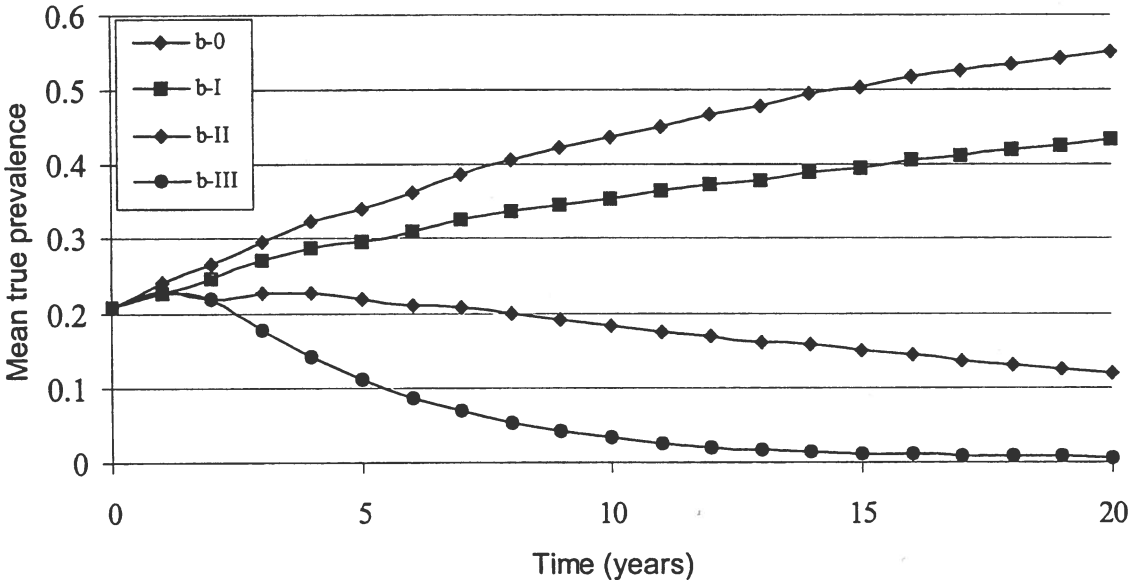


Fig. 3 Mean true animal prevalence over all Dutch dairy farms (infected or uninfected) in the second stage of the study as simulated by the 'JohneSSim' model under no control (b-0) or three different control scenarios (b-I, b-II, b-III)

Table 7 shows the losses due to Johne's disease, without any control measures being taken. Both the prevalence and the losses without control were higher in the second stage compared to the first stage, due to the adaptations of the model in the second stage. A large variation in these losses was observed between different farms. For example, in year 1 the average losses were calculated at Dfl. 1,690, but 10% of the farms had losses higher than Dfl. 5,265. The contribution of the three different categories of losses were decreased milk production (1), decreased slaughter value (2) and losses due to premature or sub-optimal culling (3). The latter accounted for almost 70% of the total losses caused by Johne's disease.

Table 7. Losses caused by Johne's disease without control, reduction of the losses (in Dutch guilders), Net Present Values (NPV) and Benefit Costs (BC) ratio's on an average Dutch dairy farm (infected or uninfected) as calculated by the 'JohneSSim' model. Losses are separated into losses due to decreased production (1), losses due to a decreased slaughter value (2) and losses due to premature culling of clinical or sub-clinical animals (3).

| | Total losses | | | | Categories of losses | | |
|---|---|---|---|---|---|---|---|
| Year | Average | 10% | 50% | 90% | (1) | (2) | (3) |
| 1 | 1.690 | 0 | 398 | 5.265 | 488 | 157 | 1.046 |
| 2 | 2.649 | 0 | 750 | 7.628 | 616 | 267 | 1.767 |
| 5 | 4.304 | 0 | 2.166 | 12.105 | 1.002 | 401 | 2.901 |
| 10 | 7.398 | 0 | 4.890 | 18.561 | 1.670 | 640 | 5.088 |
| 15 | 11.016 | 0 | 8.666 | 26.209 | 2.384 | 960 | 7.673 |
| 20 | 14.808 | 0 | 13.797 | 33.032 | 3.248 | 1.268 | 10.292 |
| Discounted | 86.484 | 0 | 69.532 | 200.539 | (22%) | (9%) | (69%) |

Table 8. Losses caused by Johne's disease without control and reduction of the losses (in Dutch guilders), NPVs, BC ratio's of the control strategies as calculated by the 'JohneSSim' model in the stage phase of the study

| | Losses without control | Reduction of losses with control | | |
|---|---|---|---|---|
| Year | b-0 | b-I | b-II | b-III |
| 1 | 1,690 | 64 | 64 | 64 |
| 10 | 7,398 | 1,277 | 4,268 | 6,096 |
| 20 | 14,808 | 3,457 | 12,010 | 14,491 |
| Total | 86,484 | 14,762 | 49,859 | 64,340 |
| Costs of control | | 19,493 | 40,274 | 61,732 |
| BC-ratio incl. | | 0,64 | 1,10 | 0.95 |
| BC-ratio excl. | | 2.22 | 2.78 | 1.58 |
| 10% - 90% [a] | | 0.00 - 6.60 | 0.00 - 5.94 | 0.00 - 3.32 |
| NPV incl. | | -4,731 | 9,584 | - 2,608 |
| NPV excl. | | 8,701 | 34,380 | 27,319 |
| 10% - 90% [a] | | -8,170 - 36,967 | -14,544 - 97,735 | - 32,405 - 102,003 |

[a] 10% and 90% percentiles on farm level

Finally, Table 8 shows the economic consequences of the different control strategies against Johne's disease, as calculated by the 'JohneSSim' model in the second stage. Because of the increase in the reduced losses (=benefits) and the decrease of the control costs, the BC ratio's and the NPV both increased considerably. The average BC-ratio's, with or without the costs of labour, for the programme which included all three management steps were 0.95 and 1.58, respectively. The average NPV, not including the costs of labour, was Dfl. 27,319 for the total 20-year period. Both the BC-ratio's and the NPV's had a large variation, signifying the large difference between the benefits of Johne's disease control per dairy farm.

## DISCUSSION

The design of a new Johne's disease control programme was initially mainly focussed on 'test-and-cull' strategies. However, the results of the 'JohneSSim' model showed that eradication was not possible with any of the simulated 'test and cull' strategies. An important reason for this, was the low sensitivity of the available tests for Johne's disease, especially for infected, but not clinically affected animals. In addition, none of the simulated control strategies were on average economically attractive. The results of the first stage of the simulation study indicated that eradication within 20 years was not possible. However, strategies based on improved calf hygiene seemed more promising.

In the second stage of the study, the focus changed to improving the management of calf hygiene. The new programme that was defined was called Paratuberculosis Programme Netherlands (PPN). In PPN, the order of implementation of measures was arranged in a logical order, following the course of life of the calf. Some testing can be performed to get more insight

in the Johne's disease status of the farm. However, because PPN is mainly based on the implementation of the necessary management practices, its effect will depend on the farmer's motivation to control Johne's disease. Making PPN a compulsory programme would not be very useful because of the impossibility of monitoring many of the critical management adaptations. Additionally, a compulsory programme might also have a negative effect on the motivation of the farmers, which is the key to the successful control of paratuberculosis. One difficulty is acquiring and maintaining the farmers' motivation to perform all measurements required to effectively reduce the prevalence of Johne's disease (Benedictus, 1984). The epidemiology of the disease and the reasons for the need to implement the measures required are often difficult for farmers to comprehend. It is therefore very important to inform and educate and hence try to change the attitude and behaviour of farmers. An educational instrument, the ParaInformer (ParaWijzer), has been designed as part of PPN. This gives detailed information about the disease, the ways of controlling it and also contains a checklist for designing a control plan which is specific for each farm. A pilot study has been started on 1,500 farms in The Netherlands to determine the implementation rate of the different management practices (Hesselink, 2000).

The average true prevalence in year 0 in the first stage of the study was slightly lower than in the second stage. A reason for this is the more detailed simulation and higher probability of introduction of infected animals. In addition, the stochastic nature of the model can potentially lead to slightly different results. The faster increase in the average prevalence without control in the second stage of the study was caused by the two most important additions to the model (increasing herd-size and the refinement of the introduction of Johne's disease infected animals to the dairy farm). From field data, it is known that the prevalence of Johne's disease is higher in larger herds (Ott et al., 1999; Jackobsen et al., 2000). Furthermore, the more detailed simulation of infected cows is more accurately represented by the practice of animal introduction on dairy farms in The Netherlands.

Simulation of several variants indicated that management adaptations are a more effective and economically more attractive strategy to the control of Johne's disease than test-and-cull only (Table 8). Furthermore, the results indicate that implementation of all necessary management adaptations is critical. For instance, if step 3 is not taken the average true prevalence will not decrease to zero within a 20-year period (Fig. 3).

The results of both stages of the study showed that separation of calves between 7-12 months from adult animals had a significant impact on the average true prevalence. One reason for this is that in The Netherlands the contact between 7-12 months old calves and older cows was found to be high (Muskens et al., 1999). Therefore, a high contact rate was assumed in the model. Separation of the older calves from the animals older than two years reduced this contact rate and therefore resulted in a significant impact on the average true prevalence.

Epidemiologically, the defined 'ideal test' was considerably more effective in reducing the mean Johne's disease prevalence. However, economically, this was a very expensive strategy, caused by the large number of infected animals that had to be culled. A proportion of those infected animals may never have become an excretor of the organism and would never have experienced any production losses. Culling of those animals would therefore lead to high control costs with only small benefits (reduction of losses). It might even result in a lack of replacement heifers, because of the temporary high culling rate caused by the culling of test positive latently infected animals. If a programme was to be based on such an 'ideal test', political decisions would therefore need to be made on which group should suffer the costs

associated with the early culling; farmers, consumers or government. In addition, a pool of Johne's disease free herds should be available to provide uninfected replacement heifers. However, a test with the properties as defined for this 'ideal test' will probably not be available within a measurable timescale.

The output of the 'JohneSSim' model depends on the quality of the assumptions and parameters used. Real data were used wherever possible. However, some parameters or distributions had to be based on the best guesses of experts. Validation of the model with field data was difficult because no *M. a. paratuberculosis* infected herds have been monitored intensively for an extended period of 20 years. This extended time periods would be required because of the slow spread of Johne's disease. However, the model has been validated with field data from 21 Dutch dairy herds and by face value validation by Johne's disease experts (Groenendaal et al., 2001).

In conclusion, the 'JohneSSim' model can be considered a flexible, appropriate and useful tool to evaluate different Johne's disease control strategies, combining the current knowledge of Johne's disease in an optimal way. It has proved to be a valuable tool in the process of defining and deciding upon a new national Johne's disease control programme in The Netherlands by predicting the effectiveness and the economical attractiveness of this programme. It has caused a fundamental change in the design of the Dutch paratuberculosis control programme, from a focus on 'test-and-cull' to a focus on 'stepwise improvements of calf hygiene' strategies, which now forms the key of the new national voluntary control programme. This decision making process has been greatly supported by the 'JohneSSim' model.

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# MULTIVARIATE TECHNIQUES USED IN A CROSS-SECTIONAL STUDY OF FEATHER PECKING AND VENT PECKING IN LAYING HENS IN ALTERNATIVE SYSTEMS

C.J. PÖTZSCH, K. LEWIS, C.J. NICOL AND L.E. GREEN

## SUMMARY

The aim of this cross-sectional study was to identify and quantify management associations with feather pecking and vent pecking in adult laying hens in alternative systems on commercial farms in Great Britain.

The data were derived from a postal questionnaire carried out in 1998. Of 200 farmers, 56.1% reported that feather pecking had occurred in the last depopulated flock, 36.9% reported vent pecking and 33.3% had both problems. The two outcomes, feather pecking and vent pecking, were compared with management exposures using univariate statistics and associated risk factors where $P \leq 0.05$ were then tested in two logistic regression models. The final feather pecking model contained eight risk factors: <50% of the flock used the outdoor area on a sunny day, the diet was changed more than twice, inspections were done by one person, no loose litter left by the end of lay, the hen house temperature was <20°C, lights were turned up when the flock was inspected and bell drinkers were used. An increased risk of vent pecking was noted where dim lighting was used to stimulate the use of nest boxes, the diet was changed more than twice during the laying period, hanging bell drinkers were present and egg laying started before 20 weeks of age. The factors from both logistic models were then tested by survival analysis. In the vent pecking Cox proportional hazards regression model, all four factors significant in the logistic regression model remained significant. The three risk factors for feather pecking that remained in the Cox proportional hazards regression model were: <50% of the flock used the outdoor area on a sunny day, inspections were done by one person, lights were turned up when the flock was inspected. The outcome of a stratified Kaplan-Meier analysis suggested that after eliminating the effects of the risk factors from the Cox proportional hazards model, the proportion of unaffected flocks could be increased dramatically.

The results indicated that survival analysis identified similar, but not identical risk factors, as the logistic regression modelling. Survival analysis permitted the testing of the effect of removing these risks on the reduction in hazard. It also provided a useful alternative analytical technique for indicating which risk factors delayed the occurrence of an outbreak of vent or feather pecking.

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Carsten J. Pötzsch, Ecology & Epidemiology Group, Dept. of Biological Sciences, University of Warwick, Coventry, CV4 7AL, UK. Email: CPotzsch@bio.warwick.ac.uk

## INTRODUCTION

Both feather pecking (FP) and vent pecking (VP) pose severe health problems in alternative systems because they can affect large proportions of the flock and hence incur large economic losses from reduced egg production and increased feed consumption. Compared with conventional cage systems, both problems are less controllable and spread more easily because of the large flock sizes in alternative systems (McAdie & Keeling, 2000). FP can occur during the rearing and laying period but the number of birds affected often increases dramatically with the onset of lay. VP generally starts soon after the birds have come into lay (Allen & Perry, 1975).

Behavioural studies suggest that VP and FP are maladaptive behaviours with multifactorial causes that are closely related to the activity and stress of the hens (Savory & Mann, 1997; El-Lethey et al., 2000). However, most studies that have been carried out on both forms of pecking have been experimental and under controlled conditions which limits the number of birds under examination (Jones & Hocking, 1999). In 1998, a three year observational study into vent and feather pecking on commercial farms was initiated. The hypothesis was that the proportion of unaffected flocks during the laying period could be increased by identifying management practices that were predictive for these maladaptive behaviours. Variables associated with both forms of abnormal pecking were identified in logistic regression models (Green et al., 2000; Pötzsch et al., 2001). From these studies on the occurrence of FP and VP, it appeared that all flocks would eventually succumb to the conditions if flocks were not depopulated by 72 weeks of age. This study was aimed at evaluating the risk factors associated with FP and VP along with an attempt to define the number of weeks free of these conditions. This would enable a comparison to be made on the costs of removing risk factors with the likely benefits. Survival analysis was used in this assessment. This paper describes the survival analysis and discusses the results and their interpretation.

## MATERIALS AND METHODS

The data for this study came from alternative egg producers who responded to a postal questionnaire that requested details about the last flock depopulated on their farm. The questionnaire was designed using epidemiological principles and requested information on VP and FP, rearing and housing management, feed, drinker types, provision and type of nest boxes, egg production, ventilation, temperature, light management and flock health.

### Data analysis

The outcome variables for the analyses were the reported occurrence of feather and vent pecking after the point of lay. The outcomes were compared with management exposures using Chi squared test (or Fisher exact test where appropriate), Mann Whitney test and Spearman rank test, with all tests having the significance level defined as $p \leq 0.05$ (Kirkwood, 1988). Logistic regression, with a likelihood ratio chi square significance level of $p \leq 0.05$, was then adopted for exposure variables that were significant from the univariate analysis. Only variables consistent throughout the laying period were tested. As a consequence of this, flock mortality, diseases in the flock and changes in management as a response to abnormal pecking were excluded. Full details of the analytical techniques are detailed elsewhere (Green et al., 2000; Pötzsch et al., 2001).

Survival analysis was then employed to estimate the effect of the significant variables obtained from the logistic regression models with age at development of abnormal pecking defined as the failure time. The variables were tested using a Cox proportional hazards regression analysis (Collett, 1994) using a likelihood ratio chi square significance level of $p \leq 0.05$. Interactions were tested in the two final regression models. The significant variables in the Cox proportional hazards models were then assessed by Kaplan-Meier survival function plots.

## RESULTS

### Associations between FP and VP

In this investigation, 56.1% (111/196) of the flocks reported the occurrence of FP with an average within flock prevalence of 30% (IQ range 10 - 75) and a case mortality rate of less than 1% (IQ range 0 - 1). Preventive measures against FP were taken by 37.5% (69/184) of the farmers and 61.5% (67/109) implemented control measures once FP was observed in the flock.

Vent pecking was reported in 36.9% (73/198) of flocks. The median proportion of the flock affected with VP was 3.5% (IQ range 1 - 10) and the mortality that farmers attributed to VP was 1.3% (IQ range 1 – 4). Measures to reduce the risk of VP were taken by 31.7% (59/186) of the farmers.

The development of FP and VP in the study flocks from the period of lay is illustrated in Fig. 1. Both behaviours started at point of lay (about 20 weeks) and increased until the time of depopulation at approximately 72 weeks. While 33.3% of flocks showed both problems at depopulation, 23.1% were affected by FP only, while less than 5% of the flocks had VP without FP.

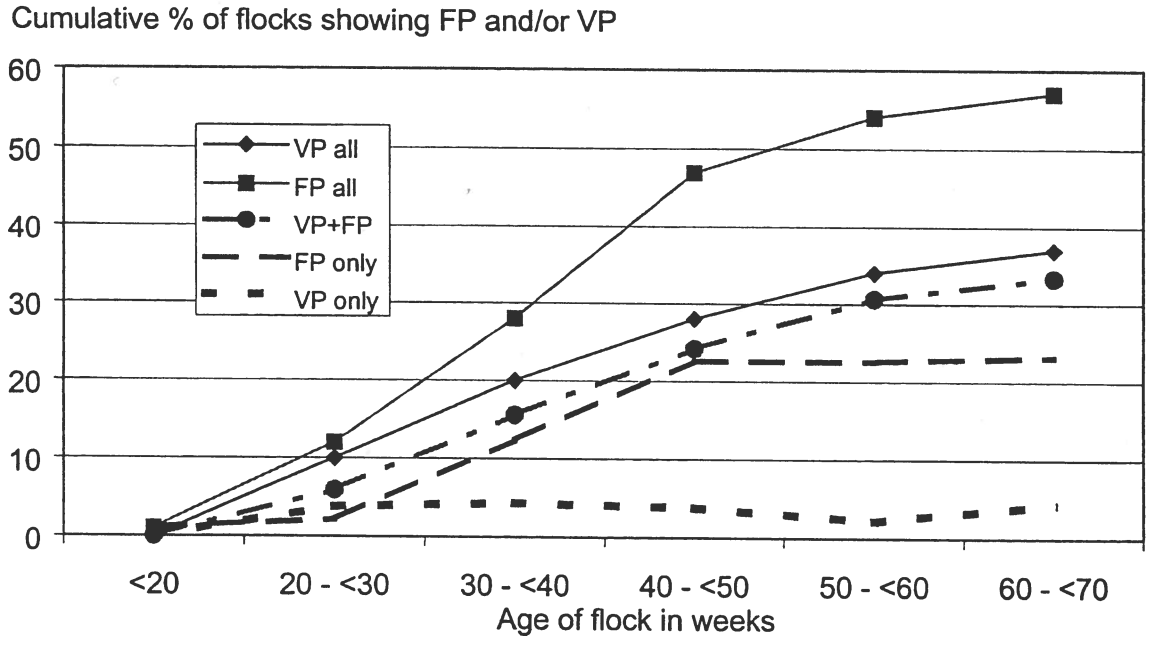


Fig. 1. Cumulative percentage of flocks with VP and FP during the laying period

There was no obvious time pattern observed to first occurrence of VP alone or FP and VP together. However, FP peaked at 40 to 50 weeks of age (Fig. 2).

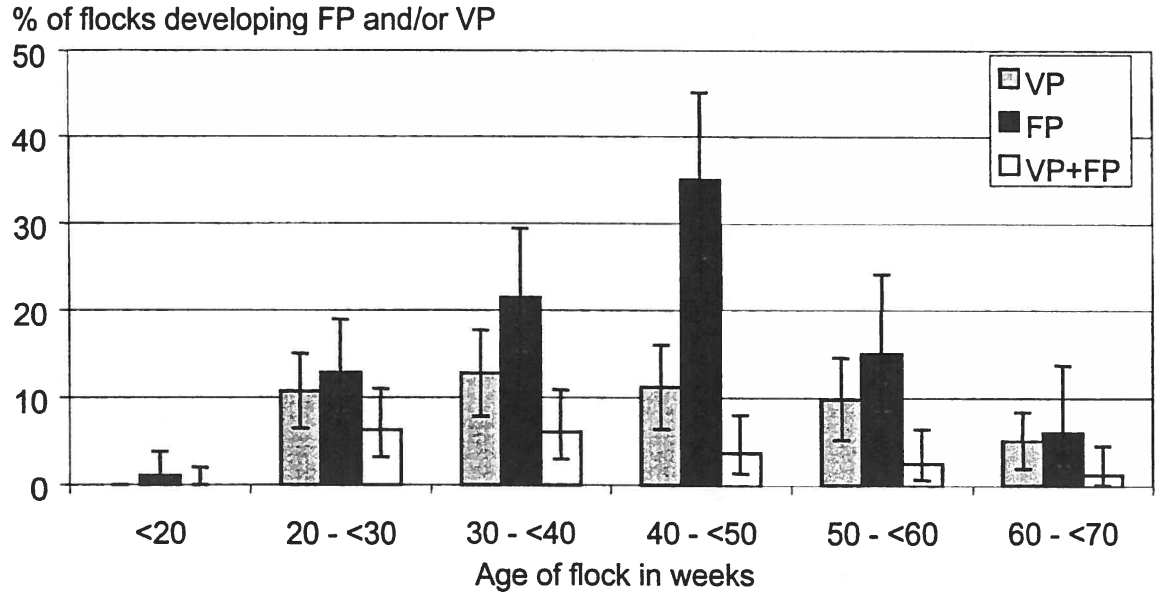


Fig. 2. Proportion of flocks developing FP and/or VP during the laying period as a proportion of all flocks and with 95% confidence intervals

The majority of affected flocks (26.3% of all flocks) had a flock prevalence of less than 10% VP whilst the flock prevalence of FP was distributed more evenly between 0% and 100% (Fig. 3).

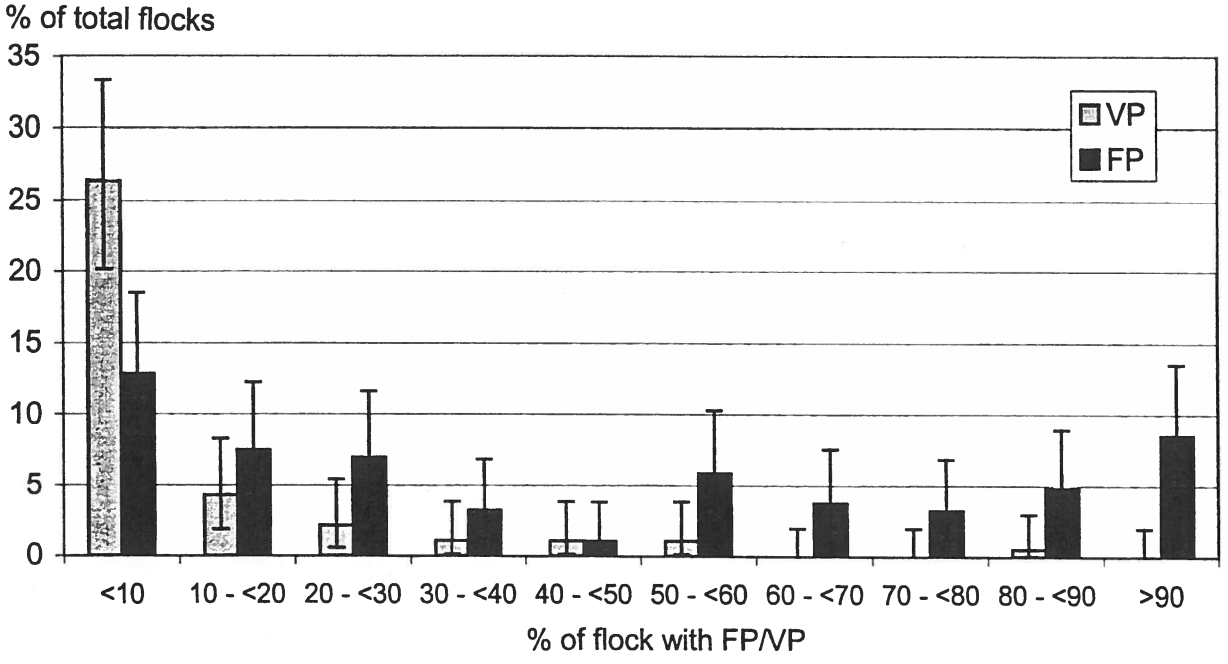


Fig. 3. Proportion of flocks affected with VP or FP by within flock prevalence and 95% confidence intervals

A positive significant correlation was found between the onset of VP and FP (Spearman rank correlation coefficient r = 0.69) as well as between the flock prevalence of VP and FP (r = 0.33). Of all flocks that showed both problems, 48% developed FP followed by VP, in 21% of the flocks VP was observed before FP while in 30.6% both problems occurred simultaneously. FP was seen approximately 8.3 weeks before VP (mean value). A late onset of FP was significantly correlated with a low prevalence in the flock (r = -0.36) while no significant correlation was found between the flock prevalence of VP and the age of the flock when it started.

Analysis of exposure variables with FP and VP

Out of the variables tested in the univariate analysis, 33 were associated with an increased risk of FP and 21 with an increased risk of VP. These variables were included in a logistic regression analysis (Green et al., 2000; Pötzsch et al., 2001).

The logistic model for FP contained the following five risk and three protective factors (odds ratios): lights turned up when flock inspected (11.51); bell drinkers used (11.35); diet changed more than twice during lay (5.86); inspections always carried out by same person (4.91); dim lighting in nest boxes (4.82); >49% of flock used the outdoor area on a fine, sunny day (0.18); loose litter at the end of laying period (0.29) and hen house temperature >20°C (0.40) (Table 1, from Green et al., 2000).

Table 1. Logistic regression model of constant management factors associated with FP

| Variable | Deviance | Coefficient | s.e. | Odds Ratio | Confidence Interval | LRS $\chi 2$ |
|---|---|---|---|---|---|---|
| >49% of flock used outdoor area on fine, sunny day | -8.9 | -1.699 | 0.68 | 0.18 | 0.05 - 0.70 | < 0.01 |
| Diet changed 3 or more times during lay | -8.0 | 1.769 | 0.59 | 5.86 | 1.83 - 18.8 | < 0.01 |
| Inspections always carried out by same person | -5.8 | 1.591 | 0.58 | 4.91 | 1.57 - 15.4 | 0.016 |
| Loose litter at the end of laying period | -6.8 | -1.23 | 0.59 | 0.29 | 0.09 - 0.92 | < 0.01 |
| Hen house> 20°C | -6.0 | -0.913 | 0.57 | 0.40 | 0.13 - 1.22 | 0.014 |
| Lights turned up when flock inspected | -4.0 | 2.440 | 1.00 | 11.51 | 1.62 - 81.5 | 0.046 |
| Bell drinkers used | -6.1 | 2.426 | 0.99 | 11.35 | 1.62 - 78.9 | 0.014 |
| Dim lighting in nest boxes | -3.9 | 1.573 | 0.80 | 4.82 | 1.00 - 23.2 | 0.048 |

s.e. = Standard error

LRS $\chi^2$= Likelihood ratio statistic chi square value

The final model for VP consisted of four significant variables (odds ratios), (Pötzsch et al., 2001), these were: the flock came into lay before 20 weeks (0.4), the use of hanging drinkers (2.32), diet changed more than twice during lay (2.36) and dim lighting was used to encourage nest box use (4.59) (Table 2).

Table 2. Logistic regression model of constant management factors associated with VP

| Variable | Deviance | Coefficient | s.e. | Odds Ratio | Confidence Interval | LRS $\chi^2$ |
|---|---|---|---|---|---|---|
| Dim lighting used to encourage the use of nest boxes | 246.9 | 1.52 | 0.47 | 4.59 | 1.83-11.52 | 0.001 |
| Diet changed 3 or more times | 226.5 | 0.86 | 0.35 | 2.36 | 1.20- 4.67 | 0.013 |
| Flock came into lay at ≥20 weeks | 217.6 | -0.93 | 0.34 | 0.40 | 0.20- 0.77 | 0.007 |
| Drinkers were hanging | 211.3 | 0.84 | 0.34 | 2.32 | 1.19- 4.53 | 0.013 |

s.e. = Standard error
LRS $\chi^2$= Likelihood ratio statistic chi square value

These variables were then tested in Cox proportional hazards models for FP and for VP. The following variables remained significant for FP (hazard ratio): >49% of flock used an outdoor area on a fine, sunny day (0.38); inspections were always carried out by the same person (1.6) and lights were turned up when the flock was inspected (1.69) (Table 3).

Table 3. Cox proportional hazards regression model of constant management factors associated with FP

| Variable | Deviance | Coefficient | s.e. | Hazard Ratio | Confidence Interval | LRS $\chi^2$ |
|---|---|---|---|---|---|---|
| >49% of flock used outdoor area on fine, sunny day | 858.4 | -0.96 | 0.29 | 0.38 | 0.21-0.67 | <0.001 |
| Inspections always carried out by same person | 858.2 | 0.46 | 0.21 | 1.60 | 1.06-2.42 | 0.023 |
| Lights turned up when flock inspected | 849.3 | 0.52 | 0.24 | 1.69 | 1.03-2.75 | 0.046 |

s.e. = Standard error
LRS $\chi^2$= Likelihood ratio statistic chi square value

All four variables that were tested in the VP-Cox proportional model remained significant (hazards ratio): dim light used to encourage the use of nest boxes (2.82); flock came into lay before 20 weeks (0.53); the use of hanging drinkers (2.2) and three or more diet changes during lay (1.72) (Table 4).

Table 4. Cox proportional hazards regression model of constant management factors associated with VP

| Variable | Deviance | Coefficient | s.e. | Hazard Ratio | Confidence Interval | LRS $\chi^2$ |
|---|---|---|---|---|---|---|
| Dim lighting used to encourage the use of nest boxes | 691.6 | 1.03 | 0.28 | 2.82 | 1.62-4.93 | 0.001 |
| Flock came into lay at ≥20 weeks | 671.1 | -0.63 | 0.26 | 0.53 | 0.32-0.89 | 0.002 |
| Drinkers were hanging | 661.9 | 0.78 | 0.26 | 2.20 | 1.31-3.69 | 0.003 |
| Diet changed 3 or more times | 623.1 | 0.53 | 0.27 | 1.72 | 1.00-2.96 | 0.045 |

s.e. = Standard error
LRS $\chi^2$= Likelihood ratio statistic chi square value

There were no interactions among the significant variables in the final logistic model or in the Cox proportional hazards model.

The effects of the variables on the survival functions were plotted using Kaplan-Meier survival curves. The survival ratio for all flocks at depopulation was 0.4 for FP and 0.62 for VP (Fig. 4 and 5 respectively).

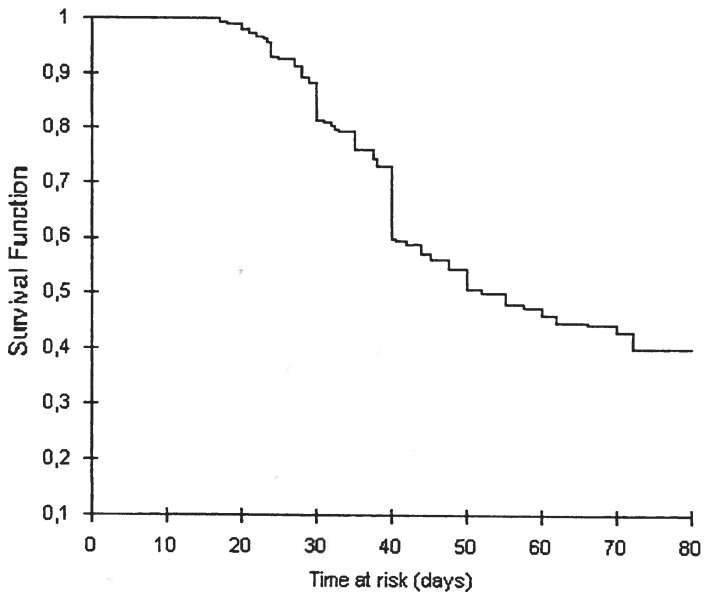


Fig. 4. Kaplan-Meier survival function for FP for all flocks

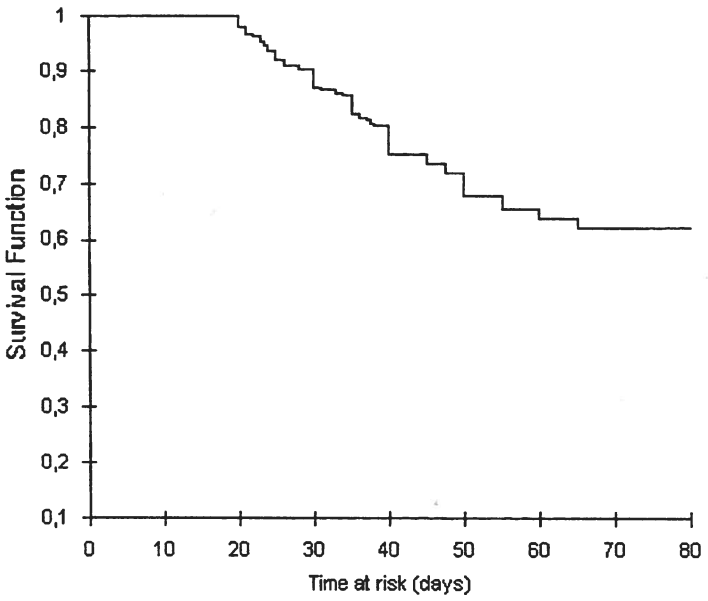


Fig. 5. Kaplan-Meier survival function for VP for all flocks

Both curves continuously drop from the beginning of the laying period (circa 20 weeks) to end of lay at approximately 72 weeks. Figures 6 and 7 show the Kaplan-Meier survival functions for FP and VP after stratification with the factors significant in the Cox regression models. When all three significant factors were applied to reduce the risk of developing FP, the survival ratio for FP increased to 0.89 from 40 weeks (group 1). When tested as three hazards, the survival ratio was 0.18 from week 42 (group 2).

The optimum combination of variables to reduce VP was: dark nest boxes, less than two diet changes during the laying period, no hanging bell drinkers, flocks came into lay at 20 weeks or later. This combination gave a value of 0.83 survival ratio from week 50 whilst, when presented as hazards, the survival ratio was 0.33 from the 30th week of age.

## DISCUSSION

### Association of FP and VP with management

Eight exposures were significantly associated with FP in the logistic regression model (Green et al., 2000). Out of these the following three fitted a Cox proportional hazard regression model for FP: more than 49% of flock used outdoor area on a fine, sunny day; flock inspections were always carried out by same person and the lights were turned up when the flock was inspected. Four exposures were significantly associated with VP in the logistic regression model (Pötzsch et al., 2001) and remained significant in the Cox proportional hazards model: lighting of the nest boxes, more than two diet changes, the use of hanging bell drinkers and flocks that came into lay at 20 weeks or later.

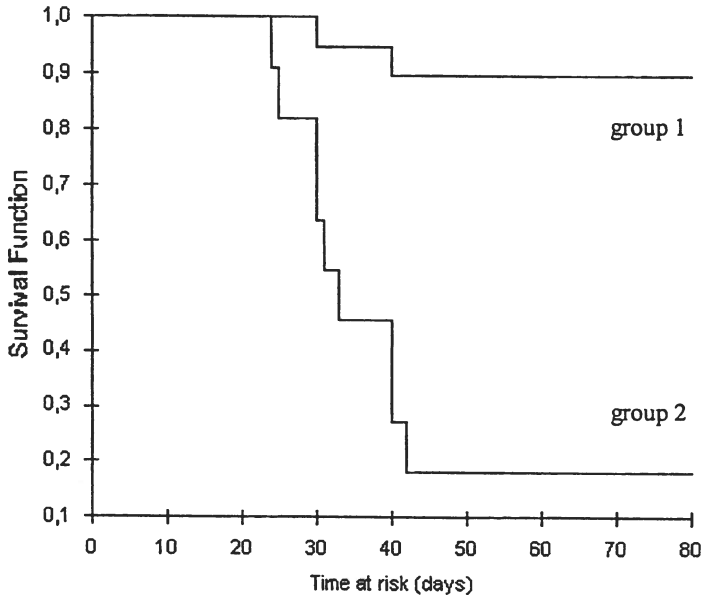


Fig. 6. Kaplan-Meier survival functions for FP for flocks stratified by management variables: group 1: 50% or more of the flock used the outdoor area on a fine, sunny day; flock inspections were not carried out by same person; lights were not turned up when the flock was inspected; group 2: less than 50% of the flock used the outdoor area on a fine, sunny day; flock inspections were always carried out by same person; lights were turned up when the flock was inspected.

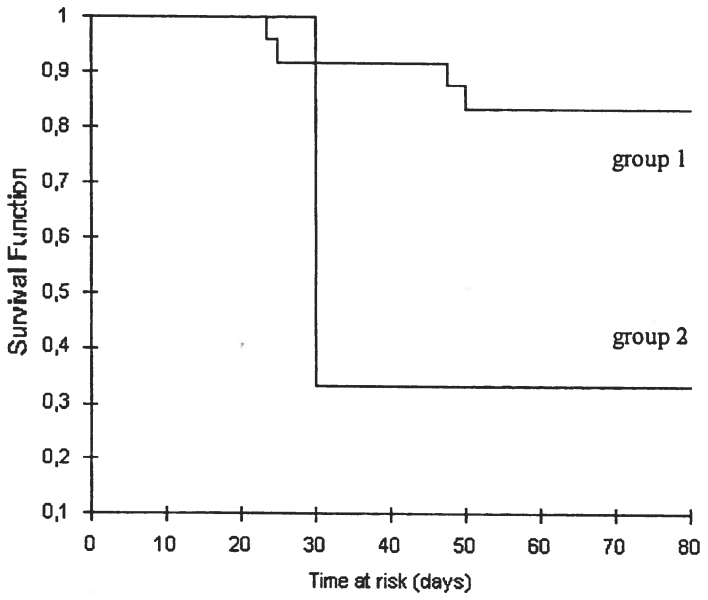


Fig. 7. Kaplan-Meier survival functions for VP for flocks stratified by management variables: group 1: dark nest boxes, less than two diet changes during the laying period, no hanging bell drinkers, flocks came into lay at 20 weeks or later; group 2: nest boxes were lit, more than two diet changes during the laying period, use of hanging bell drinkers, flocks came into lay before 20 weeks

Some of these factors might increase stress and activity, which are known risk factors for abnormal pecking (Lindberg & Nicol, 1994). When at least half the flock used the outdoor area on a bright and sunny day, then stocking density within the house will be reduced and foraging increased. These factors have been reported previously as protective for FP (Nicol et al., 1999; Huber-Eicher & Audige, 1999). Three or more diet changes during lay and the use of hanging bell drinkers may increase levels of frustration along with hunger and thirst because of irregular intake of food and water. Bell drinkers also create a focus of attention and birds have to move among their conspecifics to reach water. A low hen house temperature may also increase activity and hunger. Associations are also reported between early laying hens and increased stress (Craig et al., 1975). Finally, hens that are inspected by just one person might be less adaptable and so prone to stress. Of high importance in the FP and VP models was the lighting management. Low light intensity in the hen house is used to control VP and FP. The strongest association with VP was dim lighting to encourage the use of nest boxes in comparison with no nest box lighting, a factor that also appeared in the FP logistic regression model. In the latter model, turning up lights to inspect the flock was also a risk factor. The presence of loose litter at the end of lay reduced FP in the logistic regression model but interestingly was not significant in the Cox proportional hazard model. Loose litter allows foraging, this has frequently been reported to reduce behavioural abnormalities in experimental studies on growing birds.

Comparing the two logistic regression models, three variables were commonly associated with both VP and FP. These were lighting of the nest boxes, more than two diet changes and the use of hanging bell drinkers. This result indicated that FP and VP may be associated with some common explanatory variables. However, none of these variables remained in the final Cox proportional hazard model for FP. An alternative explanation is that these three factors were influenced by the positive correlation between VP and FP in flocks.

In contrast to the epidemiological study of Gunnarson et al. (1999) who did not find an association between VP and FP, this correlation was of high significance in the present study. The temporal relation of more flocks developing VP after FP might support the hypothesis that VP may sometimes develop from FP after onset of lay (Savory & Mann, 1997; Jones & Hocking, 1999). The results indicated that there were different patterns in attrition. There was an increased occurrence of FP at the age of 40 to 50 weeks but no such increase for VP. Few epidemiological studies have been carried out on commercial farms to investigate FP and VP. Gunnarson et al. (1999) in their investigation of 59 flocks demonstrated that early access to perches decreased VP but had no influence on FP. Huber-Eicher and Audige (1999) found a high stocking density and no access to elevated perches associated with increased FP. However, none of these studies described the time dependence of the outcome variables FP and VP.

Comparison of the logistic regression and the Cox proportional hazard models

This study showed that the use of the Cox proportional hazard model was of additional benefit to the results of the logistic regression model. All variables that were associated with VP in the logistic regression model had a significant effect on the time of onset of VP. This was not the case for the FP models. This may indicate a consistent effect of these variables and therefore a robustness of the VP logistic and Cox regression models. Only 3% of VP cases occurred in flocks without FP, so it may be that the FP models are confounded by the impact of VP. Bugnard et al. (1994) found the same risk factors in a logistic and a Cox proportional regression for one data set, all factors from the logistic model remained in the Cox model.

Methodological considerations

Unfortunately, these results do not come from a random sample of producers. It was estimated that 90% of independent producers in the United Kingdom were contacted, but a sampling frame for all free-range flocks was not available. This has to be taken into consideration when interpreting the results of this study. The data were derived from a postal questionnaire, so this study relied on the farmers' judgement of FP and VP. Different perceptions of pecking, different levels of experience, the retrospective nature of the study and a possible tendency to hide welfare problems might have contributed to inaccuracies. However, the fact that we identified different risk factors for these behaviours and that VP but not FP was associated with higher flock mortality levels indicate that farmers did differentiate the two classifications. There was also only one data point per flock for each failure due to FP or VP. In effect, this is a retrospective survival analysis and is therefore vulnerable to all the known risks for retrospective data sets.

## CONCLUSIONS

Many associations detected in the logistic and survival analysis models have also been reported in experimental studies, such as the use of an outdoor area and the impact of lighting. This strengthens the argument that these factors may be causal and should be tested by intervention studies to establish whether modification of the exposures affect the level of abnormal pecking in a commercial flock.

## ACKNOWLEDGEMENTS

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# POPULATION DYNAMICS OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) IN BREEDING HERDS

G. NODELIJK[1], M.C.M. DE JONG[1] AND J.H.M. VERHEIJDEN[2]

## SUMMARY

In this paper results of earlier studies are reviewed and combined, to discuss the occurrence of outbreaks, persistence and fade-out of PRRSV in Dutch breeding herds. Starting from the dynamical character of PRRSV infection in endemically infected herds, some aspects of strategies aiming to control PRRSV spread are discussed.

## INTRODUCTION

Understanding the transmission of porcine reproductive and respiratory syndrome virus (PRRSV) in pig populations is essential to the development of effective porcine reproductive and respiratory syndrome (PRRS) prevention and control strategies. Knowing where and to what extent the virus circulates in pigs under field conditions will provide new insights into the clinical relevance of PRRS, and will enable the targeting of interventions.

A longitudinal study of a major outbreak of PRRS in a Dutch breeding herd with 115 sows described the subsequent persistence of infection at herd level and the final fade-out five years after the introduction of PRRSV (Nodelijk et al., 2000a). The observational data was analysed using statistical methods and Monte Carlo simulations based on stochastic SIR models. The transmission parameter ($R$) in sows and rearing pigs was estimated at 3.0. From the simulations of a fictive breeding herd of equal size, the average time-to-extinction was estimated at six years, but for a herd of twice the size, it was estimated that the virus would persist for up to 80 years. These findings indicated that, when PRRSV is not reintroduced from outside, the infection can become extinct in small sow herds relatively quickly but, that it can persist for a very long time in large sow herds. In the model, certain assumptions were made about the underlying transmission process. These assumptions and their implications in relation to the field situation will be discussed in this paper.

A cross-sectional study of 32 Dutch breeding farms revealed seropositive weaned pigs in almost all of these farms, proving that PRRSV was still endemic five years after the virus was discovered in the Netherlands in 1991 (Nodelijk et al., 1997). The aim of this study was to determine whether under Dutch field conditions, PRRSV infections occur in young pigs before the finishing period. The seroprevalence of PRRSV in 4–5 week old pigs was significantly higher than in 8–9 week old pigs, indicating the presence of maternal antibodies in the younger

[1] Quantitative Veterinary Epidemiology, Institute for Animal Science and Health (ID-Lelystad), P.O. Box 65, 8200 AB Lelystad, the Netherlands

[2] Department of Farm Animal Health, Faculty of Veterinary Medicine, University of Utrecht, P.O. Box 80151, 3508 TD Utrecht, the Netherlands

pigs. Based on an average decay function of maternal antibodies, an estimated seroprevalence of 20% or higher in a unit of 8–9 week old pigs was considered to correspond to circulation of PRRSV. Thus, PRRSV infection before the start of the finishing period could be confirmed in 23% of the units with 8–9 week old pigs. It was concluded that under Dutch field conditions most pigs entering the finishing facilities will be PRRSV seronegative. However, high PRRSV seroprevalences (95-100%) were found in 6 month old finishing pigs (Nodelijk et al., 1997). This supported the findings of Wensvoort (1994), that most Dutch pigs are PRRSV seropositive at the end of the finishing period.

Several strategies aimed at controlling the spread of PRRSV have been applied in the field, but these were not always successful. To develop a better understanding of these inconsistent results, the occurrence of outbreaks, persistence and fade-out of PRRSV in Dutch breeding herds are discussed in this paper. In particular, the dynamic character of PRRSV infection in endemically infected breeding herds and the assumed risk factors for (re)introduction of PRRSV, such as the purchase of rearing pigs or finishing pigs, are detailed.

## ROUTES OF TRANSMISSION

### Spread between farms

The first outbreaks of PRRS in the Netherlands were observed in 1991, after which the condition spread rapidly throughout the country. Spread between farms was mainly explained by infectious pigs being transported between farms and by airborne transmission. Circumstantial evidence indicated that airborne transmission was an important factor in local spread of PRRSV. However, airborne transmission between farms is probably not important during the endemic phase of the infection because less aerosolised virus is present. Furthermore, semen has also been suggested as a possible source of an outbreak of PRRS. Experimental studies support the potentiality of PRRSV transmission by semen.

In the case of the breeding farm from a longitudinal study (Nodelijk et al., 2000a), the possibility of tying the purchase of infectious pigs from outside to the observed major outbreak of PRRSV was excluded, because the breeding herd was closed. All sera tested for antibodies to PRRSV were negative in March 1991 and as the first positive sera were found in April 1991, it was assumed that PRRSV was introduced during the first sampling interval. During this sampling interval, artificial insemination was performed and relatively more sows than rearing pigs seroconverted. Therefore, introduction of PRRSV via semen could not be excluded. It remains an open question as to whether PRRSV was introduced from outside by semen and/or aerosol in this breeding herd.

### Spread within farms

Within farms, PRRSV spreads rapidly by movement of infectious pigs from one compartment to another and by close contact between infectious and susceptible pigs. Horizontal transmission by direct contact between inoculated and sentinel pigs has been demonstrated under experimental conditions (Terpstra et al., 1992; Albina et al., 1994). PRRSV has been recovered from nasal swabs, oropharyngeal swabs, saliva, faeces and urine of infected pigs, indicating a wide variety of possible routes of virus shedding (Yoon et al., 1993; Wills et al., 1997). On the other hand, several studies have demonstrated that PRRSV is heat and pH labile, and it is quickly inactivated in the absence of moisture or in hostile solutions. Therefore, PRRSV is unlikely to survive in the environment for extended periods of time. Although

aerosol transmission has been demonstrated experimentally, further studies are required to define the rate of aerosol transmission under different environmental. Moreover, vertical transmission of PRRSV to foetuses has been demonstrated by transplacental infection in sows (Terpstra et al., 1991).

Non-porcine hosts

Non-porcine hosts of PRRSV also exist, but their role in the epidemiology of PRRSV is uncertain. Although rodents are not susceptible, some avian species, such as mallard ducks, are susceptible to PRRSV.

## OCCURRENCE OF OUTBREAKS

In a naive population, pigs of all ages are susceptible to PRRSV. The transmission of an infectious agent generally depends on the susceptibility and infectivity of individuals and the rate and intensity of contacts between susceptible and infectious individuals. Transmission can be quantified by the reproduction ratio $R$, which is defined as the average number of secondary cases infected by one typical infectious case (Diekmann et al., 1990). An infection cannot spread extensively unless $R$ is larger than one. When $R > 1$, the introduction of a pathogen in a closed population might result in a major outbreak. However, a minor outbreak can also occur since an early stage of infection may fade-out purely by chance. Persistence of infection can occur when $R > 1$ and new susceptible animals are added to a population at a sufficiently high rate.

After introduction of PRRSV in a naive Dutch pig breeding farm, the virus spread rapidly leading to a major outbreak with high seroprevalences in sows (86-95%) and gilts (100%) within one to two months (Nodelijk et al., 2000a). Subsequently, the infection persisted at herd level until final fade-out five years after PRRSV was introduced. The transmission ratio $R$ in sows and rearing pigs was estimated at 3.0 (95% CI: 1.5 – 6.0). Assuming that this value of $R$ was also valid for other Dutch breeding herds, a major outbreak was expected to have occurred at most of these farms after the first introduction of PRRSV. Moreover, the probability of herds with only a minor outbreak is expected to be low as the number of introductions to a herd during the epidemic was probably higher than one.

In endemically infected breeding herds, the occurrence of PRRSV outbreaks in young pigs before entering the finishing period may depend on the presence of maternal immunity. It has been demonstrated experimentally that the transmission of pseudorabies virus (PRV) among pigs with maternal immunity ($R = 0.2$) was significantly lower than the transmission of PRV among pigs without maternal immunity ($R = 6.3$) (Bouma et al., 1996). Although this has not been quantified for PRRSV, it could explain why PRRSV infections are common in finishing pigs. If maternal immunity has some protective effect against transmission, the extent of PRRSV spread in young pigs under field conditions would partially depend upon the presence and the level of antibodies in their dams (Nodelijk et al., 1997). The longitudinal study in an endemically infected breeding herd revealed that the number of seropositive and infectious sows, based on the prevalence and the incidence of PRRSV respectively, varied over time (Nodelijk et al., 2000a). Assuming that maternal immunity reduces transmission, these findings can explain why in the cross-sectional study: (1) active PRRSV infection in young pigs was confirmed in only 23% of the units of 8–9 week old pigs; (2) not all pigs in these units were

found to be seropositive; (3) serological profiles of young pigs at breeding farms varied within herds over time.

Under Dutch field conditions in compartments with finishing pigs, the introduction of infectious pigs can be expected to lead to major outbreaks. Most pigs will be susceptible to PRRSV infection, because they are seronegative at the start of the finishing period (Nodelijk et al., 1997). Moreover, the transmission parameter $R$ in finishing pigs is expected to be larger than in sows since the housing system for finishing pigs allows more frequent and more intense contact between pigs. Indeed, high seroprevalences (95-100%) in pigs at the end of the finishing period were found in the cross-sectional study and in other Dutch finishing herds (Wensvoort, 1994).

## PERSISTENCE OF PRRSV IN BREEDING HERDS

From the simulations for a fictitious closed breeding herd, it followed that fade-out of PRRSV infection can be expected predominantly in small sow herds and, that the probability of PRRSV persistence at herd level increases exponentially with herd size. Moreover, in reality, the probability of persistence can be increased by multiple reintroductions of PRRSV.

Persistence of PRRSV infection in a breeding herd may result from different mechanisms. Basically, a sufficient number of susceptible and infectious pigs needs to be present to maintain the chain of infection. After the initial outbreak of PRRSV, the infection does not necessarily lead to seroconversion of all the pigs within the herd (Terpstra et al., 1992; Albina et al., 1994), but some pigs remain susceptible and can subsequently be infected at any time. Indeed, in the longitudinal study (Nodelijk et al., 2000a), it was shown that those sows that initially escaped infection, seroconverted later on. New susceptible pigs can be added to a population by replacement, by birth of piglets from seronegative sows, by loss of passive immunity in young pigs born from seropositive sows, or by loss of active immunity in previously infected pigs. Dependent on the status of the incoming replacement stock, purchase of gilts can result in the introduction of new susceptible pigs, but there is also a risk of infectious pigs being introduced into a herd. Maternal immunity confers a protective effect on piglets (Albina et al., 1994; Chung et al., 1997). However, loss of passive immunity at various ages based on antibody levels (Nodelijk et al., 1997) provides a constant stream of pigs susceptible to PRRSV. Although field and experimental studies have shown that re-exposure of previously infected sows failed to induce clinical disease (Albina et al., 1994), they have no lifelong protection against re-infection by circulating PRRSV. Based on the serological profiles of individual sows in the longitudinal study (Nodelijk et al., 2000a), re-susceptibility of sows for infection was demonstrated directly (by detection more than one seroconversion for the same sow over time) and indirectly (by the maintenance of high antibody levels in individual sows for years).

Infectiousness of an individual pig depends on the duration of the infectious period and the amount of virus that can be transmitted. Infectious pigs can be added to a population by replacement, by infection or re-infection of susceptible pigs and by birth of infectious piglets born from sows infected during pregnancy. Studies have shown that PRRSV can persist in individual pigs for an extended period of time (Albina et al., 1994; Wills et al., 1997; Benfield et al., 1999), but how long pigs remain potentially infectious is still not known. PRRSV has been isolated from oropharyngeal samples of individual pigs for up to 157 days after experimental infection but it was not proven that it could lead to transmission (Wills et al., 1997).

Transmission of PRRSV to susceptible contact pigs has been detected up to 56 days after experimental infection (Terpstra et al., 1992).

## FADE-OUT OF PRRSV IN BREEDING HERDS

Fade-out of PRRSV infection under Dutch field conditions has been described in one breeding farm (Nodelijk et al., 2000a). Although it would be expected that more breeding farms have become seronegative after the initial major outbreak of PRRSV, the actual number of these farms has not been quantified. From the simulations for a fictitious closed breeding herd, it followed that fade-out of PRRSV infection can be expected predominantly in small sow herds, and that the probability of PRRSV persistence at herd level increases exponentially with herd size (Nodelijk et al., 2000a). To extrapolate these simulations to the field situation, the officially registered Dutch breeding farms ($n$ = 8273) can be divided into the following categories of herd size: (1) <100 sows ($n$ = 2804, 34%); (2) 100-200 sows ($n$ = 2654, 32%); and (3) >200 sows ($n$ = 2815, 34%). Based on herd size only and assuming a closed herd, it can be expected that PRRSV has faded-out in most farms of category 1, fade-out or persistence of PRRSV is present in farms of category 2, and PRRSV still persists in most farms of category 3. However, the simulations were undertaken using the stochastic SIR model under the assumption that the fictitious breeding herd was completely closed and that after an initial introduction of PRRSV, the virus was not reintroduced.

Therefore, in reality the probability of fade-out could have been diminished by multiple reintroductions of PRRSV. Although risk factors for reintroduction of PRRSV on Dutch breeding farms have never been quantified, it is assumed that the purchase of rearing pigs or finishing pigs are important risk factors and that the risk from the use of artificial insemination and airborne transmission between herds are of less importance (Mousing et al., 1997). When rearing stock is purchased from endemically infected farms, there will be a risk of introducing new infectious pigs into the sow herd of the recipient breeding farm. A questionnaire survey carried out on 32 randomly selected breeding farms that were involved in the seroprevalence study demonstrated that none of these herds could be considered as being closed since gilts and/or boars were purchased from other farms (Nodelijk, unpublished data). Purchase of rearing stock can be regarded as a common practice as boars were purchased at 29 of the farms (91%), and sows were exclusively replaced by gilts from their own rearing stock on only three of the farms (9%). Furthermore, when breeding farms have their own (on-site) finishing facilities but finishing pigs are purchased for efficiency reasons, there could be a risk of PRRSV transmission by non-animal contacts from the finishing herd to the sow herd as a result of the occurrence of major outbreaks of PRRSV in the finishing pigs. Official figures show that more than 50 finishing pigs were present in 40-50% of the Dutch breeding farms, indicating a potential risk of reintroducing PRRSV into the sow population of these farms. Based on herd size, together with the presence of risk factors on most breeding farms for multiple reintroductions of PRRSV, most of the Dutch breeding farms can be expected to be endemically infected with PRRSV. The results of the cross-sectional study (Nodelijk et al., 1997) were in accordance with this expectation, as seropositive weaned pigs were found on almost all of these farms.

## ASPECTS ON PREVENTION AND CONTROL

Throughout the world, several management strategies have been used to prevent and control PRRS. Basically, in PRRSV-free herds these strategies are focused on preventing the

introduction of PRRSV. In PRRSV-infected herds, the focus is on the control of the disease by trying to prevent new introductions of PRRSV, to reduce PRRSV within herd spread or to induce protective immunity of pigs against clinical signs. Several American field studies report on the possible effects of certain management techniques on virus spread and performance of pigs (Dee et al., 1997; Dee and Philips, 1998). However, the scientific value of these reports is often limited by the low number of herds involved, inadequate sample size of pigs, relative short observation periods to evaluate long-term effects and lack of standardised conditions and controls. Moreover, it is questionable whether the results of these American studies can be extrapolated to European conditions. Nevertheless, some aspects of the prevention and control measures mentioned in these studies will be discussed alongside the new insights gained in our studies on the population dynamics of PRRSV infections.

Efforts to control PRRS in the field have been based on the use of strategies utilised in controlling other diseases of swine . However, results were inconsistent with this approach. As the scope of this paper is the population dynamics of PRRSV infection, further discussion will be focused on several strategies aiming to control PRRSV spread.

PRRSV-infected herds

In test and removal strategies, the identification and culling of carrier pigs demands highly sensitive and specific diagnostic tests, in order to minimise false negative and false positive test results. Test qualities are important as culling of false positive pigs implies extra costs and the presence of false negative pigs implies risk of transmission of virus. Therefore, it is unlikely that an endemically infected breeding herd can be rebuilt into a PRRSV-free herd by the use of the currently available diagnostic tests. To make a herd infection-free by test and removal requires that infected animals are removed before they infect an average of less than one other animal. If test and removal strategies were to become technically feasible so as to achieve this criterion, repeated testing will eventually result in an infection-free herd (cf. Gerritsen et al., 1994). However, apart from the technical aspects, the test and removal strategy should be also economically feasible.

Several management techniques are based on efforts to control PRRSV spread by pig flow strategies, such as all in/all out, medicated early weaning, segregated early weaning and nursery depopulation (Dee et al., 1993, Christianson et al., 1994, Bruna et al., 1997; Dee et al., 1997). Although the production of seronegative pigs has been reported in some of these American field studies, none of these techniques has the ability to consistently eliminate PRRSV in young pigs. In view of the dynamic character of PRRSV infection in endemically infected breeding herds, inconsistent results can be explained by the fact that due to chance processes, transmission of the virus among sows might result in transplacental infection of foetuses or infection of piglets prior to weaning. Other routes of virus introduction could also not be excluded in these field reports.

Whether vaccination can be used to control virus spread within and between herds, will depend on the capacity of the vaccine to reduce virus transmission among vaccinated pigs. If vaccination can reduce virus transmission to a degree that an infected animal infects on average less than one other animal, indicating $R < 1$, the virus will be eradicated. However, failure to reduce $R$ below one does not imply that vaccination has no practical relevance. A reduction of $R$ by vaccination can still result in fewer major outbreaks, and in outbreaks of a smaller size among vaccinated individuals (De Jong, 1995). Moreover, using a mathematical model for pseudorabies virus infection, Van Nes (1999) concluded that to have an impact on transmission between herds in a region, it is not necessary for $R$ of vaccinated pigs in a herd to be $< 1$,

provided that $R$ between herds is < 1. In a recent study, set out to investigate whether vaccination reduces transmission of Lelystad virus (the first isolate of PRRSV in the Netherlands) in pigs under experimental conditions, data of three consecutive vaccination-challenge experiments were analysed (Nodelijk et al., 2000b). It was concluded that under the specific experimental conditions the vaccine used did not significantly reduce transmission and that the $R$ of vaccinated pigs was significantly larger than 4.9. Thus far, no quantitative studies have been reported evaluating any PRRSV vaccines' capacity to reduce transmission of PRRSV under field conditions. Therefore, it is not clear whether the currently available PRRSV vaccines can be used to control virus spread in herds.

PRRSV-free herds

Progress has been made in a better understanding of the epidemiology of PRRSV but it should be noted that not all routes of virus introduction into herds are completely understood. Quantitative studies on the risk factors for PRRSV introduction into a herd are lacking. however, the movement of infectious pigs between farms has been well established to be an important risk factor for PRRSV introduction.

Although the actual number of PRRSV-free Dutch breeding farms is unknown, they do exist (Nodelijk et al., 2000a). If these farms are not closed herds, maintenance of their PRRSV-free status could be hampered by purchase of replacement stock or finishing pigs. Therefore, pigs should only be purchased from known PRRSV-free farms which participate in a PRRSV certification programme. Furthermore, until the risk of virus introduction by semen is clarified, semen should only be purchased from AI centres known to be free of PRRSV. More research is needed to establish the feasibility of a future certification programme for PRRSV, some aspects of which will be discussed here.

The same line of reasoning as for test and removal for individual animals can be applied to herds, that is an infected certified herd infects on average less than one herd so that a pool of certified herds can be maintained (Graat et al., 1999). To form a pool of infection-free herd by a certification programme, requires protocols for entrance of herds into the programme and for surveillance of the certified herds. The entrance protocol should assure that newly certified herds pose no increased threat for the other certified herds. The surveillance protocol should assure that a certified herd that becomes infected will typically infect on average less than one other herd.

A longitudinal study demonstrated that major outbreaks can occur in breeding herds with only PRRSV-naive pigs, but also that circulation of PRRSV may occur in breeding herds with a low seroprevalence (Nodelijk *et al.*, 2000a). Therefore, it will be complex to design a proper surveillance programme. If only major outbreaks at herd level are an important risk of the spread of infection to other herds, a surveillance scheme can be designed (Graat et al., 1999). It has been demonstrated that a low PRRSV seroprevalence did not exclude virus circulation, which makes such a herd potentially infectious to other herds. A possible surveillance scheme will, therefore, not only depend on the frequency and the number of contacts between certified herds, but also on the infectivity of herds with a low seroprevalence. If contacts between herds are rare and characterised by a long interval, the long time interval to detection will not jeopardise the certification programme. Therefore, more information about the sensitivity of the tests and about the transmission between herds is required to enable an informed decision about the technical feasibility of certification. Specificity of the tests, required sampling intervals and

costs of the infection will eventually be needed to determine the economic feasibility of such a certification programme.

## CONCLUDING REMARKS

The information gained from the observational studies, infection experiments, mathematical models and analyses, discussed in this paper has contributed to a better understanding of the population dynamics of PRRSV infections in Dutch breeding farms. Monte Carlo simulations based on quantitative information concerning PRRSV transmission in breeding herds showed that when PRRSV is not reintroduced from outside, the infection can 'rapidly' become extinct in small sow herds, but it can persist for a very long time in large sow herds. In conclusion, although spontaneous fade-out of PRRSV will have occurred on some breeding farms, most Dutch breeding farms will still be endemically infected since the introduction of PRRSV into the Netherlands in 1991. Moreover, from a population dynamics point of view, it can be expected that future developments in the pig industry, such as an increase of herd size and group housing of sows, will enhance the probability of PRRSV persistence at herd level. On the other hand, the expected increase of closed herds, will reduce the risk of reintroduction from outside these farms. Additional research is needed to define and quantify the possible risk factors of PRRSV introduction into pig herds.

To control PRRSV spread in endemically infected herds, vaccination seems to be potentially an important intervention measure, particularly since none of the currently available management strategies has been proven to be consistently effective. Derived quantitative information on PRRSV transmission (Nodelijk et al., 2000a) can be used to design field trials set out to determine whether vaccination reduces PRRSV transmission between pigs. However, the limitations of available diagnostic tools should be borne in mind in such vaccination studies, as discrimination between infected and uninfected pigs is essential to a proper analysis of transmission. So far, there have been no quantitative studies reported evaluating the effect of currently available vaccines on PRRSV transmission under field conditions.

Although the economic losses as a result of the PRRSV epidemic in the Netherlands in 1991 have been established, the impact of endemic PRRSV infections on herd performance seems to be less evident. Therefore, further research is needed to assess the economic losses caused by endemic PRRSV infections in breeding herds. Furthermore, to assess the impact of new outbreaks in PRRSV-free herds, it is important to study the virulence of current Dutch field strains of PRRSV. The outcomes of such studies will determine the economical feasibility of certain intervention measures, such as vaccination, and that of any future PRRSV certification programme. If economical feasibility is established, then the issue of technical feasibility of a certification programme will need to be addressed by further research.

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# BIOSECURITY ON DAIRY FARMS: THE ECONOMIC BENEFITS

G. VAN SCHAIK[1,*], M. NIELEN[1] AND A.A. DIJKHUIZEN[1]

## SUMMARY

A more closed farming system can be a good starting point for eradication of infectious diseases. However, the economic implications of biosecurity measures are not always obvious to farmers. The management decisions regarding biosecurity may be related to different parts of the farm and are farm specific. A model to support such management decisions was developed as a first attempt to model the economic consequences of certain biosecurity measures. A simple model, which is static and deterministic in design, was chosen. The risk factors in the model were solely based on bovine herpesvirus 1, but losses due to introduction of other infectious diseases (bovine viral diarrhoea virus, *Leptospira hardjo* and *Salmonella dublin*) were also added into the model. The economic consequences of these biosecurity measures for various risk factors are shown.

## INTRODUCTION

Infectious diseases cause economic losses, one of which is the potential future loss of market access caused by the risks to animal or public health (Wells et al., 1998). One way of achieving a higher animal health status is through disease eradication. The responsibility for eradication is often at the farm level which implies that individual farmers are responsible for the health level of their own animals. In the Netherlands, a considerable number of farmers participate in eradication programmes for bovine herpesvirus type 1 (BHV1), bovine viral diarrhoea virus (BVDV), *Leptospira interrogans* serovar *hardjo* (*L. hardjo*) and *Salmonella enterica* subsp. *enterica* serotype *Dublin* (*S. dublin*). For a successful eradication programme, farms should remain disease-free and should take adequate biosecurity measures to prevent reinfection of the herd.

In practical terms, a dairy farm cannot become completely closed as there are always necessary contacts with the outside world, such as with veterinarians, AI-technicians or cattle grazing outside. In this paper, professional visitors are visitors that enter the animal area of the barn and come into contact with cattle. Protective farm clothing is defined as overalls/overcoats and boots that are provided by the farmer for use by visitors before their come into contact with the cattle. A sanitary barrier is a covered area outside the barn in which visitors can change into protective farm clothing. A sanitary barrier has a 'dirty' side,

[1] Farm Management Group, Wageningen University, Hollandseweg 1, 6706 KN, Wageningen, The Netherlands.

[*] Corresponding address: Department of Population Medicine and Diagnostic Sciences, Cornell University, PO Box 26, Ithaca, NY, 14853, USA. E-mail: gv25@cornell.edu

where visitors change clothing and a 'clean' side, where visitors wear the dedicated protective clothing and can then enter the barn.

The economic implications of biosecurity measures are not always obvious to farmers. Management adaptations need to be made for different parts of the farm and tend to be farm specific. The management measures required to become a more closed system will differ in effectiveness in terms of risk reduction and costs. Furthermore, the possible benefits of biosecurity measures, as expressed by the losses avoided from the decreased risk of disease introduction, will also differ depending on the characteristics of the farm. An economic model can provide better insight into this complex management problem. For effective on-farm decision support, the inputs for such an economic model have to be farm specific. It should represent the situation on the farm, and it should be able to evaluate a wide range of strategies. Furthermore, the output of the model needs to be recognisable and applicable to the farmer (Jalvingh, 1992).

Management strategies can be evaluated using simulation or optimisation. Optimisation models are generally developed for a specific situation and are less suited to study the consequences of a wide range of management strategies (Jalvingh, 1992). Furthermore, the goal of the current economic model was to give farmers an insight into the possibilities of a more closed farming system. The final solution of the model does not necessarily have to be the optimal solution from a financial or risk perspective. Therefore, a simulation model was preferred to model the economic consequences of biosecurity measures.

A deterministic design is the most straightforward and simplest modelling approach. Other, more elaborate approaches are probabilistic or stochastic modelling. In probabilistic modelling, probability distributions are included to model uncertainty. Random number generators are added when a stochastic modelling approach is used. In the deterministic approach, the resulting average performance of the farm is always equal for the same input (Jalvingh, 1992). Chance and uncertainty are important features of disease introduction. However, the present model was developed as a first attempt at modelling the economic consequences of biosecurity measures and therefore a simple, static, deterministic design was chosen.

The objective of the present study was to describe and discuss the results of an economic model for on-farm decision support. The economic consequences of several biosecurity measures aimed at preventing the introduction of certain diseases will be shown.

## MATERIAL AND METHODS

An economic model was developed to calculate the costs and benefits of a more closed system on dairy farms. The economic model is a static model, which means that time was not included as a variable. Furthermore, the model is deterministic and contains no probability distributions to model uncertainty in the behaviour of the system. The inputs for the model were obtained from previous studies that focused on introduction of BHV1 (Van Schaik et al., 1998, 1999a, 1999b, 2000a, 2000b). The odds ratios (ORs) of the risk factors in the model were therefore solely based on BHV1, but were assumed to be the same for introduction of the other infectious diseases, BVDV, *L. hardjo* and *S. dublin*. The model contained the potential losses as a result of introduction of BHV1, BVDV, *L. hardjo* and *S. dublin*, as calculated by Van Schaik et al. (1999b), Groenendaal (1998), Bennett (1993) and Visser et al. (1997),

respectively. The potential losses were used to calculate the benefits (or avoided losses) of certain biosecurity measures. The costs of the biosecurity measures were calculated by partial budgeting (Dijkhuizen and Morris, 1997). The net revenue of every management measure was calculated per year for the Dutch situation. Changes over time and uncertainty of the costs were not included in the calculations. Each biosecurity measure was assumed to reduce the risk of a risk factor by a certain percentage set between 0% and 100%. The risk reduction of each biosecurity measure was not based on scientific results, but was estimated based on common sense and discussions with farmers and experts.

The model was divided in four modules. A module for general farm characteristics ('farm-input module') contained information on BHV1 status, farm size, farm intensity, number of cattle sold and the distance to neighbouring cattle farms. A module for the management measures ('management module') consisted of numerous biosecurity measures, which may eliminate or reduce the risk of the risk factors. In the 'losses module', the losses as a result of introduction of infectious diseases were calculated. In the 'results module', the chance of introduction, costs of the management measures and losses of introduction of BHV1, BVDV, *L. hardjo* and *S. dublin* were combined to calculate the possible benefits of the biosecurity measures. A more thorough description of the model is available (Van Schaik, 2000c).

Several biosecurity measures incorporated into the model to counteract the various risk factors are presented in this paper. The results show which measures are profitable and under what conditions. The benefits of the biosecurity measures were calculated as follows:

Benefit = total disease introduction losses*(1–remaining risk) – costs of biosecurity measures

where remaining risk = $OR_{farm}$ after biosecurity measures / $OR_{farm}$ with initial management

with $OR_{farm} = e^{(\beta 1+\beta 2+\beta 3+\beta 4+\ldots+\beta n)}$ and

$\beta_n$ = regression coefficient of the $n^{th}$ risk factor

The total losses from the introduction of an infectious disease were calculated for a fictitious farm as described previously (Van Schaik et al., 2000c). These losses were kept constant at 9531 Dutch guilders (Dfl.). This hypothetical farm was used in all the analyses presented in this paper. The costs and benefits were calculated over a 5 year period.

## RESULTS

### Input of economic model

The ORs of all risk factors in the farm-input module are shown in Table 1. The fictitious farm used for the calculations was a 55 cow dairy farm, at 350m distance from another cattle farm with an average of two professional visitors per week to the cattle barn. The herd is free of BHV1, BVDV, *S. dublin* and *L. hardjo*. The farm only sells young bull calves for fattening and does not sell breeding bulls or heifers. The losses due to the introduction of the infectious diseases are shown in Table 2.

Table 1. Risk factors for a fictitious 55 cow dairy herd in the farm-input module

| Risk Factor | OR |
|---|---|
| Distance to other cattle farms (in 100 metres) | 0.70 [a] |
| The number of purchased BHV1-free heifers (per year) | 1.32 [a] |
| Participation in cattle shows (yes/no) | 3.54 [a] |
| Cattle returned to the farm e.g. rejected for export (yes/no) | 4.59 [b] |
| Cattle are grazed at other farms or other cattle at the home farm (yes/no) | 1.28 [c] |
| Hectares of land where cattle were grazed adjacent to neighbouring cattle (ha.) | 1.22 [c] |
| Young stock are served by a purchased bull (yes/no) | 1.28 [c] |
| The number of professional visitors in the barn (per year) | 1.004 [a] |
| Has a temporary employee that also works at other farms (yes/no) | 3.27 [a] |

[a] Van Schaik et al. (1998)
[b] Van Schaik et al. (1999a)
[c] estimated based on the univariable results of Van Schaik et al. (1998, 1999a, 2000a)

Table 2. Potential losses from the introduction of infectious disease onto the fictitious dairy farm (Dfl.)

| Current management | IBR | BVD | *L. hardjo* | *S. dublin* | Total |
|---|---|---|---|---|---|
| Total loss over 5 years | 8882 | 28215 | 18975 | 24585 | |
| Probability of introduction[a] | 11% | 10% | 3% | 21% | |
| Risk before measures | 100% | 100% | 100% | 100% | |
| Average loss over 5 years[b] | 977 | 2822 | 569 | 5163 | 9531 |

[a] Van Schaik et al. (2000b)
[b] average loss = total loss * probability of introduction * risk before measures

The benefits of biosecurity measures

The economic benefits of biosecurity measures to eliminate or reduce the risk of the risk factors in the model on the fictitious dairy farm are shown in Tables 3 – 8. The tables contain various current management options as well as the potential biosecurity measures that can be taken. The economic comparison (costs of measure and benefits of the additional biosecurity measure) is given in the body of the tables in Dutch guilders[1] over a 5-year period. Positive benefits mean that the measure is profitable compared with current management practices. When the benefits are negative, biosecurity measures are not profitable compared with current management practices. The remaining risk was stated as a percentage and is the remaining farm risk after the implementation of the biosecurity measures divided by the initial risk before biosecurity measures. The initial risk was based on the risk factors of the current farm situation.

In Table 3, the farmer purchases two cows per year and to eliminate or reduce the risk of purchasing cows, the farmer may take certain biosecurity measures. The economic benefits of several potential measures are shown.

[1] Dfl. 1 = 0.45 EURO = US$ 0.41 = GB£0.28 (Dec. 2000)

Table 3: Benefits of certain biosecurity measures taken to reduce the risk from purchasing two cows per year (Dfl.)

| | Current management | | | | | |
|---|---|---|---|---|---|---|
| | Purchase of any two cows | | | Purchase of two BHV1-free cows | | |
| Biosecurity measures | Costs | Risk | Benefit | Costs | Risk | Benefit |
| Purchased cows tested for BHV1 and quarantined till result. | 3000 | 59% | 932 | 1000 | 97% | -732 |
| *Stop purchasing cows by:* | | | | | | |
| Rear 2 extra heifers for which forage has to be purchased and housing and labour costs are incurred. | 13300 | 57% | -9214 | 11300 | 95% | -10781 |
| Suboptimal replacement of dairy cows. | 3100 | 57% | 986 | 1100 | 95% | -581 |
| Not filling the milk quota at end of year. | 1500 | 57% | 2586 | 500 | 95% | 19 |
| Leasing part of quota to other farm. | -16050 | 57% | 20136 | -14050 | 95% | 14569 |

In the situation where the farmer purchases two cows without knowing their disease status, most biosecurity measures are profitable because the overall farm risk is reduced greatly by such measures. In other words, the purchase of 2 cows contributes a lot to the overall farm risk and the benefit of reduced risk of introduction of infectious diseases outweighs the costs of the measures. The only exception to this was when the farmer stops purchasing and has to incur extra costs from purchasing forage, housing and labour to rear two more heifers. In the second option in Table 3, the farmer already purchases BHV1-free cows. In this situation, the overall risk of introduction of BHV1 was much smaller and therefore most biosecurity measures were not profitable. The only economically attractive options in this situation were, not filling the milk quota (Dfl. 19) at the end of the year or leasing the surplus milk to other farmers (Dfl. 14569).

Table 4 illustrates the situation where a farmer enters cows into cattle shows and compares no biosecurity measures with a scenario in which the farmer tests and quarantines the cows after the show. Both biosecurity measures that the farmer could take were profitable. However, not participating in shows might cause a reduction in the potential value of the price received for any pedigree heifers that are sold. However, if the value of the heifers that are sold on a yearly basis exceeds Dfl. 1128, then the benefits of participation in cattle shows with testing and quarantine afterwards would be greater than the benefits of not participating (=Dfl. 1128).

Table 5 depicts a farm that grazes ten heifers at other farms with cattle from those farms or grazes the heifers separate from other cattle. In both situations, it was not profitable to purchase extra land so that cattle can be grazed at the home farm, or when additional costs were incurred for forage, housing and labour. However, it was profitable for the farmer to graze the heifers with other BHV1-free cattle or for the heifers to be quarantined and tested before returning them to the herd.

Table 4. Benefits of biosecurity measures to reduce the risk from participation in cattle shows by a pedigree farm selling heifers (Dfl.)

| | Current management | | | | | |
|---|---|---|---|---|---|---|
| | No measures | | | Test and quarantine after show | | |
| Biosecurity measures | Costs | Risk | Benefit | Costs | Risk | Benefit |
| Cows are BHV1 tested after show and quarantined | 500 | 32% | 5964 | N/A. | N/A. | N/A. |
| Farm stops participating in cattle shows | 0 | 28% | 6827 | 0 | 88% | 1128 |

Table 5. Benefits of biosecurity measures to reduce the risk from ten heifers that are grazed at other farms (Dfl.)

| | Current management | | | | | |
|---|---|---|---|---|---|---|
| | Grazing with cattle of another farm | | | Grazing separate from other cattle | | |
| Biosecurity measures | Costs | Risk | Benefit | Costs | Risk | Benefit |
| Heifers are grazed with BHV1-free cattle | 2500 | 11% | 6026 | N/A. | N/A. | N/A. |
| Heifers are BHV1 tested and quarantined | 5000 | 11% | 3526 | 5000 | 37% | 1024 |
| *Stop grazing on other farms:* | | | | | | |
| Purchase of extra land | 12000 | 8% | -3252 | 12000 | 29% | -5200 |
| Rearing on own farm for which costs for forage, housing and labour are incurred | 10650 | 8% | -1902 | 10650 | 29% | -3850 |

Table 6. Benefits of biosecurity measures to reduce the risk of grazing close to cattle of other farms (Dfl.).

| | Current management | | | | | |
|---|---|---|---|---|---|---|
| | Grazing cattle within 3m distance of cattle from another farm | | | Grazing cattle at least 3m apart of cattle from another farm | | |
| Biosecurity measures | Costs | Risk | Benefit | Costs | Risk | Benefit |
| Field that borders neighbours is converted to grow cereals | 16200 | 55% | -11900 | 15300 | 86% | -13973 |
| Cattle graze least 25 m apart from other cattle | 900 | 55% | 3400 | 0 | 86% | 1327 |
| A permanent double fence is built that keeps cattle at least 3 m separate | 2010 | 62% | 1623 | 1110 | 97% | -882 |
| Field is not used when other cattle graze at 3 m distance of the field | 900 | 64% | 2553 | N/A. | N/A. | N/A. |

In Table 6, cattle were grazed close to cattle from other farms. In the first scenario, this was within a 3m distance and all biosecurity measures are profitable except converting a field to grow cereals. In the second scenario, cattle grazed at least 3m apart from other cattle and, in this case, the only profitable measure a farmer can take to further reduce the risk is to keep cattle at a distance of at least 25m.

Van Schaik et al. (2000a) found that cattle escaping from their fields and mingling with other cattle was an important risk factor for introduction of BHV1 to certified BHV1-free farms. Therefore, biosecurity measures to reduce the risk of this factor were all highly profitable (Table 7).

Table 7. Benefits of biosecurity measures aimed at reducing the risk of one cow per year escaping and mingling with cattle from other farms (Dfl.)

| | Current management | | |
|---|---|---|---|
| | No measures; one animal escapes per year | | |
| Biosecurity measures | Costs | Risk | Benefit |
| Field that borders neighbours is converted to grow cereals. | 2700 | 15% | 5433 |
| Field is not used when other cattle graze at a 3 m distance of the field. | 150 | 24% | 7122 |
| Construction of a permanent double fence that keeps cattle at least 3 m separate. | 335 | 22% | 7144 |
| Cattle are grazing at least 25 m apart from other cattle. | 150 | 15% | 7983 |

Table 8 shows that when more professional visitors enter the farm on a weekly basis then it was more profitable to employ measures to reduce the risk from those visitors. The use of protective farm clothing will be profitable when more than one professional visitor enters the cattle barn per week. The construction of a sanitary barrier will be profitable when more than two professional visitors enter the cattle barn per week. When more than four professional visitors enter the barn per week, it is more profitable to have a sanitary barrier than only providing protective farm clothing (Dfl. 175, data not shown in Table 8).

Table 8: Benefits of biosecurity measures to reduce the risk of professional visitors at the farm (Dfl.).

| | Current management | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | One professional visitors per week | | | Two professional visitors per week | | | Three professional visitors per week | | |
| Biosecurity measures | Costs | Risk | Benefit | Costs | Risk | Benefit | Costs | Risk | Benefit |
| Sanitary barrier is constructed. | 3415 | 84% | -1788 | 3415 | 69% | -439 | 3415 | 57% | 680 |
| Professional visitors use protective over-coats and boots before entering the barn. | 1485 | 88% | -367 | 1485 | 78% | 620 | 1485 | 69% | 1491 |

## DISCUSSION

The economic model of biosecurity measures illustrated within this paper was based on the Dutch situation. Other countries may have other diseases with different probabilities of introduction and other economic factors that required consideration. Therefore, the exact benefits calculated by the model may not be valid for dairy farms in other countries. However, the model is a good tool for providing a more educated view on the relative benefits of biosecurity measures.

In many cases, the calculations illustrated that there were profitable biosecurity measures that could be taken to reduce the risk of introduction of infectious diseases. However, for intensive farms which can only rear a limited number of replacement heifers, not purchasing replacement cattle or not grazing at other farms can be very expensive options. Nevertheless, these farms can implement other profitable biosecurity measures, such as vaccination, testing and quarantine to considerably reduce the risk of disease introduction. Reducing the risk from professional visitors or a temporary employee through the provision of protective farm clothing will almost always be a profitable option that can be easily implemented. Additionally, most farms will have more than one professional visitor per week entering their cattle barn.

### Economics

The economic model is a static model, which means that time was not included as a variable. Furthermore, the model is deterministic and contains no probability distribution to model uncertainty in the behaviour of the system. This simple modelling approach has its advantages and disadvantages. The advantage is that the model was relatively simple to build and adaptations to the model are straightforward. However, the model would become more realistic if a probability distribution of the risk estimates and the probability distribution of disease introduction were included. The probability distributions could be based on the confidence intervals obtained from studies or based on other estimates. In the deterministic model, the costs of disease introduction were spread over a five-year period and assumed that disease introduction occurred in the first year. In reality, introduction of diseases will be a stochastic process and the year of outbreak will be a random event. Therefore, the model overestimates the losses from the introduction of infectious diseases. A probabilistic or stochastic model would be more appropriate to generically determine the costs and benefits of biosecurity measures for an average Dutch dairy farm. However, the goal of the current model was to provide a simple tool to support farmers in their farm specific decisions on biosecurity measures and not to build a generic model.

Biosecurity measures usually do not significantly influence the farming system as a whole. This assumption is a precondition for the use of partial budgeting. A disadvantage of partial budgeting is that neither a specific time pattern nor a high degree of uncertainty are included in the method.

### Model input

The magnitude of the risk factors for BHV1 in the model was such that direct animal contacts were more important than other sources such as visitors. This will be true for most infectious diseases of dairy cattle. The biosecurity measures in the model were also assumed to prevent the introduction of BVDV, *L. hardjo* and *S. dublin*. Many studies show that these infectious diseases share the same risk factors as BHV1.

The reduction of risk due to the specified biosecurity measures was arbitrarily chosen. It is clear that 'no purchase' will reduce the risk of introduction by purchased animals to 0%. However, it is less clear how much the risk posed by professional visitors is reduced when the visitors use protective clothing. Previous studies of Van Schaik et al. (1998; 1999a; 2000a) gave an indication of the reduction in risk when professional visitors use protective clothing, but the exact amount is hard to quantify and, in the model, the risk reduction was set at 60%. Information on the reduction of risk is difficult to obtain. The 'success' of a biosecurity measure in reducing the risk will also depend on the quality of management of the farmer. For example, the risk reduction of protective clothing will be smaller when protective farm clothing is not consistently used. It is worthy of note that the figures used in the model can be easily modified to suit an individual farmer.

The probability of introduction of infectious diseases used in the economic model was assumed to be equal to the average probability of infectious disease introduction of a cohort of BHV1-free Dutch dairy farms (Van Schaik et al., 2000b). The remaining risk was relative to the initial risk, which was based on the initial biosecurity status of the farm. Therefore, the avoided losses of infectious disease introduction were dependent on the initial risk and the final risk of a particular farm situation. However, the relative probability of introduction of the four infectious diseases was kept constant (11%, 10%, 3%, and 21% for BHV1, BVDV, *L. hardjo*, and *S. dublin*, respectively). The benefits of biosecurity measures will vary depending on the probability of introduction of the specific diseases. The results of the economic model are most valid for a situation in which the relative probability of introduction of the infectious diseases is similar.

When a farm is at risk from more diseases, biosecurity measures will become more beneficial. Furthermore, an eradication programme for an infectious disease may also enhance the benefits of biosecurity measures. It is costly to eradicate a disease once it has been introduced. On the other hand, an eradication programme will decrease the probability of introduction of the disease since the national prevalence will decrease as a result of the programme. The economic model will allow for replacement of the probability of introduction, losses from the introduction of BHV1, BVDV, *S. dublin* and *L. hardjo,* and inclusion of other diseases. The four diseases that were included in the model should be seen as an indication of the costs of introduction of infectious diseases. The model indicated that the benefits were maximal for farms that are already relatively closed and that are at risk from BHV1, BVDV, *L. hardjo* and *S. dublin*. Conversely, the economic benefits of implementing the biosecurity measures will be lower for farms that are less closed or for farms that are not at risk from the introduction of diseases (i.e. diseases are exotic to area or are already present at the farm).

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# OPEN SESSION

# ECHINOCOCCOSIS IN TUNISIA: AN ECONOMIC ANALYSIS

M.M. MAJOROWSKI, H. CARABIN, M. KILANI AND A. BENSALAH

## SUMMARY

*Echinococcus granulosus* infection is a preventable zoonosis of human and veterinary public health importance in Tunisia. Few authors have attempted to comprehensively quantify human and animal echinococcosis losses in a developing country. The originality of this analysis was to include age stratified rates and losses, productivity losses (including those not formally employed) and Monte Carlo sampling to represent the uncertainty inherent to some epidemiological and economic values.

Echinococcosis in Tunisia causes significant direct and indirect losses in both humans and animals, ranging from approximately US$ 10-19 million annually. These estimates are based on numerous methodological improvements over previous studies and are of considerable consequence comparable to Tunisia's US$ 21.2 billion gross domestic product. A cost-benefit analysis of control programmes using the methodological advances presented here and regional comparison to other endemic diseases are warranted. These may provide information to assist policy decision makers in prioritising the allocation of scarce resources.

## INTRODUCTION

*Echinococcus granulosus* infection is a preventable zoonosis of human and veterinary public health importance that remains problematic in several developed and developing countries. Significant direct and indirect negative health impacts and their economic burden in both humans and animals could be avoided since strategies to control, or even eliminate the disease, exist (Roberts & Gemmell, 1994). Successful control programmes in Iceland, New Zealand, Tasmania and Cyprus have illustrated this potential (Economides et al., 1998). Extensive sheep farming practices, free movement of herds and human demographics as well as religious customs involving the home slaughter of animals, contribute to a high disease burden (Gemmell, 1990). Relative to other countries in the region, Tunisia has a high rate of reported echinococcosis human surgical cases (annual mean 15/100,000) and the design of an effective national control programme would have many benefits (Anon., 1993).

Any disease programme, particularly within developing nations, must compete for scare resources that fall severely short of demand (Schwabe, 1984). Cystic echinococcosis (CE) control programmes require veterinary, agricultural and educational efforts (McCullagh, 1996). The cycle of the parasite starts with the adult worm shedding eggs in the faeces of the definitive host, the dog. Amongst others, humans, sheep, cattle goats and camels can then become

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*Meghan Majorowski, University of Oxford, Magdalen College, Oxford, OX1 4AU, UK.
Email: meghan_majorowski@hotmail.com

infected when they ingest the infective larvae contained in the dogs' faeces (by direct contact or by water contamination) and these immediate hosts eventually develop CE. The prevalence of CE depends on many factors. The population of the definitive host reservoir (canids), its worm burden, infection rates in the intermediate host reservoirs (livestock populations and occasionally man) are all important (Gemmell, 1990; Dar et al., 1997). These epizootiological factors, as well as socio-cultural ones, all contribute to the human load of this debilitating disease. The scarcity of resources available to government departments makes addressing zoonotic diseases, such as echinococcosis, even more difficult. (Martin et al., 1987).

One tool that can help governments establish priorities when allocating scarce disease control resources is economic valuation (Drummond et al., 1997). As early as 1978, the World Health Organization (WHO) recommended that socio-economic evaluation should be an essential part of all programmes for the control of parasitic zoonoses (WHO, 1979). A cost-benefit analysis can be particularly useful when approaching the problem of zoonoses generally and with echinococcosis in particular. By expressing all losses in uniform monetary units, it becomes possible to assess the consequences of echinococcosis on diverse species. The proposed monetary evaluation will help to illustrate the magnitude of this disease's costs on Tunisian society and emphasize the need for its control.

Few authors have attempted to comprehensively quantify human and animal echinococcosis losses in a developing country. Moreover, attempts to include quantified indirect costs to society of any zoonoses are rare. The originality of this analysis was to include age stratified rates and losses, productivity losses (including those not formally employed) and Monte Carlo sampling to represent the uncertainty inherent to some epidemiological and economic values.

## MATERIALS AND METHODS

The study was aimed at guiding decision making in regions where echinococcosis is endemic. A societal approach was adopted throughout to represent the monetary impacts of the infection and disease on the society as a whole (Drummond et al., 1997).

There is a lack of available basic epidemiological and economic information on which to found estimates of monetary losses due to *E. granulosus* infection and disease. The first step in conducting an economic analysis is to assign monetary values to all the consequences of the infection. Therefore, one needs to estimate the incidence rate of infection (or disease if infection data are not applicable or available) in all species affected by the disease. Ideally, one would then obtain the rate of occurrence of complications (or symptoms) within the infected population. An average cost to treat specific symptoms of the disease would then be evaluated. Usually, productivity losses will occur before, as well as during, the occurrence of disease symptoms whereas costs associated with treatment will occur only in symptomatic patients. In reality, all these data are very difficult to obtain, especially data on disease incidence.

Therefore, the data used in the present study were from notifiable disease registry (incidence rate of surgical procedures in humans) and abattoir data (prevalence of condemned offal in animals). The epidemiological and economic parameters used in this analysis are explained in Table 1. For zoonoses in general, monetary losses should be calculated by the following expression (Eq. 1):

$$\sum_{s=1}^{S} \int_{a=1}^{A} \left( N(a,s) \cdot \beta(a,s) \cdot \left( \sum_{x=1}^{X} \alpha(x,a,s) \cdot C_{xas} \right) \right) \partial a \qquad \text{Eq. (1)}$$

Where $s$ represents species ($s$=1-$S$), $a$ represents host age ($a$=1 to $A$), $x$ represents the symptom associated with infection ($x$=1 to $X$) and $C$ represents the unit societal cost associated with each specific symptom.

Theoretically, the total costs for echinococcosis is the result of the additive societal losses for all affected species ($S$) across all age groups ($A$). For the study population ($N$) of age group $a$, species $s$, with the annual rate of echinococcosis infection ($\beta$), there is a proportion of infected individuals with symptoms $x(\alpha)$. The total cost is calculated by multiplying the unit cost due to each symptom $x$ by the affected proportion of individuals in age group $a$ of species $s$ (direct and indirect unit cost of loss or for treating that population proportion).

Data on the cost of echinococcosis were gathered through literature review and other sources in Tunisia. Information was collected on the current status of *E. granulosus* infection from both scientific and related policy documentation published in journals, books, grey literature and on the Internet. Several government reports published by the Tunisian Ministry of Public Health on echinococcosis surgical incidence were extensively utilised (Anon., 1993; Anon., 1995). The FAOSTAT web site was used to acquire records on livestock prices, primary production and products, as well as human population demographic data (FAO, 2000). The costs associated with *E. granulosus* infection have two primary sources: human and livestock.

Humans

Epidemiology Data: Epidemiology information for human infections was gathered from a variety of sources. In this study, echinococcosis prevalence ($\beta$) was represented by the human surgery incidence rate as reported by the Tunisian Department of Public Health (Anon, 1995). Based on these records, the annual CE surgical rate (surgeries/100,000) was stratified according to 10-year age groups and sex for the year 2000 Tunisian population, using FAO human demographic information (FAO, 2000). Premature mortality associated with CE infection, which refers only to deaths occurring in reported surgical cases in this study, was likewise stratified from data reported by the Tunisian Department of Public Health (Anon, 1995) and published data (Shambesh, 1997).

Few literature estimates concerning losses due to long and short term echinococcosis disability exist. Estimated recovery rate from hydatid surgery has been approximated at 87.5% (Elldrissi et al., 1997). The remaining 12.5% were either fatalities or suffer some level of permanent disability. For this analysis, the authors used the more conservative estimates listed in Table 1. Five to ten percent of survivors of echinococcosis surgery were assumed to suffer 5-10% permanent disability (Dowling, 2000). Those not suffering permanent disability were assumed to suffer 0-10% short-term productivity reduction (for a period of 5 years).

Cost Data: For this analysis, direct human loss referred only to the costs of hospitalisation and treatment of reported surgical cases by a recognized government health professional and

these were obtained from the Tunisian Department of Public Health and published literature (Table 1). The cost in these cases was based only on the surgical intervention and consisted of two primary expenses: general expenses (hospital stay, staff, medications - 65%) and the cost of the surgical act itself (15.6%). Other components of the direct cost estimates used included radiology examinations (10.8%), laboratory examinations (6.4%), exploratory surgery (1.3%) and physiotherapy (0.9%) (Anon, 1995). These costs do not include outpatients visits prior to hospitalisation, non-surgical cases, visits to traditional healers or the use of drugs (over the counter and prescribed) outside the hospital setting. As this value accounted for only the actual hospital costs, it was not reflective of the true opportunity costs of the disease. To supplement the government reported values, the cost including work absenteeism due to hospital stay and recovery have also been estimated.

There were not obvious cost data for the indirect effect that echinococcosis can have on work productivity. Quantifying reduced productivity, work absenteeism and other indirect human losses was difficult. To estimate the cost of this lost productivity, income estimates were obtained from the World Development Indicators of the World Bank (World Bank, 2000). The income estimates and disability parameters used in this study are listed in Table 1. Losses related to premature-mortality refer only to reported surgical cases.

Most methods accounting for reduced productivity, relate only to those formally 'economically active' in society (Statistics Canada, 1995). According to the World Bank (2000) and FAOSTATS (FAO, 2000), only half of Tunisian women are economically active. Although difficult to quantify, those not formally economically employed also contribute to society productivity (United Nations, 1995; Statistics Canada, 1995). As such, the researchers assigned women who were not formally categorized as economically active as 30% productive as a formally employed individual. Retired individuals were considered 10% as productive as a formally employed individual. Similarly, afflicted children do not directly bear any productivity losses, but it was assumed that supervision would be required during their convalescence. A 30% productivity loss was assumed for each child's hospital stay and one month period of recovery.

Livestock

Epidemiology Data: For livestock, echinococcosis prevalence ($\beta$) was represented by the rate of cyst incidence at slaughter, based on abattoir data (Kilani, 2000). Livestock population figures were based on FAO data (FAO, 2000). Although disease prevalence referred only to those animals slaughtered in abattoirs, a uniform infection was assumed over the national population. Age stratified prevalence data were only available for sheep and a uniform prevalence was assumed for other species. Although livestock are considered to tolerate *E. granulosus* infection well (Urquhart et al., 1996), productivity losses are known to occur (Torgerson et al, 2000). Reductions in productivity due to echinococcosis infection have been reported regarding reductions in body weight, milk production and wool production and quality (Table 1b as applied in this study).

Cost Data: Direct costs in livestock can be measured by the monetary value of condemned offal due to hydatid infestation. Where only the number of infected offal was reported, this value was converted to total weight using Tunisia specific weight averages of each species' liver and lungs. Total condemnations were then simply multiplied by cost of offal by weight to determine the direct cost (Kilani, 2000). Unit costs of offal were obtained from Tunisia market prices (Kilani, 2000) and published data (Lahmar et al, 1999) and these are listed in Table 1b.

Table 1a. Data used to assess the cost of human echinococcosis (All prices in year 2000 US$).

| Parameter | Estimate | Source |
|---|---|---|
| 2000 Population Data | 9,586,000 | FAO, 2000 |
| Prevalence/incidence of infection | | |
| Reported surgical incidence | 15/100,000[ab] | Anon., 1993 |
| *Premature Mortality | | |
| Age and sex specific data | 1.2%[ab] | Anon., 1993 |
| Annual average | 3.30% | Shambesh, 1997 |
| *Lost Productivity (Disability) | | |
| Hospitalised cases | | |
| Permanent disability | 5-10% | ElIdrissi et al., 1997; Dowling, 2000 |
| Productivity reduction | 5-10% | ElIdrissi et al., 1997; Dowling, 2000 |
| Short-term disability | 90-95% | ElIdrissi et al., 1997; Dowling, 2000 |
| Productivity reduction | 0-10% | ElIdrissi et al., 1997; Dowling, 2000 |
| *Income Distribution | | |
| Agricultural worker income | $968 | World Bank, 2000 |
| Average national income | $2,050 | World Bank, 2000 |
| Industrial worker income | $3,599 | World Bank, 2000 |
| *Average Hospital Stays | | |
| Lower quartile | 16 days | Anon., 1993, 1995 |
| Average Stay | 23 days | Anon., 1993, 1995 |
| Upper quartile | 25 days | Anon., 1993, 1995 |
| *Average Cost of Surgery | | |
| | $2,419[c] | Bchir et al., 1987 |
| | $813[c] | Anon., 1995 |

*Uncertain parameters expressed with Monte Carlo sampling (see Table 2).
a: Annual CE surgical rate (average 15 surgeries/100,000) and premature-mortality were sex and age stratified into 10-year age groups for year 2000 Tunisian population.
b: Annual average from 1988-1992. c: US$ NPV 2000 using 3% annual inflation.

Livestock productivity losses were calculated using methods similar to those used to determine the human cost estimates. Indirect livestock losses in this analysis were confined to reduction in milk, meat, and wool yield. Unit costs of these livestock products were obtained from FAOSTATS (FAO, 2000) and are also described in Table 1b.

Data Standardisation

This study evaluated the net present value (NPV) of losses associated with identified echinococcosis cases for the year 2000 in Tunisia. However, there is little current data available on the societal cost of CE. All charges used in this study were therefore first converted into 2000 US$, using depreciation and inflation rates of 3% using the following equation:

(Adapted from Drummond et al., 1997) - Eq. (2)

$$NPV = \sum_{n} F_n (1 + r)^{-n}$$

NPV = Net Present Value
$F_n$ = Future cost at year n
r = annual interest (discount) rate

Three percent was chosen because it is now standard practice of World Bank and WHO cost analyses to assume this rate (Drummond et al., 1997). Additionally, this rate falls near the actual value of Tunisia inflation (3.3%) (CIA, 1999). Because it was assumed that temporary disability would take approximately five years to resolve, the one-year cost estimates for productivity losses due to these sequelae were replicated over the years and then discounted at 3% to the base year.

Table 1b. Data used to assess the cost of livestock echinococcosis (Adjusted to year 2000 US$).

| Parameter | Measure | | | | Source |
|---|---|---|---|---|---|
| Prevalence/incidence | | | | | |
| Cattle | 8% | | | | Kilani, 2000 |
| Sheep | 85% | | | | Lahmar et al, 1999 |
| Lambs | 15% | | | | Lahmar et al, 1999 |
| Goats | 2% | | | | Kilani, 2000 |
| *Lost productivity (% reduction) | | | | | |
| Value of finished carcass | 2.5-5% | | | | Polydorou, 1981 |
| Milk yield | 2.5-10% | | | | Polydorou, 1981 |
| Fleece quality | 20% | | | | Kenzhebaev, 1985 |
| Production | (MT) | | (Price/MT) | | |
| Meat | | | | | |
| Cow meat | 50,400 | | $2,220 | | FAO, 1995 |
| Ovine meat (25% lamb) | 45,700 | | $2,326 | | FAO, 1995 |
| Goat meat | 8,300 | | $2,612 | | FAO, 1995 |
| Milk | | | | | |
| Cow milk | 565,000 | | $349 | | FAO, 1995 |
| Sheep milk | 14,500 | | $349 | | FAO, 1995 |
| Goat milk | 10,500 | | $349 | | FAO, 1995 |
| Wool | | | | | |
| Sheep wool | 6,525 | | $1,903 | | FAO, 1995 |
| Lamb wool | 2,175 | | $1,903 | | FAO, 1995 |
| | (Kg) | | (Price/kg) | | |
| Offal (liver/lung) | | | | | |
| Cattle | 140,000 | 41,250 | 10 | 2 | Kilani, 2000 |
| Sheep | 70,000 | 29,400 | 8 | 1 | Kilani, 2000 |
| Goat | 6,000 | 6,750 | 8 | 1 | Kilani, 2000 |
| Donkey | 20 | 2 | 3 | 0 | Kilani, 2000 |

*Uncertain Parameters expressed with Monte Carlo sampling (see Table 2)

Sensitivity Analysis

A lot of the data required to establish itemised costs of echinococcosis to society or its control were unvalidated, unavailable or only available from very small, socio-economically distinct databases. The uncertainty inherent to many of the values was represented by using

probability distributions (Table 2). The 5th and 95th percentiles (95% CI) were calculated as part of the simulation and represent the proportion of Monte Carlo iterations falling within these boundaries. Based on available data, multivariate parameter distributions were defined and the simulation performed with 5,000 iterations each over two separate trials. In Trial Run I, all uncertain parameters were varied based on a uniform distribution. The second set of iterations, Trial Run II, generated results by sampling the uncertain parameters with normal and lognormal probability distributions (Table 2).

Table 2. Uncertain Parameters expressed with Monte Carlo sampling

| | Run I: Uniform Distribution | | Run II: Defined Distributions | | |
|---|---|---|---|---|---|
| Human | Min | Max | Mean | SD | Distribution |
| Lost productivity (disability) | 5% | 10% | 5% | 5% | *Normal* |
| Permanent productivity reduction[a] | 5% | 10% | 5% | 5% | *Normal* |
| Short-term productivity reduction | 0% | 10% | 5% | 5% | *Normal* |
| Premature mortality[b] | 1.2% | 3.3% | 1.2% | 1.2% | *Lognormal* |
| Income distribution | $968 | $3,599 | $2,050 | $2,050 | *Normal* |
| Average hospital stays | 16 days | 25 days | 23 days | 25d | *Normal* |
| Average cost of surgery | $813 | $2149 | $813 | $707 | *Normal* |
| Animal | | | | | |
| Lost productivity (% reduction) | | | | | |
| Value of finished carcass | 2.5% | 5.0% | 2.5% | 2.5% | *Normal* |
| Milk yield | 2.5% | 10% | 2.5% | 7.5% | *Normal* |
| Fleece quality | 10% | 20% | 10.0% | 10.0% | *Normal* |

[a]Researchers assumed all those not permanently disabled may suffer limited temporary disability as listed.
[b]Monte Carlo sampling occurred using the age and sex stratified data as described in Methods.

## RESULTS

Estimated losses due to echinococcosis in Tunisia averaged US$ 14.7 million (95% CI $10.4-$19.0 million) with uncertain parameters varied across a uniform probability distribution. For the analysis using uncertain parameters with varied probability distributions, the loss averaged US$ 10.7 million (95% CI $3.4-$18.8 million). Detailed estimates with confidence intervals for Run I and Run II are listed in Table 3a and Table 3b respectively.

Losses calculated in Run I were split relatively evenly between human and animal categories at 43% and 57% of the total societal cost respectively. In both human and livestock, direct costs make up 14% of the total national losses. Nearly 70% of the average estimate for this analysis compromised of indirect costs. Within indirect costs, losses due to reduced meat yield are the largest cost burden at 21% of the total listed losses. Work absenteeism also contributes significantly to the total average loss with an estimated US$ 2.29 million (95% CI $1.82-$2.75 million) societal costs.

Table 3a. Costs associated with echinococcosis infection.

| | Run I: Uniform Distribution of Parameters | | | |
|---|---|---|---|---|
| | *5th Percentile* US$ 2000 | *Average* US$ 2000 | *% Total* | *95th Percentile* US$ 2000 |
| Human direct (treatment-surgery-hospital stay) | 1,259,968 | 2,122,556 | 14.4 | 2,983,378 |
| Human indirect (income loss) | | | | |
| Work absenteeism | 1,824,302 | 2,287,689 | 15.5 | 2,748,026 |
| Lost productivity | 600,833 | 1,139,212 | 7.7 | 1,680,056 |
| Premature mortality | 582,205 | 682,975 | 4.6 | 784,917 |
| *HUMAN TOTAL* | *$4,322,646* | *$6,324,333* | *43.0* | *$8,302,909* |
| Animal direct (condemned offal) | 2,046,312 | 2,046,312 | 13.9 | 2,046,312 |
| Animal indirect | | | | |
| Meat | 2,190,602 | 3,139,646 | 21.3 | 4,103,093 |
| Milk | 587,390 | 1,284,582 | 8.7 | 1,989,427 |
| Wool | 1,292,208 | 1,920,349 | 13.1 | 2,599,290 |
| *ANIMAL TOTAL* | *$6,116,512* | *$8,390,889* | *57.0* | *$10,738,122* |
| **TOTAL** | **$10,439,158** | **$14,715,222** | **100** | **$19,041,031** |

Table 3b. Costs associated with echinococcosis infection.

| | Run II: Defined Distribution of Parameters | | | |
|---|---|---|---|---|
| | *5th Percentile* US$ 2000 | *Average* US$ 2000 | *% Total* | *95th Percentile* US$ 2000 |
| Human direct (treatment-surgery-hospital stay) | 203,378 | 2,122,556 | *19.8* | 2,983,378 |
| Human indirect (income loss) | | | | |
| Work absenteeism | 1,252, 589 | 2,287,689 | *21.3* | 2,748,026 |
| Lost productivity | 296,685 | 1,139,212 | *10.6* | 1,680,056 |
| Premature mortality | 352,662 | 682,975 | *6.4* | 784,917 |
| *HUMAN TOTAL* | *$852,725* | *$4,725,539* | *44.0* | *$5,983,858* |
| Animal direct (condemned offal) | 2,046,312 | 2,046,312 | *19.1* | 2,046,312 |
| Animal indirect | | | | |
| Meat | 340,991 | 2,111,865 | *19.7* | 5,601,000 |
| Milk | 119, 400 | 534,075 | *5.0* | 3,109,629 |
| Wool | 184,838 | 1,338,287 | *12.5* | 3,773,174 |
| *ANIMAL TOTAL* | *$2,572,141* | *$6,005,660* | *56.0* | *$12,782,055* |
| **TOTAL** | **$3,424,866** | **$10,731,199** | ***100*** | **$18,765,913** |

Losses for Run II (Table 3b), where uncertain parameters were sampled over defined distributions, showed a wider range of estimated societal echinococcosis costs. Losses were again split relatively evenly between human and animal sources at 44% and 56% of total losses respectively. Using the normal and lognormal distributions for uncertain parameters produced a much broader 95% CI for all values. Trial Run II direct costs were substantially more than in Run I making up nearly 40% of the total average societal cost of US$ 10.7 million. Within the indirect costs, losses due to work absenteeism made up the largest contribution to total cost (21%). Losses due to reduced livestock productivity in meat and wool yield also contribute significantly to the total average loss with an estimated US$ 2.11 million (20%) and US$ 1.34 million (12.5%) societal costs respectively. Lost human productivity due to disability also contributed notably to the total with an average of US$ 1.14 million making up over 10% of the overall total.

## DISCUSSION

This evaluation of the direct and indirect costs of echinococcosis in both humans and animals in a developing country is one of the most detailed and comprehensive ever produced. The originality of the present study was the addition of several cost parameters and the use of Monte Carlo sampling techniques to simultaneously represent the uncertainty inherent to valuing the frequency and costs of this infection.

In Tunisia during 2000, the human and animal consequences of echinococcosis were thought to incur an average of US$ 14.7 million (95% CI $10.4-$19.0 million) to the country when uncertain parameters were varied uniformly. This value is slightly higher than previously estimated in economic analyses where average consequences were valued at US$ 8.98 million and US$ 3.35 million in Uruguay and Jordan, respectively (Torgerson et al., 2000; Dowling, 2000). Comparative analyses using normally distributed uncertain parameters, generated results closer to these estimates at an average of US$ 10.7 million (95% CI $3.4-$18.7 million). While previous analyses obtained lower comparable total losses, those estimates lie within the 95% confidence intervals calculated in the present study. The slightly higher point estimate of the latter could be partially attributed to the incorporation of age stratification and the use of Monte Carlo method for the simultaneous sampling of uncertain values.

Only two other papers are known to address both human and livestock direct and indirect costs of CE. These two original papers presented conservative direct and indirect average losses of US$ 8,987,394 (range $2.89 - 21.61 million) for Uruguay (Torgerson et al, 2000) and the equivalent cost of US$ 3,351,837 (range $966,481 - $5,771,379) in Jordan (Dowling, 2000). However, as the totals for Uruguay and Jordan were not presented with a year of reference, it is difficult to compare these national values to the current results. In addition, the currency conversion rate was not clearly mentioned in one of the studies (Dowling, 2000). While Tunisia and Uruguay have comparable total gross domestic product (GDP $21.2 and $20.2 billion respectively), their per capita values differ $2,100 and $5,900 (World Bank, 2000). Jordan has a considerably lower GDP and per capita gross net product (GNP) valued at $7 billion and $1,500 respectively (World Bank, 2000).

In the current analysis, attempts were made to refine some aspects of previous studies. In terms of human related costs, the most important addition was to incorporate sex and age-stratified rates of echinococcosis infection and surgery related mortality. According to published data, mortality and rates of infection vary between age groups and sexes (Anon.,

1993, 1995). Hence, these factors should be included in a realistic economic assessment of losses due to hydatid disease. Therefore, more precise estimates of losses could be calculated relating to lifetime lost earnings (for premature mortality) and for disability. Other methodological improvements included assessing the work absenteeism caused by surgery and recovery, as well as including the lost productivity of those who are not formally considered 'economically active'.

The large variation in loss during and after surgery was represented by sampling a range of plausible values instead of using a single fixed average hospital stay or wage. The calculation of work absenteeism attempted to integrate the varying lengths of patient hospital stay and a recovery period, as well as to reflect the different income losses possible with varying associated wages. Work absenteeism accounted for 36% of human costs and 16% of total combined societal costs. Inclusion of those not formally considered 'economically active' increased the estimates by approximately 20%. Previous studies did not include work absenteeism estimates nor productivity losses attributable to individuals who were not considered as formally economically active.

Finally, both Torgerson et al. (2000) and Dowling (2000) presented results in a three level fashion of minimum, intermediate and maximum results. Minimum results in humans incorporate only an average static charge for the direct cost of human surgical costs. The intermediate and maximum values incorporate two static values of additional losses linked to death, disability and, in one study, subclinical cases. The range of values presented within this paper incorporated constantly varying sets of parameters that were believed to more accurately reflect the actual fluctuations of costs in Tunisia. On the other hand, a value to represent losses associated with non-surgical cases was not included, which was one of the limitations of the present study. However, it would have been extremely difficult to assess these costs as no literature is currently available on this topic. Torgerson et al. (2000) assumed that asymptomatic cases would be associated with a decrease of 2% in work production. However, this estimate seems high. This cost was excluded from the current analysis, which would underestimate the true consequence of the infection. The Tunisian direct human losses (US$ 2,122,556; 95% CI $1,259,968 - $2,983,378) presented were based on the range of reported surgical hospital costs. It was considered that the constant varying of parameters for age-stratified mortality and lost productivity also added to the robustness of our reported range of cost values.

The animal related echinococcosis costs presented also include developments beyond previous studies. Again, the introduction of even basic age-stratified rates of infection in sheep is significant. Published data indicate that the prevalence of cysts at slaughter varies greatly with age in sheep (Lahmar et al., 1999). Based on offal condemnation, Tunisian sheep have a CE prevalence at slaughter of 85% while lambs infection prevalence is only 15% (Lahmar et al., 1999). Only ewes are milked and have lambs, while lambs constitute the bulk of animals killed for meat. Hence the reduction in carcass weight would be more applicable to the 15% of infected lambs and reduction in milk yield and reduced reproductive capacity would apply only to the 85% of infected ewes. Unfortunately, it is not possible to obtain stratified data on the prevalence in goats and cattle. As the reported prevalence of cysts at slaughter is not indicative of all CE infections, the number of cases presented and hence the total direct and indirect losses, are likely an underestimate of the true echinococcosis costs in livestock.

Age structure should be incorporated into both animal and human indirect loss estimations. This is particularly important if one includes indexes such as lambing and other national fecundity parameters into estimates. Based on the methods used by Torgerson et al. (2000) and

Dowling (2000), indirect costs due to fecundity losses would have accounted for over seven million US dollars if they had been incorporated into the results presented in this paper. However, it was believed that the parameters used to support these losses were not robust enough to warrant the inclusion of such a significant cost into this study. This is mainly due to the confounding factors of co-morbidity with other worm infections. Nutrition may also be to blame, and so it was decided not to include indirect losses due to fecundity into this model until more research is completed and methods for accounting for this issue are developed. The overall impact of this omission is that the current estimate is probably an underestimate of the true losses.

The complexities of addressing diseases that cut across veterinary and human health sectors should not be underestimated (Schwabe, 1984). Zoonotic diseases constitute a worldwide public health problem and have a particularly heavy burden in developing countries (Perry & Randolph, 1999). Unfortunately, diverse expertise and differing priorities may inhibit meaningful dialogue between the intrinsically interconnected fields of human and animal health. Economic analyses can be extremely useful and can often be the only common denominator that illustrates the combined consequences of a disease that affects both human and animal hosts (Drummond et al., 1997). Echinococcosis is an example of a controllable disease that may receive increased attention and resources now that it has been economically evaluated.

Many data that are needed to establish itemised costs of echinococcosis or its control are unvalidated, unavailable or available only from very small and socio-economically distinct databases. Few up-to-date data are available on the cost to society of a human case of echinococcosis. Due to the high degree of uncertainty across all aspects of this type of study, it is important to perform some type of sensitivity analysis to accompany any cost estimate (CCOHTA, 1997). Sensitivity analyses that simultaneously vary factors and give upper and lower bounds for point estimates by way of 95% confidence intervals, such as Monte Carlo simulation, are encouraged (Doubilet et al., 1985; CCOHTA, 1997).

In the two studies discussed previously, 'sensitivity analysis' was performed (Torgerson et al., 2000; Dowling, 2000). However, in both cases, this is accomplished by presenting three different estimates of losses: 1) direct costs only (minimum estimate) 2) direct costs with a conservative or low indirect loss estimate and 3) a liberal or more high estimate for indirect costs. Parameters for conservative and liberal estimates are static, fixed and based on the national averages without being stratified for age or sex. The analysis presented in this paper build and expand on these methods. Although direct costs alone (Tables 3a and 3b) were provided, a range of values that might result from simultaneously varying all the uncertain parameters and 95% confidence intervals. The use of the Monte Carlo sampling methods attempted to represent a situation where costs were the result of the simultaneous varying of costs and productivity reductions around official reported averages. The current ranges of estimates are believed to be more representative of the uncertainty created by the unavailable data on CE. However, these values should only be viewed as a framework for the possible costs associated with the disease.

Very few of the neighbouring communities of the Mediterranean region report a higher annual echinococcosis surgical incidence than Tunisia. In addition, Tunisia is one of the most economically advanced countries of North Africa. Although this may be a function of more accurate reporting practices, the high rate signifies a serious problem that could be ameliorated with effective control measures. The estimated costs to society presented in this paper are

unfortunately difficult to compare or interpret due to the lack of a standard method of estimating zoonotic losses (Perry & Randolph, 1999).

The transferability of our results is limited by the basic epidemiological data that formed the foundation of the analysis. Our use of surgical rate as the basis of our human costs significantly underestimates the true costs. These values are not true indications of prevalence but an indication of some of those who received surgical care. These values do not account for individuals with inoperable cysts, patients who for other reasons could not be operated on, undiagnosed individuals or asymptomatic individuals. All these cases may include additional occurrence of work absenteeism, disability (temporary or permanent) and premature mortality. Furthermore, the studies providing the surgical incidence rates stressed that the reported values were also underestimates: diagnoses and surgical procedures were often unreported as were cases discovered at post-mortem (Anon., 1993, 1995).

These underestimations can only increase the cost associated with human losses presented here. Furthermore, not only has this study underestimated cases of infection, true human echinococcosis costs will also be higher than those reported. The analysis does not include costs due to care before and after the surgical operations, costs of care for undiagnosed or non-surgical cases and other societal costs such as transportation to receive medical care, medications or visits to traditional healers.

Another limitation of this study was the reliance of incidence of cysts at slaughter as a reflection of infection in livestock. These data are based on offal periodically collected at eight abattoirs over a period of three years for ovine data, and on voluntary (and unregulated) reports of local veterinarians for bovine and caprine data. Although it represents the best available estimate of echinococcosis in Tunisia, these figures are not indicative of actual infection rates.

One challenge inherent in the estimation of livestock disease in Tunisia is the fact that only an estimated 40% of all livestock slaughter occur within reporting abattoirs (Kilani, 2000). While it was assumed that the rates of cyst incidence at slaughter would occur at equal rates in home slaughter, this assumption may affect our results. Although it is impossible to presuppose the effect of this bias, it can be assumed other factors resulted in an underestimation of the total cost associated with livestock CE losses. Incidence of cysts at slaughter only reflects infections where a cyst occurs in a harvested organ (liver and lungs in this study), and is detected and reported by the reviewing veterinarian. Cases of infection with cysts occurring at other sites, cases of cysts that have not reached a detectable size or cysts otherwise overlooked, will all contribute to an underestimation of true infection rate. Of course, all costs, both direct and indirect, associated with these missed cases would also act to increase the estimate.

Finally, the incidence of CE infection within the national livestock carries with it a certain stigma relating to the perceived quality, and hygiene relating to regions and their food products. Estimates of the financial losses associated with such stigma and other intangible losses would be arbitrary at this stage. However, these losses would further increase the total losses and should be considered when evaluating echinococcosis losses.

In this study, annual national loss estimates from two different trials were reported. Although the average total loss of the second trial was somewhat less than in Trial Run I, it still fell within the 95% CI of the first estimate. Discussion focused on the analysis featuring the sampling using a uniform distribution of uncertain parameter estimates because insufficient data exists on which to found assumptions of distribution. However, for all uncertain parameters,

research should focus on obtaining sufficient data to decide on the probability distribution of the input variables which could either replace the assumed distributions or be added to the model to further refine the results. This is particularly pertinent for uncertain parameters affecting indirect costs as these values constitute the majority of losses and are likely to be most questioned by decision makers.

Echinococcosis in Tunisia causes significant direct and indirect losses in both humans and animals, ranging from approximately US$ 10-$19 million annually. These estimates were based on considerable methodological improvements over previous studies and are of considerable consequence comparable to Tunisia's US$ 21.2 billion GDP (World Bank, 2000). A cost-benefit analysis of control programmes using the methodological advances presented here and regional comparison to other endemic diseases are warranted. These may provide information to assist policy decision makers in prioritising the allocation of scarce resources.

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# RISK FACTOR ANALYSIS OF *ESCHERICHIA COLI* O157 ON DUTCH DAIRY FARMS: PRELIMINARY RESULTS

J.M. SCHOUTEN*, M. BOUWKNEGT*, A.W. VAN DE GIESSEN**, K. FRANKENA* AND E.A.M. GRAAT*

## SUMMARY

Faecal samples (17,413) were collected in 2,217 pooled samples from herds on 524 dairy farms throughout The Netherlands between September 1996 and January 2000. During sampling, questionnaire information about farm characteristics was obtained from the farm manager. The questionnaire yielded 53 variables for investigation of associations with *Escherichia coli* O157 infection. Faecal samples were tested for *E. coli* O157. Data from the months December to April were removed from the study (n=164) since no positive herds were found in this period in consecutive years. In total, 32 of the 360 herds (8.9%) showed at least one positive pool sample. Univariable analysis was performed on 53 variables, whereby *E. coli* O157 positive herds were compared to *E. coli* O157 negative herds. Variables with a *P*-value<0.25 entered multivariable analysis. Six models were created to compare all variables. Conclusively, one final model was created based on the outcome of the six previous models, in which administering beet pulp (OR=3.65), presence of non-farm animals outside the stable (OR=0.32) and location of a herd in the western part of The Netherlands compared to the mid/eastern part (OR=0.22) showed significant associations with the presence of *E. coli* O157 (*P*-values<0.10). Presence of at least one horse on the farm and purchase of animals are considered confounders for the region in which a herd is located. Type of animal housing is considered confounder for the season in which samples are taken.

## INTRODUCTION

Infection with verocytotoxin producing *E. coli* O157 can result in a variety of human diseases, including non-bloody to severe bloody diarrhoea, haemorrhagic colitis and the potential lethal haemolytic-uraemic syndrome which is the most important cause of acute renal failure in children (Riley et al., 1983; Karmali et al., 1985; Tesh and O'Brien, 1991; O'Brien and Kaper, 1998). Cattle are regarded as main natural reservoir for *E. coli* O157 and other verocytotoxic *E. coli* (Chapman et al., 1993). Faecal shedding of the bacteria can lead to contamination of milk, crops, surface water, rodents and insects and hence can subsequently enter the human population.

* Quantitative Veterinary Epidemiology Group, Wageningen Institute of Animal Sciences (WIAS), Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands
** National Institute of Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands

Until now, little is known about environmental factors inhibiting or facilitating infection of animals with E. coli O157. In 1997, a surveillance programme was started in The Netherlands to study prevalence and risk factors of the zoonotic pathogens Salmonella, Campylobacter and E. coli O157 on, amongst others, Dutch dairy farms. In this study, data obtained from this surveillance were analysed to identify and quantify risk factors for E. coli O157 on Dutch dairy herds.

## MATERIALS AND METHODS

### Data and case definition

As part of a national surveillance programme in The Netherlands, carried out by the National Institute of Public Health and the Environment (RIVM), every week faecal samples were collected on a number of farms throughout the Netherlands by an inspector of the Inspectorate for Health Protection, Commodities and Veterinary Public Health. The Animal Health Service in the Netherlands was assigned to compile the study population. Randomly selected farms were requested to participate in the surveillance programme for a one-time sampling.

Depending on farm size, a number of faecal samples were collected from the stable floor in a plastic bag. Samples were pooled up to a maximum of 13 different samples per pool, with a maximum of five pools per farm. At the same time as sample collection, information about farm characteristics was collected by means of a questionnaire, which was completed by the inspector. In total, data from 524 dairy herds, collected during the period October 1996 through to December 199,9 were present in the database.

Microbiological analysis of the faecal samples was performed at RIVM. *E. coli* was isolated by culture, subsequent Immuno Magnetic Separation (IMS; Dynal, Oslo, Norway) (Wright et al., 1994) and inoculating on CT-SMAC (Zadik et al., 1993), MUG and EMB. Conclusively, the suspected *E. coli* O157 colonies were tested with the Wellcolex agglutination latex kit (Murex, Kent, UK) according to the firm's manual in order to ascertain the authenticity of the colonies.

For each pooled sample, it was determined whether *E. coli* O157 was present or not. A farm was considered positive if *E. coli* O157 was cultivated from at least one of the pool samples.

### Statistical Analysis

Statistical analysis was performed according to the principals of a case-control study (Thrusfield, 1995). *E. coli* O157 positive herds were compared to *E. coli* O157 negative herds in relation to potential risk factors. The procedure of logistic regression was performed according to the method described by Hosmer and Lemeshow (1989). Fifty-three variables derived from the questionnaire were subjected to univariable analysis (SAS, 1996). Dummy variables were created for categorical variables. Variables with a prevalence of zero in one or more of the categories were analysed on the basis of exact statistics (Rothman, 1986) with use of the statistical programme, LOGXACT (Cytel, 1993).

All variables with a $P$-value<0.25, other than variables with a prevalence of zero, were put in the multivariable model. Incorporation of variables with a prevalence of zero in the

model can interfere with a correct model estimate (SAS, 1996). To be able to compare all combinations of variables, six multivariable models were investigated. The variables 'season' and 'region' were expected to be confounders and/or effect modifiers and were added to every model. No correlation between any of the variables in the multivariable analysis was found. Correlation was considered to be present when the correlation coefficient between two factors had a value exceeding |0.5|. Variables were excluded one by one from the multivariable analysis by descending *P*-values, until all variables obtained a *P*-value<0.10. The exclusion of variables was checked for confounding by monitoring the change in regression parameters ($\beta$; $\Delta\beta<25\%$ or $\Delta\beta<0.1$ if $-0.4<\beta<0.4$). Per model, two-way interactions were tested for significance by monitoring the change in -2 LOG LIKELIHOOD ($P<0.05$). The other variables and interactions in each model were put in a final multivariate model, in which the backward procedure was repeated.

## RESULTS

In total, 32 of 524 herds were found *E. coli* O157 positive. No positive herds were found in the months December to May in the study years (Fig. 1). Therefore, herds sampled in this period (n=164) were not used in the logistic regression analysis. The sample population (n=360) represents 1.1% of all Dutch dairy herds (CBS, 1999).

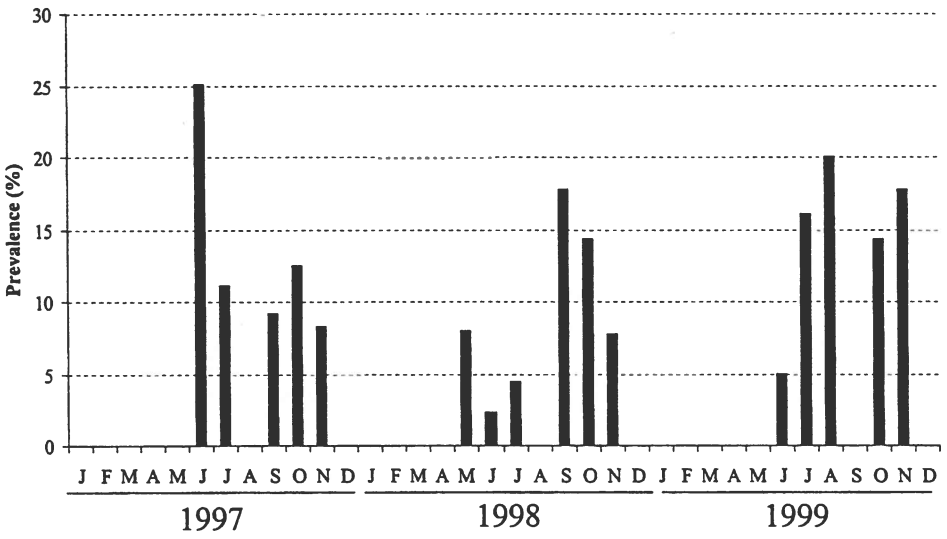


Fig. 1 Prevalences per month for the period January 1997 up to December 1999

Results of the univariable and multivariable model are presented in terms of odds ratios (OR). Univariable analysis resulted in the selection of 19 variables for the multivariable analysis (Tables 1 and 2). These include (where appropriate, a '+' indicates a risk factor and a '-' indicates a preventive factor) type of animal housing, presence of at least one pig on the farm (+), presence of at least one horse on the farm (+), pigs on at least one farm within a range of 1km (+), horses on at least one farm within a range of 1km (-), purchase of animals (+), distribution of beet pulp to the herd (+), distribution of maize to the herd (-), amount of boot disinfection per month (-), amount of overall washings per month, non-farm animals outside the stable (-), non-farm animals within the stable (+), the region in which a farm is

Table 1. Variables in univariable analysis with a *P*-value<0.25 (except variables with a prevalence of zero), together with the division in categories and the frequencies (Freq), prevalence (Prev) and the P-value per category (n=360).

| Variable | Category | Freq (n) | Prev (%) | *P*-value |
|---|---|---|---|---|
| Animal housing | Tying stall | 54 | 13.0 | 0.1930 |
| | Loose housing | 217 | 7.4 | |
| | Other | 9 | 11.1 | 0.6794 |
| At least one pig on the farm | Absent | 271 | 7.8 | |
| | Present | 72 | 13.9 | 0.1112 |
| At least one horse on the farm | Absent | 265 | 7.6 | |
| | Present | 78 | 14.1 | 0.0806 |
| Pigs on at least one farm < 1km | Absent | 164 | 6.7 | |
| | Present | 164 | 11.6 | 0.1298 |
| Horses on at least one farm < 1km | Absent | 285 | 9.8 | |
| | Present | 43 | 4.7 | 0.2846[a] |
| Purchase of animals | No | 150 | 6.7 | |
| | Yes | 128 | 10.9 | 0.2106 |
| Beet pulp supplied | No | 220 | 7.7 | |
| | Yes | 34 | 17.7 | 0.0686 |
| Maize supplied | No | 214 | 10.3 | |
| | Yes | 40 | 2.5 | 0.1490 |
| Boot disinfections (per month) | 0 - 30 | 80 | 13.8 | |
| | 31 - 60 | 40 | 5.0 | 0.1632 |
| | > 60 | 11 | 9.1 | 0.6710 |
| Overall washings (per month) | 0 – 4 | 63 | 14.3 | |
| | 5 – 8 | 49 | 4.1 | 0.0906 |
| | 9 – 12 | 41 | 4.9 | 0.1454 |
| | 13 – 16 | 10 | 30.0 | 0.2250 |
| | > 16 | 35 | 5.7 | 0.2130 |
| Non-farm animals outside stable | No | 33 | 15.2 | |
| | Yes | 313 | 8.0 | 0.1722 |
| Non-farm animals in the stable | No | 50 | 4.0 | |
| | Yes | 293 | 9.6 | 0.2138 |
| Region | North | 66 | 9.1 | 0.8434 |
| | Mid/East | 110 | 10.0 | |
| | West | 68 | 2.9 | 0.0979 |
| | South | 101 | 10.9 | 0.8325 |
| Season | Spring | 77 | 3.9 | |
| | Summer | 148 | 8.1 | 0.2397 |
| | Fall | 135 | 12.6 | 0.0488 |

[a] *P*-value of -2 LOG LIKELIHOOD < 0.25.

located and season in which sampling occurred (Table 1). Variables with a *P*-value<0.25, but with a zero-prevalence in at least one of the categories can not be analysed in the multivariable analysis. These variables were treatment of a *Fasciola hepatica* infection within the last year before sampling, breed of the animals, concentrates supplier, presence of a *Salmonella*-infection at sampling and amount of manure-removals from the loose housing per day (Table 2).

Table 2. Variables in univariable analysis with a *P*-value<0.25 and a prevalence of zero in one category, together with the division in categories and the frequencies (Freq), prevalence (Prev) and the *P*-value per category based on exact statistics

| Variable | Category | Freq (n) | Prev (%) | *P*-value |
|---|---|---|---|---|
| Treatment of *F. hepatica* within | No | 252 | 9.5 | |
| The last year before sampling | Yes | 28 | 0.0 | 0.1114 |
| Breed of the animals | HF/FH | 225 | 8.4 | |
| | MRY | 23 | 0.0 | 0.2921[a] |
| | Mixed | 88 | 11.4 | 0.5483 |
| | | | 14.3 | 0.9452 |
| Concentrates supplier | 1[b] | 34 | 17.7 | |
| | 2 | 31 | 9.7 | 0.5732 |
| | 3 | 45 | 15.6 | 1.0000 |
| | 4 | 30 | 6.7 | 0.3455 |
| | 5 | 30 | 0.0 | 0.0359 |
| | 6 | 19 | 5.3 | 0.4014 |
| | 7 | 14 | 14.3 | 1.0000 |
| | Other | 110 | 8.2 | 0.2146 |
| | More than one | 30 | 0.0 | 0.0359 |
| *Salmonella* infection at sampling | Absent | 349 | 9.2 | |
| | Present | 8 | 0.0 | 0.7660[a] |
| Amount of manure removal | Other | 7 | 0.0 | 1.0000[a] |
| From loose stall per day | 0 – 1 | 33 | 9.1 | 1.0000 |
| | 1 – 2 | 170 | 7.7 | |
| | > 2 | 19 | 0.0 | 0.4807 |

[a] *P*-value of -2 LOG LIKELIHOOD < 0.25
[b] Companies are coded to preserve the identity

In the final model of this study, 243 herds were left, of which 22 were cases. Variables associated with *E. coli* O157 positive herds in the final model (*P*<0.10) were administering beet pulp (OR=3.65), presence of non-farm animals outside the stable (OR=0.32) and location of a herd in the western part of The Netherlands compared to the mid/eastern part (OR=0.22). Presence of at least one horse on the farm and purchase of animals within the last two years before sampling were confounders for the region in which a herd was located. Type of animal housing was confounder for the season in which samples were taken. All three variables re-entered the model (Table 3). The majority of interactions could not be tested in the final model due to the low prevalence. Interactions that could be calculated did not show statistical significance.

Table 3. Variables included in the final model with the division in categories and the frequencies (Freq), prevalence (Prev), Odds Ratio (OR), 90%-confidence interval (90%-CI) and the *P*-value per category (N=243)

| Variable | Category | Freq (n) | Prev (%) | OR | 90%-CI | *P*-value |
|---|---|---|---|---|---|---|
| Animal housing | Tying stall | 49 | 14.3 | 2.38 | 0.94 – 6.03 | 0.1256 |
| | Loose housing | 186 | 7.5 | 1.00 | | |
| | Other | 8 | 12.5 | 2.91 | 0.45 - 19.09 | 0.3495 |
| At least one horse on the farm | Absent | 189 | 8.5 | 1.00 | | |
| | Present | 54 | 11.1 | 1.20 | 0.49 - 2.97 | 0.7408 |
| Purchase of animals | No | 131 | 7.6 | 1.00 | | |
| | Yes | 112 | 10.7 | 1.49 | 0.67 - 3.33 | 0.4114 |
| Beet pulp supplied | No | 210 | 7.6 | 1.00 | | |
| | Yes | 33 | 18.2 | 3.65 | 1.44 - 9.26 | 0.0225 |
| Non-farm animals outside the stable | No | 28 | 17.9 | 1.00 | | |
| | Yes | 215 | 7.9 | 0.32 | 0.11 - 0.95 | 0.0860 |
| Region | North | 51 | 9.8 | 0.80 | 0.28 - 2.28 | 0.7253 |
| | Mid/East | 77 | 10.4 | 1.00 | | |
| | West | 48 | 4.2 | 0.22 | 0.05 - 0.94 | 0.0860 |
| | South | 67 | 10.5 | 0.95 | 0.34 - 2.65 | 0.9367 |
| Season | Spring | 65 | 4.6 | 0.34 | 0.11 - 1.11 | 0.1333 |
| | Summer | 99 | 9.1 | 0.79 | 0.33 - 1.88 | 0.6501 |
| | Autumn | 79 | 12.7 | 1.00 | | |

## DISCUSSION

In this study, farm characteristics associated with infection with *E. coli* O157 on Dutch dairy farms were investigated. Due to the small number of *E. coli* O157 positive herds and large number of missing values, a relative low number of case herds could be used in the multivariable analysis. Furthermore, several variables with a prevalence of zero could not be analysed in the multivariable models. It would have been interesting to include these variables since they may be strongly associated *E. coli* O157 negative farms. Future research could therefore be directed to investigation of these variables and also the incorporation of zero-prevalence variables in multivariable models generally.

Randomness might have been violated due to the voluntary basis of this study. The willingness to participate in the surveillance programme can be related to presence of an *E. coli* O157 problem on the farm, herd size, type of management or other factors. In which direction, however, remains unclear. Therefore, prevalences in the study may have been either overestimated or underestimated.

There were no indications that the presence of *E. coli* in collected faecal samples is not representative for its presence in the herd, because *E. coli* O157 is able to survive in faeces outside the host for considerable time (Fukushima et al., 1999). However, although the number of samples taken was adjusted to herd size, individual cases may have been missed with this type of sampling. This could have resulted in underestimating the number of herds infected with *E. coli* O157 and, as a consequence, potential risk factors were missed.

Each farm is only visited once for collecting faecal samples. *E. coli* O157 infections are known to be transient, indicating that strong fluctuations in prevalence can occur (Hancock 1997; Mechie et al., 1997). An overall positive farm could have tested negative, through which potential risk factors were assumed to be preventive factors in the analysis. Sequential sampling with a monthly interval has shown to be a good measure of dealing with the transient nature of *E. coli* O157 (Hancock et al., 1997). Therefore, in the current study an underestimation of the prevalence could have occurred.

The removal of data from the period December to April in the study years resulted in a higher contrast between positive and negative farms since potential risk factors were not determined for farms sampled during this time period. The assumption made that conditions during this time of the year are not conducive enough for *E. coli* O157 to be found has been suggested previously as Mechie et al. (1997), Hancock et al. (1997) and Heuvelink et al. (1998) found a similar seasonal pattern.

In this study, feeding sugar beet pulp to dairy cattle was positively associated with an *E. coli* O157 infection ($P$-value<0.05). In previous reports, an association between volatile fatty acids (VFA) content of the intestines and *E. coli* O157 infection was reported (Kudva et al., 1995; Kudva et al., 1997; Diez-Gonzalez et al., 1998; Russel et al., 2000). Large amounts of carbohydrates in animal feeds are known to cause an increase in the number of VFA and a decrease of the pH in the intestine (Maynard et al., 1979). Sugar beet pulp has a carbohydrate content of approximately 82% and is known to decrease the pH in the intestine shortly after intake (Haaksma, 1993). The level of acid tolerance of some *E. coli* O157 strains is high (Benjamin and Datta, 1995; Russel et al., 2000). Due to this lower susceptibility to lower pH values, *E. coli* O157 could be more capable of surviving gastric acidity in a host. The bacterium may be able to pass through the stomach more easily than non-acid resistant pathogens and enters the intestine. Therefore, cattle that are fed sugar beet pulp, or in general, feeds with a high carbohydrate content, could be more susceptible to *E. coli* O157 infection.

This study demonstrated a significant ($P$<0.10) association between the incidence of *E. coli* O157 and administering beet pulp, presence of non-farm animals and location of a herd in the western part of The Netherlands compared to the Mid/East. Although a rational explanation for the findings regarding beet pulp is given, associations between variables and prevalence of *E. coli* O157 on dairy farms are to be interpreted cautiously. In this type of study, it is difficult to demonstrate causal relationships. Variables can be positively associated with faecal shedding of *E. coli* O157 and considered risk factor, while it is the result of actions taken by the farm manager due to the recognition of a potential *E. coli* O157 problem. Besides that, interpretation of questions asked in the questionnaire, giving designed answers by farm managers and interpretation of answers by researchers may lead to distorted conclusions. Therefore, and due to the low prevalence of *E. coli* O157, most results

presented in this study should be considered as indications for further research rather than the revelation of causal factors associated with *E. coli* O157 infection.

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# THE TRANSMISSION DYNAMICS OF SCRAPIE: ANALYSES USING MATHEMATICAL MODELS

T.J. HAGENAARS, N.M. FERGUSON, C.A. DONNELLY AND R.M. ANDERSON

## SUMMARY

Following the BSE epidemic in Great Britain, and its link with new-variant CJD in humans, the control of TSEs in sheep has obtained a higher priority in view of the possibility that BSE could have established itself in the sheep population, with a clinical presentation similar to scrapie. Enhanced efforts to collect epidemiological data for scrapie are likely to facilitate more detailed analyses of the transmission dynamics of this disease using mathematical models. Such analyses are aimed at obtaining a better understanding of the processes that underlie the spread and persistence of the disease and will aid the assessment of current disease control strategies, such as selective breeding, and the design of new ones. At present, given the uncertainties surrounding the transmission characteristics of scrapie, it is important to consider a wide range of transmission scenarios. This paper discusses recent modelling analyses of the transmission dynamics under a variety of transmission scenarios.

## INTRODUCTION

Scrapie is a transmissible spongiform encephalopathy (TSE) of sheep and goats endemic in the United Kingdom (UK) and in many other countries (Hoinville, 1996). It has a typical incubation period of several years and it is apparently naturally transmitted between animals. Experimental studies have provided evidence for both maternal and horizontal transmission of scrapie. Infection may be transmitted horizontally via environmental faecal-oral contamination. The environment can act as a long-lived infectivity reservoir (Brown and Gajdusek, 1991; Hoinville, 1996). Scrapie exists in different strains, including the 'natural strain' and a number of strains referred to collectively as 'type C' scrapie. Host susceptibility is strongly influenced by genetic factors (Goldmann et al., 1994; Belt et al., 1995; Hunter et al., 1996; Bossers et al., 1996; Smits et al., 1997; Hunter et al., 1997; Hunter, 1998) and it is strain dependent. Whether apparently resistant animals are innately immune against infection or whether they can act as carriers, has not been unresolved.

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*Department of Infectious Disease Epidemiology, Imperial College School of Medicine, St Mary's Campus, Norfolk Place, London W2 1PG, UK. Email: t.hagenaars@ic.ac.uk

In 1993, the European Union declared scrapie as a notifiable disease and since then, between 235 and 543 cases have been reported annually in the UK. This corresponds to an incidence of less than one reported case per 100,000 sheep. However, the degree of underreporting is believed to be high (Hoinville et al, 1999; Hoinville et al., 2000). The recent BSE crisis in GB has resulted in a general acceptance of the need to monitor, control and perhaps eradicate TSEs in sheep. BSE has been shown to be transmissible to sheep by oral challenge with infected bovine brain tissue (Foster et al., 1996), and many sheep in Britain have been fed contaminated meat and bone meal (MBM) prior to the ruminant feed ban in 1988 and the final ban on the use of MBM in all animal feeds in 1996.

Currently scrapie control strategies are mainly based on breeding for resistance. A better understanding of the transmission dynamics of scrapie will enable an assessment of the cost-effectiveness of existing strategies and the design of alternative ones. Mathematical modelling provides general insights into the transmission dynamics of the disease, taking into account available data and importantly allowing for present uncertainties, and also allows for quantitative studies of specific control strategies.

Clearly, future progress in understanding the epidemiology and control of scrapie will rely most crucially on improved surveillance and carefully designed field studies and experiments. Reports on a recent postal survey and of preliminary results of ongoing epidemiological studies have been published (Hoinville et al., 1999, 2000; McLean et al., 1999; Baylis et al., 2000). Ongoing and future studies will be greatly helped by recently developed tests for the diagnosis of scrapie infection in live animals (Schreuder et al., 1996, 1998; O'Rourke et al., 1998, 2000).

Key aims of modelling work on scrapie include understanding the population level consequences of various hypotheses about between animal transmission characteristics and deducing underlying transmission characteristics from epidemiological data obtained from outbreaks of scrapie. Studies pursuing the first aim include those by Stringer et al. (1998), Woolhouse et al. (1998), Matthews et al. (1999) and Hagenaars et al. (2000, 2001a), and studies analyzing outbreaks have also been published (Woolhouse et al., 1999; Hagenaars et al., 2001b).

The first mathematical model for the transmission dynamics of scrapie in a sheep flock was developed by Stringer et al. (1998), in the form of a system of partial differential equations (PDE) with respect to time, age and infection load. This paper also presented numerical analyses of the model dynamics, emphasizing two important features: scrapie outbreaks are likely to be of long duration (in the order of decades) and lead to a reduction of susceptibility allele frequencies. Using the same model, Woolhouse et al. (1998) explored outbreak patterns and the potential impact of different control measures under transmission scenarios with and without an environmental reservoir of infectivity and with and without carrier genotypes. Additionally, Matthews et al. (1999) derived an expression for the basic reproduction number ($R_0$) for scrapie, and studied its sensitivity to a range of epidemiological parameters including susceptibility allele frequencies and the degree of inbreeding.

A slightly different PDE model framework has been introduced (Hagenaars et al., 2000). This model also expressed $R_0$ and the mean generation time between infections in terms of model parameters. Also, in order to obtain insight into how the duration of scrapie outbreaks depends on the transmission scenario, the epidemic growth rate was expressed in terms of $R_0$ and cumulants of the generation-time distribution. The disease-generations perspective was used to study the interplay between horizontal and vertical transmission during disease invasion and endemicity. Analytically tractable model simplifications were employed to obtain insight

into characteristics of the endemic state. The study of scrapie persistence properties, necessitating the use of stochastic transmission models, has recently was started (Hagenaars et al., 2001a). This model concentrates on how patterns of disease persistence and extinction depend on the transmission scenario and the flock size.

The first transmission model analysis of a natural scrapie outbreak within a flock of Cheviot sheep was carried out by Woolhouse et al. (1999). Hagenaars et al. (2001b) analysed an outbreak of scrapie in Romanov sheep in the south of France which was previously reported by Elsen et al. (1999). In order to estimate confidence intervals for key transmission parameters, stochastic models were fitted to the outbreak data. Additionally, an analysis of a further outbreak of scrapie in Cyprus is in preparation (Gravenor et al., 2001).

## MATERIALS AND METHODS

The considerable current epidemiological uncertainties regarding, for example, the precise role of the environment and the possible role of apparently resistant animals in transmission, necessitate a modelling approach that explores a variety of biological scenarios. This can be done by varying the model design and by sampling parameters from broad ranges, such as using Latin Hypercube sampling, within each particular model design. Such scenarios differ from each other for example in the relative amounts of direct horizontal transmission, indirect transmission via environment and maternal transmission. They may also differ in the way the transmission via a particular route is assumed to occur.

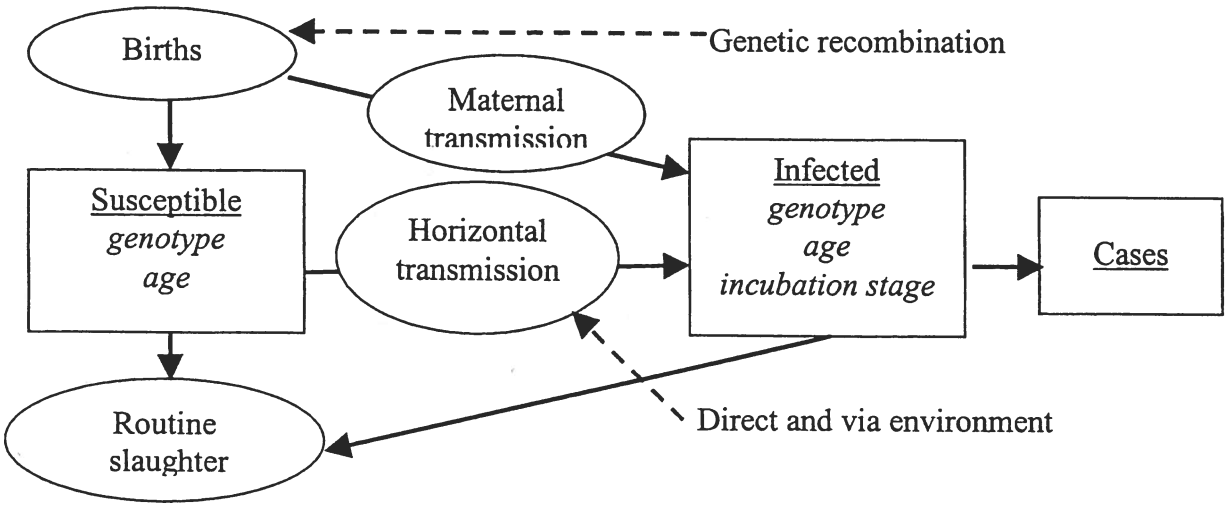


Fig. 1 Mathematical model framework

Figure 1 illustrates the structure of the compartmental model framework at the flock level. More details of this framework are available (Hagenaars et al., 2000). The main mathematical difference to the framework used by Woolhouse et al. (1998) is that this model does not incorporate the notion of an infection load. Incubation is modelled using incubation stages, which for stage-independent per-head transmission rates results in the incubation period distribution taking the form of a (time-delayed) gamma distribution. In the models used by Woolhouse et al. (1998), variation in the incubation period results from a distributed initial infection load.

## RESULTS

The results presented in this paper serve as an introduction to the recent work on the transmission dynamics of scrapie. Full results and more detailed discussions are available elsewhere (Hagenaars et al., 2000, 2001a, 2001b).

### Outbreaks in an open flock

In Fig. 2, the time-evolution of an outbreak resulting from the introduction of a single infection in an open flock of 800 animals is illustrated. An open flock was defined as having external rams that have a fixed Hardy-Weinberg equilibrium genotype distribution. $R_0$=5.0 was selected along with a one-locus two-allele situation in which the susceptibility allele frequency of the rams was 0.5. The mean incubation period equalled 2.5 years and infectiousness in the last incubation stage was assumed to be 100 times as high as in earlier incubation stages. The initial genotype frequency of the ewes in the flock equals that of the rams. The time-evolution observed was very similar to that calculated for another open flock within another PDE model (Woolhouse et al., 1998). The growth phase of the epidemic of infections was seen to have a duration of more than a decade. Disease-induced mortality lead to natural selection for resistance, that is, a rise in the resistance allele frequency (thin line). Due to the permanent injection of genetic susceptibility into the flock by the external rams, the susceptibility allele did not become extinct and hence the disease became endemic. The small periodic oscillations observed in Fig. 2 arise as a result of birth rate seasonality pattern.

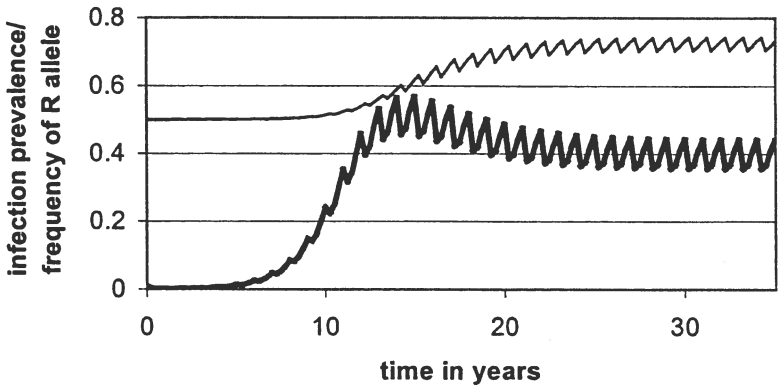


Fig. 2 Time-evolution of an outbreak in an open flock. The fat line represents the infection prevalence and the thin line the resistance allele frequency

### Effects of demographic stochasticity

As the curves in Fig. 2 were calculated using a deterministic model, they are only meaningful if fluctuations due to demographic stochasticity do not have an important effect on the epidemic pattern. In Fig. 3, this was verified by using the stochastic analogue of the model. The thin lines represent different realizations of the time evolution. In total, 10 realizations were

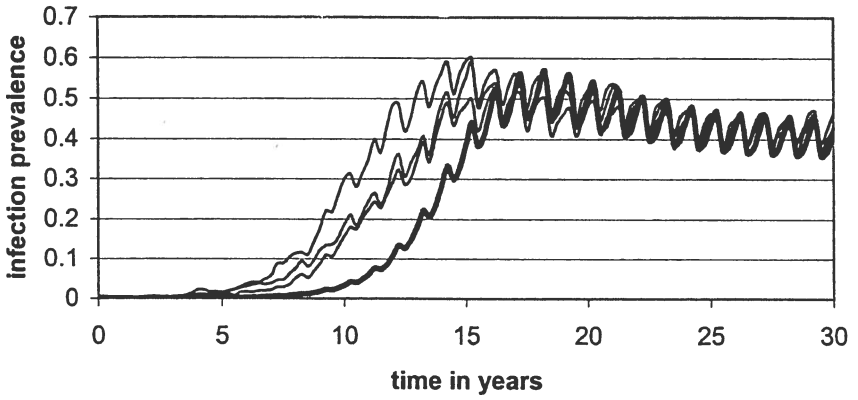


Fig. 3 Comparison of 10 stochastic realizations (thin lines) with the deterministic approximation (fat line) shown earlier in Fig. 2

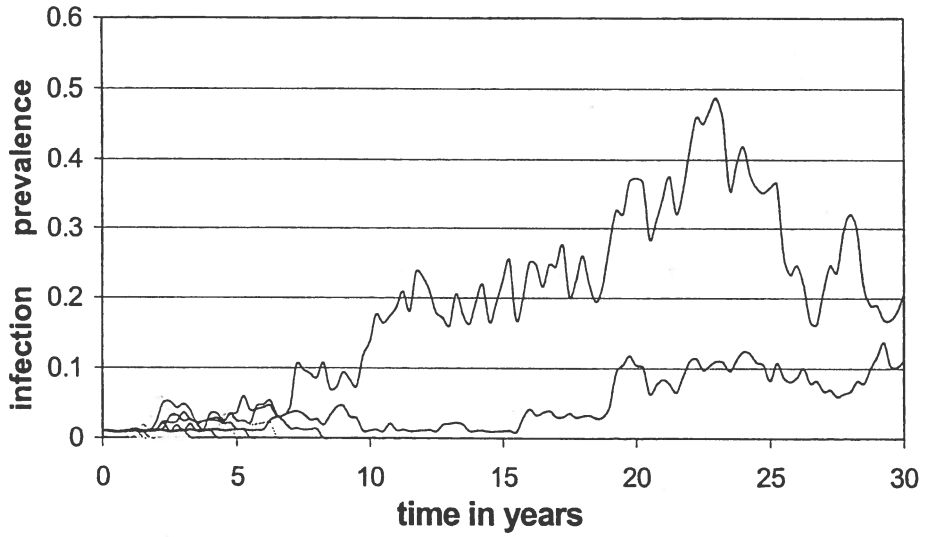


Fig. 4 Fifteen stochastic realizations for a transmission scenario with $R_0$=1.5 in a flock of 200 animals

calculated, out of which 7 display early disease extinction (these are invisible on the scale of the figure) and 3 display a major outbreak (followed by the establishment of a quasi-stationary endemic state). Therefore, conditional on the occurrence of a major outbreak, the deterministic approximation gives a good account of the typical time evolution of an outbreak. This is in contrast with the case, depicted in Figs 4 and 5, of outbreaks in an open flock of 200 animals and with $R_0$=1.5, and with all other characteristics the same as above. In the latter, it is clear that demographic stochasticity has important effects and as a result the deterministic approximation fails.

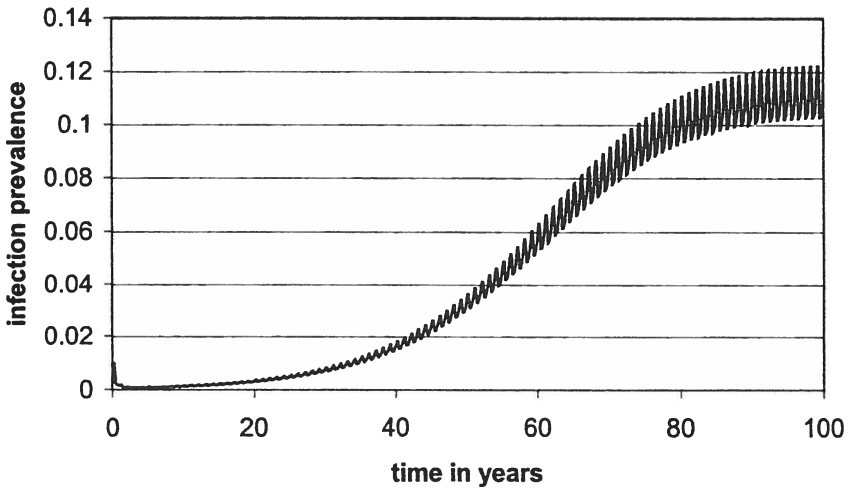


Fig. 5 Deterministic approximation for the same transmission scenario ($R_0$=1.5) and flock size (200 animals) as studied in Fig. 4

## CONCLUSION

This paper has briefly reviewed the recent mathematical modelling studies on the transmission dynamics of sheep scrapie that have been developed. The time evolution of outbreaks in an open flock has been described by both a fully stochastic transmission model and its deterministic counterpart. It is anticipated that significant progress in the understanding of scrapie epidemiology will occur in the near future and mathematical models on the transmission dynamics will provide an important tool in assisting with this progress.

## ACKNOWLEDGEMENTS

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# SOCIETY FOR VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

# SOCIETY FOR VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

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**SOCIETY FOR VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE**

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Please enclose the membership fee (£20 sterling) along with this application form. Overseas members without British bank accounts are requested to pay 2 - 3 years in advance. Cheques should be in £ sterling and drawn from a British bank. British members should pay future dues by standing order (forms are available from the Secretary or Treasurer). Payment can also be made by credit card; the appropriate form is available from the Secretary or Treasurer.

Please send this form to the Society's Treasurer:

**Dr. L.E. Green**
**Department of Biological Sciences**
**University of Warwick**
**Coventry**
**CV4 7AL**

☎ **+44 (0)2476 523797**
**FAX +44 (0)2476 524619**
**E-mail laura.green@warwick.ac.uk**

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## INTEREST GROUPS

Please tick appropriate boxes to indicate your interests:

- ☐ Analytical Epidemiology (Observational Studies)
- ☐ Quantitative Epidemiology & Statistical Techniques (Incl. Statistical/Mathematical Modelling)
- ☐ Herd/Flock Level Disease Control Strategies
- ☐ National/International Disease Control Policy
- ☐ Sero-Epidemiology
- ☐ Herd Health and Productivity Systems
- ☐ Disease Nomenclature and Epidemiological Terminology
- ☐ Economic Effects of Disease on Animal Production
- ☐ Veterinary Public Health and Food Hygiene
- ☐ Computing, including data logging
- ☐ Computer Programming *per se*
- ☐ Population and Animal Disease Databases
- ☐ Information System Design
- ☐ Geographical Information Systems (GIS)
- ☐ Risk Analysis

# CONSTITUTION AND RULES

## NAME

1. The society will be named the Society for Veterinary Epidemiology and Preventive Medicine.

## OBJECTS

2. The objects of the Society will be to promote veterinary epidemiology and preventive medicine.

## MEMBERSHIP

3. Membership will be open to persons either actively engaged or interested in veterinary epidemiology and preventive medicine.

4. Membership is conditional on the return to the Secretary of a completed application form and subscription equivalent to the rate for one calendar year. Subsequent subscriptions fall due on the first day of May each year.

5. Non-payment of subscription for six months will be interpreted as resignation from the Society.

## OFFICERS OF THE SOCIETY

6. The Officers of the Society will be President, Senior Vice-President, Junior Vice-President, Honorary Secretary and Honorary Treasurer. Officers will be elected annually at the Annual General Meeting, with the exception of the President and Senior Vice-President who will assume office. No officer can continue in the same office for longer than six years.

## COMMITTEE

7. The Executive Committee of the Society normally will comprise the officers of the Society and not more than four ordinary elected members. However, the Committee will have powers of co-option.

## ELECTION

8. The election of office bearers and ordinary committee members will take place at the Annual General Meeting. Ordinary members of the Executive Committee will be elected for a period of three years. Retiring members of the Executive Committee will

be eligible for re-election. Members will receive nomination forms with notification of the Annual General Meeting. Completed nomination forms, including the signatures of a proposer, seconder, and the nominee, will be returned to the Secretary at least 21 days before the date of the Annual General Meeting. Unless a nomination is unopposed, election will be by secret ballot at the Annual General Meeting. Only in the event of there being no nomination for any vacant post will the Chairman take nominations at the Annual General Meeting. Tellers will be appointed by unanimous agreement of the Annual General Meeting.

## FINANCE

9. An annual subscription will be paid by each member in advance on the first day of May each year. The amount will be decided at the annual general meeting and will be decided by a simple majority vote of members present at the Annual General Meeting.

10. The Honorary 'Treasurer will receive, for the use of the Society, all monies payable to it and from such monies will pay all sums payable by the Society. He will keep account of all such receipts and payments in a manner directed by the Executive Committee. All monies received by the Society will be paid into such a bank as may be decided by the Executive Committee of the Society and in the name of the Society. All cheques will be signed by either the Honorary Treasurer or the Honorary Secretary.

11. Two auditors will be appointed annually by members at the Annual General Meeting. The audited accounts and balance sheet will be circulated to members with the notice concerning the Annual General Meeting and will be presented to the meeting.

## MEETINGS

12. Ordinary general meetings of the Society will be held at such a time as the Executive Committee may decide on the recommendations of members. The Annual General Meeting will be held in conjunction with an ordinary general meeting.

## GUESTS

13. Members may invite non-members to ordinary general meetings.

## PUBLICATION

14. The proceedings of the meetings of the Society will not 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*