SOCIETY FOR VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

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CONTENTS

INFECTIOUS DISEASE IN TIME AND SPACE

RISK ASSESSMENT

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Society for Veterinary Epidemiology and Preventive Medicine

The Gareth Davies Lecture

ART, SCIENCE AND MATHEMATICS REVISITED: THE ROLE OF EPIDEMIOLOGY IN PROMOTING ANIMAL HEALTH

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My major intent in delivering this paper is to ask and propose answers to the question, "what is the role of epidemiology in promoting the future health of food animals?" Of course in obtaining these answers we need to be aware that one cannot discuss epidemiology independently of other health disciplines. Also, in seeking answers we need to focus on a broad collection of factors that might have major impact on the food animal industry, especially those factors over which veterinarians (particularly veterinary epidemiologists) have, or can have, the interest and skills to exert control. As a general approach to answering this question I would like to revisit one particular paper, by Gareth Davies, published in 1985 entitled "Arts, science and mathematics: ---". In that paper, Gareth developed a number of principles and concepts, including a brief overview of the change from art to science, and a thorough discussion of the need to pursue research and develop animal health technologies within the context of explicitly defined animal health problems. In the final paragraph of the paper he listed his main points as: measuring disease and not infection, building herd health programmes, environmental control, the use of economic (cost-benefit) analyses, setting targets for disease and production and using operational research. I believe every veterinary epidemiologist should read this paper, and I would like to use Gareth's major points as building block topics, also. Nonetheless, interestingly, outside of the references, Gareth used the term epidemiology only once in the entire paper. Some years ago, I asked him about this, as I was puzzled by it. Gareth said he had not noted this or seen it as an "apparent" deficit, nor had he particularly planned that fact. Without meaning to criticize his paper, from my perspective, discussing the maintenance of animal health without explicit reference to epidemiological concepts and methods would be an extremely difficult task. Whereas, the discipline of epidemiology does not provide a panacea for solving animal health problems, it provides many of the essential concepts and tools to help define, prioritise, and research health problems.

As this is an inaugural Gareth Davies Lecture, I believed it necessary to learn more about the ideas of this man and be guided by them when appropriate. Hence, I perused a number of Gareth's recent papers on a variety of topics including; rabies policy in the UK (Davies 1994b), eradication of epidemic pig diseases, and foot and mouth disease from the EU (Davies, 1994a; Maragon et al, 1994), risk assessment (Davies, 1993), and the role of the public sector in controlling epidemic diseases of livestock (Davies 1996). Many of the examples he used in his 1985 paper reflected his prior experiences, especially at the Central Veterinary Laboratory, and most of his papers are concerned with epidemic diseases of national and international concern in which the public sector has a reasonably well defined role to play. I would seek your indulgence, in my discussions of the role of epidemiology in the food animal industry, as my topics will begin with an academic or educational slant, and most examples are concerned more

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with endemic diseases for which the role of the private practitioner is crucial and the role of the public veterinary sector is either less direct, or less well defined.

With respect to the change from art to science, Schwabe (1982; 1991) has presented an excellent overview and I would recommend his papers. Rather than repeat his discussion I would prefer to accept Schwabe's viewpoints and focus the initial section of my paper on the issue of how to educate veterinary students since they will pursue issues of food animal health in the future. My views are based on the belief that the food animal industries of the future will need highly skilled private practitioners, or indeed similarly talented farm-employed veterinarians, with considerable training epidemiology. These individuals will form the backbone of a national animal health system, and can contribute to a national health monitoring system also. Whereas, publicly employed veterinarians working at the national or industry wide level can help provide an umbrella of protection against epidemic diseases, it requires well-informed veterinarians working at the farm level to ensure that the industry can develop and function to its optimal level. I believe Gareth expressed these views by noting that in order for veterinarians "to maintain our privileged position as advisors on the health and welfare of animals" they will need to develop a number of new skills.

EDUCATING VETERINARIANS FOR THE FUTURE

As an educator it is essential that I am aware of the future, not the current, needs of the food animal industries in terms of welfare and health matters. Further, I am pleased to see that curricular issues are being formally discussed at this meeting. Defining the needs would be a relatively easy task if the industries functioned in a relatively static environment; however, it is readily apparent that the food animal industries are undergoing unparalleled change. For example, the increase in herd size, and changes in herd structure, and management of animals, will undoubtedly alter the important health problems to be faced in the future, and as well will as dictate that new approaches may be needed if we are to develop appropriate responses to them (this is the first of the numerous examples of the need for our technologies for disease control to fit into the real world context of the industry). Yesterday's approaches to disease control will not likely suffice. In general, the future food animal veterinarians will need much more knowledge in the areas of nutrition, genetics, epidemiology, economics, animal management, and information management than we currently provide.

In addition to the changes in structure of individual farms (fewer farms with more animals), we must be aware that the industries themselves are changing rapidly. At least in North America there is a dramatic concentration of ownership on a variety of fronts: fewer but larger feedstuff suppliers, fewer animal health biologics suppliers, fewer (but very technically advanced) slaughtering plants, and a very widespread food products distribution system. Whereas there are many advantages to these changes, the risk of catastrophic events may also increase because of them. For example, recently, 40 tons of Canadian hamburger were destroyed because the meat was found to contain *E coli* 0157. Regardless of whether BSE originated from sheep scrapie, or a BSE-infected cow, the centralization of rendering and feedstuff manufacturing undoubtedly contributed to the spread of this agent throughout the UK. Of course there are numerous examples of salmonella contaminated eggs or egg products becoming widely distributed in a short time period through the market channels. Thus, these larger complex components of the food industry demand increased vigilance to prevent large

scale problems. Furthermore, as the industries themselves become more self reliant, and as they are expected to contribute a larger percentage of costs to national animal health programmes, they will, quite rightly, demand much more control over the design and performance of these programmes. Under these conditions, veterinarians need to be prepared to function as part of the animal health teams.

Two other features that will impact on the structure and management of farms, and also impact on educational issues at the DVM and postgraduate level, are animal welfare and environmental concerns. Both are at least partially susceptible to investigation and resolution using epidemiological techniques. Veterinarians should be leading the industries in terms of research into how to house, feed and manage food producing animals consistent with excellent levels of animal welfare. However, examples of this happening are few and far between. To be sure the questions are difficult to answer and go far beyond purely scientific issues, but it is also clear that we must treat inappropriate animal welfare as, or more, seriously than the occurrence of clinical disease. Whereas some clinical disease can be tolerated, very little inappropriate welfare can be similarly endured. For example should we condone the feeding of finely ground pig feeds on the basis of their increased feed efficiency, or condemn them because they lead to more gastric ulcers? As Preben Willeberg (1997) asked, in Paris, should one condone the use of BST because of its effects which increase production levels and efficiency or condemn it because of increased disease occurrence? (Of course we need to recognize some disease frequencies and production levels have been positively correlated without the use of BST. Perhaps what we need to do is to search for subsets of the population where this correlation is not present and try to understand how production can increase without a concomitant increase in disease occurrence.) How does one establish the most welfare supportive management programmes? Can we support segregated early weaning of pigs with its associated behavioural problems? I don't have answers to these or other welfare questions but as a profession we must devise satisfactory ways of resolving these issues, and it will be crucial that the public is involved in these discussions.

The issue of environmental degradation is even more complex to resolve, it takes us outside of the bounds of focusing only on the health of farm animals and their owners, and it is less clear what roll the veterinary profession should play here. On one hand, manufacturing plants can have detrimental effects on food animals, and veterinarians should be knowledgable of the potential health problems, and how to investigate and resolve suspected "pollutant problems". On the other hand large intensive farm operations can have down sides in terms of concentrations of pesticides and fertilizers, animal wastes and the impact on water availability and quality. Two examples might suffice. One concerns the effects of effluent from fresh or salt water fish farms. Not only is the discharge high in organic solids, but also in inorganic pollutants such as phosphorous and residues such as antimicrobials and disinfectants. Hence, we are now seeing serious concerns and demands for action to prevent further degradation of local water. The second relates to the citing of large farms; dairy farms place a heavy demand on water supply, and swine herds place a heavy demand on waste disposal (including untoward aromas). Thus, the future production units will have to be carefully managed to prevent environmental problems. In addition, there may be a conflict of interest, for the private veterinarian, between the farm owner's desires and the welfare of animals, the protection of the environment and issues of food safety. Avoiding these conflicts requires very careful specification of tasks, responsibilities and lines of authority for individual veterinarians. Unfortunately, the downsizing of governments, has lead to a decreased ability for publicly

funded veterinarians and other personnel to monitor the industries and enforce activities for the "public good", thus we will have to trust that the industries themselves will be good environmental stewards, and our profession needs to develop a well-thought out stance on these matters.

At the same time as these industry changes are occurring, it is well to remember that the overwhelming majority of our population (and hence of our students) is of urban origin, that in developed countries the practice of companion animal medicine is attracting a growing majority of our graduates, and that the public, if not many of our students, is either ignorant of, or not especially supportive of, many of the common practices used in rearing food animals. Against these backgrounds, I would like to briefly discuss the educational ramifications of the changes in the food animal industries and cite two specific reports which deal with the topic of delivering animal health services to food animal industries.

The first report (Botterell, 1976), whose senior author was a medical neurologist commissioned by the Ontario Ministry of Agriculture and Food, was published in 1976 and clearly identified the need to train veterinarians (both DVM students and practising veterinarians) in the herd approach. (The contents of this early report were very instrumental in supporting the formation of the Department of Population Medicine which I have had the privilege of chairing for 11 years.) The more recent report (Pritchard, 1988) strongly advocated that veterinary colleges attempt to graduate veterinarians with more focused in-depth skills (areas of emphasis) and move away from the generalist graduate. Of course, how far and how quickly one moves on these suggestions is constrained by issues of veterinary licensure including the ability of graduates to pass national (international) examinations which continue to stress a broad-based generalist education, as well as by the views of members of our profession and the views of the faculty themselves. Like most schools in North America, the Ontario Veterinary College (OVC) undertook serious discussions concerning possible curricular revision, following the release of the PEW report, in the late 1980s. With respect to teaching epidemiology Canadian veterinary colleges have had a relatively large number of contact hours in their DVM programmes so only minor changes were made in the teaching of this discipline. Today, at OVC, we are continuing an evolutionary curricular change process (many small changes to the curriculum and teaching strategies have been made in the interim) with a view to completing the first phase of major curricular revision in the year 2000. Some of the North American schools have already developed, independently, or jointly with other schools, a defined "tracking" curriculum which includes a number of focused areas of emphasis (food safety/public health, food animal medicine, companion animal medicine, biomedical research, etc). Other schools, like the OVC, have agreed to keep graduating generalists while allowing students increased freedom to pursue areas of emphasis (the details are spelled out in a competency-based curriculum document; OVC, 1996). In recent years it appears that many of our schools have focused more on the "how to" of teaching/learning strategies (eg developing learner-centred programmes which help students learn, integrate and utilize essential factual material from the growing "knowledge explosion") rather than refine the competencies required by the food animal sectors. While I support these "educational" moves, I am concerned that the needs of the food animal industries are changing much more rapidly than our ability to respond, let alone lead, on this front. We may be training veterinarians for today, rather than tomorrow. For example, in the same time it takes us to educate one cohort of veterinary students (4 years) we, in Canada, will go from a supply managed dairy system to an open market dairy system with all the concomitant changes in structure, management and health problems that this will

produce. Generalist veterinarians will be of little value in this setting. Years ago the veterinary profession failed to respond to the needs of the poultry industry, and today we run the danger in the swine industry. We have known for decades that only a small proportion of swine producers have a regular relationship with a veterinarian. Yet, we act as if that is their problem, not ours. Nonetheless, deciding on an appropriate educational strategy is made more difficult by increased tensions within veterinary schools over the allocation of shrinking resources. Indeed many schools will be forced to make difficult judgements about their primary purposes, because if the majority of funding flows with student interest (largely in companion animals), it will necessarily mean a shrinking budget for the food animal sector of the curriculum. And, in these discussions we need to be mindful that it is by no means clear that the public would prefer to support food animal medicine rather than companion animal medicine. What we have in our favour is that most veterinary schools obtain significant funds through departments of agriculture so there is a reasonable understanding of the roles we play in food production; we need to elaborate on that role and capitalize on that advantage.

Taking all the above matters into account, it is my belief that schools of veterinary medicine must take immediate steps to ensure that they have the ability to graduate a sufficient number of skilled students for private practice in the area of food animal health. Given the lack of enthusiasm for longer DVM programmes, this will likely entail a reduced emphasis on individual animal care and companion animal medicine and surgery to allow students sufficient time to obtain the requisite skills as outlined earlier. Failing this, our colleges must be prepared to provide an additional intensive period, perhaps of one semester's duration immediately after graduation, in which appropriate courses in species specific nutrition, genetics/breeding, and experience with delivering herd health programmes (including a strong component on data handling, epidemiology and farm level economics) can be obtained. Failure to graduate veterinarians without a firm grounding in these subjects will almost certainly result in our losing our valued placed in the food animal industries. Beyond meeting our needs for a cadre of veterinarians functioning at the farm level, and in order to meet the needs at the industry, or national level, veterinary schools will also need to provide masters and doctoral level training in the broad areas of economics, epidemiology, and farm level food safety issues applied to food animal health, and food safety. Let me now turn to the issues raised in Gareth's 1985 paper

ELEMENTS OF PROMOTING FOOD ANIMAL HEALTH

Measuring disease and not infection

To this title I would add, measuring health, not disease, and not infection. Our clear purpose must be to optimize health (perfect health is illusionary) and we need to develop new concepts and methods for doing this. The same problem arises in monitoring human health care, in that whereas we want to promote health and recognize the need to quantify our level of success, most of our quantitative measures focus on disease and not health----and one is not the mirror image of the other. In veterinary medicine, various production level, production efficiency and economic parameters are used as surrogates of health. I support the use of these but it is becoming vitally important to develop comprehensive indices which better reflect the whole spectrum of outcomes we currently measure, and to incorporate better measures of animal welfare. I remain to be convinced that the best monetary choice for the producer is necessarily the best for the welfare of the animal(s), and we will face a public that will increasingly push

producers to emphasize the latter. Having said that we need measures of health, we will continue to monitor specific diseases as components of health as they allow for problem detection and effective focused action within the herd, and are really useful provided we don't forget the overall health of the herd. In a similar vein we will continue to monitor for infectious agents and will undoubtedly develop more sensitive and specific methods for herd level monitoring. It is important that we learn about the incidence and prevalence of these agents, not so much in a pathogenetic sense, but more so as factors whose presence can alter the health status of the farm. In particular we need to use the methods similar to the "Knox" approach of relating attrition rates (the rate of transfer from susceptible to immune state) to the specific clinical outcome frequency. My guess is that few of these will be linear, and we will need to bear this in mind when developing our control programmes.

Despite our ongoing struggle to find better measures of health, it needs to be remembered that monitoring is an essential aspect of any ongoing field programme. However imperfect the data, the need to make decisions (How to prevent the entry of disease?, Which diseases are important risks?, Where should we invest research dollars?, Are we meeting our goals/targets?, etc) is real. Trade, at all levels, cannot be sustained in the absence of a well defined and academically sound monitoring system. Likewise, for private practice optimal decision making for herd health programmes in the absence of data at the farm level is not possible. Technology is making monitoring easier, one can certainly visualize and see the benefits of a real time version of HandiSTATUS (Bernardo, 1997), as an example. In human medicine, web-based observational studies are already underway (people's health and exposure status are monitored over time and all data are automatically entered into structured data bases). Hence our challenge in the future is not how to implement an animal health monitoring system, but how to implement an effective system at minimal costs.

Building herd health programmes

Schwabe *et al.* (1977) have described the tight linkage between epidemiology and modern herd health programmes as "Herd health means disease control means field research means epidemiology". While perhaps simplistic, this statement nonetheless indicates that herd health programmes must be based on sound epidemiological principles. If one examines the contents of two major texts on herd health (Brand *et al.*, 1996; Radostits *et al.*, 1994) it is evident that epidemiological principles are intertwined explicitly and implicity throughout the material. Arie Brand *et al.* (1996) note that most herd health programmes already include descriptive epidemiological techniques and virtually all use the "production deficit" approach described by Gareth as a means of detecting ill health at the herd level. Although national targets are extremely useful as global goals, veterinary practitioners appreciate that every farm owner is different and it is his or her goals for the herd that must be taken into account when setting targets and when developing health maintenance strategies (again part of the essential social interactions described by Davies). The use of quantitative epidemiological techniques to identify risk factors has in the past often been part of "one off" research projects; however, these, together with on-farm research, will become an integral part of, and regular activity within, future herd health programmes. It is sobering to examine the rapidity of change in this area. Ten years ago many of us "epitypes" and a few so-called herd health veterinarians talked about the use of computers, epidemiological techniques, formal monitoring for production deficits and developing appropriate responses to health problems as an essential feature of herd

health programmes. Today, in Ontario, all of the DHI customer service representatives (previously known as field technicians) carry notebook computers to over 5000 farms to input data on the farm and to produce on the spot reports which identify health deficits. Once detected, specially trained DHI field people are available to help resolve the problems, or the farmer can seek help from his/her veterinarian. Interestingly, when this programme started, many veterinarians were upset because they believed that non veterinarians were treading on their turf. However, at the same time from the farmer's perspective, too few veterinarians had the skills, or the time to help them. Clearly here is a problem (which presents a wonderful opportunity) that we as a profession need to resolve if we are to retain our valued spot in the food animal industries.

To continue this subject and since there is an element of prediction in my topic about how epidemiologists will contribute to the area of animal health, one method of making such predictions is to extend a previous historical record. For example, if one examines the series of ISVEE proceedings, one notes that there is a continued interest in epidemic diseases and the related national and international control programmes, but that there is a growing emphasis on programmes aimed at endemic diseases. The latter are focused at the farm level and the methods and results are of direct interest to private veterinary practitioners (including the cadre of young veterinarians referred to earlier). Indeed we now have a subset of epidemiologists who claim that individual farms are their populations of interest and these individuals pursue within farm risk factor analyses on behalf of individual farmers. Whether or not this latter assertion is true, scientifically, I believe that it is much more worthwhile to use the structured analytic approaches developed earlier for groups of farms and apply them to individual farms over time, than to continue with an informal unstructured approach that has often passed as "heard health" in previous eras. Thus, it is a good bet that studies aimed at individual farms, at groups of farms, and at the national level will continue into the future. The diseases at the national level tend to be those where the agent and its transmission are of primary interest and importance. These endemic diseases have a much more multifactorial causation in that the agent is usually present but certain complexes of risk factors allow the agent to tip the agent-host balance in its favour leading to occult disease or, more usually, subclinical but economically important production decreases.

Environmental control

Control of environment is a common tool used for both the promotion of health as well as the prevention and control of disease. For example in his papers, Gareth outlines many of these practices in the context of swine fever and foot and mouth disease control, and also describes some of them under the roles of publicly funded veterinarians. Some of the more important appear to be: cleaning and disinfection of affected premises, ensuring that vehicles (and humans) are disinfected (more positively ensuring good biosecurity for farm entry), reducing the density of farms per unit area, control of swill feeding, and perhaps identify and controlling the as yet unknown environmental factors that are related to the type of control programme a nation selects. In the situation of endemic disease control, environmental risk factor control is even more important (Brand *et al.*, 1996; Radostits *et al.*, 1994). In fact, it is likely true that the overall gains in disease control have been through this method. Recognizing this, most research centres are pursuing studies to identify the key environmental risk factors for specific disease prevention and control as well as for the more general promotion of health.

If we can learn from our human health colleagues (Evans *et al.*, 1994), we will be aware that effective health promotion activities may become the most effective overall way of positively influencing the health. This approach does not target specific diseases, nor for that matter specific risk factors, but rather attempts to build an overall lifestyle, or management strategy, that in the truest sense actually promotes health. Effective disease care, early detection and treatment of selected diseases, and preventing exposure to risk factors for disease will help improve help, but these are not sufficient for a healthy population. It is, of course, in this context that we are beginning to recognize that exposure to these risk factors, in humans, is so strongly motivated by socio-cultural beliefs and the social-physical environment. For this reason, appeals to individuals to reduce exposure to risk factors will probably not be successful; perhaps this observation has some analogues in veterinary medicine where producers may choose to continue high risk management practices. Should we not know more about what motivates people? I like Gareth believe that we must factor sociological factors into our programmes in a more formal manner.

Operational research

I am not totally sure of what Gareth meant by this term within the context of animal health programmes. However, my understanding is that it includes elements of biologic/context knowledge, elements of economics (including social factors), and elements of epidemiology (also including social factors). If this is true, then I agree that this combination is particularly useful at the farm, industry and national/international levels. For sure there is a need for closer and ongoing collaboration among the "disease specialists", the "species specialists", the epidemiologists with their observational study and on farm trial skills, with "modellers"(theoretical epidemiologists), and with economists. And, it is vital that these working groups are able to help decision makers at both the farm and industry (or higher) levels. For example, one of our deficits in Guelph is that we have all of the pieces to put the operational research puzzle together except for the modelling and econometric skills (I should explain that we have excellent economists at Guelph, but few are interested in animal health and even fewer in developing economic models useful at the farm level). Our lack of the economic skills applicable to animal health is a real drawback to the future development of our herd health programmes at the farm level. Hence we are trying to close the circle by better collaboration with economics groups like Aalt Dijkhuizen's at Wageningen and George Gettinby's modelling group at Strathclyde. We suspect that we are not alone in having incomplete within school resources to research or teach the broad base of skills necessary for successful animal health programmes. Thus, in the likely absence of additional resources, many veterinary schools will have to build collaborative linkages with each other. Again, technological advances are making such long distance communication and collaboration much easier than in the past.

Thoughts on disease control

Finally, a disease control matter that Gareth discussed in his 1985 paper was whether or not to vaccinate against BVD. He noted the similarity between this disease and rubella in humans; in both instances the major objective is to prevent the breeding age (especially pregnant) females from being exposed to the agent. Well BVD has been endemic in Canadian dairy herds

for many years, but in the 90s we have had an epidemic of mucosal disease suggesting the presence of many PI animals in our herds that are for some reason undergoing exposure to the BVD virus. Other herds are experiencing the less serious clinical diarrhoeic form of BVD. One of the reasons we think this epidemic has occurred is that farmers have typically used killed vaccines to vaccinate breeding age heifers, but in doing so have tended to give only the first dose of the vaccine. This practice lowers the efficacy of the vaccination programme to the point that it probably protects fewer individuals, and the level of immunity in vaccinated individuals is lower than is desired, thus leaving them open to strong challenges with the virus. Thus, in many of the herds the herd immunity is too low to stop the circulation of the BVD virus. As a result we have recommended ensuring that all heifers get vaccinated with the appropriate two dose routine, that adults are routinely revaccinated, and that new herd additions are fully vaccinated at least one month before entering the herd. The effects of this programme on the age-specific incidence of infection are unknown, and the programme we suggest represents only one version of an array of vaccination programmes. Since we don't know the effects of our programme on infection rates, nor for that matter what proportion of breeding age heifers is vaccinated, it might be advisable to vaccinate all calves at 3-4 months of age and to revaccinate heifers prior to breeding; most males would likely be revaccinated at 6-8 months of age when they enter feedlots. In any event, the situation requires investigation as it would be devastating if our recommended programme actually made the infection rate in susceptible breeding females higher than the earlier haphazard policy. Of course the side issue to this is that since BVD is endemic there is no provincial or national policy; rather, individual herd owners must decide whether to vaccinate and what their programme should be----this is a clear area calling for epidemiological research, so that the veterinary profession can speak with one well-informed voice on the matter.

The above points represent the main features, I think, of Gareth's 1985 paper. The following subjects also deserve mention, albeit briefly.

FOOD SAFETY AND ANIMAL HEALTH

Food safety is usually taught as a subject related to public health but not very directly connected to the private on-farm practice of veterinary medicine. This was particularly true for infections such as many of the salmonella infections in poultry; these did little harm to the poultry but posed a serious public health concern. Now with organisms such as the multiple drug resistant phage type 104 salmonella, with the ever widening *E coli* 0157 story, (all in the wake of BSE) and with increasing potential public health concerns arising from *M. paratuberculosis* and its possible role in Crohn's disease (Morgan, 1997), in addition to concern over drug resistance in humans traceable to on-farm drug usage, any distance between food safety as a subject and the practice of clinical medicine is shrinking. Veterinarians must walk the difficult middle road between meeting the welfare needs of sick animals while not forgetting that they are really treating "living food". Recent studies in Ontario have shown an association between cattle density per county and the rate of reported human *E coli* 0157 cases (the association did not hold up in the more northerly areas of the province where both human and cattle densities are low). These studies have also indicated more potential for human to human spread that previously thought possible, adding a new dimension to on-farm control programmes (Wilson et al, 1997). Recent studies have also been conducted to identify the effects of preslaughter shipment on numbers of *E coli* shed at the slaughter plant and also to

investigate the roles of hide contamination versus faecal shedding on carcass contamination. Another example linking on-farm veterinary practises to food safety relates to bovine respiratory disease in feedlot cattle. Despite much research, including epidemiological studies, and the development of new vaccines, this health problem persists. One of the more effective ways to reduce the frequency of clinical respiratory disease is to mass treat, once or twice, with broad spectrum antimicrobials. This should pose little risk of a residue because of its timing relative to slaughter (although tissue damage may be present when slaughtered; Van Donkersgoed et al, 1997), but its use has a perceived health risk and we are unsure of its effect on antimicrobial resistance in the general population. In recent studies, at Guelph, an effect on antimicrobial resistance was not found. However, epidemiologists at Guelph have developed a mechanism to screen large numbers of microorganisms and identify the proportion that are resistant (Dunlop et al, 1998). Clearly, when designing disease control programmes, in addition to the welfare of farm animals we must now incorporate practices designed to reduce the risk of residues, enhance food safety and reduce the risk of antimicrobial resistance.

CHALLENGING ACCEPTED PRACTICE

An issue that Gareth raised in light of recent discussions about rabies prevention in the UK was to question whether or not "accepted practice" had any real basis in fact, or if the environment had changed to the point that longstanding regulations were now out of date. Now the point of this is not that we should become obstreperous, but that we should question current practice and dogma in our search for better health promotion. Mastitis research might be a similar case in point in dairy cattle. Over the years a variety of approaches to the treatment of clinical cases have been studied to evaluate their efficacy (Drug A vs Drug B etc). Of course, antibiotic residues in milk are also a real public health concern so only the most effective programmes are justifiable. Recently the idea that many cases of mastitis will self cure without antimicrobial use has been discussed and currently trials are in progress to evaluate test kits designed to screen affected quarters in an attempt to classify the causal organism into those that should be treated and those that don't need antimicrobial therapy. Initially it seemed heretical to suggest that clinical cases of mastitis should not receive antimicrobial therapy --- I suppose the same is true for the suggestion that most cases of human upper respiratory tract disease do not need, or benefit from, antimicrobial therapy.

In conclusion, it should be apparent that epidemiology has many roles to play in the future food animal health programmes. In many respects its value is largely limited by our lack of insight and imagination. There is a need for broader training of all food animal practitioners in epidemiology and related population oriented disciplines; veterinary schools must soon make some crucial decisions on this issue. We do need to be careful that we focus on solving problems and not on the technologies used to solve these problems; else veterinary epidemiologists may also fall prey to the "limits of epidemiology" (Taubes, 1995). Opportunities for graduate trained epidemiologists exist in both the employ of industries as well as with governments and international agencies. Through his activities and publications Gareth Davies has lead the way and given us some paths to follow as we embark on preparing for tomorrow. It has been a privilege for me to be able to deliver this talk in his honour.

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THE ERADICATION OF TUBERCULOSIS FROM CATTLE HERDS

TUBERCULOSIS CONTROL IN LIVESTOCK IN NEW ZEALAND

THE CURRENT SITUATION AND FUTURE DIRECTIONS

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Taking a long term historical view of tuberculosis infection of livestock in New Zealand (NZ), one might comment 'a foreign disease in foreign species'. Further, that a number of quite unique factors have resulted in a complex and very difficult disease control situation being established.

The small landmass that NZ developed from drifted away from the ancient super-continent, Gondwana Land, many hundreds of millions of years ago, before the mammals evolved (Stevens, 1980). Thus apart from a bat, which presumably flew the infant Tasman sea, there were no land mammals; vegetation evolved without heavy grazing pressure. Approximately 1000 years ago man arrived. First, the Polynesians with fire (they also introduced a Pacific rat and dogs). Second, the Europeans, about 150 years ago, with their efficient tools to systematically attack the forest and turn it into grasslands, and with their domestic and wild animals.

The land has been transformed. An extensive pastoral industry (sheep, dairy and beef cattle, and later deer) has been developed. A multitude of exotic wild and feral animals were introduced and some thrived in this new environment. Among these the Australian brushtail possum (*Trichosurus vulpecula*) which was introduced as early as 1837 in an attempt to establish a fur industry. Others of importance in the tuberculosis story are deer (initially introduced for sport but later domesticated), pigs (domestic but then they escaped into the forest), and the mustelids (ferrets, weasels and stoats, in an attempt to control rabbits which had been introduced earlier).

This paper concerns tuberculosis. However, it should be noted that many of these exotic wild and feral animals have had serious environmental impacts. Browsing possums have killed many native trees; they compete with native birds for food and also destroy nests and eat eggs and chicks. Likewise, deer can destroy the forest leading to erosion. Ferrets, like rats, prey on ground nesting native birds.

There is little doubt that the 'pest' *Mycobacterium bovis* was introduced into NZ via cattle imported from Europe. In Australia, tuberculosis has never been observed in possums. Tuberculosis in deer was not diagnosed until later this century (The first report of what appears to be tuberculosis was in 1954, the first confirmed case was in 1970).

Early reports of state veterinary authorities point to extensive tuberculosis in cattle, especially dairy cattle. In 1945, for public health purposes, a voluntary control scheme for 'town milk' suppliers was introduced. In 1956 this became compulsory. During the 1960's the cattle industry and government recognised the growing requirement to meet minimum disease standards if access to export markets for meat and dairy products was to be maintained. To this end, two major national eradication schemes, for brucellosis and tuberculosis, were started. The campaign against brucellosis has been a success. Initially

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progress in the tuberculosis scheme was also very promising, and it was confidently expected that within a short period the disease would be eradicated from cattle.

The first 'problem herds' were reported, in the late 1960's and early 1970's, in three different localities (West Coast of the South Island, Central North Island (Taupo/Taumarunui) and South East North Island (Wairarapa)). All these areas have extensive forests, with, by then, a plethora of wild and feral animals. Gradually the association between problem herds and infected possums unfolded. Very early, all those directly involved recognised the potential for spread from tuberculous possums *in extremis* with generalised disease and weeping fistulae. Such possums were found wandering around open pasture during the day (possums are normally strictly nocturnal). Recent studies have confirmed that cattle are attracted by possums showing such aberrant behaviour (Paterson and Morris, 1995). In these areas tuberculosis was also found in wild deer and feral pigs.

In 1982 tuberculosis in ferrets was reported. Ferrets are found in high numbers in the South Island rabbit prone areas. In some areas the disease appeared to occur in the absence of possums, or at least tuberculosis infected possums. Further, prevalences as high as 15 to 17% were found (Ragg et al., 1995). After controlling ferrets, disease levels in cattle and deer were able to be reduced. Clearly here was an additional complicating factor.

Over the last decade, there have some profound social and economic changes in NZ and these have had a direct effect on national disease control schemes. From being a welfare state, NZ society has been taken into the world of 'the market'. Government has progressively withdrawn from running 'businesses' and has encouraged competition. Users of services have had to start paying full costs, and the agriculture sector has lost its once privileged position in terms of assistance and subsidies.

THE CURRENT SITUATION

Infrastructure

The Biosecurity Act:

The framework for national disease control schemes is now provided by the Biosecurity Act. This reflects current political doctrine, especially having regard to 'user pays'. Under this, a 'Pest Management Agency' for the control of bovine tuberculosis has been established outside of government. This is the NZ Animal Health Board (AHB); it has representation from the beef, dairy and deer industries and from central and local government. The Board has a small executive of around 10 people.

The AHB's first task was to develop the so-called 'Pest Management Strategy for Bovine Tuberculosis' (Anonymous, 1995). This is known locally as the TB-PMS. This is a legal document. It defines, currently in a very comprehensive manner, how the pest (ie *M.bovis*) is going to be controlled. (There are moves to take the detailed operational aspects out of the PMS, to enable a more flexible approach. However, a change in the legislation will be required before this can be adopted.)

Financing of the strategy is also defined within the TB-PMS. As might be expected, beef, dairy and deer farmers are identified as the primary 'beneficiaries' and therefore it is concluded that a significant portion of funding should be derived from them. However, they also argue that 'those who are engaged in industries associated with cattle and deer farming also benefit'. Further, that there is an element of public good. On these grounds they suggest that local and central government should also contribute.

So-called 'exacerbators' are also identified. This term was invented with the verb to exacerbate (ie to aggravate) in mind. For example, central government (ie the Crown) is named a Class One exacerbator because of the extensive public lands that harbour infected wildlife adjacent to farmland. Exacerbators are also expected to contribute monies.

An important aspect is that a variety of funding arrangements are possible. One very striking difference is the decision by the deer industry for deer farmers to pay individually for testing and to have no compensation for reactors. In contrast, the cattle industry adopted a levy system, with testing costs and compensation paid in bulk from this fund. (Both cattle and deer farmers contribute to administration, regulatory, research, management and vector control costs.)

National Science Strategy Committee (NSSC):

In recent years government research activities have also been restructured. During this, a procedure was introduced whereby the Minister of Research, Science and Technology could bring together specialists to consider important problems and recommend research strategies. To this end a NSSC on 'possums and bovine tuberculosis' has been formed.

The mission of the committee is 'to identify, coordinate and promote research on possum and bovine tuberculosis in order that the threats to NZ's export markets and to conservation values can be eliminated'. It has identified five key areas of research: (1) tuberculosis management (2) possum damage (3) conventional control of possums (4) new methods of possum control and (5) social issues. Via recommendations to the Minister, NSSC influences priorities for government funded research.

Funding:

The result of negotiations after the TB-PMS had been finalised is shown in Table 1. The Animal Health Board provisional budget for the 1997/1998 is shown in bold; ie $44.6 million. The balance of research monies is from the 1995/1996 year.

Table 1. Tuberculosis control programme operations and research budget. Budget (NZ$) in millions, exclusive of GST.

Activity	Budget	Agency			
	millions	Industry	Government	Regional Councils	Other
Disease control (testing, compensation, data systems, etc)	**16.22**	100%			
Vector Control on Crown Land	**6.46**		100%		
Vector Control on private land	**19.12**	29%	37%	34%	
Total Operations	*41.8*	*52%*	*32%*	*16%*	
Animal Health Board allocated research	**2.86**	41%	45%	14%	
Government allocated research	9.16		100%		
Research - private agencies	1.24				100%
Total Research	*13.26*	*9%*	*79%*	*3%*	*9%*
Total Expenditure	*55.06*	*42%*	*43%*	*13%*	*2%*

One could comment that the introduction of 'users pays' has not lead to simple planning and budgeting.

Service contractors:

After having settled methods and funding, the Animal Health Board lets contracts for the activities under its control.

A decade ago the Ministry of Agriculture and Fisheries (MAF) was divided into policy and operational groups. The former is known as the MAF Regulatory Authority (MRA), the latter as MAF Quality Management (MQM). MQM has no direct government funding; it must seek contracts with MRA and other agencies such as the Animal Health Board. The MRA contracts (eg disease surveillance) are progressively being made contestable (ie opened for competition).

MQM is the major Animal Health Board contractor for disease control services. These are diverse and include technical consultation, database management, district veterinary investigations and management, laboratory services, cattle testing and regulatory activities (eg movement control).

MQM para-veterinarians (ie livestock officers) test all the cattle. Traditionally veterinary practitioners test deer herds. In some areas MQM livestock officers also test deer.

The other major AHB operational item is vector control, especially of possums. This has been a function of local government in most districts, in particular regional councils. Over many years pest control methods have been refined and this is generally regarded as a specialist area. However, there are moves to also include private hunters, the rationale being that in the case of possums the skin can be harvested. In addition this provides work in rural areas where unemployment is often a problem.

Research is mainly contracted to the Crown Research Institutes and the universities. Other groups, such as MQM and private consultants, also take on minor research contracts.

Current disease control policies

The initial tuberculosis eradication campaign was a traditional one, with herd testing and slaughter of reactors. With the discovery of a wild life reservoir of infection, this has necessarily been modified.

There has always been an attempt to base control policies on an understanding of the epidemiology of *M.bovis* in the New Zealand environment, be it at times more hypotheses than known facts. This basic information is still the focus of intense research, and as new data comes to hand there will be appropriate changes in policy. When attempting to define the relationships between animals, DNA fingerprinting (ie DNA restriction endonuclease analysis (REA)) has been a very valuable epidemiological tool (Collins and de Lisle, 1985).

The epidemiology of *M.bovis* in NZ:

The basic outline of the current concept is illustrated in Fig.1. Data from tuberculosis breakdowns in livestock and from direct sampling of wild life populations is used by MQM veterinarians to map areas where wild life are infected and where they are not. The former are termed vector risk areas, the latter vector free areas.

Infection can be maintained in wild life independently of farmed cattle and deer. There is good evidence, which is supported by current research, that possums are the key species. It is accepted that possums are a reservoir host of tuberculosis. Conversely, there is a growing body of data that suggests ferrets, feral pigs and possibly wild deer are spillover hosts.

The epidemiology of tuberculosis in possums has been investigated both in the field and by modelling. An important element is the development of chronic clusters of infection, 'hot spots', within the possum population. Modellers have found it difficult to develop systems that show this patchy infection. One very important outcome from the early models, is that by reducing and holding possum populations at 40% of carrying capacity, tuberculosis will not be maintained (Barlow, 1995). This is the rationale for using long term possum control to eradicate infection from an area.

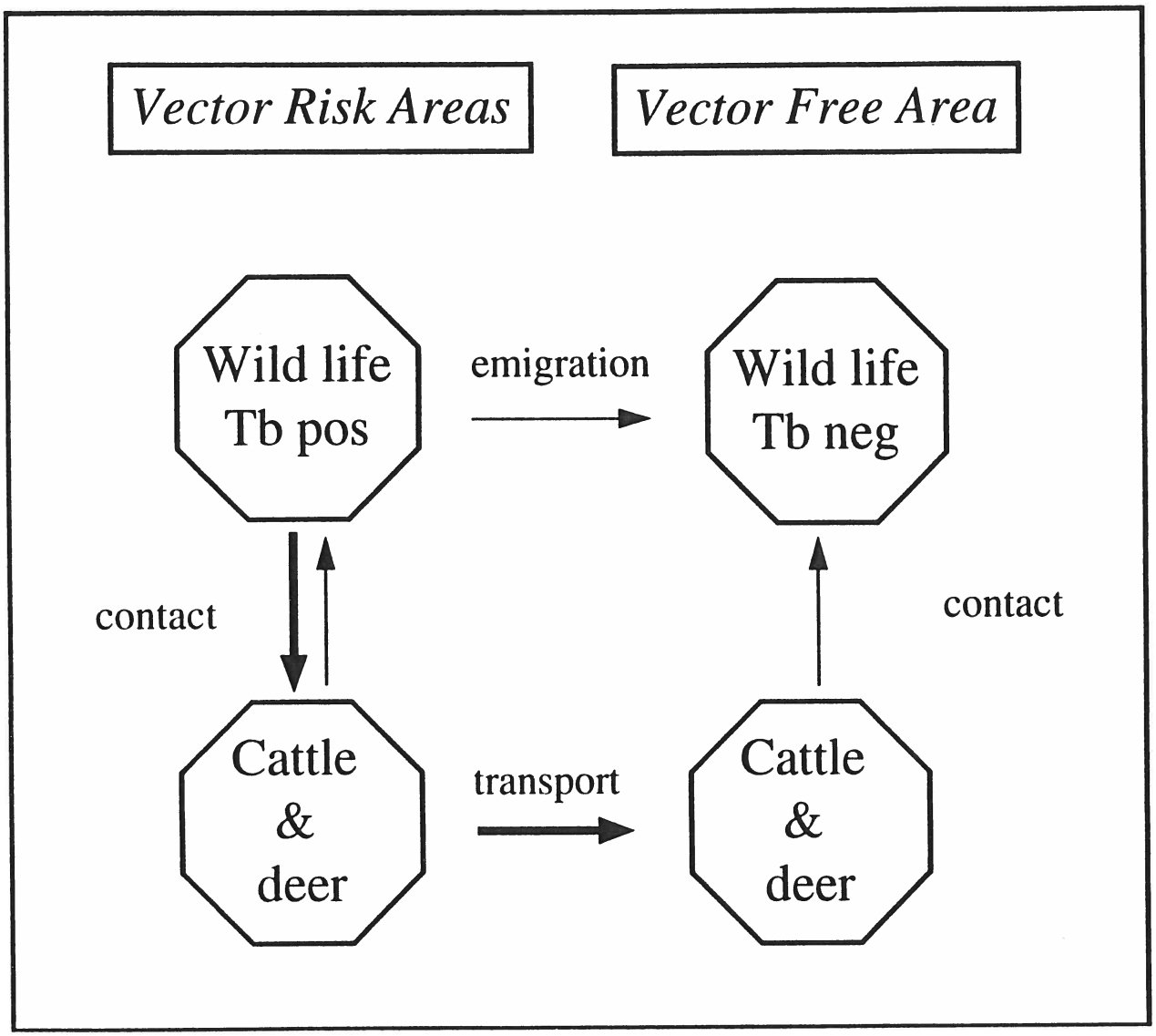

Fig.1 Schematic representation of the epidemiology of *Mycobacterium bovis* in NZ.

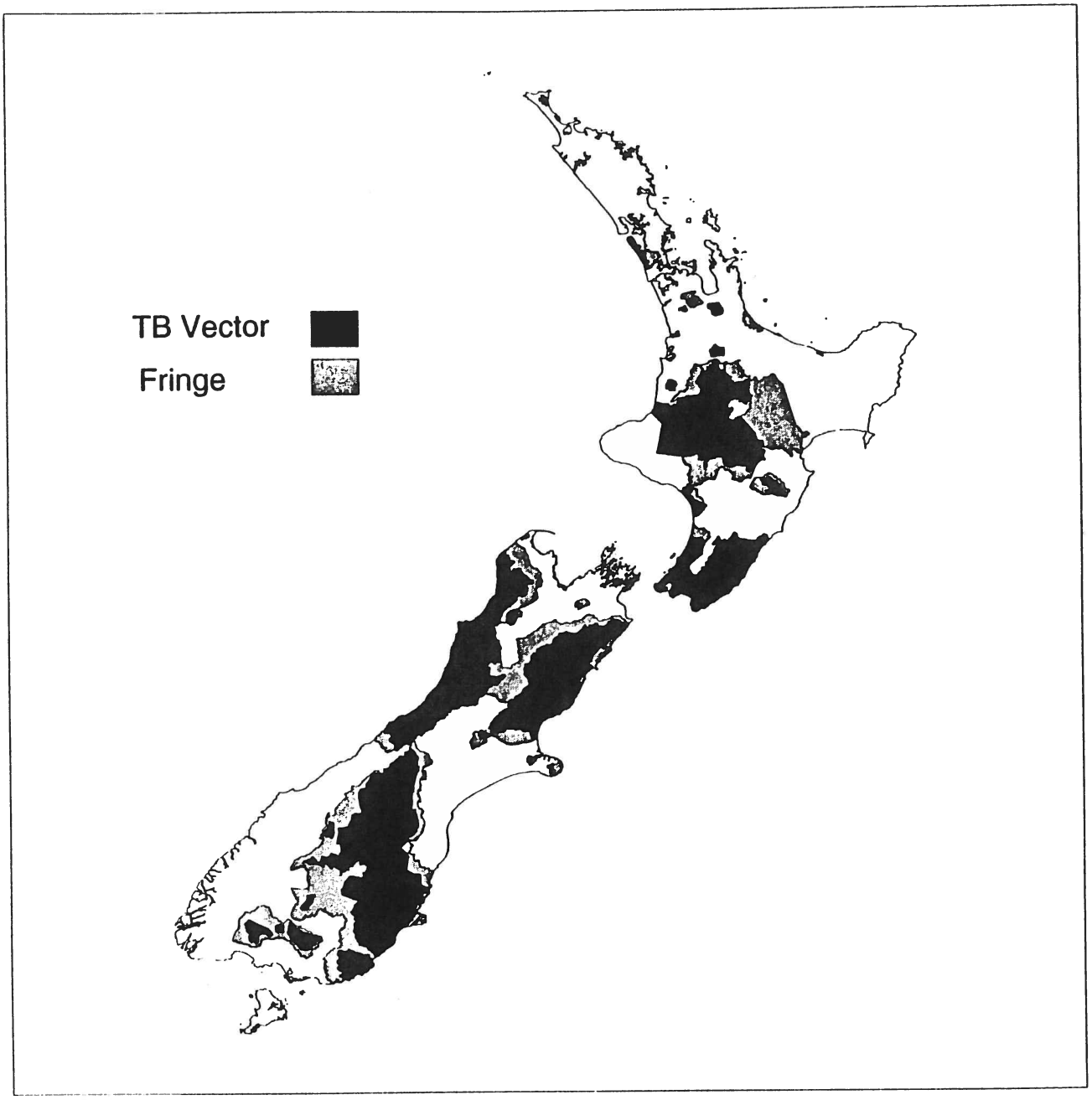

Fig.2 TB vector areas (shaded black) in NZ as at June 1997. Fringe zones (shaded grey) are also shown (see disease control strategies below).

In vector risk areas, there is widespread and regular transmission of infection from wild life to cattle and deer. The main species responsible is the possum, but ferrets are an important source in some areas. We believe that transmission in the reverse direction is rare, but that infected farmed deer may have an important role. The same can be said for vector free areas.

The vector risk areas have gradually expanded over the last 25 years. The current situation is shown in Fig.2. The primary factor thought responsible is short to medium range (1 to 5 km) movement of infected wild life, in particular juvenile male possums. Longer range (5 to 30 km) transmission via infected feral deer appears a possibility, but there is scant data to support this. The initiation of new vector risk areas via the deliberate illegal movement of possums (to establish areas for trapping), and deer (for hunting) is also possible and appears to have occurred.

Until recently we considered the epidemiology of *M.bovis* in farmed cattle and deer to be quite simple. Under annual confirmatory testing (ie testing of clear herds to confirm freedom of infection), which is the policy in vector risk areas, cattle to cattle (and deer to deer) transmission was considered an uncommon event. Under biennial and triennial confirmatory testing (the policy in vector free areas) the potential for transmission within a herd was recognised. Infection could be transferred between herds via livestock. Clearly the transmission from herds in vector risk zones to vector free zones was considered most important. The imposition of area movement control in vector risk areas should have reduced the importance of this pathway. In the absence of vectors, skin testing alone was seen to be an efficient and very effective means of eradicating tuberculosis from infected herds (eradication testing).

In 1995 a national case control study of breakdowns was conducted (Ryan et al., 1995). Significant risk factors associated with cattle movements on and off properties were found. Of some interest was the result that the variable 'number of young beef cattle moved on' was also significant. Many beef cattle breeding farms are located in vector risk areas, and this movement of young animals was seen as an important way that tuberculosis was being spread from vector risk areas.

Unexpectedly the study also showed that prior infection was a risk factor. This has prompted other studies of the epidemiology of tuberculosis in intensively farmed vector free areas. The movement of cattle in such a district, the Waikato, was surveyed and various models of tuberculosis developed. The magnitude of cattle movements surprised all; 26% of the total cattle population was moved between farms over one year (Ryan et al., 1996). The models suggested that with this amount of movement, under current disease testing policies, tuberculosis would be maintained in the cattle population independently of infected vectors (Barlow et al., 1998). Later, investigations of the performance of skin testing (see below) also suggested that the tuberculosis problem is not entirely due to infected vectors.

Disease Control Strategies:

The Animal Health Board's long term aim is to eradicate bovine tuberculosis from NZ. It is seen that this is not practicable within 5 years with current technology, and therefore it has set the following objectives.

1. To reduce the number of infected herds in vector free area to 0.2%

2. To prevent the establishment of new vector risk areas and the expansion of current vector risk areas.

3. To decrease the number of infected herds in vector risk areas to 11%

4. To encourage individuals to take action against tuberculosis on their properties and in their herds.

To achieve these objectives an interlocking programme of both livestock testing and vector control has been developed. In addition, there are elements directed at individual livestock owners taking more

responsibility.

The initial very important task for MQM veterinarians is to define the vector risk and free zones. This can be difficult; generally a border is mapped that errs on the side of freedom from infection. A zone around each vector risk area is then drawn with reference to the likely short to medium range emigration of infected vectors. This is called the fringe area. There is intensive surveillance for tuberculosis within the fringe zone to detect, as quickly as possible, infected vectors moving out of the vector risk area.

In vector free areas, so-called special testing zones are set up if wild life tuberculosis is suspected. As in fringe areas, there is heightened surveillance for tuberculosis, including survey of wild life.

The balance of the vector free area is termed the surveillance zone.

In each of the different zones appropriate livestock testing, quarantine and vector control policies are set. For example, around each large vector risk area, usually within the fringe area, a vector buffer would be set up. Buffers are generally from 3 to 4 km wide and are subject to very intensive and frequent vector control. The goal is to sweep up any emigrating infected vectors.

Within vector risk areas, the disease control objectives are either control or eradication of infection. With small ones, it is total eradication of tuberculosis. With large ones, the aim is eradication at the margins, (ie 'roll back'), and control in the balance. It is envisaged that eradication zones within vector risk areas will be established in the future. Livestock testing policies are more conservative where the goal is eradication rather than control.

In surveillance areas, either biennial or triennial confirmatory testing is usually practised. If a high prevalence breakdown is detected, especially in deer, BLIP vector control is conducted (Brief Local Initiated Possum control). This is to reduce the risk of a new focus of vector infection.

Infected herds are quarantined. Deer from infected herds cannot move to other properties. Cattle are subject to a more complex movement control procedure. Cattle and deer moving from vector areas where there is a high level of infection (breakdowns > 10 herds per 10,000) are subject to area movement control.

When cattle and deer are moved, a tuberculosis status declaration card must be sent with them. This lists information on the tuberculosis status of the herd and area.

Reactor compensation for cattle is currently set at 65% of fair market value. This will be reviewed shortly and may be reduced.

The Animal Health Board maintains an active communications, education and technology transfer programme, both locally and nationally.

Management information systems

The tuberculosis control campaign is supported by a nationally networked management information system, National Livestock Database (NLDB). NLDB consists of 6 modules (business, herd/flock and farm registration, surveillance, quarantine, testing management, pathology and vector). All the elements noted above can be recorded against either farms or herds, as appropriate. Twenty disease control offices have access to NLDB; it is used for day to day management, for planning and for retrospective epidemiological analyses. Regional and national specialist staff can also access the system.

Progress

In 1977, movement control of cattle was introduced. Throughout NZ there had been very good progress, and it was expected that with movement control, within 5 years tuberculosis would reduce to very low levels. Indeed numbers of infected herds in vector free areas decreased considerably. However, there was a concurrent increase in the size of the vector risk areas and numbers of associated infected herds. During this early period MAF attempted to remove the newly identified infected populations of possums. The control operations were very successful, as measured by herd testing. It was considered that the problem has been resolved and finance for the control of possums was thus reduced (Fig.3).

Unfortunately infected possums returned and continued to spread to new areas. Over the next 10 to 15 years the number of infected cattle herds gradually increased. Most (75% to 85%) were in vector risk areas. Likewise the number of cattle reactors increased, again most coming from vector risk areas.

In the late 1980's funds for possum control operations again became available, and has been gradually increased over 10 years (Fig.3). Largely due to the scope and effectiveness of these operations, especially with the toxin 1080, there has been a marked improvement. Numbers of infected cattle herds have been progressively reduced over the last three years. In some of the vector areas there have been dramatic reductions in tuberculosis, both in infected herds and reactors.

A compulsory tuberculosis control scheme for deer was introduced in 1990. In all areas numbers of infected herds have decreased, but gains in vector risk areas were less than in vector free areas. As seen with cattle, over the last three years there has been better progress in vector risk areas.

One area of concern is the increasing area classed as vector risk. From small foci these have gradually increased over the last 25 years (Fig.4). Buffer vector operations have reduced the rate of extension, but isolated new areas have occurred and the existing ones have expanded.

Using NLDB one can conduct intensive analysis of testing data. Some long term trends have been observed; eg an increase in the specificity of caudal fold (CF) skin testing in cattle. There has also been a reduction in the proportion of CF test-positive animals retested. Such changes clearly have flow-on effects on such parameters as proportion of lesion positive reactor cattle. It is beyond the scope of this paper to report on these issues in detail. A recent reference on this subject is Ryan & Cameron (1995). Summary testing data for the 96/97 year is shown in Table 2.

Table 2. Testing data for cattle and deer or the 1996/1997 financial year

Species	No. primary tested	Primary test positives	No. Ancillary tested	Reactors*	Lesion reactors	Non-reactor lesion animals**
Cattle	4,555,736	10,119	6,794	4,488	1,968	889
Deer	520,621	7,865	7,022	1,387	244	224

* Animals slaughtered as a result of positive tests. ** This group is termed culls.

These data show a developing problem with diagnosis of tuberculosis in deer. The high cull rate with deer, in comparison with cattle, is due in part to deer farmers electing to slaughter infected groups of animals rather than attempt eradicating by testing.

THE FUTURE

Future strategies are invariably linked with current research on tuberculosis and with technological advance in general. In NZ, NSSC has encouraged a broad range of research, in terms of both subject and expected period to implementation. The products of the short to medium term projects are now becoming available.

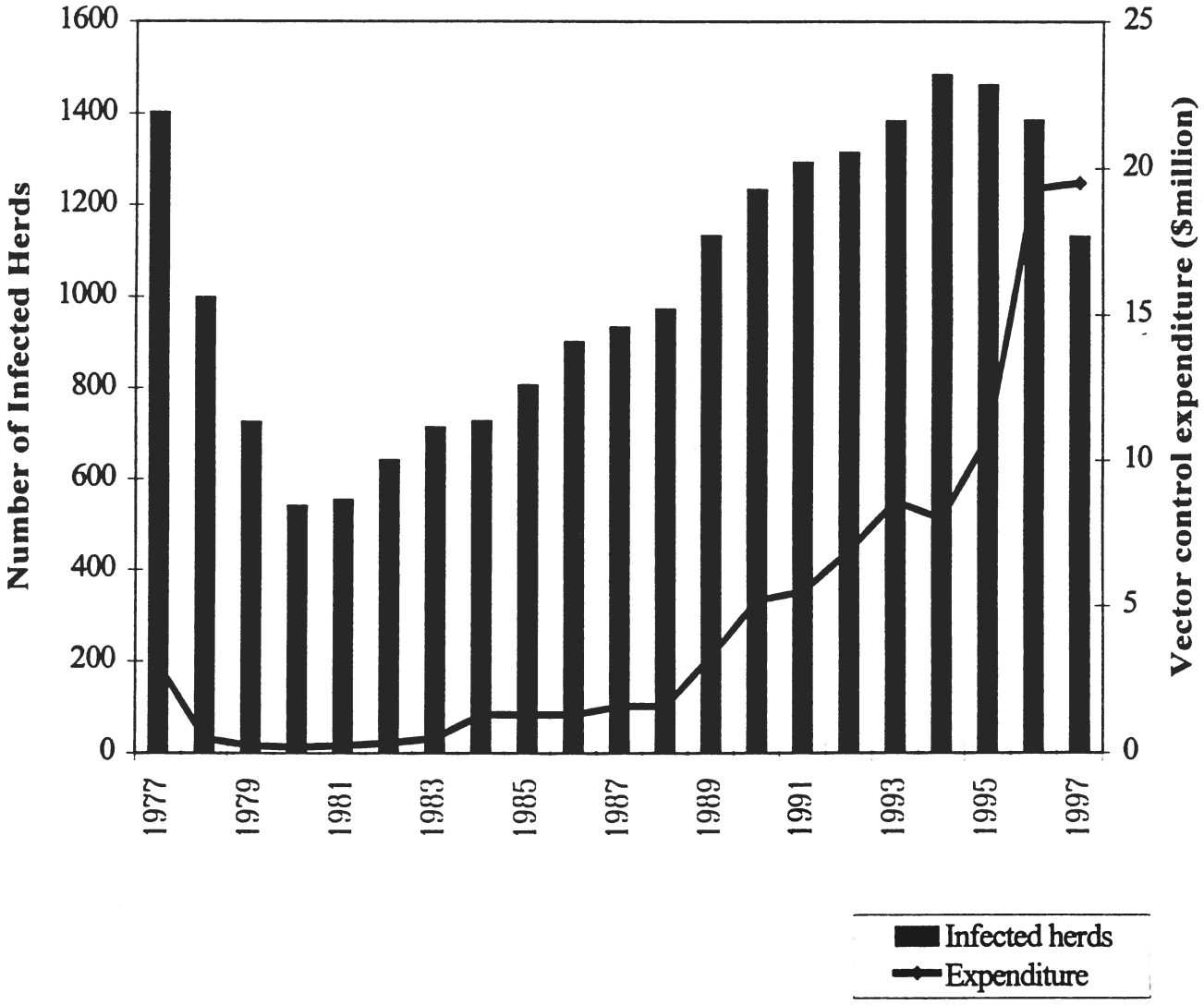

Fig.3. Number of infected cattle herds and annual expenditure on vector control for the years 1977 to 1997. Expenditure is corrected to 1990 dollars.

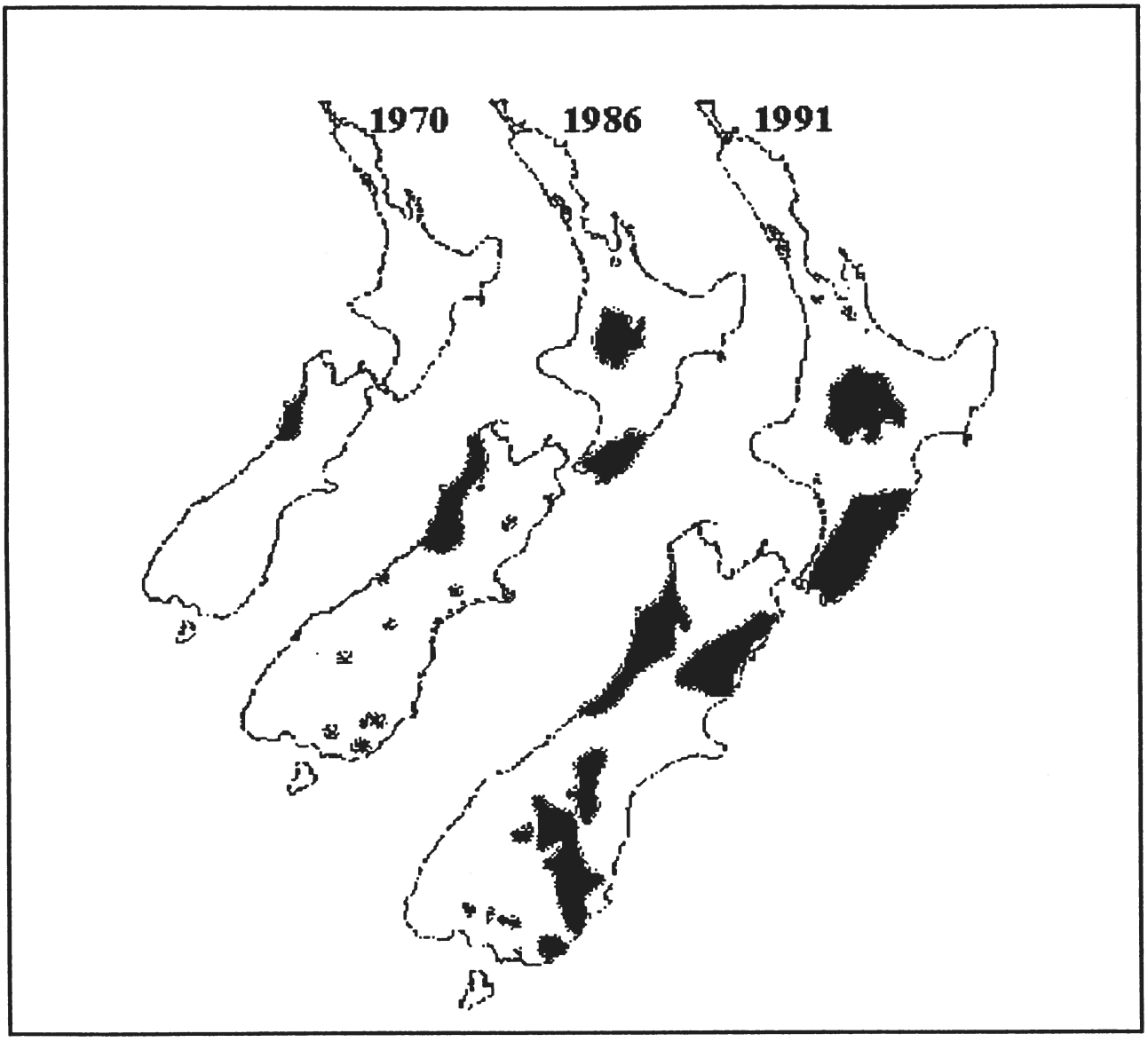

Fig.4. Vector risk areas in NZ (shaded black). The situation in 1970, 1986 and 1995 from left to right, respectively.

Traditional strategies

Testing:

Skin testing has been the mainstay of all national tuberculosis eradication schemes, and much has been achieved internationally. In NZ, in areas distant from infected vectors, the disease has been reduced to very low levels with skin testing. However, fieldwork has shown the sensitivity of caudal fold testing in cattle is generally only 'moderate' (85% ± 10%). Isolated cases of severe failure have also been reported. Further, the desensitization that follows skin testing is inconvenient; eg serial retesting has to be delayed 60 days. For these reasons, alternative diagnostic tests for cattle have been an active area of research for 5 years. The important findings are as follows:

1. The accuracy of the (-interferon assay (BOVIGAM® CSL) was found to be similar to caudal fold skin testing. It was concluded that the additional cost and inconvenience of blood testing in the confirmatory test programme could not be justified (Ryan, 1994). (Note: Bloods in this trial were cultured after overnight transport, not within 12 hours as recommended by the manufactures of this test.)

2. We have demonstrated that satisfactory sensitivities and specificities can be obtained with BOVIGAM, and with some lymphocyte transformation assays, when used as a serial test, 10 to 30 days after caudal fold testing. Again bloods are cultured after overnight transportation to a laboratory. The preliminary data from this investigation (more animals will be tested this year) is shown in Table 3.

Table 3. Preliminary results from an investigation of serial testing with interferon, transformation and ELISA assays in cattle, 10 to 30 days after skin testing (Tests conducted by AgResearch Wallaceville and the Otago Deer Research Laboratory).

Test	Sensitivity %	Se 95% CL**	Specificity %	Sp 95% CL**
Interferon, day 0	89	81 to 94	87	78 to 94
Interferon, day 1	88	82 to 94	90	82 to 95
Transformation, LT	91	84 to 96	81	72 to 88
Transformation, LR	84	75 to 91	87	78 to 92
ELISA AgResearch	22	14 to 31	94	88 to 98
ELISA* AgResearch	32	23 to 42	94	86 to 98
ELISA Deer Lab	20	12 to 29	92	85 to 97
ELISA* Deer Lab	56	46 to 66	92	84 to 97

* Johnes Disease vaccinates removed. ** CL = Confidence Limit.

1. The value of parallel testing problem herds with both cellular (BOVIGAM and transformation) and antibody (ELISA) assays is also under investigation. In a very consistent manner the blood tests have detected infected skin test negative animals. In some herds the results have been quite dramatic (eg 22 skin test negative blood test positive animals with 18 exhibiting gross lesions). In others, skin testing has been shown to be accurate. The reasons for these failures of skin testing are not clear.

Interestingly, the *in vivo* cellular assays rather than ELISA have been more useful parallel tests.

As a result of these findings, BOVIGAM has been approved for use as a short interval serial test in cattle. Field data suggests that it is very much more sensitive than comparative cervical testing; a policy change allowing only BOVIGAM testing in herds where there are less than four skin reactors is likely to be introduced.

The parallel testing investigation is continuing. A probable outcome is that problem herds (high prevalence breakdowns or chronic infection) will be required to undergo a blood test prior to achieving official clear status.

Vector Control:

A very broad range of research into vector control is underway. Examples are the development of new toxins, new methods of application of toxins and systems of monitoring the effectiveness of operations. Contracts to define the ecology and epidemiology of tuberculosis in wild animal populations have been let; the goal being to define which wild life species should be the control target. Field methods, using large scale field operations, are also being investigated. The findings from these very diverse projects are being used enhance the effectiveness of vector control operations.

Decision support systems (DSS):

Tuberculosis in NZ is a very complex problem. Management decisions are often difficult and can involve large sums, perhaps millions of dollars. To support national managers and local people, various researchers are investigating decision support systems. At Massey University a group is bringing together various models of tuberculosis in possums into a system called EPIMAN-TB. One part is directed at identifying likely 'hot spots' which could then be subject of intense and containing control. Some parts are currently being prototyped and will delivered over the next 2 to 3 years.

Other strategies

Vaccination:

Internationally there is much interest in the development of better vaccines for tuberculosis for use in man and other animals. A number of groups in NZ are working on this, some in collaboration with overseas institutes. The performance of BCG in livestock (cattle and deer) is also being investigated. Workers from Massey University and AgResearch Wallaceville are also evaluating BCG efficacy in possums.

There has been much debate about possible vaccination strategies. In NZ we have to consider the effect that vaccination would have on the ability to export meat and dairy products. On balance it would seem that vaccination of wild life, in particular possums, offers more potential in the medium term than vaccination of livestock.

Biological methods of control of possums:

Of course the ultimate solution to both the disease and environmental problems of possums would be to find a self-sustaining biological method of reducing numbers. Various searches for and studies of pathogens and parasites, both in NZ and Australia, are been conducted. Studies of the reproductive physiology of possums are also underway. The goal would be to find a mechanism to interrupt the reproduction of this normally very fecund animal; eg immuno-contraception.

CONCLUSIONS

Only 150 years ago, NZ was a land of mainly forest and birds. There is now a diverse range of wild, feral and domestic animals interacting in a very modified environment making a ideal niche for *M.bovis*. At the centre of this is the brushtail possum. Ironically in Australia tuberculosis never moved into possums from cattle. The epidemiology of tuberculosis in cattle and deer in NZ is a classical example, on a national scale, of the triad of disease determinants in action.

Many are looking for a simple solution to the problem. The reality is that multi-facet control will most likely be required. For example, cattle owners may have to modify their management, especially the manner in which they shift animals from farm-to-farm. However, the trends seen over the last few years and the early results of research have engendered much optimism.

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DNA FINGERPRINTING OF *MYCOBACTERIUM BOVIS* ISOLATES USING SPOLIGOTYPING - EPIDEMIOLOGICAL ISSUES

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In Great Britain, control of bovine tuberculosis (TB) in cattle depends primarily upon a programme of surveillance using the single intradermal comparative tuberculin test and meat inspection. As part of the veterinary investigation of any confirmed case, the most likely origin of infection is established by looking at such things as stock movements into the herd, the infection status of contiguous herds and the presence or absence of wildlife species known to be natural reservoirs of infection, particularly badgers. Despite these investigations, a source of infection may only be ascribed through a process of elimination and association or else remain undefined altogether (Report, 1997). Molecular typing is a valuable tool for investigating potential sources of infection and has recently been recommended as a means of providing 'conclusive evidence on whether, and to what extent, badger to cattle transmission takes place' (Krebs, 1997).

For genetic fingerprinting methods to be of benefit in large-scale epidemiological studies they must produce a degree of discrimination which allows for epidemiologically meaningful temporal and spatial patterns to emerge. Ideally, the techniques also need to be cheap, quick and easy to perform, and independent of bacterial culture. So far, three principal methods have been developed for differentiating *Mycobacterium bovis* isolates, Restriction Endonuclease Analysis (REA), Restriction Fragment Length Polymorphism (RFLP) and Spoligotyping (Spacer-Oligonucleotide).

REA is used exclusively in New Zealand and has been shown to enable differentiation of *M. bovis* isolates into a large number of types. However, high resolution is needed to identify slight but real changes between patterns, making interpretation more demanding than for the other methods. REA has been used in New Zealand to type *M. bovis* isolates from various species, particularly cattle, deer and possums, as part of the investigation of disease outbreaks (Collins et al, 1994). In one example, a single REA type was associated with multiple cattle herd breakdowns and possums in the same localities, while in another example typing suggested that further investigation into stock movements was warranted. Typing is now incorporated into the New Zealand TB control programme, although the cost of the method does not permit all isolates to be typed prospectively.

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RFLP typing using the insertion element IS6110 has been extremely useful for epidemiological studies of *M. tuberculosis* since there may be 25 or more copies of IS6110 in the chromosome (Small & van Embden, 1994). In contrast, *M. bovis* isolates from cattle usually harbour only a single copy of IS6110 in the chromosome, so limiting strain differentiation by IS6110 RFLP (van Soolingen et al., 1994). Other probes, such as the GC-rich repeat sequence (PGRS) and the direct repeat sequence (DR), give better discrimination (Cousins et al., 1993; Skuce et al., 1994). Several epidemiological studies of *M.bovis* have been based on RFLP. In Argentina and the USA, the zoonotic implications of cattle infection were investigated (Cave et al., 1992; Romano et al., 1996). In Northern Ireland, isolates, especially from cattle and badgers, were typed and the potential use of the methodology in epidemiological studies evaluated (Skuce et al., 1996). In Sweden investigations of an outbreak of TB in farmed deer incorporated RFLP, and the type found indicated origin of infection from imported deer from GB (Bolske et al., 1995). The main drawbacks are that the method is labour-intensive and requires purified DNA from large-scale cultures.

Spoligotyping is designed to detect the presence or absence of unique spacers of the direct repeat (DR) locus of the *M. bovis* genome by hybridization with a membrane at present containing 43 oligonucleotides derived from spacer sequences of *M. tuberculosis* H37RV and BCG (van Embden et al., 1995). Spoligotyping is PCR-based and so is intrinsically more rapid, as well as being simpler and cheaper to perform than RFLP. Large numbers of isolates can be typed at the same time. In addition, the method generates data which are more suitable for computer analysis than that derived by RFLP. Spoligotyping was the basis of a study of *M. bovis* isolates from various species (especially cattle, goats and cats) in Spain, with particular emphasis on the interaction between species (Aranaz et al., 1996).

In 1996, we introduced spoligotyping methodology to our laboratory with funding from the Ministry of Agriculture, Fisheries and Food. The aim was to validate and develop the technique for typing *M. bovis* isolates and to assess its usefulness as an addition to current bovine TB control procedures. This was to be achieved by applying spoligotyping to historic and prospective *M. bovis* isolates, primarily derived from cattle and badgers in the course of statutory TB control. Previous results from this study were supplied to an independent scientific review group (Krebs, 1997). The current paper summarises our findings to date.

MATERIALS AND METHODS

Mycobacterium bovis isolates were derived from a) cattle either slaughtered in the course of routine surveillance, primarily as reactors to the skin test or as dangerous contacts, or found with suspicious lesions at slaughter, b) badgers killed in removal operations, conducted in response to herd breakdowns, c) badgers with clinical samples (faeces, tracheal aspirates and urine) collected during a prospective study of a naturally infected badger population (see Rogers et al., 1997), and d) other wildlife and exotic species where there was a suspicion of bovine TB.

Cultures of *M. bovis* were prepared by standard methods and stored at -20°C until required or else taken directly from culture medium for DNA extraction. Preparation of DNA and spoligotyping were performed as described previously (Aranaz et al., 1996). If an isolate was

found to have a previously unseen combination of oligotides it was ascribed the next sequential spoligotype number. Gels were analysed using GelCompar (Applied Maths BVBA, Belgium).

An ACCESS database was prepared whereby each isolate had an individual record detailing the species of animal involved, the Ordnance Survey map reference, the herd identifier and any associated badger control operation number, the date the sample was taken, the spoligotype and the reason why the sample was taken. The map references allowed the spatial distribution of spoligotypes to be displayed using a geographic information system (ARCVIEW, Environmental Systems Research Institute, Inc., USA).

RESULTS

To date, 34 unique spoligotypes have been found in 2668 samples collected mainly during the years 1996 and 1997. Their distribution by species is summarised in Table 1. Certain types predominate, with 70% of samples represented by types 9 and 17. So far 16 of the 34 types are unique to particular species, although nine of these are based on a single isolate.

Of the 2002 samples from cattle, 1974 were associated with a particular herd. In all, 709 known herds were represented. Figure 1 represents the geographical distribution of types 9 and 17, where 20x20 km squares have been coded according to the type which has been detected most frequently. In Fig. 2 the squares have been coded according to the most frequent spoligotype found, excluding types 9 and 17 and types identified less than three times. Two or more samples from a herd were typed in 331 instances. Up to five different spoligotypes were found within a single herd. The distribution of herds by number of samples typed and number of spoligotypes detected is summarised in Table 2.

Mycobacterium bovis isolates from cattle were typed from a total of 734 breakdowns, as defined from the national skin test data recording system. A number (43) of herds were represented by isolates from more than one breakdown, with 37 herds having two breakdowns, and six herds having three. Where herds were considered to have more than one distinct breakdown, in other words movement restrictions were lifted after the required herd skin retests and then reimposed after reactors were again detected or lesioned animals were found at slaughter, at least one spoligotype matched each time except on five occasions.

For the 734 breakdowns, an ascribed origin of infection was recorded in 426 instances. Badgers were considered the most likely source of infection on 334 occasions, purchased cattle 41 times, imported Irish cattle 13 times, spread from contiguous premises five times and unknown after investigation on 33 occasions. Otherwise, the source of infection was unassigned at the time of preparing this paper. The number of isolates according to spoligotype and source of infection is detailed in Table 3. So far isolates from seven breakdowns have been linked to those from herds which supplied animals to the breakdown herds. The types matched in six of the cases.

To date *M. bovis* isolates taken from badgers killed in the course of control operations following herd breakdowns have been paired with cattle isolates from the originating herd breakdown in 99 instances. In 90 of the breakdowns at least one badger spoligotype matched

Table 1. Spoligotypes found in different species

Species	Spoligotype:																
	4	9	10	11	12	13	15	17	18	20	21	22	23	25	27	28	31
Alpaca									4								
Badger		196	6	53	14	26	2	272		16	9	10		9			1
Cat					1		5				1					5	1
Cow		794	20	157	29	16	77	543		42	34	172	5	60			
Deer	1		1			1		5			1						
Fox		2															
Goat												1					
Horse		1															
Human																1	
Tapir										1							
Unknown		1													1		
Totals	1	994	27	210	44	43	84	820	4	59	45	183	5	69	1	6	2

Species	Spoligotype:																	
	32	34	35	36	39	40	43	44	45	46	47	49	51	53	54	55	59	Totals
Alpaca																		4
Badger	1		3		1				1					1				621
Cat		1	2												1			17
Cow	1		29	2	1	1	2	1		1	2	2	9			1	1	2002
Deer			2												2	3		16
Fox																		2
Goat																		1
Horse																		1
Human																		1
Tapir																		1
Unknown																		2
Totals	2	1	36	2	2	1	2	1	1	1	2	2	9	1	3	4	1	2668

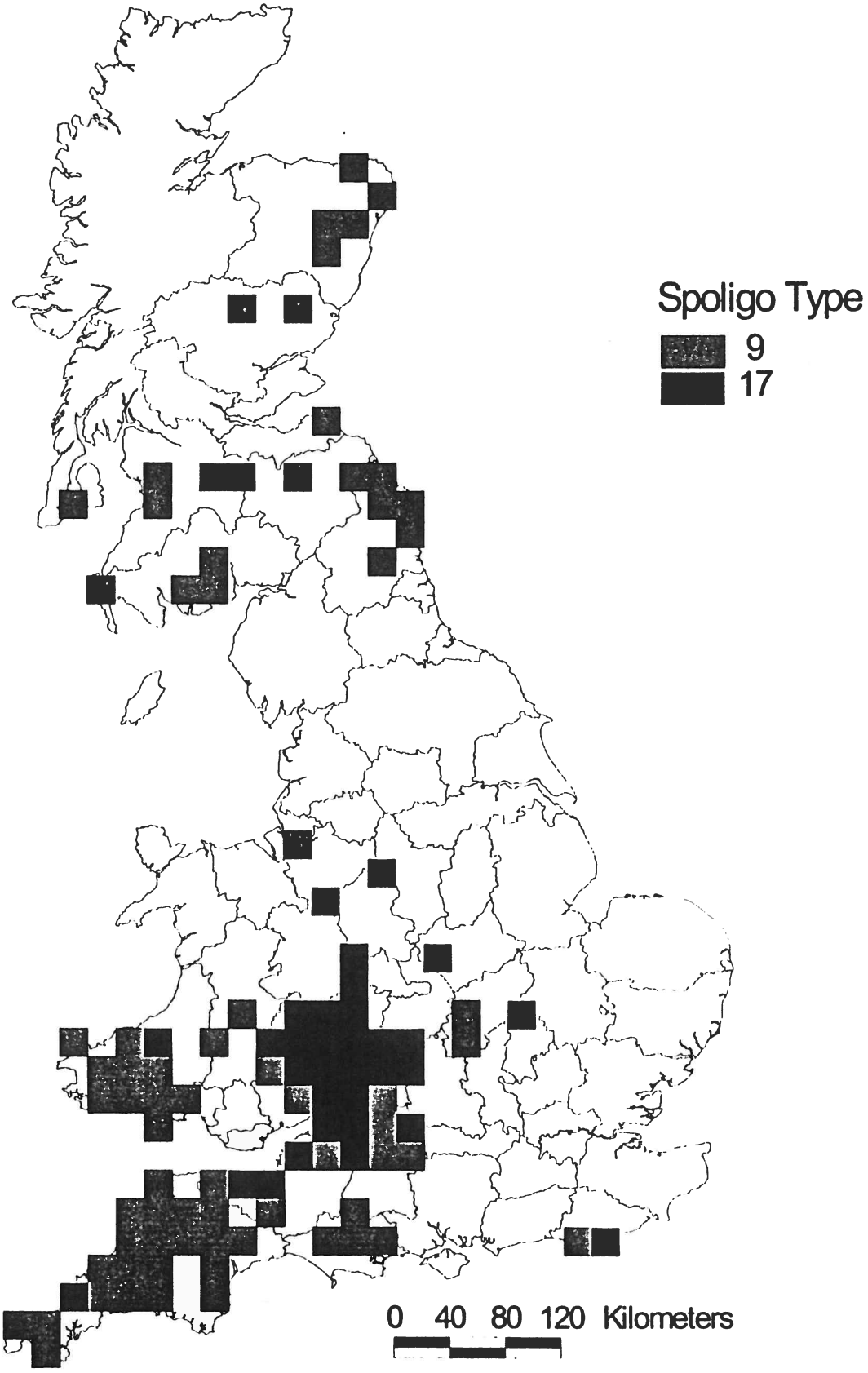

Fig. 1 Spoligotypes 9 & 17 from Cattle Isolates

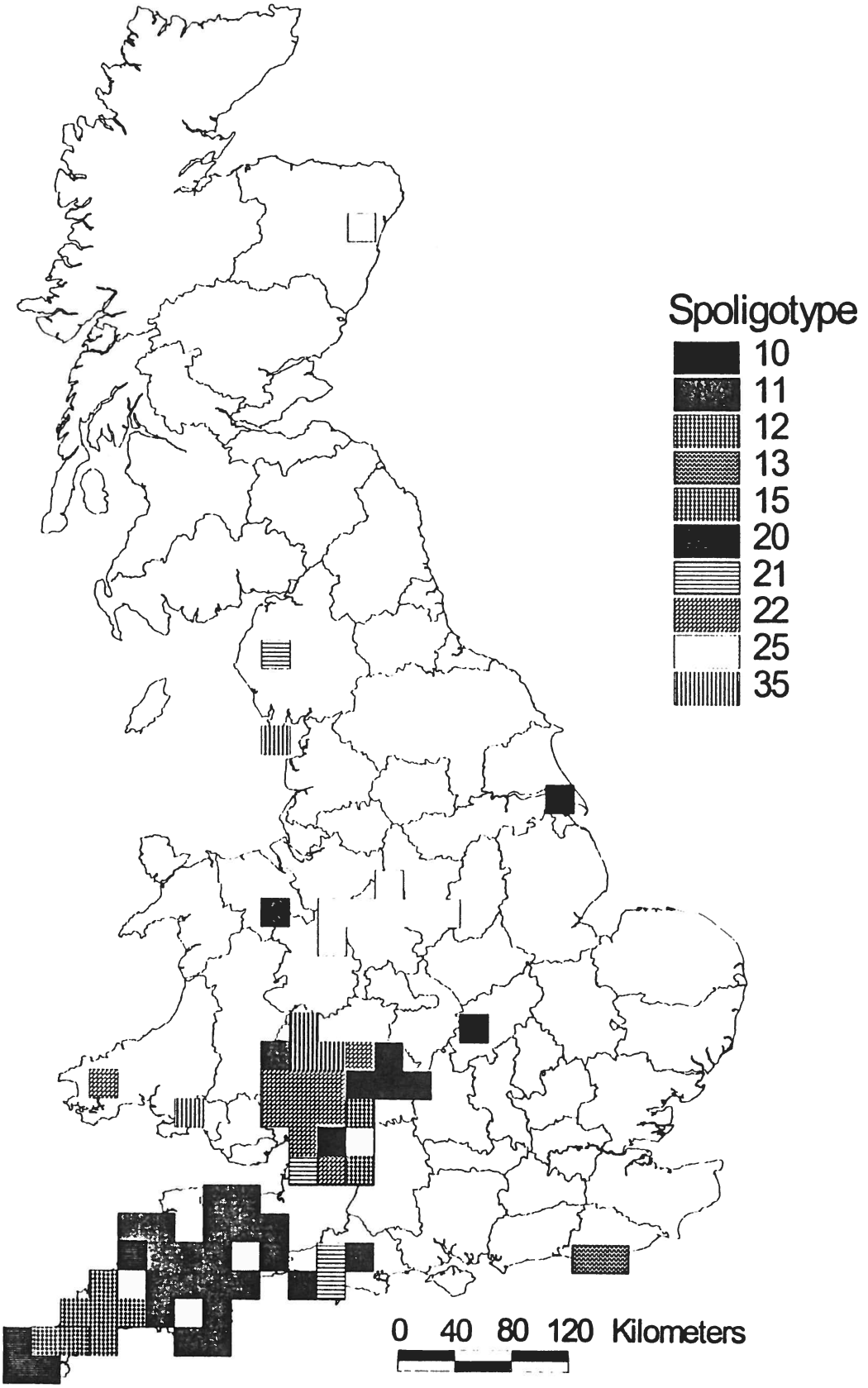

Fig. 2 Spoligotypes other than 9 & 17 from Cattle Isolates

those in the cattle, while in nine cases there was no match. Figures 3 and 4 have been derived for badger isolates as Figs. 1 and 2 have been for herd isolates.

Table 2. Samples typed from cattle

Samples per herd	Number of herds	Number of spoligotypes: 1	2	3	4	5	Samples typed
1	378	378					378
2	119	98	21				238
3	52	39	11	2			156
4	34	22	11	1			136
5	19	16	3				95
6	25	17	5	3			150
7	18	14	3	1			126
8	17	13	4				136
9	14	10	3	1			126
10	9	6	2	1			90
11	6	1	3	2			66
12	3	2		1			36
13	4	3	1				52
14	2	1			1		28
15	1		1				15
16	1			1			16
17	1	1					17
18	3	1	1			1	54
19	1			1			19
20	2	1		1			40
Totals	709	623	69	15	1	1	1974

Isolates were typed from badgers in 18 of 35 social groups at the Woodchester Park study area, spanning a period from 1988 to 1997. Altogether, 65 isolates from 46 animals were spoligotyped. All were type 17 except for one type 11 isolate from a badger in a boundary social group without evidence so far of infection with type 17.

DISCUSSION

For several spoligotypes, especially in species apart from cattle, sample numbers need to increase before conclusions about species and geographical distribution can be validly made. For example, the majority of badger isolates were collected from animals killed in the same geographical locations as herds with TB breakdowns. Similarly, spoligotype 18 may be an exotic type, unique to alpacas, but small sample numbers from the alpaca and from other species in GB preclude such conclusions.

Table 3. Number of isolates from cattle according to spoligotype and source of infection

Breakdown origin	Totals	Spoligotype: 9	10	11	12	13	15	17	20	21	22	23	25	32	35	36	39	40	43	44	46	47	49	51	55	59
Badger	392	153	10	37	9	2	11	127	16	4	12		2		2	2			2	1	1	1				
Contiguous	5	5																								
Irish	14	12											1													1
Purchased	44	27		9	1		1	4		1					1											
Unknown	34	14	1	1			3	10	1		4															
Unassigned	345	98	2	20	2	4	9	96	4	5	69	1	21	1	7		1	1				1	1	1	1	
Totals	834	309	13	67	12	6	24	237	21	10	85	1	24	1	10	2	1	1	2	1	1	2	1	1	1	1

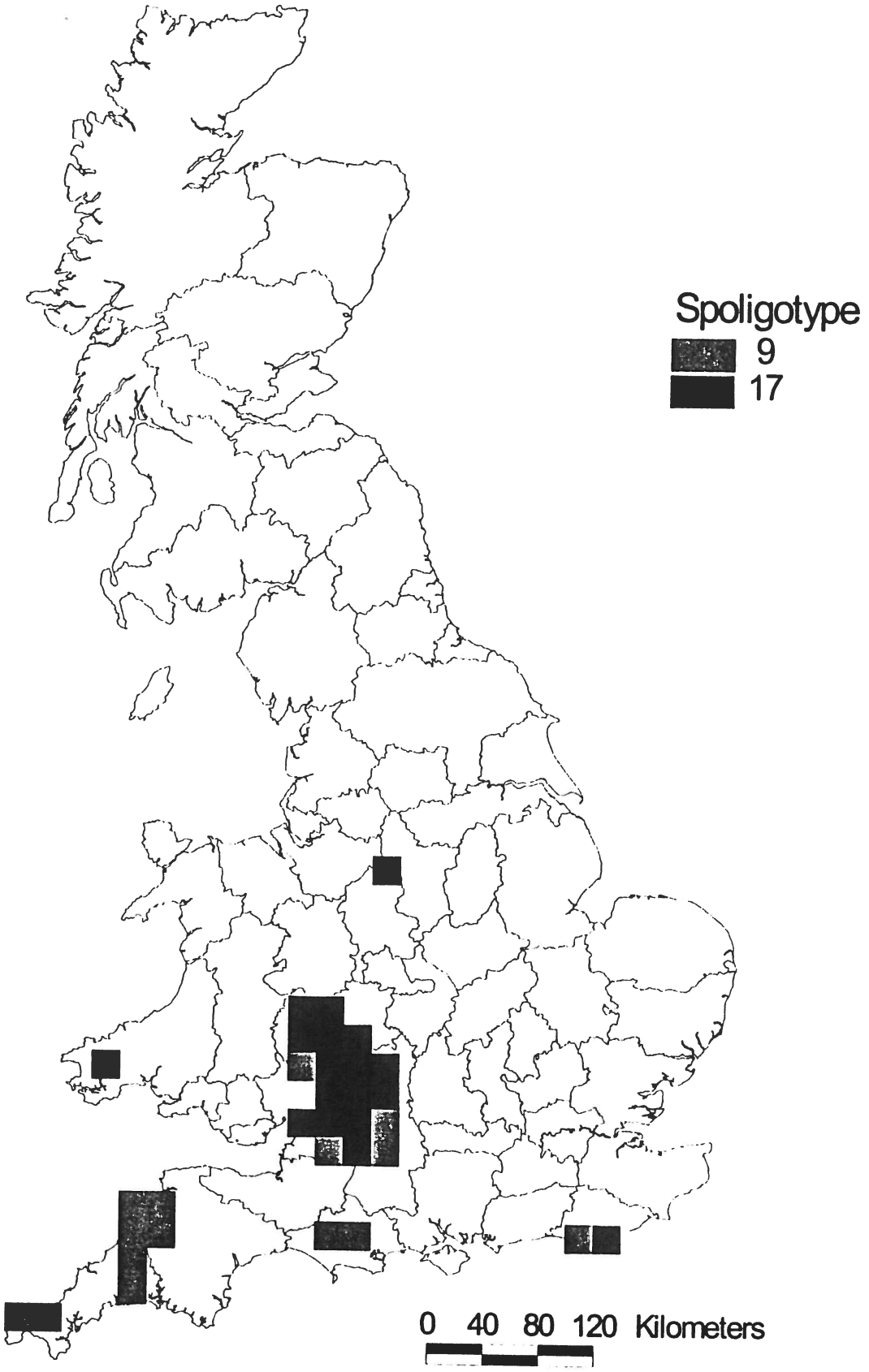

Fig. 3 Spoligotypes 9 & 17 from Badger Isolates

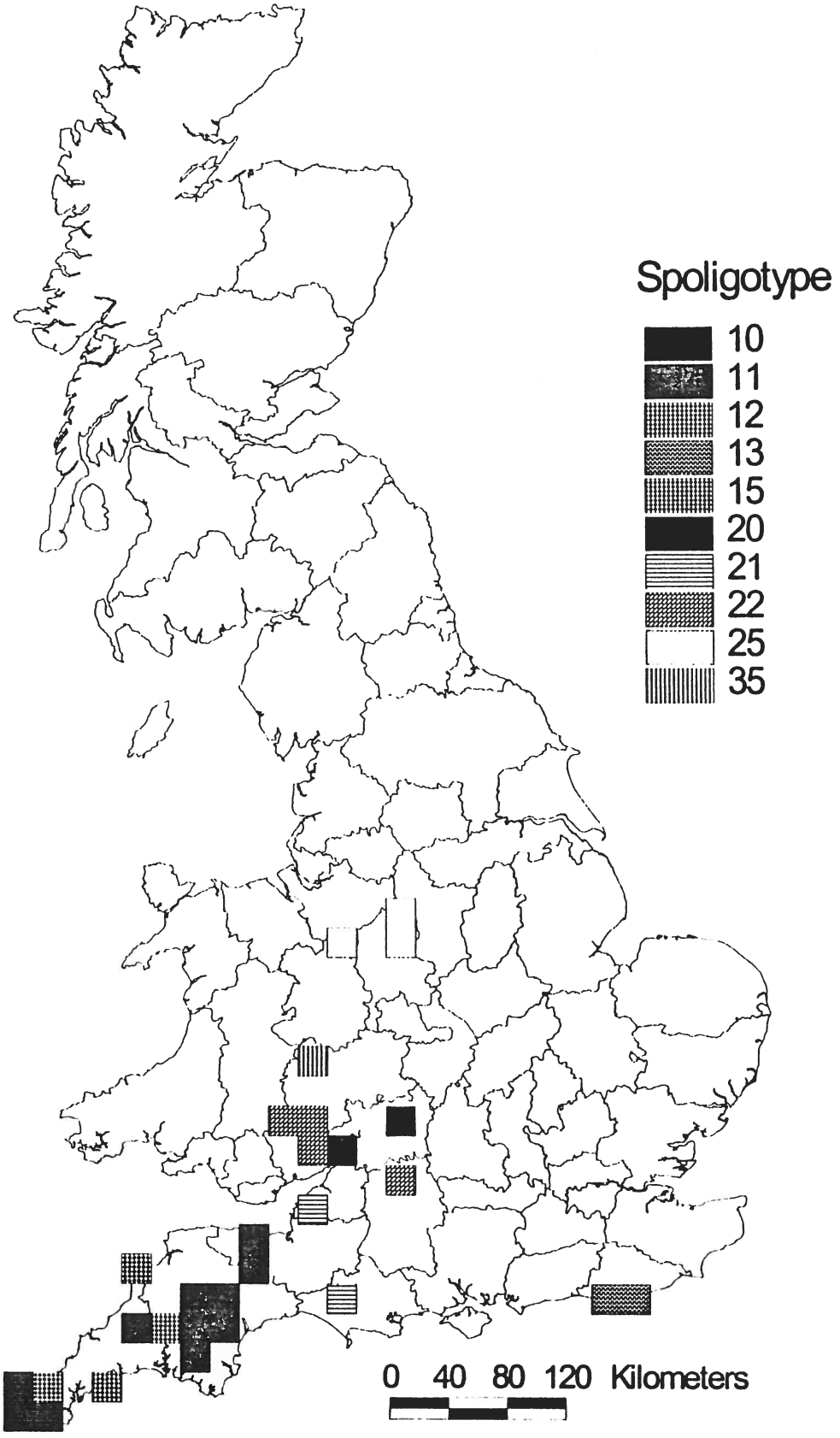

Fig. 4 Spoligo Types other than 9 & 17 from Badger Isolates

However, there are several key questions which need to be asked of any strain typing system, if it is to be useful for epidemiological purposes. In this study, three questions have been addressed, a) is the extent of differentiation sufficient for the intended application? b) are the defined strains stable in time and space? c) do the results give any insights into the spread of disease? The results are now beginning to provide some answers.

Thirty four unique spoligotypes have been observed, 25 of which are in cattle. This provides a reasonable basis for strain differentiation for epidemiological studies, especially as spatial clustering of some spoligotypes is becoming apparent. However, two types, 9 and 17, predominate. Similarly, in a naturally infected possum population in New Zealand various REA types were detected over a 22 month period, with some types having a dominant position while others entered part of the study site but failed to become established within the population (Pfeiffer, 1994). At Woodchester Park, all isolates except one over a ten year period were type 17. Within the population, infection remained within some social groups over many years whereas in others it appeared but failed to become established. It might be expected over the ten year period that other *M. bovis* strains would enter the badger population, as happened in the possum study. Further differentiation of spoligotypes might be achieved by applying alternative fingerprinting techniques, such as RFLP. We currently have work in progress to examine this possibility.

The Woodchester Park data, so far comprising samples over a ten year period from 46 animals, provide some evidence that types may remain relatively stable within a geographical location over a period of time, both within a defined badger population and within individual animals within the population. Apart from stability within a population, the evidence that multiple isolates over several months taken from individual animals gave the same spoligotype perhaps indicates a degree of type stability within an animal. However, the possibility remains that multiple types could co-exist within one animal if there was no selective advantage between the types.

Where recurrent breakdowns have occurred, the same spoligotype has been detected in most cases, while additional types have been found in some instances. From an epidemiological point of view this is open to various possible interpretations, for example that the original source of infection, whether within the herd or not, was not removed. If infection is exogenous to a herd, then any local wildlife reservoir of *M. bovis* could be carrying more than one spoligotype. That up to five spoligotypes have been associated with one herd argues for this but also cautions against being too dogmatic in interpreting possible associations between spoligotypes derived from different sources. In some cases unexpected results may merely be the effect of sampling. However, in other situations, typing may suggest routes of transmission which would otherwise have been ignored (for example, Collins et al., 1994).

The distribution of typed isolates so far in Great Britain suggests that a marked degree of clustering exists for several of the spoligotypes, for example type 25 which has principally been found in samples from Staffordshire and Derbyshire. In addition, one type 25 sample was from a Scottish herd where the origin of infection was considered to have been an Irish import. One explanation of the clustering could be the evolution of strains with different spoligotype patterns in a relatively immobile reservoir species, such as the badger. Sporadic appearances of other types could be explained by longer distance movements, in particular of infected cattle being sold between herds, as in the case of the Scottish herd. This indicates the need to establish in

some detail the past and current distribution of *M. bovis* types within GB so that unusual types within any geographic area and any temporal change in pattern can be identified.

Spoligotyping looks a promising fingerprinting technique for *M. bovis*, and studies are now progressing to compare it with other methods and establish its full epidemiological potential.

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TUBERCULOSIS IN CATTLE: CLASSIFYING BREAKDOWN EPISODES AS A BASIS FOR DECISION MAKING IN ERADICATION PROGRAMMES

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The analysis of progress of national programmes for the eradication of tuberculosis in cattle has been based on quantitative data relating to the number of test positive animals identified and the number of herds placed under movement control. This approach provides information with limited capacity to describe the evolving nature of tuberculosis as it affects individual animals and herds. Historically, the results of each year's testing activity in Ireland's national programme have been presented as quantitative data, e.g. number of herds restricted each year, reactor animals identified per 1000 animal tests (APT) in official statistics of the Department of Agriculture and Food (DAF). However, there is a qualitative aspect to breakdowns which is not described in the annual returns.

The tuberculous lesion rate is a measure of the number of animals deemed "reactor" which had either a tuberculous lesion identified at gross post-mortem inspection or which had tuberculosis confirmed by laboratory tests. While the presence of a laboratory-confirmed lesion is proof of tuberculosis in an animal, the converse is not the case (Corner, 1994).

This paper describes a means of qualitative assessment of the pattern of breakdowns based on data relating to breakdown episodes during 1989 - 1994 and an analysis of animal data, in relation to the lesion rate which was commenced in 1995. The primary objective of the analysis was to determine which variables influenced the lesion rate. This approach utilises the apparent prevalence of tuberculosis at herd level along with the rate of gross lesions disclosed at slaughter in tuberculin-reactor animals. The concept of disease episodes is introduced; this is proposed as an appropriate unit of analysis and reporting format for the future.

MATERIALS AND METHODS

Based upon DAF summary testing records, 50,387 individual restrictions were issued to herds over the period, 1989 - 1994. Any given herd may have been restricted more than once over the period. A completed episode is defined here as the interval from the time when a herd received a restriction notice to the time that notice was revoked and the herd returned to trading status. The database currently contains fully matching test and animal data for 45,385 episodes, representing 90.1% of the total episodes for the period of study.

Data on 205,989 reactor animal identified during 1989 - 1994 provided the basis for the analysis of the association between skin reactivity and the rate of disclosure of tuberculous-like lesions at slaughter.

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Basis for classifying episodes

A modified classification system was chosen to demonstrate a potential use of the database. Completed episodes were evaluated using two parameters:

1 Number of Standard Reactors to the tuberculin test identified during the episode, and

2 Number of animals with gross tuberculous lesions identified at slaughter during the episode.

The classification system uses six groupings of herds, the definitions of each of which are tabulated in Table 1. This system excludes the confounding influence of herd size. The influence of herd size on the probability of a herd having a positive single intradermal comparative tuberculin test (SICTT) is recognised (O'Keeffe, 1993), and will be incorporated in future classification systems intended for use in the field or laboratory. The present system utilises this simplified format for demonstration purposes only at this stage.

Table 1. Criteria for classification scheme.

	Number of Standard Reactors[1]	Number of cattle with tuberculous lesions
Group 1	2 or more	1 or more
Group 2	1	1 or more
Group 3	0	1
Group 4	3 or more	0
Group 5	1 - 2	0
Group 6	0	0

RESULTS

For the remainder of this paper the testing results of the 45,385 matched episodes, for which the data capture process is complete, are assessed, using this classification system.

The flexibility of the database is demonstrated in Table 2, where a selection of sub-groups within Group 1 are presented. Any combination of standard reactors or lesions may be chosen, as desired.

Of the episodes between 1989 - 1994 for which fully matched data is available, 5,217 (10.5%) had four or more standard reactors, with at least one animal with a tuberculous lesion identified or confirmed. The fact that larger breakdowns are the exception is a reflection of the success of the eradication regime employed over past decades.

[1] The number of standard reactors may be less than the number of total reactors identified during the episode.

Table 2. Sub-groups of episodes within the Group 1 classification.

Total standard reactors	Total no. of cattle with tuberculous lesion(s)	Number of Episodes	% of Group 1 Episodes
4 or more	1 or more	4,415	39
4 or more	1	802	7
3	3	460	4
3	2	694	6
3	1	937	8
2	2 or more	241	2
2	2	1246	11
2	1	2532	23
		11,327	100

Group 1 Episodes

This group is the classical manifestation of a fulminating outbreak of tuberculosis in a herd of cattle. The criteria for this group are (i) a minimum of two animals positive to the standard interpretation of the SICTT and (ii) one or more animals with gross tuberculous lesions identified following gross post-mortem examination and/or where tuberculosis was laboratory confirmed. Overall, this group accounts for 11,327 (25%) of the matched episodes for the period 1989 - 1994. As a given herd may have had more than one episode, the 11,327 episodes reported occurred in a lesser number of herds during that period.

Group 2 Episodes

This group of herds represents a common manifestation of tuberculosis, where a comprehensive tuberculin test system is being applied. Tuberculosis has either been laboratory- confirmed, or a lesion has been found in one or more animals deemed "reactor" following a SICTT. A single animal only was positive to the standard interpretation of the SICTT during the episode involving these herds. There were 8,952 episodes (19.7%) which fulfilled the criteria for this group over the period, 1989 - 1994. The greater majority of these episodes (94%) had a single lesion identified/confirmed, with 576 episodes having a single animal test-positive to the standard interpretation and more than one animal with a tuberculous lesion. This evidence confirms the SICTT as an effective screening test.

Group 3 Episodes

While small, at 1532 episodes (3.4%) 1989 - 1994, this is an interesting and informative group. Details of sub-groups within the overall group are presented at Table 3. These are episodes where tuberculosis had been confirmed or a tuberculous lesion had been found in one or more animals and where no animals positive to the standard interpretation of the SICTT were identified. The majority of these episodes were initiated by the disclosure of a tuberculous lesion at slaughter in an animal from a herd considered to have been free of tuberculosis.

Table 3. Sub-groups of episodes within the Group 3 classification.

No. of Standard Reactors	Total no. of cattle with tuberculous lesion(s)	Number of Episodes	% of Group 3 Episodes
0	2 or more	24	1
0	2	105	7
0	1	1403	92
		1,532 episodes	100

All such lesions were sent to the Veterinary Research Laboratory at Abbotstown for confirmatory testing. If the specimen proved positive for tuberculosis, the herd is restricted and tuberculin tested. On average, 85% of such herds have no test reactors identified during the testing carried out over the remainder of the episode. These comprise the bulk of the episodes depicted in Table 3. The fact that 92% of these episodes are confined to a single animal with a confirmed lesion is further evidence supporting the SICTT as an effective screening test under Irish conditions.

Table 4. Subgroups of episodes within the Group 4 classification.

No. of Standard Reactors	Total no. of cattle with tuberculous lesions.	Number of Episodes.	% of Group 4 Episodes
4 or more	0	323	22
4	0	318	21
3	0	856	57
		1497	100

Group 4 Episodes

This is again a small grouping, comprising 1,497 (3.3%) episodes 1989 - 1994, but is also an interesting group. Sub-groups are presented at Table 4. A concern with this group is that intercurrent infection with agents other than *M. bovis* may account for the skin reactivity of animals at the SICTT. Earlier work showed that there were other mycobacterial organisms associated with sphagnum mosses, e.g. in counties Clare and Donegal, which could have resulted in cross reactivity with the SICTT in animals in those areas. It is likely that there are such agents, notably *Mycobacterium hiberniae* (Monaghan et al. 1991), which are capable of causing reactivity in other geographic areas also. This group of episodes warrants a special investigative programme, the objective being to establish or exclude the presence of tuberculosis in these cattle populations.

Group 5 Episodes

This group includes some herds which qualify for the new policy on "singleton" herds introduced by the DAFF this year on an experimental basis. The group comprises 16,283 (35.9%) of episodes during the period 1989 - 1994, of which 13,467 (83%) had a single standard reactor animal identified.

Table 5. Sub-groups of episodes within the Group 5 classification

No. of Standard Reactors	Total no. of cattle with tuberculous lesions	Number of Episodes	% of Group 5 Episodes
2	0	2,816	17
1	0	13,467	83
		16,283	100

The accuracy of the tuberculin test is called into question in cases where a single animal is deemed "reactor" and (i) where tuberculosis is not subsequently confirmed or (ii) where a gross tuberculous lesion has not been found at post-mortem examination. Where such episodes occur in herds with prior clear testing histories in areas with historically low levels of tuberculosis, this concern is justified, in view of the relatively low sensitivity of routine post-mortem examination as conducted under commercial conditions (Collins and Hannan, 1994).

Group 6 Episodes

In common with Group 5 episodes, some herds within this group also qualify for the new DAFF policy in relation to "singleton" herds. This group comprises 5,794 episodes with (i) no standard reactor identified or (ii) where no tuberculosis-like lesion was identified at gross post-mortem examination of animals deemed "reactor" or was confirmed during follow-up testing at the Central Veterinary Laboratory, Abbotstown. A sizeable sub-group within this group, comprising 25% of the episodes, were attributed to inconclusive animals deemed "reactor" at an inconclusive retest.

Frequency of the breakdown episode groupings from 1989 to 1993

During the interval 1989 - 1993 there was a reduction in the number of breakdown episodes recorded from 9,764 in 1989 down to 6,683 in 1993. The reduction in the occurrence of Group 5 and 6 episodes was most pronounced (Fig.1). The occurrence of Group 1 and Group 2 episodes remained quite constant over the interval. The reduction in the overall numbers of breakdowns episodes was not uniformly represented between groups. This variation is due to a change in policy which resulted a raising of the cutpoint at which a herd was placed under movement control in 1991 from that which was applied previously.

Frequency of Group 1,2,5 and 6 Episodes during the period 1989 - 1993

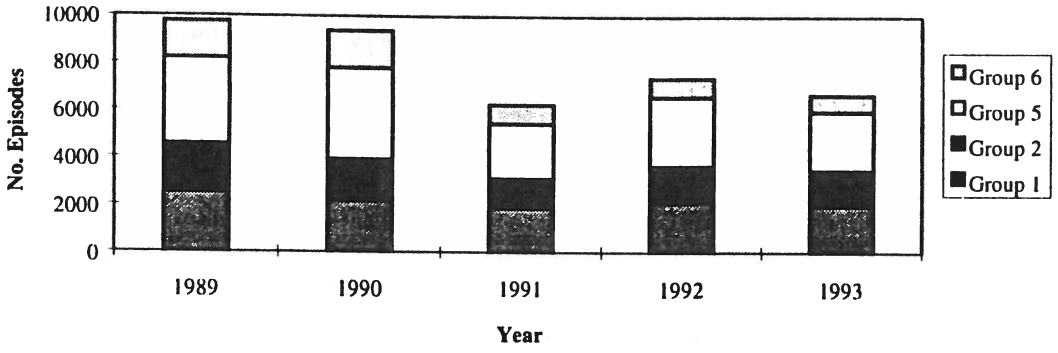

Figure 1 Details of breakdown episodes, 1989 - 1993.

Lesion Rate: 1989 - 1994

The preliminary analysis of the animal data was concerned with establishing the overall lesion rate for the period 1989 - 1994. It was also decided to segregate animals deemed "reactor" into groups[1] based on the severity of the interpretation applied with the single intradermal comparative tuberculin test (SICTT), as all animals deemed "reactor" are not directly comparable. It was important to establish a common baseline so as to ensure that comparisons between animals deemed "reactor" were meaningful. The initial two by two table of these groups is presented in Table 6.

Table 6. Lesion Rate by tuberculin reactor class, 1989 - 1994.

Reactor Class	No. of lesion negative animals	No. of lesion positive animals	Total no. of animals
Standard	77,980	52,821	130,801
%	59.6	40.4	*63.5*
Non-standard	62,885	12,303	75,188
%	83.6	16.4	*36.5*
Total	140,865	65,124	205,989
%	68.4	31.6	

The "basket" of reactors for the period, 1989-1994, was comprised of 63.5% of animals which were positive on standard interpretation, and 36.5% of which were deemed reactors on

[1] The groups chosen were Standard reactors and Non-Standard reactors. Standard reactors have a bovine minus avian skin difference of 5mm. or more to the SICTT (i.e. positive on standard interpretation).

non-standard interpretation. The lesion rate overall was 31.6%, and varied between 40.4% for the standard reactors and 16.4% for non-standard reactors.

Table 7. Reactor animals by year and type, including lesion rate.

Year	No of Standard reactors (lesion rate %)	No. of Non-standards (lesion rate %)	Overall totals (lesion rate %)
1989	24,412 (41.8)	14,470 (17.2)	38,882 (32.6)
1990	24,152 (36.7)	15,876 (14.4)	40,028 (27.8)
1991	21,538 (38.7)	14,561 (14.1)	36,099 (28.8)
1992	21,356 (40.1)	11,809 (16.0)	33,165 (31.5)
1993	19,967 (42.6)	9872 (19.0)	29,839 (34.8)
1994	19,376 (43.2)	8600 (19.9)	27,976 (36.0)
Total	130,801 (40.4)	75,188 (16.4)	205,989 (31.6)

The lesion rate, broken down by year into the standard and the non-standard reactor components, is presented in Table 7. The overall lesion rate varied between years, with the greatest variation proportionately in the non-standard component of animals deemed "reactor".

The positive relationship of a low lesion rate and a low proportion of standard reactors observed here makes intuitive sense, as the non-standard reactors, with a lesion rate less than for standards (16.4% compared to 40.4%), would be expected to reduce the overall lesion rate. Interpretation policy is, therefore, a key factor affecting the lesion rate. The lesion rate is dependent on the composition of the group of reactors on which it is based.

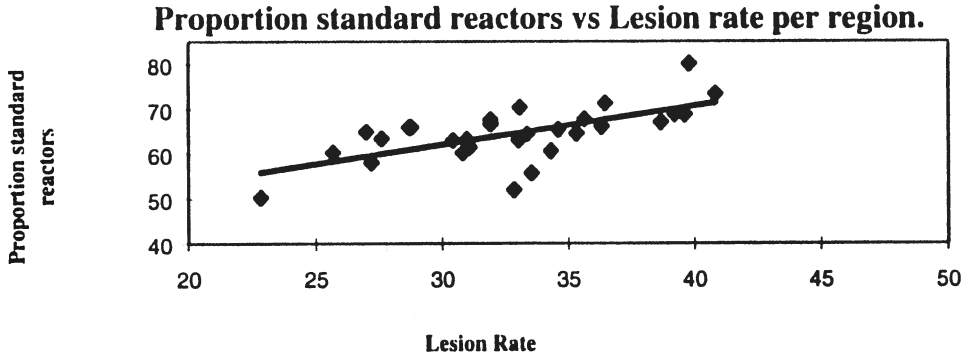

Figure 2. Proportion standard reactors vs Lesion rate for each of the 27 DVO areas, 1989 - 94.

There is a high correlation ($r = 0.65$; $p > 0.0001$) between the lesion rate and the proportion of standard positives in the overall "reactor" mix (Figure 2). There are differences evident in the mode of interpretation used and, consequently, in the proportion of standard reactors, as well as in the lesion rate between counties. The more serious the disease situation encountered

locally, the more severe is the interpretation mode of the SICTT applied, leading to the consequences described above.

The strong relationship between the interpretation policy and lesion rate was examined further by sub-dividing all the animals deemed "reactor" over the period, 1989-1994 into groups based on each individual animal's recorded "skin measurement difference" to the SICTT. The "skin measurement difference" was calculated using the following formula:

The skin increase at the bovine injection site minus the avian increase at the avian site,where the skin increase at the bovine injection site equals the 72hr. skin measurement at the bovine tuberculin injection site minus the initial skin measurement at the bovine site, and where the avian increase at the avian site equals the 72hr. skin measurement at the avian tuberculin site minus the initial skin measurement at the avian site.

In this case no account is taken of the clinical nature of the response to tuberculin at either site.

These results are presented at Table 8.

Table 8. The lesion rate in groups of reactor cattle, as defined by their "skin measurement difference".

Skin difference (mm)	Lesion rate %
Less than 0	13
0	11
1 - 2	14
3 - 4	20
5 - 9	30
10 - 19	46
20 - 39	56
40 or more	61

There was a strong relationship between the recorded skin measurement difference for animals deemed "reactor" and the lesion rate. This relationship demonstrates that the lesion rate *per se* is a secondary effect, and a reflection of the recorded skin measurement difference of animals deemed "reactor". The skin measurement difference indicates an animal's immunologic response to an intradermal injection of tuberculin. A tuberculin-responsive animal is presumed to have been been previously sensitised to tuberculin by being infected with *M. bovis*. This relationship suggests that the lesion rate might be highly predictable, using an appropriate confidence interval around the measured skin difference for a majority of animals. This relationship may be relevant to the quality assessment of tuberculin testing procedures and of in-plant post-mortem examination procedures.

DISCUSSION

The concept of "episode" is an important one in relation to our understanding of tuberculosis in the Irish context. The close relationship that exists between lesion rate and the skin responsiveness of animals to the SICTT has potential as the basis for extending the classification of breakdowns beyond the six groupings used in this study.

The classical disease, as represented here by Group 1 episodes, is manifest in only 25% of total episodes. This is not to say that the problem of tuberculosis in this country is limited to this group. There is to some degree a tuberculosis problem in each of the six groups, with Group 6 episodes gives rise to the smallest proportion of problems attributable to *M. bovis* infection. The clear segregation between Groups 1 and 2 on the one hand and Groups 5 and 6 episodes on the other, delineates two important herd groupings within our national herd.

The impact of strategies aimed at eradicating tuberculosis in cattle should be evaluated independently for their effect in each of these groupings. Successful strategies should be defined as those leading to a demonstrable reduction in Group 1 and 2 episodes over time, without adding to the Group 5 and 6 "false positive" episodes. Episodes in Groups 5 and 6 mask a high level of artifactual components dispersed among the true disease-related component. The artifactual effects are in effect the false-positive element of animals deemed "reactor" and lead, as a consequence, to a proportion of herds being mis-classified as tuberculosis-positive. Successful strategies should deal effectively with such artefacts.

The EU legislation relating to tuberculosis in cattle requires animals that are twice inconclusive to the SICTT be deemed "reactor", irrespective of the outcome of the results of epidemiological investigations of the herd. This rigid policy should be reviewed because, at present, it results in an excessive degree of "overkill" in some areas of the country, and leads to unwarranted restrictions in some cases. If an inconclusive animal is (1) home bred, (2) in a herd with a clear testing history and (3) in an area with low levels of tuberculosis among herds, then it is very unlikely that the observed reactivity to the test is solely attributable to a response to infection with *M. bovis*.

CONCLUSIONS

The database described here provides a detailed and comprehensive classification system which can be applied to herds which are actively infected with *M. bovis*. The validity of the system can be further evaluated, using hypothesis testing. Once validated, the system can be used to estimate likely future outcomes of policy options in the national bovine tuberculosis testing program.

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WILDLIFE

GEOGRAPHIC INFORMATION SYSTEM-AIDED ANALYSIS OF FACTORS POTENTIALLY INFLUENCING THE SPATIAL DISTRIBUTION OF *ECHINOCOCCUS MULTILOCULARIS* INFECTIONS OF FOXES

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Alveolar echinococcosis, caused by the larval stage of the cestode *Echinococcus multilocularis*, is considered as the most dangereous autochthonous parasitic zoonosis in Central Europe (World Health Organization, 1990). The obligate two-host parasitic cycle of *E. multilocularis* is predominantly silvatic in Central Europe, where the red fox represents the main definitive host with prevalences of more than 60% in some regions (Lucius & Bilger, 1995). *E. multilocularis* infections of dogs and cats are rare (Worbes, 1992) even in areas where the infection occurs in foxes. Different species of rodents are involved in the parasitic cycle as intermediate hosts. They get infected by oral uptake of oncospheres ("eggs") which the definitive hosts shed with their faeces after a prepatent period of nearly four weeks. Human alveolar echinoccosis represents a rare disease with an estimated average annual incidence of 0.02-1.4 per 100,000 inhabitants for the Central European endemic area (Eckert, 1996).

A previous study has shown heterogenous spatial distribution patterns of *E. multilocularis* in an endemic area in northwestern Brandenburg, Germany (Tackmann et al., 1998). The municipalities could be distinguished by prevalence estimation in a high endemic area with two foci (23.8% prevalence) and a low endemic area with a prevalence of 4.9%, respectively.

Since elevated temperature and dessication are known to reduce the infectivity of the oncospheres (Frank, 1989; Veit et al., 1995), microclimate and habitat can be suspected as factors potentially influencing spatial heterogeneity. Geological and climatic factors as well as vegetation types seem to be of utmost importance for the survival of the oncospheres and may thus represent key factors for the parasitic cycle and the distribution patterns of the infection.

Geographic Information Systems (GIS) are computer-based tools that can rapidly combine and analyse areal data, providing insight into complex spatial phenomena at multiple scales of resolution. They include spatial data in the form of geographical coverages (maps) and descriptive data in the form of a relational database associated with the mapped features (Paterson, 1995). The GIS was used to associate the dispersion pattern of *E. multilocularis* with

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a large number of physical, biological and demographic factors.

MATERIAL AND METHODS

Sampling

To determine the distribution of *E. multilocularis* in two rural counties in the Northwest of the Federal State Brandenburg, 3,797 foxes were sampled between 1995 and 1997. The study area is situated between 11.5°-13.0°N and 52.5°-53.5°E and covers approximately 4,450 km^2. The sex of the hunted foxes was recorded and the animals parasitologically examined for infections with *E. multilocularis* (Tackmann et al., 1998). Hunters were asked to mark the location where each fox was shot in a topographical map (scale 1:50,000). This information, as well as the date when the animal was shot and the laboratory results were recorded with the help of a programm written in CLIPPER (Computer Associates International Inc., New York, USA) in a DBASE (5.0 for WINDOWS, Borland International Inc., Scotts Valley, CA, USA) file.

Geographical data

The geographical positions of the hunted foxes were digitized on screen as point coverages with the Geographical Information System tool ArcView (ESRI, Redlands, USA). A relational database associated with the fox positions was generated.

Vectorised data of lakes, rivers, settlements, streets, forests, elevation contours and elevation points of the Geological Survey of the Federal State Brandenburg (TK 50; reference map scale 1:50,000; GB-D 27/94; May 1995) were used to describe the topography of the landscape. A Digital Terrain Model (DTM) of the area was generated in ARC/INFO (ESRI, Redlands, USA) from the vectorized contour lines and elevation points.

To identify different land-use classes of the positions of the hunted foxes with a higher resolution vector data of the CORINE Land Cover (CLC) project were used (European Communities-Commision; 1992). Computerised thematic maps on 44 different land cover categories (e.g. coniferous forest, heath, pasture, crop irrigated, swamp, town) were generated for Germany on the basis of satellite images of Landsat TM and KFA 1000, topographical maps (TK 50 and TK 100) and panchromatic aerial photos. The minimal digitized land cover unit had to cover an area of 25 hectares. For our study the CLC-data categorization were spatially simplified in four classes (urban/water, crop, pasture and forest) in ARC/INFO.

To identify soil moisture variation a georeferenced Landsat 5 Thematic Mapper (TM) satellite image (185 x 175 km) with 25 m resolution recorded on 6 October 1994, was obtained from the German Remote Sensing Data Center (DFD). We assume that the handling of wetlands did not change in recent years in the investigation area. The Kauth-Thomas tasseled cap transformation of a satellite scene was used to separate the spectral data variation into brightness, greenness, haze and wet data structures (Crist & Kauth, 1986; Lillesand & Kiefer, 1994). To elimimate false classified canopy of trees (Lillesand & Kiefer, 1994), lakes, rivers and small waters like fish farms in the wetness image these pixels were erased automatically by overlay analysis of the vector data (TK 50) or corrected by hand in the image processing software ERDAS Imagine (Egham, UK) or the Spatial Analyst Module of ArcView. The reclassified five wetness categories (very dry to wetland) were verified by local knowledge of colleagues, field examination and slope data derived from the Digital Terrain Model to identify sinks, bassins and flat areas. To minimise errors in overlay or spatial analysis we projected all the vectorized datasets to the Landsat TM projection (Universal Transverse mercator, zone 32).

Spatial and statistical data analysis

The digitized fox positions and the vectorized data of the Geological Survey of Brandenburg were used to calculate the relative position of the foxes to the different topographical categories. For each infested or non-infested fox we calculated the distance to the nearest line feature in the coverages rivers and streets or to the next border line in the polygon coverages lakes, settlements and forests. The search radius for this algorithm was restricted to the average diameter of the home range of a fox which was assumed as 5 km (Tackmann et al., 1998). Furthermore, overlay analysis was used to identify the different land-use categories (CLC-data) and the wetness index for the positions of the hunted foxes. A GIS function was used to quantify the landscape proportions surrounding each hunted fox. We generated a buffer zone extending 2.5 km from the position of the foxes. These buffers were then intersected with the CLC landscape map to calculate the land cover proportions surrounding each animal. In addition, the land cover proportions for the buffer zones of 25,000 randomly generated geographical positions were calculated. Raster- and vector-based analysis were carried out in the Spatial Analyst Module of ArcView or ARC/INFO.

The resulting datasets of the spatial databases was imported into Statistica 5.1 (StatSoft, Inc., Tulsa, OK, USA). The distributions of the distances to the different topographical categories of infected foxes were compared with those of non-infected foxes applying Kolmogorov-Smirnov two-sample test. The analysis of the intersection of fox positions with the CLC-data and wetness raster data were carried out for different fox groups (e.g. by infection status or sex) using cross-tabulation tables, which were evaluated using the Pearson chi-square statistic.

RESULTS

In the investigation period of 40 months, *Echinococcus multilocularis* was detected in 83 out of 3,521 foxes investigated parasitologically (2.4%; Table 1). More male than female foxes (1.2 : 1) were shot by the hunters. Nevertheless, the ratio between infected and non-infected foxes (1.034 : 1) is nearly equal for males and females ($\chi^2 = 0.02$, $P > 0.1$).

Table 2 shows the mean and median distances of the hunted foxes to the different topographical categories. When the distributions of distances to the examined topographical categories for *E. multilocularis*-infected and non-infected foxes were compared, only the category "rivers" showed significant differences (Kolmogorov-Smirnov two-sample test, 99%).

We also tested whether different land cover classes (crop, pasture and forest) and wetness categories were associated with *E. multilocularis* infections. Screening these landscape classes we found significant prevalence differences ($\chi^2_{CLC} = 21.24$, $P = 0.00002$). The prevalence of *E. multilocularis*-infected foxes on pasture was higher than the prevalence in any other land cover category (2.3%; 4.4%; 0.6%). The prevalence differences between the different wetness categories were statistically significant ($\chi^2_{WET} = 10.52$, $P = 0.032$). By contrast, the sex distribution produced no significant prevalence difference ($\chi^2_{CLC} = 0.75$, $\chi^2_{WET} = 1.14$, $P > 0.1$) between the tested factors.

In addition, we quantified the landscape proportions in a buffer zone surrounding each fox with a diameter of 5 km. Comparing the landscape proportions for *E. multilocularis*-infected and non-infected foxes we found high differences in terms of pasture and forest (Fig. 1). The area proportions of the negative foxes reflect nearly the proportions of the randomly generated buffer zones and the absolute proportions of landscape classes in the investigation area.

Table 1. *Echinococcus multilocularis* infections and sex distribution of the hunted foxes (prevalence 2.4%)

Sex	*E.m.*-negative	*E.m.*-positive	Total
Female	1561	37	1598
Male	1877	46	1923
Total	3438	83	3521

Table 2. Distributions of the distances to the different topographical categories of *E. multilocularis*-infected or non-infected foxes

Landscape classes	Total examined[a]	No. infected (%)	Mean distance [m]		Median distance [m]		P-value[b]
			infected	non-infected	infected	non-infected	
Rivers	3360	83 (2.4)	239	341	149	237	0.0062
Lakes	2086	54 (2.6)	630	671	645	685	$p > 0.10$
Forests	2819	70 (2.5)	402	332	279	194	$p > 0.10$
Villages	3293	78 (2.4)	545	579	512	549	$p > 0.10$
Streets	3384	80 (2.4)	586	523	532	465	$p > 0.10$

[a]Differences in the sample size results from the limited search radius
[b]Kolmogorov-Smirnov two-sample test

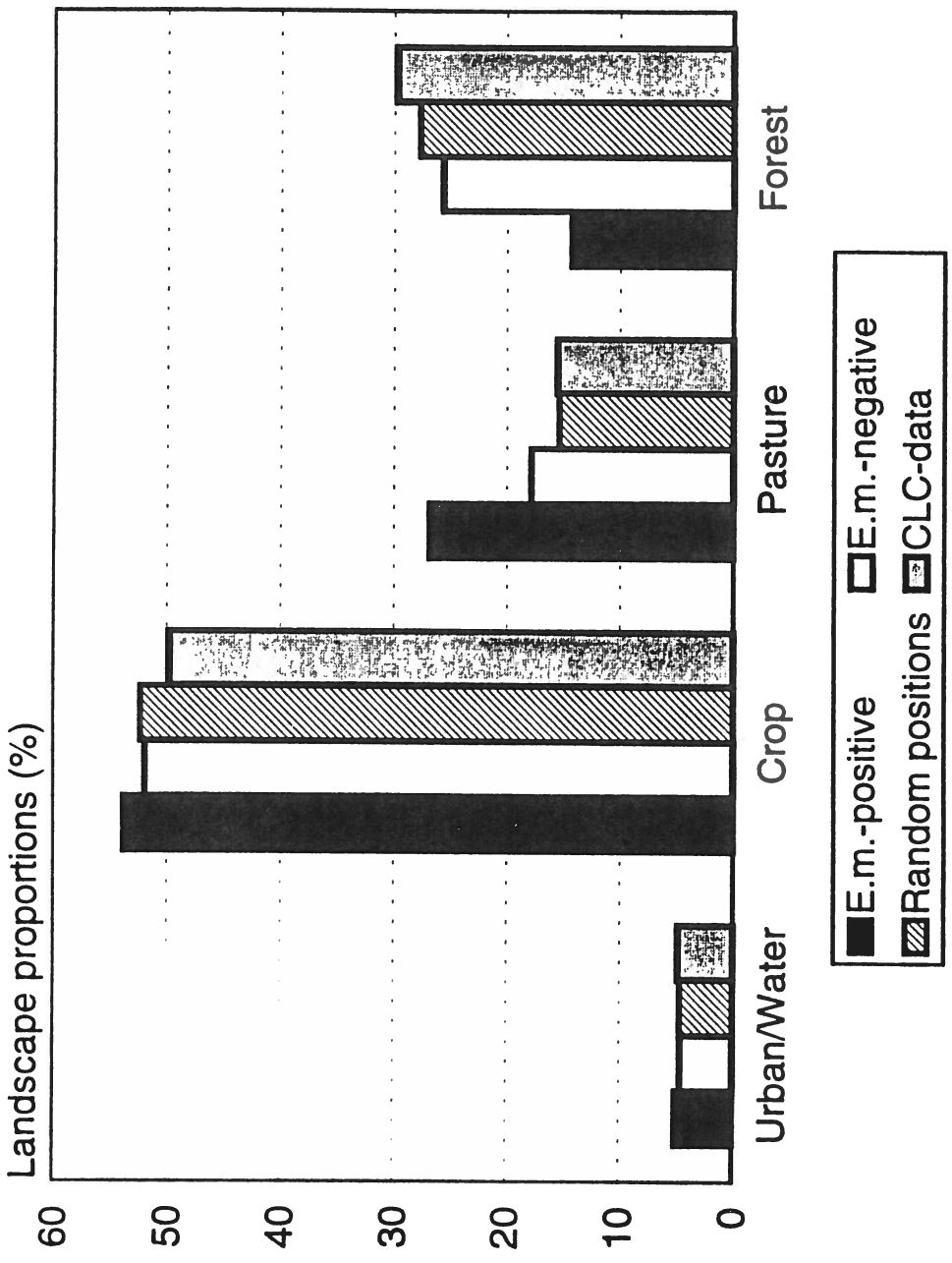

Fig. 1 Landscape proportions surrounding each hunted fox and the CLC area data

DISCUSSION

A previous study in the northwestern part of Brandenburg shows the existence of heterogeneous spatial distribution patterns of *E. multilocularis* on the scale of municipalities. The decreasing risk of infection around an anchore point in the endemic foci could be verified by repetition of the calculations with the precise coordinates of the hunted foxes (Tackmann et al., 1998). Heterogeneous spatial distribution patterns of intermediate and definitive hosts appear to be a particular epidemiological feature of the parasite observed by several investigators (Kritsky & Leiby, 1972; Deutz et al., 1995; Eckert et al., 1991).

Deteriorating environmental conditions could have a limiting influence on the parasitic cycle. It is known that the tenacity of the parasite oncospheres outside the body is limited by elevated temperature or aridity (Frank, 1989; Veit et al., 1995). Studies conducted in France also propose the influence of vegetation types, geological and climatic factors on the spatial distribution of the parasite (Aubert et al., 1987; Gilot et al., 1988).

Geographic information systems and remote sensing have demonstrated potential applications in a number of public health problems, e.g. malaria, trypanosomiasis, trematodes, ticks or Lyme disease (Hugh-Jones, 1989; Arámbulo III & Astudillo, 1991). GIS and remote sensing data can describe spatially a large number of geographical factors. Overlay analysis allow us to associate the distribution patterns of the disease vector with these environmental conditions (Kitron et al., 1991; Nicholson & Mather, 1996).

LANDSAT Thematic-mapper (TM) imagery is ideal for characterising the status of wetland environments. Because of the high spatial resolution of the nonthermal bands, and the inclusion of the middle infrared bands 5 or 7, the images can be even more useful than the data of other high-resolution satellite sensors such as the SPOT multispectral scanner (Pope et al., 1994; Washino & Wood, 1994). The tasseled cap transformation allows to identify rapidly vegetation and soil components in the spectral data. But this unsupervised generation of data should be interpreted carefully and data should be always verified by ancillary data or field investigations (Lillesand & Kiefer, 1994). For this reason and to identify different land-use classes with a higher resolution, we shall classify the Landsat TM image for the investigation area. Also other geostatistical methods which also consider spatial autocorrelation will be adressed in further studies (Kitron et al., 1992).

For the first time, conditions favouring (open landscape with higher humidity) or limiting (larger coherent forest regions) the life cycle of the parasite in this region were verified using field data. Several factors may be associated with occurence of *E. multilocularis*. These include the presence of pasture or low soil humidity and the proximity to rivers. Since these three factors are often associated, their effects are difficult to separate at this point.

The integration of GIS and remote sensing into epidemiological studies allow us to unterstand and model more realistically the spatial and temporal structure of the parasite-host systems. Further understanding of the association of ground measures with spectral data will assist in better interpretation and possible extrapolation of our results. GIS also permit to proof and develop new hypotheses regarding factors affecting the spread of the disease. The description of the spatial distribution pattern of infected foxes plays especially in newly recognized endemic regions an important role. The data collected in the GIS may prove valuable for the study and management of several other diseases as rabies or Lyme disease.

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THE EPIDEMIOLOGY OF BAT RABIES IN THE UNITED STATES: WITH EMPHASIS ON *LASIONYCTERIS NOCTIVAGANS*, THE SILVER-HAIRED BAT

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For almost 45 years, the prevalence of rabies has been on the increase in bats; whether this is a real increase or the result of bias due to greater interest in these fascinating creatures and/or better reporting systems is unclear.

The first report of a rabid bat in the United States occurred in June, 1953 in the Tampa Bay, Florida area where a yellow bat, *Lasiurus intermedias*, bit a young boy (Scatterday & Sutan, 1954). That same year, two additional bats were found positive, one each in Pennsylvania and Texas (Baer & Smith, 1991). A survey of bats later that year in Florida revealed 8 of 384 (2.1%) apparently normal bats were rabid (Venters, *et al.*, 1954). Since these early reports, indigenous bat rabies has been reported from 49 states in at least 37 of various insectivorous species in North America (Brass, 1994; Constantine, 1979); only Hawaii has not reported indigenous cases of rabies in bats (Krebs *et al.*, 1996). From 1973 through 1992, the average annual number of rabid bats reported by the Centers for Disease Control and Prevention (CDC) has been 712. California and Texas reported the most cases with an annual average of 135 and 77 respectively. Depending on the year, bats accounted for 8.2% (1992) to 27.1% (1976) of all wildlife rabies reports in the United States (CDC, 1973-1994). In general, 1-3% of normally appearing migratory bat species (primarily *Taderida* sp.) and from 2-4% of non-migratory bat species have been diagnosed with rabies (Girard *et al.*, 1965; Brass, 1994; Tramarchi & Debbie, 1977; Childs *et al.*, 1994).

Few recent reports are available which summarize regional or local information concerning bat rabies in the United States. A 5 year review of laboratory submissions from 1988 to 1992 in New York state found 4.6% of 6810 bats to be positive for rabies, with strong seasonal components (Childs, *et al*, 1994). The big brown bat, *Eptesicus fuscus*, comprised 62% of all bat species identified with 6.3% of these being rabies positive. In a 13 year review of 246 bats submitted to Michigan laboratories from 1981 to 1993, all the bats submitted were big brown bats, of which 6.2% were found to be rabid (Feller *et al.*, 1997). From 1965 to 1988, 2,433 bats were submitted to the Illinois laboratories for rabies testing and species identification, 6% of which were positive for rabies (Burnett, 1989). The hoary bat, *Lasiurus cinereus*, with an 11% positive rate was the species with the highest for prevalence of rabies. We recently reviewed nearly 3000 records of bat submissions in 9 southeastern states for the 5 year period 1990 to 1994. We identified 333, or 11.3 % of the submissions to be positive for rabies. Of these 9 states, only 3 identified bats by species, Virginia, West Virginia, and Arkansas. Again, *E. fuscus*, the big brown, was the species most often submitted, comprising 43% of the 567 submissions, and having a rabies positive rate of 10.2%.

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Antigenic typing with monoclonal antibodies (MAb) and genetic analysis have demonstrated that distinct rabies virus variants are found within the various species of bats in North America (Rupprecht *et al.*, 1987; Smith, 1988; Smith, *et al*, 1995; Smith, 1996). These variants differ considerably from those found in terrestrial animals. Rabies virus variants in migratory species are not confined to a given geographic area but are found throughout the migratory range of a given bat species. Within limited geographic regions, multiple rabies variants may be found in resident non-migratory species (Smith, 1988). Although transmission is generally intra-species, other wildlife and domestic animals have been occasionally infected with bat rabies variants (Baer & Smith, 1991). For example, in 1993 and 1994, rabies was reported from 4 foxes in Oregon, a state free of terrestrial rabies for decades (Smith *et al.*, 1995). In all 4 cases, the rabies virus variant was nearly identical to the variant found in western *Myotis* bats.

Of particular concern is the increasing number of recent human rabies deaths due to rabies virus variants associated with bats. Of 36 humans who succumbed to rabies in th 17 year period 1981 through 1997 in the U.S., 21 (58%) were associated with bat rabies virus variants, 14 were associated with canine variants (12 of these from exposures occurring outside the U.S.), and 1 was associated with a skunk variant (Krebs *et al*, 1997). Twenty-one of the 36 (58%) deaths occurred in just the last 5 years, 1993-1997. Based on antigenic typing and genetic analysis, 15 of the 21 bat-related cases (71%) were associated with the variant commonly found in silver-haired bats, *Lasionycteris noctivagans* and Eastern pipistrelle bats, *Pipistrellus subflavus* (referred to as the silver-haired bat variant, the variant has only been found in but a few Eastern pipistrelle bats), 3 were associated with Mexican free-tail bats, *Taderida braziliensis*, 2 associated with *Myotis* sp. bats, and 1 death was due to the rabies virus variant commonly found in the big brown bat, *Eptesicus fuscus* (Krebs *et al.*, 1997; Krebs, personal communication). Of these 21 bat associated human deaths, only one was as a result of a documented bat bite. However, at least 8 of the other 20 individuals did have a history of some contact with a bat (Childs *et al.*, 1997).

The silver-haired bat, *Lasionycteris noctivagans*, appears to be the enigma of bat rabies epidemiology in the United States. The silver-haired bat is an uncommon solitary dwelling bat, widely distributed in the U.S. and the southern portions of Canada (Tuttle, 1988; Kunz, 1982). They also have been reported in the Bahamas, Bermuda and northern Mexico, immediately south of the Texas border (Tuttle, 1988; Hall, 1981; Nowak & Paradiso, 1983). Their migratory habits are not well known, but they seem to shift their range northward in the spring and southward in the autumn (Nowak & Paradiso, 1983). They were reported to be the most common bat in upper New York state in the 1880s and into the early 1900s. However, in the review of bats submitted for rabies testing in New York from 1988 to 1992, silver-haired bats comprised only 0.35% of submissions (25/7047); 2 of the 25 (8%) were reported as rabid (Childs, *et al.*, 1994). Eight of 210 (3.75%) silver-haired bats submitted to the Illinois laboratories for the period 1965 to 1988 were positive for rabies (Burnett, 1989). These 210 submissions were 9% of all bats submitted, and identified to species, for the 21 year period reviewed. In our review, only 6 of 567 bats (1.1%) submitted to the state labs in Arkansas, Virginia, and West Virginia were the silver-haired species; none of which were positive for rabies.

The silver-haired bat has a cryptic coloration, usually black with silver-tinged hair on the back, though older individuals may lose the silver appearing hairs to develop a yellowish tint (Hall, 1981; Kunz, 1982; Tuttle, 1988). Relatively small in size compared to other insectivorous bats with a body length 92 to 115 mm in length, a tail 35 - 45 mm in length, a wingspan of 270-310 mm and a body mass of 8 - 11 grams (Kunz, 1982; Tuttle, 1988). *L. noctivagans* is a late flyer, usually appearing after other species have begun feeding, usually 1 1/2 to 3 hours after sunset, although reports vary (Reith, 1980; Kunz, 1982). These bats usually live primarily in old-growth forested areas, often found in crevices or cavities (loose tree bark or hollows in pine and fir trees or various hard-wood species such as willows and maples, piles of lumber, woodpecker holes, and beneath rocks), and is seldom seen in buildings except, occasionally, when hibernating in the winter months (Hall, 1981; Kunz, 1982; Nowak & Paradiso, 1983; Tuttle, 1988) They prefer large short-leaf pine trees, or similar pines with loose bark (Saugey *et al.*, 1996). On occasion, very small groups have been found, primarily during hibernation and migration, or in nurseries (Hall, 1981; Parsons *et al.*, 1986). The mean age of silver-haired bats has been reported as 2 years of age, ranging to 12 years; females generally have one or two pups; average of 1.7 volant young (Kunz, 1982).

The first report of a death associated with a silver-haired bat occurred in 1958 in California (Humphrey, 1960). A woman bitten on the finger August 2 received post-exposure treatment from September 2 - 26, but died on November 4th.

The small size, secretive behavior, and cryptic coloration may account for the observation that silver-haired bats are seldom reported in contact with humans; bites could actually occur, but be unrecognized and accepted as an insect sting or thorn puncture, or other laceration. Recent studies have indicated that the silver-haired bat variant of the rabies virus may possess a unique ability to replicate at lower temperatures than variants isolated from some terrestrial animals (Morimoto *et al.*, 1996). This may allow the small amount of the virus introduced by an insectivorous bat to amplify at the inoculation site, the surface of the body, enhancing the probability of penetrating a nerve fiber and eliciting successful transmission. As noted by Childs *et al.* (1994), *L. noctivagans* is not commonly submitted to laboratories for rabies examination. This was confirmed in our review as we identified only 6 silver-haired bats submitted for the 5-year period in the states of AR, VA, and WV; none of which were rabid.

The 11.3% overall prevalence of bat rabies we found in the southeastern states is considerably more than the prevalence reported for the reviews reported by Burnett (1989), Childs *et al.* (1994), and Feller *et al.* (1997); 6.0%, 4.6%, and 6.2% respectively . There were at least 1150 reports of human contacts with bats of the 3159 total case reports we reviewed (36.4%) from the southeastern states; 131 (11.4%) involved a rabid bat. When animal contacts were included, over 73% of the case reports involved an exposure of some kind. In the New York state report by Childs et al. (1994), 12% of the bats had contact of some kind with humans or domestic animals. Slightly over 50% of the rabid bats in Michigan had a history of exposure to humans (Feller *et al.*, 1997). Most state diagnostic laboratories in the Southeast require human or animal exposure criteria prior to specimen submission. This is also true in Michigan according to Feller *et al.* (1997). Thus, the relatively high prevalence of rabies reported in bats from the southeastern states may reflect a bias in case submissions. This is not necessarily true in New York or in a few other states that may have less stringent submission criteria. Generally, as stated earlier, about 1-4%, or less, of randomly sampled bats are rabid.

Other factors contribute to bias in the submission of certain bat species. For instance, our review of the data from AR, VA, and WV, showed that solitary bat species accounted for 31.6% of submissions, but had more than twice the prevalence of rabies positives (20.1%) than did the colonial species. The colonial bats accounted for 68.4% of submissions, but only 8.2% were reported to be rabid. Others have reported similar solitary bat:colonial bat submission ratios (Girard *et al.*, 1965; Trimarchi & Debbie, 1977; Baer & Smith, 1991). One would expect that the social behavior of colonial bats would allow for greater opportunities for the transmission of the virus.

The big brown bat, *E. fuscus*, accounted for the majority of submissions in the Michigan, Illinois, the 3 southeastern states in our study, as well as a number of other states (Burnett, 1989; Feller *et al.*, 1997). The big brown bat does not migrate and remains active to late in the fall or early winter, hibernating from November to March (Tuttle, 1988). Due to their large size, they can easily inflict a significant bite wound when handled carelessly. This species is one of the larger insectivorous bats, living in small colonies of a dozen or more, usually in or around buildings year around (Tuttle, 1988). These characteristics make them readily noticeable and often in contact with humans and/or other animals. The prevalence of rabies in the hoary bats, *L. cinereus*, was higher in our review, and in the Illinois report (Burnett, 1989), than other identified species. The hoary bat is one of the most widely distributed, and one of the largest bats in North America (Tuttle, 1988). However, they rarely live in buildings and are seldom encountered. This may be a reason why, when encountered, they are most apt to be found with abnormal behavior and thus are more likely to be diagnosed rabid.

Most bat submissions to state laboratories occur in early summer when migratory bats are returning from Mexico and Central America and hibernating resident species become most active. During this time, the young are born requiring the adult lactating females to forage actively. However, the peak for the greatest percent of rabid bats is in late summer when the young, born in May and June, begin to fly (Constantine *et al.*, 1968; Pool & Hacker, 1982; Baer & Smith, 1991; Childs *et al.*, 1994;).

In our review of bats submitted for rabies testing in the southeastern states,11% of which were positive for rabies, we found that where the bat was located at the time it was found or captured was usually only given in vague detail: residential areas, 624 (63 rabid); yards, 262 (33 rabid); buildings, 388 (29 rabid); commercial buildings, 42 (9 rabid); and water, 11 (2 rabid). Of 102 bats where information on location was specifically stated, the majority were found in, or near, or otherwise associated with a "school" (n=43); two of these 43 were rabid.

It is of obvious public health concern when the odds may rise to one in 10 of exposure to rabies virus when a person handles a bat that may be acting in an abnormal manner. Considerable current interest exists in back yard bat houses to attract bats. Granted, bats consume large quantities of insects, serving as a natural, non-toxic insecticide, and thus contributing significantly to the environment. Despite their clear ecological benefits, the public should be cautioned about handling bats, especially those that cannot fly or otherwise appear sick. Approximately half of the bats submitted for laboratory examination for rabies in our review of bats in the southeast (that had adequate information on the submission form) were considered as abnormal, either "sick", "aggressive", "lethargic", or "not flying normally". Domestic animals, especially cats, may also be at increased risk when they catch, play with, or eat a rabid bat (Hoff *et al.*, 1993). We found that 54 of 634 bats (8.5%) in contact with cats were

rabid as were 46 of 304 (15.1%) in contact with dogs. This emphasizes the very great need for companion animal rabies vaccinations.

A recent recommendation from the Advisory Committee on Immunization Practices of the U. S. Public Health Service (CDC, 1996) specific to bats states: "... PEP [post-exposure treatment] is also appropriate even in the absence of a demonstrable bite or scratch, in situations in which there is reasonable probability that such contact occurred (e.g., a sleeping individual awakes to find a bat in the room, an adult witnesses a bat in the room with a previously unattended child, mentally challenged person, intoxicated individual, etc.").

Bats are wonderful beneficial companions in our total environment but, like most other creatures, when acting in an abnormal manner, they must be considered as a potential health risk.

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STATISTICAL METHODS

LOGISTIC REGRESSION ANALYSIS IN OBSERVATIONAL STUDIES — POSSIBLE PITFALLS AND PRESENTATION OF RESULTS

G.J. ROWLANDS*

With the increased analytical capacity of computers and availability of advanced and sophisticated statistical computer software there has been a rapid increase in the use of logistic regression in veterinary epidemiology. At the recent International Symposium of Veterinary Epidemiology and Economics (ISVEE, 1997) there were more than 36 oral presentations which described analysis of results based on logistic regression. This was more than double that at the previous meeting (ISVEE, 1994). The central thesis in a paper by McDermott (1995) is the need to be more critical of the statistical methods used in veterinary epidemiology. It seems appropriate, therefore, to study the suitability of the methods currently being used in and the statistical inferences being drawn from the analysis of data by logistic regression. Before doing so, however, some theoretical aspects of logistical regression that will be discussed further in relation to these 36 papers will be described.

THEORETICAL ASPECTS

Linear logistic regression is used to analyse data made up of either proportions y = r/n, where r is the number of 'successful' outcomes in n observations, or binary values (0,1) where 1 indicates a 'successful' outcome and 0 not. Log-linear models are also sometimes applied (Rowlands and Booth, 1989). In veterinary epidemiology, of course, a 'successful' outcome may typically be death or disease. In either case the logistic transformation of the proportion or the binary value is modelled by a linear combination of explanatory variables. Assuming, for example, that three explanatory variables

$$s_i, i=1...m_i;\ t_j, j=1...m_j;\ u_k, k=1...m_k, \text{ where } m_i + m_j + m_k - 2 = p$$

are defined, the model for y = r/n can be written:

$$\text{logit}\,(y_{ijk}) = \log_e[y_{ijk}/(1-y_{ijk})] = \mu + s_i + t_j + u_k + e_{ijk}$$

where the y_{ijk} are the cell contents of a 3-way table defined by s, t and u and the e_{ijk} are the residuals, assumed to be binomially distributed.

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If the model is to provide a satisfactory fit to the observed proportions then the random residual variation must model that associated with a binomial distribution. In the logistic regression analysis of a model with N binomial proportions y_{ijk} and p independent parameters fitted as above the residual unexplained variation has an approximate X^2 distribution with (N-p) degrees of freedom. Assuming an underlying binomial distribution the expected value of a X^2 random variable with (N-p) degrees of freedom is (N-p). Thus, it follows that the residual variation (or deviance) for a model that fits the data well should be approximately equal to its number of degrees of freedom, or alternatively the mean deviance (equivalent to the residual mean square in an analysis of variance for normally distributed data as described by Rowlands and Booth (1989)) should be close to 1. If the model does not adequately describe the data, or if the variation in the data is greater than that under binomial sampling, the residual mean deviance is likely to be greater than 1. As the number of parameters in the model is increased, so that the mean number of observations n per cell is decreased, the residual deviance approximates less and less to a X^2 distribution, and for binary (0,1) data the approximation no longer holds. Whilst it is not possible to give any firm rule (Agresti, 1990) the approximation will tend to become poor once $\Sigma n/N$ falls below 5, particularly if the number of observations per cell is very variable. When the assumption of residual binomial variation may not be valid, as indicated by a mean deviance significantly greater than 1, the data are said to exhibit overdispersion or extra-binomial variation. Sometimes the residual mean deviance may be less than 1 and this may sometimes be due to fitting too many parameters included in the model. Martin (1997) recommended that the maximum number of parameters p to be used in a model should be less than $\Sigma r/10$.

A very readable book on the subject of logistic regression is that by Collett (1991) and he describes various ways of model checking including identifications of outliers which may contribute to overdispersion. For example, plots of residuals (representing the deviations of observations from their fitted values often expressed in the form of a 'standardised residual deviance'), either against observations put in some ranked order or against fitted values on the logistic scale, are often a good way of detecting outliers or an unsatisfactory fit of the model. If after a thorough analysis of possible causes of overdispersion the residual mean deviance remains greater than 1 then methods must be used to accommodate this overdispersion. The simplest method is to define a heterogeneity factor (equivalent to the extra-binomial variance) equal to the residual mean deviance (σ^2) and to multiply standard errors of regression parameter estimates by σ. This method is theoretically exact for the case when all n are equal but is fairly robust and can be used as a reasonable approximation when the n are not all equal. Where the n are very variable, however, an alternative quasi-likelihood procedure devised by Williams (1982) may be preferable.

Results can be expressed as regression coefficients on the logistic scale, or as odds ratios or expected (or adjusted) incidence values on the untransformed scale.

During statistical analysis one level of each explanatory variable, often the first, is usually set to zero. Thus, in the logistic model described earlier, $s_1 = 0$ and s_2, s_3 ... represent deviations from s_1. The expected values for the first two levels of s adjusted for levels of the other explanatory variables in the model are given by

$$\log_e[y_1/(1-y_1)] = \mu$$

$$\text{and } \log_e[y_2/(1-y_2)] = \mu + s_2$$

where, for simplicity, suffices of levels for the other two explanatory variables t and u are dropped.

Thus, by subtraction,

$$s_2 = \log_e[y_2/(1-y_2)] - \log_e[y_1/(1-y_1)]$$

$$= \log_e \frac{y_2/(1-y_2)}{y_1/(1-y_1)}$$

which is the logarithm of the well known odds ratio. The odds ratio itself is thus simply calculated as the exponent of the regression coefficient.

In a similar way it is possible to calculate

$$y_1 = e^{\mu}/(1+e^{\mu})$$

$$\text{and } y_2 = e^{\mu+s_2}/(1+e^{\mu+s_2})$$

which give expected proportions for levels 1 and 2 of s on the untransformed scale adjusted for levels of t and u.

Standard errors in statistical computer program outputs are usually given assuming a satisfactory binomial fit. If there is evidence of overdispersion then these should either be multiplied by σ or alternatively William's procedure applied. Ninety five confidence limits for the odds ratio derived from s_2 compared with s_1 are calculated as

$$\exp [s_2 \pm 1.96 \text{ x s.e.}(s_2)] \text{ for an assumed binomial fit}$$

$$\text{or } \exp [s_2 \pm t \text{ x } \sigma \text{ x s.e.}(s_2)] \text{ with overdispersion}$$

where t is the Student's t value with degrees of freedom associated with the residual deviance term.

Approximate standard errors can also be calculated for expected values of y_1 and y_2.

REVIEW OF ISVEE PAPERS

Observational unit

Seventeen of the 36 studies were based on data of individuals clustered within some form of grouping, e.g. herd. In four studies group was fitted as a random effect as described by McDermott and Schukken (1994), in five as a fixed effect and in eight the effect of group was ignored. In seven of these seventeen studies the observational unit was sometimes incorrectly defined. In three studies all explanatory variables described effects at the herd level which were common to all individuals in the herd and in four studies explanatory variables comprised a mixture of herd and individual effects. Thus, if overdispersion occurred at the herd level the statistical significance of the effects of these variables was likely to have been overestimated.

Ten of the remaining 19 studies were based on herd as the observational unit and nine on unclustered individuals. Many of these latter studies were associated with analysis of cases of disease from national databases. Some of the former studies included some form of herd grouping by region, for example, which was not always accommodated in the model.

In an analysis of 67 papers published in Preventive Veterinary Medicine in which individuals were clustered McDermott and Schukken (1994) found that 31 (46%) ignored clustering and predicted that changes in inference in 26 of these would result from too small standard errors for parameter estimates. In the present sample 8/17, almost the same percentage (47%), ignored cluster effects and thus gave rise to potential errors in inference.

Residual deviance and goodness of fit

In studies of individuals clustered in herds it may be reasonable to assume variation among individuals within herds to be binomial. In other words, whilst the risk of disease will be dependent on herd of origin, the particular animals within a high or low risk herd which have the disease will be independent and assumed to have been infected at random. This is the assumption we make when applying a X^2 test to a simple contingency table. Where the observational unit is a herd or an individual record drawn from a national database, however, it is more likely that extra sources of variation due, for example, to differences in herd management or to variations across regions may contribute to the residual deviance.

Only three of the 36 papers presented estimates of residual deviance. One, however, did not give the number of observations in the dataset sample so it was not possible to compare the residual deviance with a X^2 value. Residual mean deviances in the other two studies were greater than 1, but possible overdispersion did not appear to have been considered.

As described earlier it is not possible to determine evidence for extra-binomial variation when using the raw binary (0,1) values. One method that has been proposed for determining the goodness of fit of a model for data where n/N is close to 1 is that described by Hosmer and Lemeshow (1989). This method involves division of the n observations into equal groups of n/10 ordered by size of estimated probabilities. This test was applied in four of the 36 studies, although in two of these n/N was considerably larger than 1. Two other papers discussed goodness of fit but tests may have been applied incorrectly. An alternative solution is to build

multiway tables of proportions r/n and to analyse these values instead. The sizes of these tables were calculated, from information given in the papers, for the explanatory variables and their levels described in the models for the 19 studies for which observational units were not clustered. In ten the number of observations n divided by the number of cells N was greater than 5, so that direct analysis of cell proportions could be assumed to yield estimates of residual deviances approximating to the X^2 distribution. For the other nine studies the table dimension would need to be first reduced by eliminating less important explanatory variables before a satisfactory approximation to the X^2 distribution could be achieved.

In clustered studies it is often difficult to carry out such tests for the large number of herds makes this prohibitive. Inclusion of a herd as a random effect does not help as this method of analysis is also based on a binary variable. A possible option might be to select a random subset of herds and then to include herd as a fixed effect. If the residual mean deviance is close to 1 in this reduced model then it might be assumed that the same is true of the whole dataset. Another option might be to analyse the complete dataset with herd as a random effect, then to use fitted values to calculate fitted proportions for the cells of the table classified by the other explanatory variables. If by putting these fitted proportions into a model without herd the residual deviance is close to 1 then it can be assumed that no overdispersion remains.

Number of parameters

The total number of parameters p used in the model, including both explanatory variables and their levels, was calculated from model information given for each study.

In 17 studies the ratio of 'successful outcomes' Σr to p was greater than 10. In 12 studies, however, the ratio was less than 10 suggesting that too many parameters may have been fitted (Martin, 1997). The total number of cases of disease was undefined in the remaining seven studies so it was not possible to calculate Martin's ratio. There is often the temptation to collect information on as many measurements as possible in observational studies. It would appear that in this sample 12/29 (41%) of researchers could have been more selective in their choice of variables to measure, and this might have resulted in a simpler and sounder set of inferences. One remedy might be to select variables for measurement based on information gained from previous studies. When many variables are measured it is a common practice to apply a prescreening process where individual variables are dropped if they fail to meet a certain probability level of association. This was commonly done in the ISVEE papers. A more attractive option, however, might be to combine correlated variables into an index by an analysis such as principal component analysis before building the logistic regression model (Duchateau et al., 1997).

Presentation of results

Twenty papers gave results in terms of odds ratios, three as logistic regression coefficients and thirteen both ways. In none of the latter 16 studies was the regression coefficient referred to as the logarithm of the odds ratio and it may be that many researchers are unaware of this association. Seven of the papers included no measures of variation, either as 95% confidence limits for odds ratios or as standard errors for regression coefficients. In only one study were parameter estimates transformed back to the original scale to estimate disease incidences

adjusted for other parameters in the model. Indeed, in seven papers reporting on observational studies there was no information at all on average case incidence in the study. The need for better use of descriptive statistics to help qualify inferences drawn from statistical models was emphasised by McDermott (1995).

The odds ratio is the only quantity that can sensibly be estimated from the regression results of a case-control study (Collett, 1991) and this may be the reason for the popular use of this method of presentation. As shown earlier the odds ratio is also very easily derived as the exponent of a regression coefficient. In the general observational study, however, knowledge of the average disease incidence, at least at the zero or base parameter level, might allow clearer inferences to be made of the biological significance and potential impact of a particular odds ratio. This will be illustrated further in the following example.

EXAMPLE

Data

Zebu cattle in eight village herds (h_i, i=1...8) exposed to drug resistant trypanosomes in the Ghibe valley of southwest Ethiopia have been sampled monthly since 1987 for the measurement of packed blood cell volume (PCV) and for the detection of trypanosomes (Leak et al., 1993). When PCV in an individual was below 26% the animal was treated with diminazene aceturate at 3.5 mg/kg. Each month calving data were collected including information on still births defined as calves born dead or dieing within approximately one and four days later.

Still births: Data were assembled on 1697 calvings between years 1987 and 1996, inclusive, (Y_j, j=1...10) for those cows sampled on each of the monthly sampling occasions occurring during the three months prior to calving. Two hundred and thirty six of these calvings resulted in still births. Each calving was then classified by the number of occasions that the cow was detected with trypanosomes in the three samples (t_m, m=1,2,3), namely 0, 1 or 2-3. Each calving was also classified as being of first parity or not (p_l, l=1,2). Tabulation of the data by month of calving demonstrated a distinct seasonal pattern (s_k, k=1,2) in the proportion of still births between January-June (0.08) and July-December (0.19). The model to be fitted can be defined as:

$$\text{logit}\,[y_{ijklm}] = \mu + h_i + Y_j + s_k + (Ys)_{jk} + p_l + t_m + e_{ijklm} \qquad (1)$$

where y = r/n, the ratio of the number of still births to number of calvings in each category.

Frequency of detected trypanosomes: Trypanosome detection frequency was characterised as low (0 or 1) or high (2 or 3 times detected during the three-month sampling period before calving) and modelled for the effects of herd, year and season as:

$$\text{logit}\,[y_{ijk}] = \mu + h_i + Y_j + s_k + (Ys)_{jk} + e_{ijk} \qquad (2)$$

where y is the proportion of cows in each category detected 2 or 3 times with trypanosomes.

Tsetse control: Two methods of tsetse control were applied during the period of monitoring. Between June 1990 and May 1992 insecticide impregnated targets were placed in the areas grazed by cattle (Leak et al., 1996); from January 1994 to December 1996 an insecticidal pour-on treatment was applied monthly to the cattle themselves. To examine the effect of tsetse control on rate of still births the following model, ignoring herd, can be applied to the 20 proportions r/n calculated over 6-month time intervals:

$$\text{logit}\,[y_{ijk}] = \mu + s_i + p_j + e_{ijk} \qquad (3)$$

where s_i (i=1,2) is effect the of season and p_j (j=1,2) the effect of period 1 (without tsetse control) and period 2 (with tsetse control) and e_{ijk} the residual effect of year-season k within period j.

Statistical analysis

Still births (model (1)): The number of parameters p in Eq.(1) can be found by summing the degrees of freedom associated with each explanatory variable = 1 + 7 + 9 + 1 + 9 + 1 + 2 = 30. Thus, $\Sigma r/p = 236/30 = 7.9$ which is below the minimum value of 10 recommended by Martin (1997). It may be wise, therefore, to drop the interaction term from the model. Then $\Sigma r/p$ = 11.2.

The number of cells defined in the 5-way table defined by the model is given by N = 8 x 10 x 2 x 2 x 3 = 960. Thus, $\Sigma n/N = 1.8$, which is well below the minimum value of 5 proposed earlier. Therefore, whilst the dataset is suited for fitting the revised model of five explanatory variables without an interaction, it is not possible to use this full model to assess the adequacy of the binomial fit. For this latter purpose a simpler model needs to be fitted. If the model is restricted to herd, year and trypanosome category then N = 8 x 10 x 3 = 240 and $\Sigma n/N$ = 7.1. When this model is fitted to the proportions defined in the body of this table the following analysis of dispersion, when each variable is added to the model one by one, is obtained:

Source	df	Deviance	Mean deviance
Herd	7	24.877	3.554
Year	9	64.523	7.169
Trypanosome frequency	2	17.249	8.624
Residual	202	220.875	1.093
Total	220	327.523	1.489

The residual mean deviance is very close to 1 indicating that there is no evidence of extra-binomial variation among the residuals. Thus, the assumption of a binomial distribution is valid and statistical tests can be based on the X^2 test. Note that the total number of degrees of freedom of 220 is less than the value of 239 that would have occurred had all the cells been full.

Thus, even with $\Sigma n/N = 7.1$ relative sparseness of data can occur. Table 1 illustrates the distribution of r/n for Herd 1, which was smaller in size than the average ($\Sigma n/N = 4.0$).

Table 1. Distribution of still births (r) in Herd 1 as a ratio of number of calvings (n) by year and frequency of detection of trypanosomes

	Frequency of detection of trypanosomes			
Year	0	1	2-3	Total
1987	0/9	1/4	-	1/13
1988	0/4	0/1	1/2	1/7
1989	1/4	0/1	0/1	1/6
1990	1/12	0/7	0/2	1/21
1991	1/11	0/2	-	1/13
1992	1/14	0/4	2/4	3/22
1993	2/5	1/5	0/2	3/12
1994	0/10	0/5	0/3	0/18
1995	1/9	0/7	0/1	1/17
1996	0/8	0/6	0/1	0/15
Total	7/86	2/42	3/16	12/144

Having demonstrated the validity of the binomial assumption, the full model can now be fitted using binary (0,1) values. This results in the following analysis of dispersion table:

Source	df	Deviance	Mean deviance	P
Herd	7	24.877	3.554	<0.001
Year	9	64.523	7.169	<0.001
Season	1	39.286	39.286	<0.001
Parity	1	30.236	30.236	<0.001
Trypanosome frequency	2	15.686	7.843	<0.001
Residual	1676	1194.089	0.712	
Total	1696	1368.697	0.807	

It can be deduced that, when corrected for herd, year, season and parity, the more frequently trypanosomes are detected in a cow the more likely it will be that a calving will give rise to a still birth ($X_2^2 = 15.686$, $P < 0.001$).

No comment can be made on the size of the residual deviance as this is now derived from an analysis of a binary variable. As described earlier this form of residual deviance does not follow a X^2 distribution. Note, however, that the deviance values for herd and year are the same as in the first analysis of dispersion. This is because, whatever definition is used to define the individual unit for analysis, marginal totals remain the same (see Table 1).

The model might be improved by defining herd as a random effect, if this can be justified by the study design, and hence saving on the number of degrees of freedom. It might then be possible to include the $(Ys)_{jk}$ interaction.

Frequency of detected trypanosomes (model (2)): The number of cells in the 3-way table defined by Eq.(2) = 8 x 10 x 2 = 160. Thus, $\Sigma n/N = 10.6$, which is well in excess of 5. The analysis of deviance table, ignoring the interaction term, is as follows:

Source	df	Deviance	Mean deviance	F	P
Herd	7	24.225	3.461	2.30	<0.05
Year	9	104.194	11.577	7.68	<0.001
Season	1	0.817	0.817	<1	
Residual	141	212.480	1.507		
Total	158	341.716	2.163		

The residual mean deviance of 1.507 is significantly greater than 1 ($P<0.001$) and raises some doubt as to the validity of assuming binomial variation among residuals. Season, however, has been defined to comprise the same months as those used for incidence of still births. Tabulation of trypanosomal prevalence rate by month of calving, however, showed a higher frequency of detection of trypanosomes on 2 or 3 occasions in the preceding three months when the calving was in December, January or February (0.25) than over the remainder of the year (0.14). Redefining season in this way the analysis of deviance table becomes:

Source	df	Deviance	Mean deviance	F	P
Herd	7	24.225	3.461	2.42	<0.05
Year	9	104.194	11.577	8.11	<0.001
Season	1	8.350	8.350	5.85	<0.05
Residual	139	198.323	1.427		
Total	156	335.091	2.148		

Although the effect of season is now statistically significant ($P<0.05$), the residual mean deviance is only slightly reduced. Thus, it could be argued that there is some evidence of extra-binomial variation $\sigma^2=1.427$ which should be taken into account in the calculation of standard errors of parameter estimates. Thus, the standard error of the regression coefficient -0.400 which is obtained through this analysis for s_2 increases from 0.137 to $0.137 \times \sigma = 0.164$ when

the simplest method of heterogeneity correction is used as described earlier. This makes only a slight alteration to the 95% confidence limits for the odds ratio which increases from 0.51 - 0.88 to 0.49 - 0.92. However, because the mean residual deviance is not 1, the F test, calculated as the ratio of the mean deviance for a variable and that for the residual, should now be used to determine the significance of sources of variation associated with different variables in the above analysis of deviance table (Collett, 1991; Rowlands and Booth, 1989). The significance level for season is reduced from ($P<0.01$), when using a X^2 test, to ($P<0.05$).

Tsetse control (model (3)): Year-season is the observational unit for this model and it is assumed that these observational units are independent. Ignoring possible herd x year-season interactions the analysis of dispersion can simply be expressed as:

Source	df	Deviance	Mean deviance	F	P
Season	1	42.784	42.784	9.63	<0.01
Period	1	12.859	12.859	2.90	>0.05
Residual	17	75.501	4.441		
Total	19	131.144	6.902		

This time the residual mean deviance term is clearly greater than 1 reflecting additional variation among years over that described by a binomial distribution. The confidence interval for the estimated odds ratio of 0.50 for incidence of still births in periods with tsetse control compared with those without increases from 0.34 - 0.75, ignoring extra-binomial variation, to 0.20 - 1.25 when the heterogeneity factor $\sigma = 2.11$ is taken into account. When expressed on the original scale, mean incidence of still births is 8.5 ± 3.0 during periods of tsetse control compared with 15.4 ± 2.1 when tsetse control was not in operation. The standard errors for mean incidences of still births are 2.11 times those calculated assuming a binomial distribution among year-season categories. This adjustment uses all the extra-binomial variations to inflate the standard error. From the analysis of model 1 it is clear that other factors which cannot be included in this model have an important association with still births. The analysis of model 3 thus demonstrates the limitations of such a study design.

Presentation of results

As described earlier results can be expressed in different ways as illustrated in Table 2. Although it is not always necessary to present both regression coefficients and odds ratios as each can be derived from the other, it is often reassuring to the reader to see both. Likewise, if the expected incidence y_1 for the first level of an explanatory variable is known then y_2 can be calculated from the odds ratio. However, as mentioned earlier, inclusion of average disease incidence allows clearer inferences of the practical significance of an odds ratio to be made. It is also helpful to include the numbers of cases so that the frequency distribution across the different explanatory variable categories can be seen. Nine of the 36 ISVEE papers listed the numbers of observations n for each category but only four gave frequency distributions for both r and n. Comparison of proportions of still births to calvings with the expected (or adjusted) incidence values illustrates the impacts of other variables in the model. From a statistical point

of view, therefore, the preferred form of presentation is the complete contents of Table 2. This table presents all the information necessary for a reader to draw his or her own conclusions on the biological significance of the results.

It is also helpful to give the residual deviance and its degrees of freedom from an analysis based on proportions, as given earlier, so that the reader can judge for himself or herself the closeness of the fit of the residuals to the binomial distribution.

Table 2. Different ways of expressing the results of logistic regression analysis of the effects of season, parity and frequency of detection of trypanosomes incidence of still births (model(1))

	No. of calvings	No. of still births	Logarithm of odds ratio (regression coefficient)	Odds ratio	95% C.I. of odds ratio	Expected (or adjusted) incidence(%)[a]
Season						
Jan-June	834	70	-	-	-	8.5 ± 0.9
July-Dec	863	166	0.999 ± 0.161	2.72	1.98-3.72	18.8 ± 1.3
Parity						
1	384	81	-	-	-	22.5 ± 2.1
>1	1313	155	-0.929 ± 0.166	0.39	0.29-0.55	11.2 ± 0.8
Trypanosome frequency						
0	841	85	-	-	-	11.3 ± 1.1
1	546	77	0.258 ± 0.180	1.29	0.91-1.84	13.8 ± 1.4
2-3	310	74	0.775 ± 0.195	2.17	1.48-3.18	20.3 ± 2.1

[a]Expressed on untransformed scale adjusted for the effects of other variables in the model including year and herd.

CONCLUSIONS

In conclusion, I have expressed reservations on some of the approaches currently being used in the modelling of data by logistic regression. I have emphasised the need to be more critical of the approach being used in the development of a logistic regression model and have focussed especially on the need to verify the nature of the residual variation. There are inherent difficulties in drawing inferences from observational studies because of the basic assumptions of random occurrences of risk factors. This, and the assumption of randomness of individuals in the population sample, can rarely be achieved. Researchers need, therefore, to be more selective and cautious in the numbers of parameters included in a statistical model to ensure that inferences are both reasonable and interpretable. Where possible, proportions r/n calculated

from a multiway tabular classification of explanatory variables should be used during model development to test the residual variation assumption. Finally more care is needed in the presentation of results to ensure that a reader can determine for himself or herself the validity of the statistical models fitted and the inferences drawn. McDermott (1995) gave four recommendations for improving the statistical assessment of observational studies: (1) incorporate a biological framework, (2) clearly state and test assumptions, (3) use more realistic models when assumptions fail and (4) present more and better descriptive statistics to supplement the traditional results of statistical models. There is clearly an important role for the statistician to collaborate with veterinary epidemiologists in ensuring these recommendations are met.

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LOGISTIC REGRESSION, AN ILLOGICAL CHOICE?

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In epidemiologic research we are frequently faced with complex problems requiring statistical analyses using multivariable techniques. Two of the most commonly used techniques are linear and logistic regression. As with all statistical techniques, proper application of linear and logistic regression is based on satisfying underlying assumptions. Consequently, satisfaction of these assumptions often places constraints on using these techniques and may make them a sub optimal (illogical) choice. For example, in linear regression one assumption is that the distribution of residuals must be normal. With logistic regression, an additional constraint exists that the data should be dichotomous, or binary, in nature. In practice, when faced with data that do not satisfy these assumptions, the analyst may modify the data, for example by dichotomizing continuous or categorical data in the case of logistic regression. The result of these and other modifications is the loss of information, possibly associated with a decreased power, inefficiency, inconsistency, or an incorrect conclusion of the causal-effect relationship.

If only the probabilities or odds of limited and non-limited responses were desired, probit or logistic models would be sufficient. However, it is inefficient to ignore information that may be available for the value of the dependent variable. On the other hand, if the value of the dependent variable were available and there were no clustering of observations at a limit, linear regression would not be appropriate because the assumptions of the multiple linear regression model are not realized. However, when a continuous response is desired and clustering of the outcome exists, an alternative approach is recommended.

Alternative regression techniques, are available that will avoid these limitations. They have been designed for a category of dependent variables known as censored or truncated data. One of these techniques, Tobit regression (Tobin, 1958, Amemiya, 1984, Greene, 1997) also referred to as an example of censored normal regression models, was designed to analyze economic data, e.g. to evaluate consumer purchasing patterns (Tobin, 1958). Subsequent applications have been made in the fields of sociology (Fair, 1978), plant pathology (Autio et al, 1986), and medicine (Goldberg, 1981, Cohen, 1988, McConnel and Zetzman, 1993). A recent survey of the medical literature identified 20 manuscripts published between 1990-97. Examples include identification of home health use after hospitalization for acute illness (Kenney, 1993), outcomes of trauma patients (Smith, et al., 1990), identification of factors that predict home health utilization and reimbursement (Mauser and Miller, 1994), and evaluation of the relationship between severity and duration of rheumatoid arthritis

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(Leigh and Fires, 1993). However, to date only one application of this technique has been presented in the field of epidemiology (Ekstrand and Carpenter, submitted).

Common examples where the outcome is either truncated or censored in veterinary medicine include the reporting of serologic titers. This may occur if either a minimum or maximum value, e.g. titer is reported as negative if $\leq$ 1:8, or titration may not be carried out beyond a level and thus reported as the last positive level, e.g. 1:256. Also, herd prevalence may be summarized as the herd being either infected or not. No mention may be made of the number of seropositive individuals, if at least one is positive, or through the use of an imperfect test (sensitivity less than 100%) or a population sample, at least one infected individual may be undetected. Also, situations lending themselves to Tobit analysis include those not resulting from imposition of an arbitrary cutoff value or imperfect information. For example, if a significant portion (at least 15-20%) of outcomes is clumped at a limit and the remainder follow a normal (or may be transposed into a normal) distribution, the Tobit regression is appropriate. This situation recently occurred in the evaluation of a foot health program in Swedish broilers (Ekstrand and Carpenter, submitted).

The purpose of this paper is to compare alternative regression approaches, present a hypothetical example to illustrate the advantage of Tobit regression and provide information necessary to perform an analysis using this technique.

ANALYTIC OPTIONS

As stated above, the most commonly used regression techniques in epidemiologic research are multiple linear and logistic regression, with logistic being by far the most common. In order to compare the alternatives, it is necessary to examine their underlying equations. The most familiar is the linear regression model (equation 1)

$$Y = \beta_0 + \beta_1 X_1 + \ldots + \beta_n X_n + \varepsilon \quad (1)$$

where β_i = coefficients, X_i= explanatory variables, and ε = the error term.

The logistic regression model is given by equation 2

$$Y = e^{(\beta_0+\beta_1 x_1 \ldots+\beta_n x_n+\varepsilon)}. \quad (2)$$

The Tobit regression model appears in equation 3

$$Y^* = \beta_0 + \beta_1 X_1 + \ldots + \beta_n X_n + \varepsilon \quad (3)$$

The Tobit regression appears as a somewhat modified linear regression model in that it substitutes the term Y* for Y. The difference is that in the Tobit model with

censored (or truncated) data, 2 separate equations are used. If dependent variable values exceed the censored value (assuming left censoring), the traditional classical linear regression equation (1) applies. However, if the dependent variable values are censored, e.g. a value of 0 occurs, the outcome is set to 0. In other words, when $Y^* = 0$, Y is set to 0. It is then necessary to create an index (I) which is a linear function of the right-hand side variables:

$I_t = X'_t \alpha$
(4)
where α is a vector of the normalized regression coefficient (β/σ). The index I is then transformed to a predicted limited dependent variable.

ILLUSTRATION

If the data follow a normal distribution up to a point and are either censored or truncated at and beyond that point (see figure 1), traditional linear regression will violate the assumption that the resulting error term is normally distributed. In the example illustrated in figure 1, values of 5 or less are either not observed or cannot be measured. They are subsequently reported as 0. Analysis of these data could be performed with a modified linear regression model combined with a logistic model. For instance, values could be transformed as either 0 or 1, e.g. 0 if they are 5 or less and 1 if greater than 5. Alternatively, an individual may choose to categorize the data as less than or equal to the mean, median, or some other arbitrary value and then apply logistic regression to the data. The result would be clearly less than optimal. One improvement would be to categorize the data into a few groups and then apply polychotomous logistic regression to them. Or, using the results of the logistic model, observations having a nonzero value could be further analyzed in a linear regression. Traditional approaches are however inefficient. The Tobit model is an efficient alternative that uses the entire data set to identify the conditional probability of a "censored" value occurring and then quantify the relationships between explanatory and noncensored outcomes.

Figure 2 illustrates the results of the 3 approaches. The linear model (OLS) is greatly influenced by the 0 values occurring as the explanatory variable takes on values of 5 and more. The result is a biased prediction, first low then high, of the outcome. In addition, the error term is clearly not normally distributed. The logistic regression (LR) model satisfactorily predicted the probability of an outcome being nonzero (represented by a value of 1 being a 100% probability), being 0 (represented by a value of 0), or in between 0 and 100% (occurring with values of the independent variable between 4 and 6). In contrast, the Tobit regression model accurately and in an unbiased fashion predicts the values of the outcome variable.

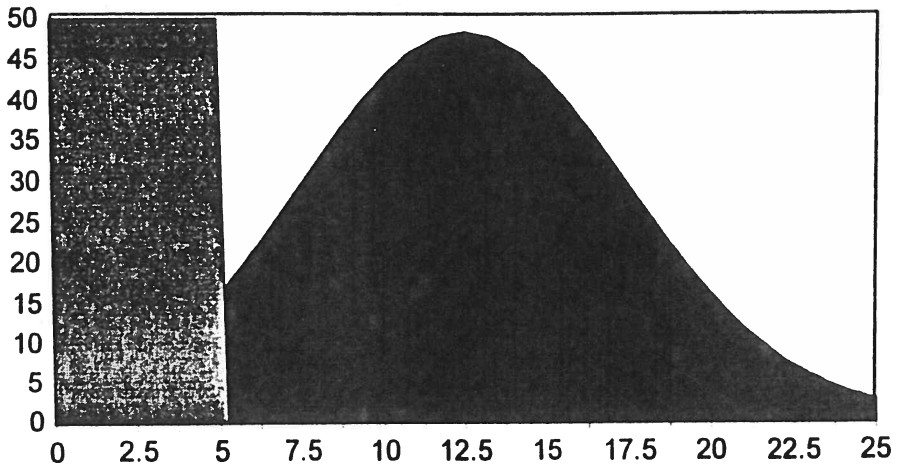

Figure 1. Hypothetical censored normal distribution.

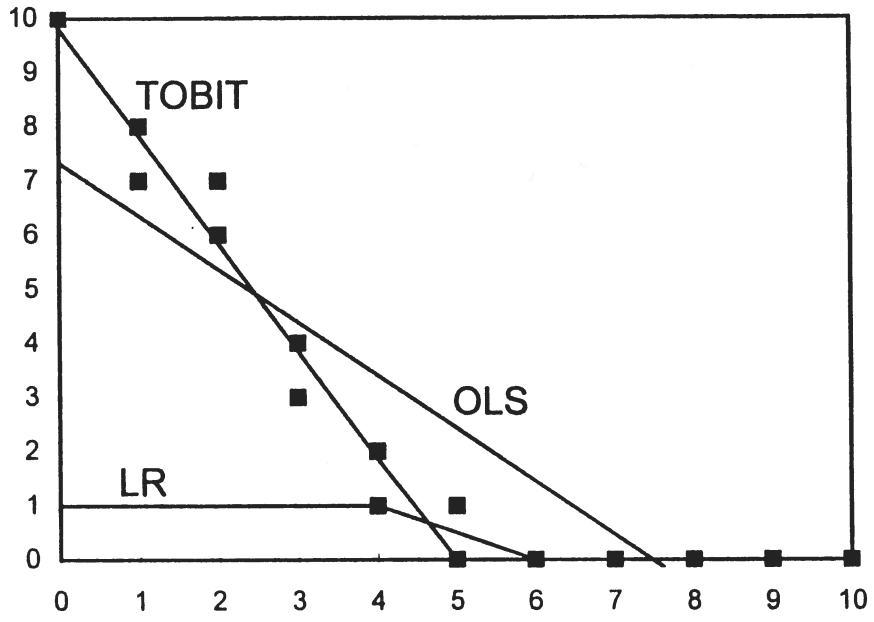

Figure 2. Comparison of Tobit, ordinary least squares (OLS) and logistic regression models fitting a set of hypothetical data.

TOBIT CALCULATIONS

Calculation of significance of regression coefficients is somewhat more complicated with a Tobit analysis and may vary depending on the software package used. In general, in a Tobit analysis, the standard error of the regression model is

analogous to the estimated root mean square error (MSE) reported in a linear regression.

Assuming there are T observations and the first N are constrained, there are T-N nonconstrained, or nonlimited, observations. The parameters α and σ are estimated through maximum likelihood estimation (MLE) of the log-likelihood function with the individual constrained and nonconstrained components as illustrated in equation 5:

$$L = \sum_{t=1}^{N} \log[1 - F(\sigma Y_t - I_t)] + \sum_{t=N+1}^{T} \log f(\sigma Y_t - I_t)$$

(5)

where **F** represents the normal cumulative distribution function (CDF) and **f** represents the normal probability density function (pdf).

The values of **F** and **f** are calculated at the point I_t and are then used to compute the conditional expectation of Y_t, given I_t, using equation 6.

$$E(Y_t|I_t) = \sigma I_t F(I_t) + \sigma f(I_t)$$

(6)

The expectation of nonconstrained predicted variables (Y) are calculated using values calculated for the standard errors of the estimate and the CDFs and pdfs for the Index variable (see equation 7):

$$E(Y_t | I_t, Y_t > 0) = \sigma I_t + \frac{\sigma f(I_t)}{F(I_t)}$$

(7)

Finally, an estimated value of the predicted variable ($\hat{Y}$) may be calculated using estimated values of α, σ and I_t as seen in equation 8:

$$\hat{Y}_t = \hat{\sigma}\hat{I}_t F(\hat{I}_t) + \hat{\sigma} f(\hat{I}_t)$$

(8)

Note this approach is applicable to situations that have a single level relationship between the risk factor and outcome. That is, if a risk factor is present, the greater the risk factor the greater the associated outcome, increasing at a linear rate. For example, a risk factor value of 4 represents twice the risk than a risk factor value of 2, etc. This has important implications for infectious disease processes within populations. The Tobit regression model assumes that the resulting population outcome is dependent on the level of the risk factor responsible for the introduction of the infection into the population. It does not account for a secondary response, that would regulate transmission of infection within the population, different from that which was responsible for the initial insult, or introduction. In other words, the Tobit model assumes that if a management or other control variable were responsible for introduction of the infection, the level of this variable could be used to predict the

final outcome in the population. If on the other hand, a second management or other type of variable were responsible for the spread of infection, once introduced, the Tobit model would be inappropriate.

Commercial software to run Tobit regression is readily available and commonly used in econometrics. Two of these include LIMDEP® and SHAZAM® (Greene, 1991; White, 1993). The availability of software able to perform tobit regression is more limited within programs typically used by epidemiologist. In fact in a recent survey, only one package, STATA® was found to contain this feature (StataCorp, 1997).

In conclusion, an alternative regression analysis technique may be appropriate to supplement those used currently. Although most situations are still amenable to analysis by the traditional multiple linear and logistic regression techniques, some are not. When facing a problem with a limited dependent outcome, either due to censoring or truncation, these techniques which are traditionally accepted in epidemiology may not be valid. They may either violate the assumption of a normally distributed error term (OLS) or be an inefficient approach (LR). In such cases, an alternative, Tobit regression, is preferred. While software availability may dissuade the analyst, it is a minor inconvenience compared with the expense of proper study design, data collection, and ultimately program intervention, which may be recommended by the analysis.

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UNBIASED POINT AND VARIANCE ESTIMATES OF A PREVALENCE BY MIXTURE DISTRIBUTION ANALYSIS

M GREINER* and D. BÖHNING**

A notorious problem of diagnostic testing in both medical and veterinary disciplines is the test validation in the absence of available reference methods (gold standards). The interpretation of results from non-validated tests in a clinical or epidemiological setting remains vague and may not justify the application of expensive tests. The methods that deal with the missing-gold-standard problem are usually based on latent class models and require multiple tests per subject (Faraone and Tsuang, 1994; Baker, 1995). We focus here on the epidemiological context where a prevalence estimation based on a single test procedure is considered. The lack of reliable estimates of sensitivity and specificity invalidates the Bayesian approach of correcting the apparent (i.e. according to test results) prevalence (Rogan and Gladen, 1978). In the first part of this paper we outline a new approach of prevalence estimation based on a single quantitative test by mixture distribution analysis. The technique is referenced in the OIE Manual of Standards for Diagnostic Tests and Vaccines (Jacobson, 1997) and involves a so-called 'intrinsic cut-off' value (Greiner et al., 1994). The procedure is based on the analysis of the latent mixture of distributions of the test data by maximum likelihood methods and is exemplified using serological data from a cross-sectional survey on bovine trypanosomosis in Uganda.

In practice we are concerned not only with the bias of the point estimate but also with its precision and with the pattern of disease occurrence. Prevalence data typically derive from a cluster (herd) sampling design. It is well recognised that a positive intracluster correlation inflates the naive sample variance of the overall prevalence (design effect). Various approaches differ in the way the extra-binomial variance is estimated. For empirical reasons we may argue for distributions of cluster-level prevalences that do not follow the beta distribution which is often used for modelling purposes. To our knowledge, a classification of farms based on prevalence heterogeneity has not yet been described. In the second part of the paper we show how mixture distribution analysis can be used for diagnosing heterogeneity of cluster-level prevalences. The example data are from the above mentioned survey.

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MATERIALS AND METHODS

Example data

The data stem from a cross-sectional study on trypanocide resistance in dairy cattle in Mukono County, Uganda, which has been conducted in June 1994. A total of n=487 cattle from k=50 farms was sampled. Sera were available from 457 animals. The lacking of serum samples was due to mis-labelling, loss during transport and shortage or deterioration of the material and was assumed to have occurred at random. The sampling procedure and the parasitological and serological (*Trypanosoma* antibody ELISA) methods used were described elsewhere (Greiner et al., 1997). A group of 86 non-infected cattle from Germany were used to establish a "conventional" cut-off value (mean plus three standard deviations). Parasitological and serological results were available on the level of individual animals as binary (trypanosomes detected; yes/no) and continuous (single-point measurement of the optical density) variables, respectively. For the sake of simplicity we ignore sampling weights and stratification and assume a representative cluster sampling design.

Visualisation and descriptive statistics of the empirical distributions

The distribution of the serological data on animal-level (ELISA values) and the distribution of parasitological data on farm-level (farm prevalences) is visualised in frequency distribution histogrammes (Fig. 1 and 2). The bin width (b) of the histogrammes was standardised using the standard deviation (s), the interquartile range (IR) and the sample size (n; 50 in case of the prevalence data) of the respective sample according to a formula suggested by Kairisto (1995)

$$b = 0.9\ [\min(s, IR/1.34)]\ n^{-0.2}.$$

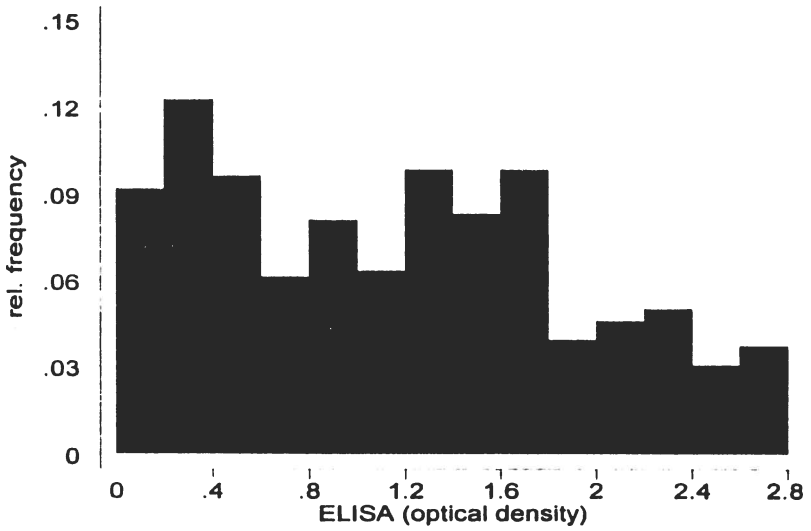

Fig. 1. Frequency distribution of *Trypanosoma* antibody ELISA values from cattle from Mukono County, Uganda (data from 1994; n=457).

A test of normality of the ELISA data basing on skewness and kurtosis was performed as described by D'Agostino et al. (1990) using Stata (StataCorp., 1997). Overdispersion in the prevalence data was assessed by a chi-square test (with $df=k-1$) based on the (observed) calculated variance of the proportions divided by the expected value for binomial distribution. Furthermore, the C (alpha) test was used which is more specific in its alternative hypothesis (distribution is beta-binomial) and Z-distributed under the null hypothesis (distribution is binomial). The programme BBD (Beta-Binomial Distribution Fitting Program; Madden and Hughes, 1994) was used for both tests.

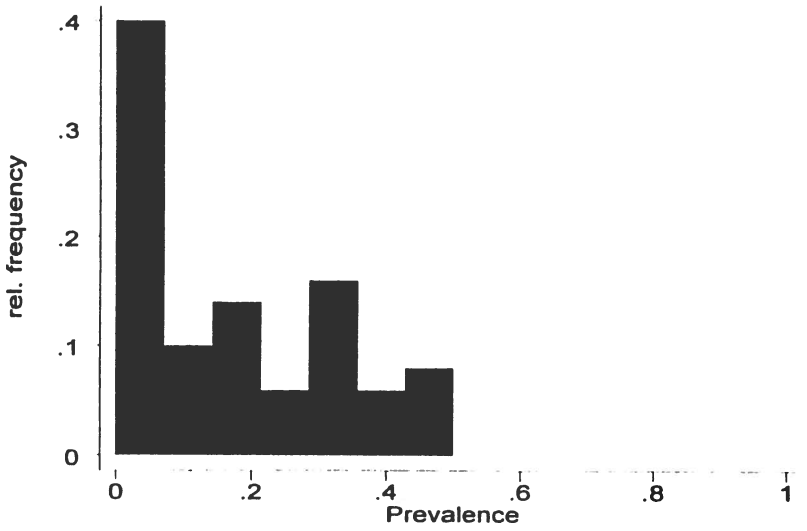

Fig. 2. Frequency distribution of trypanosomosis farm prevalences in Mukono County, Uganda (data from 1994; n=50).

Mixture analysis

The continuous ELISA data (n=457) were subjected to the Computer-assisted mixture analysis (C.A.MAN, Böhning et al., 1992)[1]. Normal distribution within the subpopulations was assumed but no prior assumption has been made concerning the number of subpopulations. The programme identifies the number of subpopulations in the data and their means and weights by maximum likelihood estimation. In case of two identified subpopulations of test data from a population sample, mixture analysis can be used to derive a cut-off value (referred to as *intrinsic cut-off*) that classifies each observation as belonging to either the subpopulation with low or high mean ELISA value (Greiner et al., 1994).

Likewise, we analysed the prevalence data (k=50) by mixture analysis without explicit prior assumption of heterogeneity. Heterogeneity of the farm-level prevalences (p) can be interpreted as the distribution of p in the (super-) population of farms that consists of subpopulations with different mean parameter values. These subpopulations are having (prevalence) parameters p_1, p_2, ..., p_m and the (super-) population is partitioned into these subpopulations according to the

[1] http://www.medizin.fu-berlin.de/sozmed/caman.html.

weights w_1, w_2, ..., w_m. The parameters p_1, ..., p_m and w_1, ..., w_m as well as the number of subpopulations m are estimated by maximum likelihood using the computer package C.A.MAN as described elsewhere (Böhning and Greiner; manuscript submitted).

Variance of the prevalence under heterogeneity

The results of mixture analysis can be used to estimate the variance of the overall (pooled) prevalence $\hat{P}_{pool}$. Following the approach by Böhning and Sarol (1997) we first estimate the mean ($\hat{p}$) and variance ($\hat{\tau}^2$) of the prevalences across subpopulations as

$$\hat{p} = \Sigma\, w_j p_j$$

$$\hat{\tau}^2 = \Sigma\, w_j (p_j - \hat{p})^2$$

where j=1, 2, ..., m. In the case of heterogeneity, the term $\hat{\tau}^2$ becomes positive. Let n_i be the sample size of the ith cluster and $N=\Sigma n_i$. The estimator of the variance that accounts for heterogeneity is then given as[2]

$$VAR_h(\hat{P}_{pool}) = \hat{p}/N + \hat{\tau}^2 [\Sigma n_i^2 / N^2].$$

RESULTS AND DISCUSSION

Analysis of ELISA data

Diagnosis of heterogeneity: The distribution type of the quantitative results of a *Trypanosoma* antibody ELISA in cattle from the endemic area in Uganda is not normal as can be seen from the frequency distribution histogramme (Fig. 1). According to the skewness (0.32), kurtosis (2.06) and the joint statistic of normality (chi-square=58.23, df=2, p<.001) the null hypothesis of normal distribution could be rejected. A plausible reason for non-normality is the occurrence of unobserved subpopulations of ELISA values. One might expect that the sample includes naive and infected (or recently infected) animals with unknown infection prevalence. Furthermore, the mean ELISA value is assumed to be higher for the infected than for the naive subpopulation. The heterogeneity was confirmed by mixture analysis. Two subpopulations and an intrinsic cut-off value of 1.27 (ELISA optical density) could be identified.

Estimation of seroprevalence: According to the intrinsic cut-off, 45.3% (40.7-49.9% binomial 95% confidence interval) of the cattle were from the subpopulation of animals with elevated antibody titres. This estimate of seroprevalence is higher than the parasitological prevalence of trypanosomosis in the sample (17.9%; 14.6-21.6%) but considerably lower than the estimate of seroprevalence based on the conventional cut-off value (77.2%; 73.1-81.0%). The analysis of influential factors for infection and for antibody response gave evidence that the intrinsic rather

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[2] Note that (a) the Poisson rather than the binomial variance is used and (b) that under homogeneity ($\hat{\tau}^2$=0) we obtain the simple Poisson variance. A free software for these computations is available (http://city.vetmed.fu-berlin.de/~mgreiner/clusDATA/clusdata.htm).

than the conventional cut-off was suitable to define infection related antibody levels (Greiner et al., 1997). Classification based on mixture analysis of quantitative test data is useful to resolve mixed distributions of low and high responders in the sample (Greiner et al., 1994). This classification may not adequately represent the infection prevalence due to antibody persistence or serological cross-reactions. However, it can be thought of being a less biased estimator of prevalence than a classification based on an arbitrarily defined cut-off value.

Analysis of prevalence data

Diagnosis of heterogeneity: The maximum likelihood estimator of the overall (pooled) parasitological prevalence is 17.9% (87/487). Both graphical and numerical methods were used to assess whether the single binomial parameter sufficiently characterises the distribution of farm-level prevalences (p) or whether there is evidence for extra-binomial variation (overdispersion). A frequency distribution histogramme shows that there is considerable heterogeneity in the distribution of the farm-level prevalences (Fig. 2). The distribution is approximately L-shaped whereby the classes around p=0.18 and p=0.3 showed higher frequencies than expected under assumption of a beta-binomial distribution. Formal tests of binomial distribution (chi-square=97.02, df=49, p<.001 and Z=10.35, p<.001) confirmed the occurrence of overdispersion. By mixture analysis we could identify a latent mixture of three groups of farm-level prevalences with mean prevalences (and weights) zero (.169), 11.6 (.475) and 31.9% (.356), respectively. The variance of p across subpopulations of farms was estimated as $\hat{\tau}^2$=0.01428.

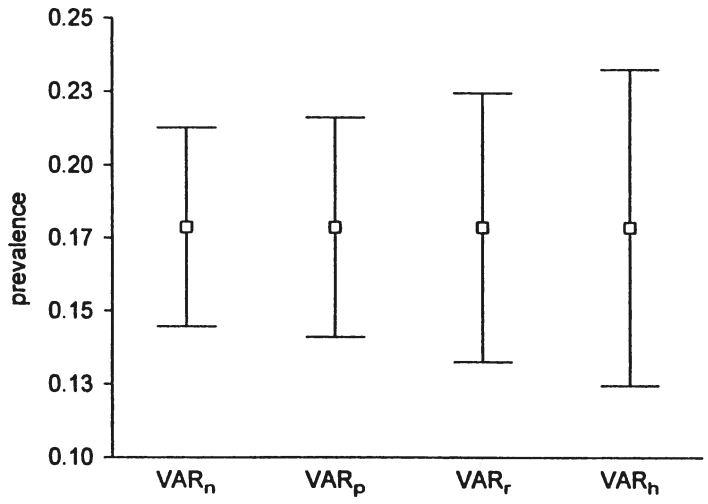

Fig. 3. Confidence intervals for the parasitological prevalence of bovine trypanosomosis in Mukono County, Uganda (data from June 1994, total sample size 487, 50 farms, mean farm size 9.74, intracluster correlation 0.0939) with 95% confidence intervals (CI, normal approximation). The underlying variance was estimated under assumption of simple random sampling (VAR_n), Poisson approximation (VAR_p), cluster sampling (VAR_r) and parameter heterogeneity (VAR_h) (see text for further explanation).

Variance estimation under heterogeneity: In a cluster sampling design the simple random sampling variance (VAR_n) of a binomial parameter usually underestimates the true variance by the term $c=1+\rho(n-1)$, where ρ and n denote the intracluster correlation coefficient and the average cluster sample size, respectively (see for example McDermott and Schukken; 1994)[3]. However, the underlying assumption of a beta distribution of farm-level prevalences may not always – as in our example – be justified. The beta distribution provides a suitable model for peaked, L-shaped, J-shaped and U-shaped distributions but not for distributions with more than two modes or bimodal distributions with modes others than zero and one. Thus, the beta distribution is not suitable to fit our data.

According to the estimate of the variance across subpopulations of farms ($\hat{\tau}^2$) we obtain $VAR_h(\hat{p}_{pool})=0.00076$. The practical relevance of the different methods for variance estimation is visualised by the widths of naive (assuming simple random sampling), design-based (accounting for cluster-sampling) and data-based (accounting for heterogeneity) confidence intervals for the prevalence under consideration (Fig. 3). Our data suggest that the confidence intervals of proportions may be underestimated in the presence of prevalence heterogeneity even when a positive intracluster correlation has been taken into account. A formal argument for this observation is provided elsewhere (Böhning and Greiner, manuscript submitted).

CONCLUSIONS

Two applications of mixture distribution analysis in the seroepidemiology context have been outlined. In the first example the technique is used to establish a so-called intrinsic cut-off value for a *Trypanosoma* antibody test. This cut-off provides a new approach for the definition of serological "low and high responders" in exposed animal populations. The approach is useful in the absence of representative reference populations and reduces the bias in the estimation of seroprevalence.

The second example deals with the estimation of a variance of a proportion (e.g., prevalence) under cluster sampling. Cluster sampling in veterinary epidemiology is unavoidable but also necessary to investigate the distribution of disease. The pattern of cluster-level prevalences can be assessed by mixture distribution analysis. The identification of heterogeneity in the cluster-level prevalences is of interest for the variance estimation of the proportion. Moreover, the diagnosis of heterogeneity should be the starting point to investigate explanatory factors for different cluster-level prevalence levels. Stratification of clusters according to such factors could potentially both enhance the statistical power of hypothesis testing and improve the understanding of the underlying biological background.

ACKNOWLEDGEMENTS

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[3] Note that $VAR_n\ c = np(1-p)\ [1+\rho(n-1)]$ is the variance of the beta-binomial distribution.

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MODELLING OF DISEASE SPREAD AND ITS IMPACT

EPIDEMIOLOGICAL AND ECONOMIC EVALUATION OF DISEASE CONTROL STRATEGIES USING STOCHASTIC AND SPATIAL SIMULATION: GENERAL FRAMEWORK AND TWO APPLICATIONS

A.W. JALVINGH, A. VONK NOORDEGRAAF, M. NIELEN,

H. MAURICE AND A.A. DIJKHUIZEN[1]

Outbreaks of contagious animal diseases, such as Foot and Mouth disease (FMD) and Classical Swine Fever (CSF) can be very costly, especially for an exporting country such as the Netherlands. In case of an outbreak of a contagious animal disease, a realistic event as shown by the recent outbreak of CSF in the Netherlands, rapid and adequate elimination of all virus sources has the highest priority. People involved in control strategy decision making are faced with many uncertainties regarding the development of the outbreak, expected efficiency of control strategies and possibility of export bans set by other countries. Despite these uncertainties they have to decide on what control strategy to apply (e.g. size of areas under movement control, degree of pre-emptive slaughter). Experimenting with different control strategies is hardly or not possible during an outbreak. Computer simulation can be an adequate tool for the analysis of the epidemiological and economic consequences of different control options, either during an outbreak, beforehand when preparing for such an event or afterwards when evaluating an outbreak.

Simulation of the epidemiological and economic consequences of disease control strategies requires a modelling approach that simulates: (1) disease spread between farms, (b) direct costs of eradication and (c) indirect costs due to export bans. In that, the outcome of the modelling of disease spread serves as the basis for the economic calculations. (Berentsen et al., 1992) worked out this modelling approach for FMD and used it to compare different strategies on stamping out FMD in a vaccinated and an unvaccinated population. More recently, the modelling approach developed for FMD was revised. The state-transition model simulating disease spread between farms, as used by (Berentsen et al., 1992), was replaced by InterSpread (Jalvingh et al., 1996), which simulates disease spread between farms much more realistically.

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The conceptual model of InterSpread was developed in New Zealand (NZ) by (Sanson 1993) as part of EpiMAN, a decision support system for the control of FMD outbreaks. InterSpread simulates within a region from day to day the spread of FMD between farms. Starting point is data on individual farms, including animal numbers and geographic location. Via three different spread mechanisms: (a) contacts (animals, people, vehicles, material), (b) local/neighbourhood spread and (c) airborne spread, the infection can be transmitted to other farms. Once the first case is diagnosed, several control mechanisms can be put in place, such as slaughter, tracing, movement control and pre-emptive slaughter. All spread and control mechanisms contain risk and uncertainty implemented through Monte Carlo simulation and act spatially by using the geographic location of farms. The applicability of EpiMAN as a whole for the European situation has been investigated within a European Union funded project (EpiMAN-EU; Jalvingh et al., 1995). Within this project InterSpread was further developed and modified to suit Dutch and EU conditions. The modifications were related to differences between New Zealand and NL/EU in (a) contingency plan, (b) structure/organisation of animal production and (c) data availability. The intended use of InterSpread by NZ and NL is different; NZ developed InterSpread for short term evaluation of control alternatives during an outbreak, NL aims on evaluating beforehand control alternatives for the outbreak as a whole. The outcome of InterSpread can be linked to an economic model developed by (Meuwissen et al., 1997), which not only calculates direct costs for the farms involved but for all partners in the livestock production chain, such as traders and slaughterhouses.

InterSpread can be considered a general framework which can be applied to other infectious diseases. Modifications will at least be necessary in the disease specific parts of the model regarding spread and control. InterSpread has recently served in the Netherlands as the basis for two studies focusing on other infectious diseases, Infectious Bovine Rhinotracheitis (IBR) and CSF. In the case of IBR (finished September 1997), the modified model provided insight into the effects of various control strategies to be applied after reintroduction of IBR into the Dutch cattle population supposing a successful eradication program starting in 1998. A major modification was the inclusion of the simulation of the spread of the disease within the farm using a deterministic state transition model using the concept of reproduction ratio. The resulting disease spread within the farm steers the rate of transmission of the different spread mechanisms between farms. In the case of CSF, a study which is currently undertaken, the model should mimic the Dutch 1997/98 epidemic, offering an excellent opportunity for validation of such models. Once available, the model should predict the future pattern of the outbreak under different control strategies, but also the effect of changing controls and events back in time. The major modifications necessary had to do with the use of real farm data and real outbreak data, such as data on diagnosed herds, pre-emptive slaughtered herds and areas under movement control.

This paper describes the general framework underlying the stochastic and spatial simulation of the spread and control of infectious diseases, as implemented for FMD (InterSpread). Then, the implementations for IBR and CSF are described.

GENERAL FRAMEWORK, AS IMPLEMENTED FOR FOOT AND MOUTH DISEASE (FMD)

Figure 1 shows the general framework of the stochastic and spatial simulation model for disease spread and control as implemented for FMD (InterSpread). In the initialisation phase,

farms of a pre-defined region are loaded into the model and the spread and control mechanisms are assigned their parameter values. Risk and uncertainty in spread and control are implemented through Monte Carlo simulation; the outcome of events, length of intervals etc. depends on the outcome of random drawings from appropriate probability distributions. As a result several replications, each representing a possible pattern of the outbreak, are necessary in order to get a good insight into the possible range of outcome.

At the start of each replication, for the index farm the interval from infection to earliest clinical signs and the interval from earliest clinical signs to detection are sampled. After that, time is moved forward in time from day to day while spreading and controlling the disease until the requested number of days has been simulated or until the epidemic is over and control measures are no longer present. Each day the actual disease control is carried out, which refers to carrying out pre-emptive slaughter, vaccination, putting farms on or off surveillance, installing or cancelling zones with movement control (Fig. 1). Most of these activities have been initialised at the time the disease has been detected on individual infected farms as will be explained later.

After disease control activities have been carried out for each of the infected farms present, several events are simulated or checked for that day (Fig. 1). In case detection of the infected farm takes place, control measures are initialised. Control measures apply to (a) the infected farm, (b) farms in radial zone(s) around the infected farm and (c) traced contact farms. Next, the disease spread of the infected farm is simulated through three mechanisms: (a) contacts, (b) local and (c) airborne spread. When this results in infection of another farm, this farm is assigned relevant dates and will be part of the list of infected herds. Next it is checked whether the farm should be slaughtered or restocked on the current day.

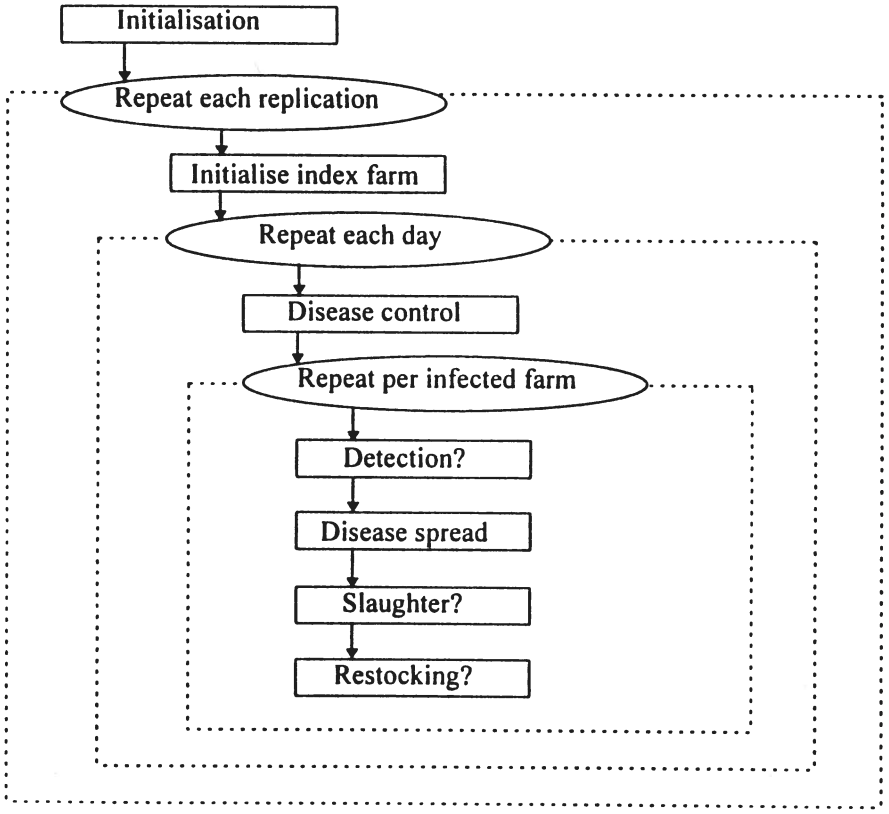

Fig. 1 Schematic representation of general framework

The main output results of the model are the number of infected and diagnosed farms, the number of days the outbreak lasts and the number of farms that face movement restrictions. The model kernel has been programmed in Borland C++. The user interface is designed using Microsoft Access. Below are more details on certain elements of the framework.

Farm data

Data on individual farms in the area involved make up the basis of the simulation: data on geographic location, animal species and the number of animals present. In the initial model for NZ, geographic locations can be entered in terms of complex polygons representing individual land parcels belonging to the farm. For the Dutch and EU situation this was not relevant since data of this level of detail is simply not available. Therefore, for the Dutch situation X-Y co-ordinates have been used to represent individual farms, preferably referring to the animal buildings. In the original model 6 different species are considered: cattle, dairy, deer, goat, pigs and sheep. For each farm and for each species the animal numbers need to be entered. The model can carry out calculations for a maximum of 32,000 farms. Until now, for the Dutch situation, calculations were carried out using a database with farm data that was generated given specific characteristics of the area, such as size, density of farms, and proportion of each farm type plus a certain combination of animal numbers per type of farm.

Infected farms

The index farm is the only farm with a fixed infection date, which is the starting date of the simulation. All other farms' infection dates are set by simulation. Once a farm is infected, index case or simulated, dates for earliest clinical signs and diagnosis are set, as a result from random drawings from specified probability distributions. The possibility exists to start not with only one farm having a fixed infection date, but to add more farms with a pre-set infection date.

Disease spread mechanisms

For each infected farm that has not been slaughtered yet, disease can spread to other farms through three different mechanisms: (a) contacts, (b) local spread and (c) airborne spread as indicated before.

Contacts: Three types of contacts off the farm are generated: (a) high, (b) medium and (c) low risk contacts. What is included under each type of contact is in fact a choice of the user. For FMD the contacts are as follows: high is susceptible animals, medium is persons and vehicles with susceptible animal contact, animal and non-animal products, and low is persons and vehicles without susceptible animal contact. For each type of contact, each day the number of contacts for that day is determined by drawing from a Poisson distribution with the average number of contacts per day as a parameter. For each contact off the farm a distance class is selected given the probability distribution for distance classes. Out of all farms within the drawn distance class one farm is randomly selected as destination farm. Whether contact results in transmission of the infection to the destination farm is determined by drawing a uniform random number and compare it with the entered probability of transmission. The probability of transmission depends on the time of contact relative to the earliest clinical signs at the infected source farm. Whether contact really takes place depends on the actual status of the source and destination farm of the contact. Being on surveillance or being present in a zone with movement control implies that all or part of the contacts are no longer allowed. When

contact is not possible solely due to the status of the destination farm, another destination farm is selected in the same distance class.

Local/Neighbourhood spread: Off each infected farm local spread to neighbouring farms is mimicked. Local spread includes spread through vermin, children, pets and other more difficult to trace contacts. All farms within a certain radius of the infected farm have each day a certain probability of becoming infected. The probability depends on the time relative to earliest clinical signs and distance from the infected farm. The probability of local spread can be modified when the farm is under surveillance by an input parameter reflecting the reduction of local spread. Local spread stops when all animals at the infected farm have been killed and are removed from the farm.

Airborne spread: Spread of FMD virus through the air is possible under certain circumstances. In the model, airborne spread is modelled as follows. The model determines whether on the current day airborne spread occurs, given the proportion of days per week with airborne spread. The plume of an individual infected farm is defined by radius, direction and width of the plume. The probability for farms under the plume of becoming infected through this route is difficult to obtain from earlier outbreaks. With the help of experts it is possible to make estimations for this rather small probability. In the calculations in this paper the airborne spread has been omitted. Part of the airborne spread can be seen to be included in local spread.

Disease control mechanisms

Disease control mechanisms are activated when the first farm is detected, and at each subsequent diagnosis additional controls are installed. Disease control affects (a) the infected farm, (b) all farms within a certain radius around the infected farm and (c) contact farms that have been traced.

Affecting the infected farm: At diagnosis the date of slaughter of the infected farm is set, which may depend on herd size and actual number of infected farms that need to be slaughtered.

Affecting all farms within a certain radius: Around each diagnosed infected farm radial zones can be implemented with different types of control: surveillance, movement control, pre-emptive slaughter and vaccination. For each type of control, specifications are needed for starting moment after diagnosis, duration (movement control and surveillance) or capacity in terms of days to complete the zone or animal numbers that can be handled per day (pre-emptive slaughter and vaccination). In the case of movement control and surveillance, the proportion of contacts allowed per contact type on and off farms is required. In the case of vaccination, additional input is required regarding the delay to immunity. This facility makes it easy to define around each infected farm a protection zone with a 3-km radius and a surveillance zone with a 10-km radius conforming with the EU regulations for FMD control, and also any alternative zone.

Affecting all traced contact farms: When detecting an infected farm, the list of contacts off the farm is checked: will a contact be traced, and if so when will it be traced. On the day the contact farm is actually traced, the farm can be put under surveillance, on movement control, or even slaughtered pre-emptively, depending on the settings chosen for the control of traced contact farms.

Results

Validation of InterSpread is an on-going process using sensitivity analysis and expert opinion. For illustration purposes some preliminary results are shown here. For an area of 100*100 km or 100 sq km with an average farm density of 1.25 per km^2, and 73% dairy, 18% pig and 9% mixed farms, calculations have been carried out for the basic control strategy as prescribed by the EU directives (slaughter of infected farms, trace contact farms and visit them, install 3-km protection and 10-km radius surveillance zone). Disease spread parameters have been set to their current basic values. Appendix I summarises the major input parameters on disease spread and control. Results of the basic strategy are compared with results of an alternative strategy in which the radius of each surveillance zone is set to 20 km instead of 10 km. A total of 100 replications have been carried out. Figure 2 shows the resulting cumulative probability distribution for the number of infected farms. In the basic strategy (10 km) an average of 81 farms are infected, in the alternative strategy (20 km) the average is 39 farms. Figure 2 shows that the alternative control strategy prevents the occurrence of very large outbreaks. As a result of the larger zones with movement control many more farms face movement controls. Additional calculations with the economic model of (Meuwissen et al., 1997) should point out whether the reduction in costs due to the reduction in the size of the outbreak outweighs the increase in costs due to the increase in farms under movement control. Additional sensitivity analysis showed that the reduction in outbreak size depends largely on the choices made for the probability distribution of contacts over distance classes.

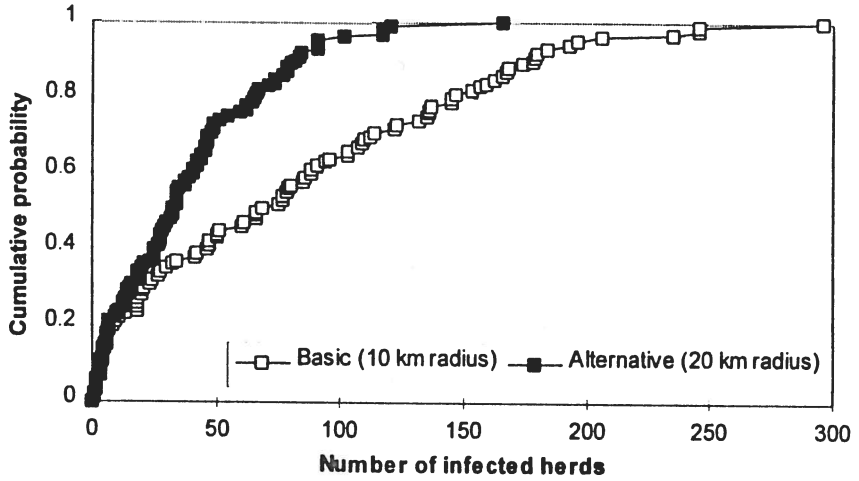

Fig. 2 Cumulative probability distribution for number of infected herds for two control strategies

IMPLEMENTATION FOR INFECTIOUS BOVINE RHINOTRACHEITIS (IBR)

In 1998, the Netherlands start a campaign to eradicate IBR in order to both reduce the on-farm costs because of reduced milk production and possible abortion and to avoid (future) export restrictions on sperm, embryos and live animals. Computer simulation was used to support policy makers in their decisions on eradication by evaluating different scenarios with respect to epidemiological and economic effects of IBR-infections and control strategies to be

applied (Vonk Noordegraaf et al., 1998). Before making the final decision on whether or not to start a compulsory program to eradicate IBR, the decision makers wanted more insight into what to expect when IBR would be reintroduced into the Netherlands after being free from the disease, and whether it would be possible to control such outbreaks at reasonable costs. For this second analysis, InterSpread was taken as the framework for the modelling of the spread of IBR between farms and the control. The complete description of this study is available in (Vonk Noordegraaf et al., 1997).

Major modifications

The expected character of IBR spread and control initiated the choice of a time step of 1 week instead of 1 day. The information on farms has been modified by the inclusion of farm type: dairy, cattle, beef, veal and miscellaneous (such as suckler herds) and by the inclusion of number of animals sold annually for live. This last parameter was obtained from the Identification & Recording (I&R) system. Within the miscellaneous herd a special group selling over 100 animals for live per year was distinguished. This was done to get more insight into their potential risk should they become infected. The model starts calculations with the introduction of virus into one of the farm types considered. Each replication concerns introduction into the same type of farm, but the actual farm varies from replication to replication.

Further spread of the virus is simulated both within the farm and between farms; in the general framework only spread between farms is simulated. A deterministic state-transition model using the concept of reproduction ratio (SIR model; De Jong, 1995) results weekly in the number of infectious animals (that shed the virus), positive animals (that carry the virus, but do not shed, but may reactivate either at the farm or during transport to another farm) and negative animals for each infected herd. This outcome steers the probability of transmission to other herds through the spread mechanisms contacts (animals and professional contacts) and local spread. The modelling of animal contacts deviates from the general framework; it concerns the modelling of selling individual animals for live. The actual number of animals sold per week is obtained by drawing from a Poisson distribution with the average number sold weekly as a key parameter. Whether the animals sold are infectious, positive or negative with respect to IBR, is determined by random samples from the outcome of the SIR model. When the virus is transmitted to another farm by one of the spread mechanisms, one can be lucky with only a small outbreak occurring, but there is also a fair chance of a major outbreak within the herd.

The control strategy for IBR is different from notifiable diseases such as FMD and CSF. Stamping out all animals in the herd and large zones with movement control after detection of the first case will not be an option. The model was used to compare different possible control strategies. In the basic strategy, bulk milk of dairy herds is tested every four weeks whereas blood samples of cattle on the miscellaneous herds are tested twice a year. Beef and veal farms are not monitored. These control measures result in probability distributions for the interval between infection and diagnosis for each farm type. As soon as a farm is tested positive, in the basic strategy a ban is imposed on the selling of live animals (proportion of animal contacts allowed is set to 0%). Moreover, all animal contacts on and off the farm are traced through the I&R system and all cattle farms within a 1-km zone are put under control. The latter means that all animals on these farms are investigated serologically (resulting in earlier detection) and

these farms are not allowed to sell live animals during four weeks (movement control). Infected farms do not need to cull their positive animals immediately, but should do so within five years. Next to the basic strategy, two alternatives were defined. The second strategy differs in this latter respect as positive animals must be culled immediately. In the third strategy, all animals on infected farms are vaccinated twice a year.

Table 1. Number of infected farms when applying the basic control strategy[a)]

	Outbreak starting on				
	Dairy farm	Beef farm	Veal farm	Miscellan. 100+	Miscellaneous
Average	0.9	0.1	0.2	21.6	1.4
25%	0	0	0	11	0
50%	0	0	0	21	0
75%	1	0	0	30	1
90%	2	0	0	38	5
95%	3	0	1	43	7
99%	6	1	3	56	14
100%	31	12	20	89	23

[a)] Number of replications: beef and veal (500), dairy and miscellan. (200) and miscellan. 100+ (100)

Major results

The major epidemiological outcome for the basic control strategy are summarised in Table 1. When the outbreak starts on a dairy farm, on average 0.9 other farms are infected. This average is even smaller when the outbreak starts on a beef or a veal farm. In the case of a start on a 'miscellaneous' farm that sells more than 100 animals for live per year, on average 21.6 other farms are infected. In all cases, there is a wide variation around these averages, as also presented in Table 1. This does not mean, however, that all these other farms face a major outbreak. In 55% of the cases, the infection is limited to the purchase of one or a few positive animals without further spread. In 10% of the cases a small outbreak occurs (less than 5% of the animals becoming infected) and in 35% of the cases almost all animals become infected.

Table 2. The average and maximum number of infected farms and the total losses due to IBR and costs of control (1000 Dfl) in the basic strategy and for a number of alternative situations (sensitivity analyses)

	Starting on a dairy farm		Starting on 'miscell. 100+'	
	Average	Maximum	Average	Maximum
Basic strategy				
Number of infected farms	0.9	31	21.6	89
Total losses and control costs	44	408	300	929
1) No control zone				
Number of infected farms	0.9	31	22.1	93
Total losses and control costs	36	240	197	624
2) 2-km control zone				
Number of infected farms	0.9	31	21.6	89
Total losses and control costs	68	819	570	1.765

	Starting on a dairy farm		Starting on 'miscell. 100+'	
	Average	Maximum	Average	Maximum
3) 4 times per year blood sampling				
Number of infected farms	0.8	12	14.6	45
Total losses and control costs	43[a)]	246[a)]	224[a)]	563[a)]
4) 3-monthly bulk milk tests				
Number of infected farms	1.5	31	28.1	89
Total losses and control costs	60[b)]	432[b)]	372[b)]	1.027[b)]
5) 10% 'illegal' animal movements				
Number of infected farms	3.8	92	67.7	224
Total losses and control costs	49	413	354	1.060

[a)] Not included is the increase in the annual monitoring costs from 5.42 million to 10.85 million Dfl.
[b)] Not included is the decrease in the annual monitoring costs from 9.97 to 3.32 million Dfl.

Strategies two (immediate removal of infected animals) and three (vaccination of all animals on infected farms) did not significantly improve the epidemiological results. This is because the infected farms are not allowed to sell any more animals for live, as long as they have infected animals. This seems to be sufficient to exclude the risks to other farms. The economic results of these strategies are worse, however, as they impose more costs than the basic strategy.

The economic outcome for the basic strategy and some results of the sensitivity analysis are summarised in Table 2, for the average and the maximum outbreak when the outbreak starts on a dairy farm and on a 'miscellaneous 100+' farm, respectively. The economic results include both losses as long as IBR is present and costs of control measures. As shown in Table 2, the economic outcome for the basic strategy is 44,000 guilders on average when the outbreak starts on a dairy farm and 929,000 guilders at maximum when it starts on a 'miscellaneous 100+' farm. These amounts are considerably less than in the case of Classical Swine Fever, as for instance, high costs have to be made for slaughter of pigs for welfare reasons in large areas with movement control. The impact of a control zone on the size of the outbreak is limited, as also shown in Table 2. No control zone, therefore, turns out to provide the best economic results. An increased frequency for blood sampling on the miscellaneous farms is to be recommended. Especially as the maximum size of the outbreaks is reduced considerably. A lower frequency of testing on dairy farms is not to be recommended. The maximum size of the outbreaks does not change (for the infected farms being traced in time in any case), but the average does. This means that it occurs less frequently that no other farms are infected. The last 'what-if' calculation concerns what is called 'illegal transports'. In the basic strategy it was assumed that a ban on live sales is completely complied with. If 10% of the sales continue, then the size of the outbreaks increases considerably in all cases. Now, the maximum size when the outbreak starts on a 'miscellaneous 100+' farm is even 224 farms. So, a responsible behaviour of farmers and a reliable and up-to-date I&R system are important factors in making the eradication of IBR economically attractive.

IMPLEMENTATION FOR CLASSICAL SWINE FEVER (CSF)

On 4 February, 1997, the first case of the Dutch CSF epidemic was diagnosed (Stegeman et al., 1997). During the outbreak the need for a tool to evaluate different control strategies arose. In the summer of 1997 a study was started which applied the general framework of stochastic and spatial modelling of disease spread and control to CSF. The objective of the model is to mimic the 1997/98 outbreak and to predict the future pattern of the outbreak under different control strategies. Next to that, and for evaluation purposes more relevant, to evaluate the effect of changing control strategies and/or certain events back in time. As for FMD, the outcome of the simulation will be linked to the economic model developed by (Meuwissen et al., 1997) in order to get economic results.

Major modifications

The use of the general framework has so far (FMD and IBR) been restricted to evaluating fictitious outbreaks, starting simulation on the day the first farm is infected (day 0). The approach implemented for CSF is different and is illustrated in Fig. 3. From day 0 until a certain moment in time, for instance the day the first farm was diagnosed, the simulation is completely steered by real outbreak data, including knowledge obtained afterwards on infection dates of farms. After this moment, called the simulation start date, new infected farms and controls will pop up as a result of simulation steered by (a) the real infected farms and control areas that are still playing a role in spread and control and (b) the parameters of the spread and control mechanisms. Due to the stochastic nature of the model, different patterns of the outbreak will be simulated (Fig. 3). The simulation start date can be varied over time, depending on the type of calculations desired.

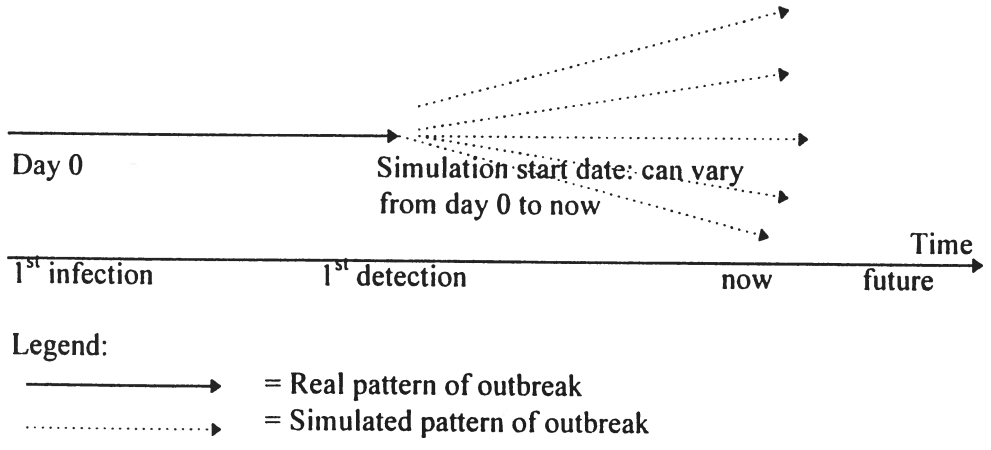

Fig. 3 Schematic representation of simulation of CSF spread and control in relation to time and use of real information on outbreak

Central in the modification towards CSF, therefore, is the use of real farm data and real outbreak data. A data set of all farms with pigs was obtained from the Animal Health Service. For each farm there is an X-Y co-ordinate and number of places for sows, fattening pigs and gilts. Real outbreak data concerns data on (1) diagnosed farms, such as date of infection

(estimated), suspicion, diagnosis and slaughter, (2) areas with movement control in place, in order to have a view on which farms are restricted in their contacts and when and for which period welfare slaughter applies and (3) list of pre-emptive slaughtered farm including slaughter date.

Assigning values to the parameters for disease spread and control is quite difficult for several reasons. Although there is an outbreak, and lots of data are available, they often do not meet the needs of the model. Together with the epidemiology group of the CSF crisis centre, values for parameters on spread and control have been determined, either after analysis of available data or by expert opinion and joint discussions. Disease spread between farms is simulated through two mechanisms: (1) contacts and (2) local spread. Contacts are defined in three categories: (1) animal, (2) transport related to animals, and (3) professional contacts (e.g. vets). The majority of controls applied in the CSF outbreak could be mimicked using the available control mechanisms. A mechanism is added to mimic the concept of purchasing pigs on farms that are under movement control because of welfare reasons: part of the transport contacts do occur although there is a movement standstill and fattening farms become empty after a certain period of time resulting in less farms that can become infected.

Results

After a phase of obtaining all necessary data from different sources, estimating disease spread and control parameters and actually modifying the model for CSF, the study is currently (January 1998) in the phase of calibration. This calibration phase aims to obtain a set of input parameters that results in median results that correspond to the real outbreak. Calibration focuses on different time periods of the outbreak: (1) from first infection till first detection, when only disease spread is important and (2) from first detection on when disease spread and control both play a role. For period 1, the simulation starting date will be set to 0.

After completion of the calibration phase, calculations will be carried out mainly to evaluate the outbreak afterwards. Back in time controls and events will be changed. For instance, the (possible) role of pre-emptive slaughter and the role of events that occurred up until the first diagnosis (moment of detection first case , peak of contacts that occurred on the day the first case was detected) will be investigated.

FINAL REMARKS

The general framework presented in this paper for the stochastic and spatial simulation of spread and control of infectious diseases has shown to be a useful tool in supporting policy makers in decision making (IBR implementation). In the coming months, the framework as applied to CSF will hopefully be useful in evaluating a real outbreak and in preparing for a similar event in the future. The application for CSF will further be developed within a project that started in January 1998 which concerns the development of a decision support system for the control of a CSF outbreak, based on the EpiMAN system as developed in New Zealand for FMD (Jalvingh et al., 1995). Within the project several people will work on obtaining more knowledge on CSF spread within and between herds and the role of data availability in the control of an outbreak. The project proposal was already in the pipeline for funding when the outbreak in the Netherlands started. The 1997/98 outbreak will give the project a flying start; a mass of data and experiences is available for further analysis. Within the same project, the FMD version of EpiMAN will be implemented for use by Dutch disease control authorities.

Data on outbreaks of contagious animal diseases as FMD and CSF is scarce, and if available often not representative for other situations. As a result many of the parameters concerning spread and control are often based on the scarcely available data and on expert opinion. This implies that sensitivity analysis should be used extensively to find those input parameters that have a large impact on the outcome. If possible, additional efforts should be put in to get more precise estimates for these unsure, but important parameters. Sensitivity analysis may as well result in modifications in certain mechanisms in order to be more consistent with reality. However, it is inevitable that there will be parameters whose values are more or less unsure. For that reason, when comparing control strategies, it is especially important to study the ranking of the control strategies in relation to different sets of input parameters ('what-if' calculations).

The strength of the general framework presented in this paper and applied to a certain disease, lies in carrying out calculations for a range of regions (variation in density of farms, distribution over type of farms) and different sets of spread and control parameters. The results of these calculations, together with the assumptions underlying the model, should be used in peace time to discuss what control strategy to apply in what situation. The aim of these discussions is to be better prepared for a real outbreak, and to lose no time during the outbreak on discussions about what control strategy to apply. The 1997/98 outbreak of CSF in the Netherlands showed once more that the threat of a contagious animal disease is there, and that it will certainly pay to be well prepared.

ACKNOWLEDGEMENTS

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APPENDIX 1: MAJOR INPUT PARAMETERS FOOT AND MOUTH DISEASE

INFECTED FARMS

Probability distributions for the following intervals:
A: infection - first clinical signs (FCS)
B: first clinical signs - detection (in case first detection has not occurred yet)
C: first clinical signs - detection (in case first detection has already occurred)

Days	0	1	2-4	5-6	7-8	9-10	11-14	15-18	19-21
A	0	0	0.001	0.134	0.526	0.294	0.045	0	0
B	0	0	0	0.100	0.400	0.133	0.267	0.057	0.043
C (cattle)	0.609	0.351	0.039	0.001	0.001	0	0	0	0
C (pigs)	0.521	0.383	0.074	0.007	0.007	0.007	0	0	0

DISEASE SPREAD MECHANISMS

Contacts off the infected farm:

Type	Average number per day (Poisson)	Transmission rate per contact	Transmission possible from
High	0.285	0.5	infection on
Medium	1.714	0.05	2 days before FCS on
Low	2.286	0.005	2 days before FCS on

Probability distribution contacts over distance classes

Distance class	0-5 km	5-15 km	15-30 km	30-60 km	60-150 km	150-250 km
Probability	0.400	0.350	0.228	0.014	0.007	0.001

Local spread:

Radius	Transmission rate per day	Transmission possible from
1 km	0.01	2 days before FCS on

DISEASE CONTROL MECHANISMS

Detected infected farm:
Slaughter is completed the day after diagnosis

Zones around infected farm:

Type	Radius	Start	Duration	Surveillance?	Proportion of contacts allowed		
					High	Medium	Low
Protection zone	3 km	Day after diagnosis	15 days	Yes	0%	25%	50%
Surveillance zone	10 km	Day after diagnosis	30 days	No	0%	50%	75%

Traced contact farms:
On surveillance for 14 days; 14 days no contacts allowed off the farm

General:
Farm on surveillance has reduction of 1 day in interval first clinical signs - detection

A STOCHASTIC APPROACH TO MODELLING POPULATION DYNAMICS AND INFECTIOUS DISEASES IN THOROUGHBRED YARDS

R. DE LA RÚA-DOMÈNECH*, S.W.J. REID*, G. GETTINBY*, A.E. GONZÁLEZ-ZARIQUIEY** AND J.L.N WOOD***

INTRODUCTION

Population models are a commonly adopted approach for the study of how production units change with time in livestock populations (Sorensen et al., 1992). In contrast, disease models typically make use of equations to represent information about the spread of a specific disease throughout the population. When population and disease models are combined the epidemiologist acquires a powerful investigative tool for testing hypotheses about the spread of the disease and for assessing the impact of control measures (McLeod, 1993). Moreover, if such models are able to include sources of random variation that typify the daily changes in the management of the production units then the model can provide a realistic framework for the provision of decision making support.

In a dynamic stochastic simulation model population changes from one point in time to another are calculated and a random component is included to enable prediction of the probability of events (McLeod, 1993). Such models are ideally suited for the study of the spread of infectious diseases in small closed or almost closed populations such as the housing arrangements commonly used in Thoroughbred yards for the rearing of racehorses.

Equine influenza (EI) is an economically important contagious disease of horses which greatly affects the performance of the horse racing and breeding industry throughout the world (Wood et al., 1993; Timoney, 1996). The disease is typically transmitted from one animal to another by direct or indirect aerosol infection. Influenza-free yards generally acquire the pathogen after the importation of latently or subclinically infected horses (Timoney, 1996). Although the spread of the infection may be restricted through the use of vaccines, there is still a great deal unknown about the infection dynamics and those parameters which increase the risk of contracting EI (Morley, 1995).

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After the widespread epidemic that affected the UK horse population in the summer of 1989, there has been evidence of minor periodic EI outbreaks. These outbreaks are most common in the summer and late autumn (Wood and Mumford, 1992) and may be related to the Flat and National Hunt racing fixtures and the arrival of young horses to the yards after the autumn sales (Wood and Mumford, 1992; Newton et al., 1997). Prevention of EI outbreaks at the yard level relies on vaccination and quarantine of incoming horses. Vaccination of horses, however, may not be sufficient to prevent EI infection or even clinical disease. Vaccine breakdown has occurred in horses with high levels of vaccinal antibody due to "antigenic drift" of the EI virus and the use of outdated vaccinal strains antigenically different from those in current circulation (Mumford et al., 1997). Horses exposed to the virus after vaccination may become asymptomatic virus shedders and contribute to the introduction of EI (Mumford, 1992).

Although much consideration has been given to modelling human influenza outbreaks (for a review see Selby, 1982), it is clear that these cannot be directly applied to horse populations because of obvious differences in their demographic structure. Stochastic models have been built for simulation of livestock populations and their diseases. However, as yet none of them has been specifically employed to address horse populations (Sorensen et al., 1992; McLeod, 1993; Tsutsui et al., 1997; González-Zariquiey, 1997). If a stochastic simulation model could be developed for EI it would be a useful aid for the assessment of efficacy in future vaccination programmes.

In order to address these points, a computer model known as EQUISIM has been under development. EQUISIM is a dynamic, discrete-time, stochastic iterative model that simulates the population dynamics of a Thoroughbred flat training yard over a 365-day period. EQUISIM was conceived as a horse population model capable of coping with population heterogeneity and random sources of variation and potentially suitable for a range of infectious diseases.

In this paper we describe how EQUISIM has been used as a tool to study the predicted course of equine influenza virus infection in a Thoroughbred training yard and to simulate the effect of some control measures on the spread of the infection.

MATERIALS AND METHODS

Description of the population model

EQUISIM has been developed as a stochastic computer model that attempts to mimic the typical management life cycle for horses within a thoroughbred flat training yard over a whole year. In summary, the model includes the following components:

1. an initial population of horses divided into age and sex strata,
2. a set of variables that contain all the relevant information needed to represent all the animals in the population at any time (state variables) and,
3. demographic parameters or decision variables controlling the transition of animals from one time step to the next.

Some of the model's main features are:

1. individual horses constitute the simulation units,
2. the model operates with time steps of one day, allowing changes in the yard structure to be followed from day to day,
3. most discrete events (such as decision for culling, actual number of daily arrivals and departures, etc.) and continuously distributed variables are stochastically generated,
4. it is amenable to a single or a large number of realisations of any given scenario and,
5. it can mimic the effect of seasonal factors and management decisions on the population structure and output.

The yard population was simulated according to the conceptual model provided in Fig. 1, representing the approximate movements of a typical flat training yard in Newmarket. The model takes into account the thoroughbred aging system, which considers all of the horses to be born the 1 January of their year of birth.

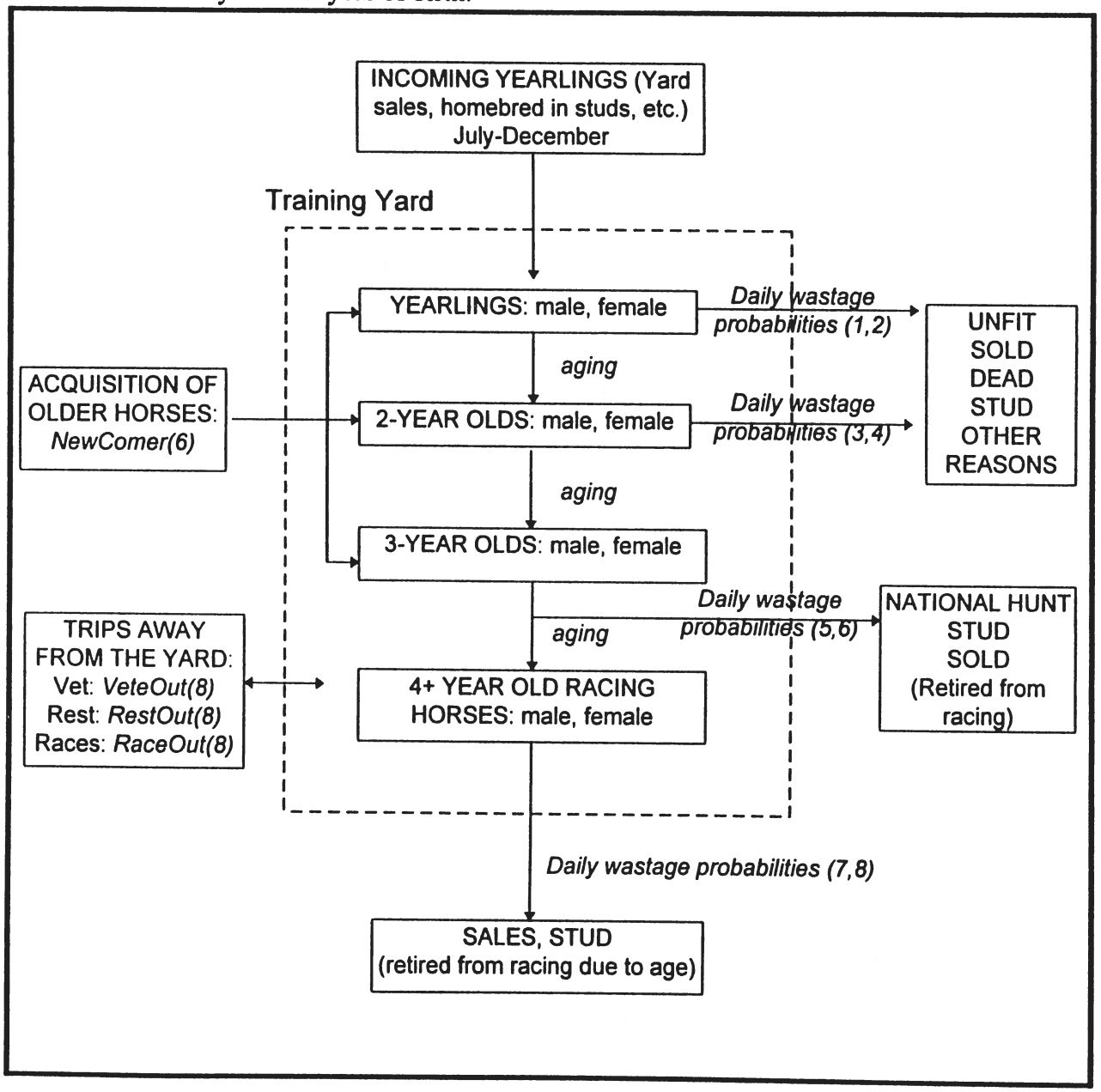

Fig. 1 Conceptual population model: compartmental representation of the flow of horses

Movement of horses out of and into the yard and the flow from one age stratum to the next one are illustrated by the compartmental representation in Fig. 1. Four age strata, with two sex categories each, making a total of eight groups of animals were considered. Animals may leave each of the age groups through wastage or aging. New animals of different origin arrive in the yard as yearlings, although a small proportion can arrive at an older age. There are no foalings on the yard. Most incoming yearlings enter the yard in the second half of the year (mainly from September to December) in large numbers. Therefore, the yearling population of the yard tends to build up at year's end. In addition, EQUISIM allows for seasonal effects in the trips outside the yard. Seasonality of racing, for instance, was built into the model by assigning higher monthly weights to the months of March through October.

"Wastage" due to limb injuries, poor performance and other reasons takes place throughout the year. The majority of horses are kept in training until the end of their fourth year (i.e. when they are still "three year olds"), although there are also sales of 2 and 3-year olds. At the beginning of their fifth year most horses end their flat racing career, and leave the yard to breed or to become National Hunt jump racers. However, a small proportion of 4-year old and older horses can be kept on at the yard and go on flat racing in events for older horses. Age and sex-specific wastage probabilities are calculated based on the composition of the initial population so that the age structure of the yard population is maintained at the end of the simulation period. The different causes of wastage (injury, death, lack of ability to race, retained for breeding, unsoundness, etc.) are irrelevant to this simulation model. Default values for the population parameters in the model were taken from data recorded at a flat training yard in Newmarket.

Description of the disease model

A simple disease model simulates day to day changes in the infection status for each animal on the yard. When a horse is exposed to the infectious agent, its infection status goes through a cycle divided into five states or compartments. As the days progress, the state variable defining the infection status changes from one state to the next state until the horse becomes susceptible again. A susceptible horse can also reach the immune state after receiving the vaccine. Table 1 summarises the five possible values taken on by the state variable for the infectious cycle.

Table 1. Description of the disease model compartments or EI infection stages

State	Mean duration in days (standard deviation)	Next stage	Indicator variable
Susceptible	Until infection or vaccination	Latent or Immune	1
Latent	1.25 (0..25)	Infectious	2
Infectious	5.50 (1)	Convalescent	3
Convalescent	25 (4)	Immune	4
Immune	105 (10), following vaccination 152 to 250, following infection	Susceptible	5

The conceptual disease model assumes that the population simulated must be homogeneous in the sense that while horses are on the yard, they mix randomly and uniformly. In other words, all horses are equally likely to contact one another so that a single effective contact rate can be applied throughout. The model only considers direct and aerosol transmission of EI, ignoring indirect transmission through contaminated fomites. It is also assumed that while a horse is on the premises it has no contact with any horses other than its yard mates. Consequently, the only way the infection can be brought into the yard is through acquisition of infected yearlings and older horses or by incoming horses which picked up the infection at a racetrack or when they were taken to a veterinary hospital or to a resting yard. In order to mimic the infection of horses which leave the yard temporarily and assign an infectious status to new incoming horses, the program must first simulate the epidemic in the general horse population and generate an array of daily probabilities of infection.

To explore vaccination parameters that influence the spread of EI, the part of the code that simulates the vaccination policy is governed by several decision variables. These include the duration of lay off from racing following vaccination, the dates of up to three yearly EI vaccinations and the mean duration of immunity following vaccination, with its standard deviation. Additionally, two key probability values can be identified in field conditions. First, the likelihood of receiving the vaccine, and if so, the probability of building an immunising response. For practical purposes, the program combines these two probabilities in a single value, the probability of not being properly immunised on the vaccination day.

The impact of the disease and the strategies implemented to control it are evaluated on a day by day basis from the final output. An epidemic is described by the daily total number of infected horses rather than the incidence of clinical disease, since a significant proportion of EI infections are subclinical (Mumford, 1992). Due to the extremely low mortality associated with uncomplicated EI, the model does not compute influenza-related deaths, although it does estimate the days of training lost due to EI infection and convalescence, plus the days of training lost following EI vaccination layoff.

Stochastic simulation program

Yard management practices simulated by EQUISIM and disease parameters are combined into a single epidemiological computer model of the spread of disease (EI) within a yard. The computer program that implements the transition logic consists of a series of population and disease procedures. Each procedure executes a specific aspect of the model by using equations and algorithms written in Visual Basic code.

The user specifies the number of horses per age group at the beginning of the simulation, along with the demographic and disease parameters. Based on that input population the program assigns an age and infection status to the horses in each age group using stochastic simulation. Additionally, another procedure calculates daily wastage probabilities for the transitions between age strata.

Every individual in the simulated population has its own record, defined by nine state variables stored in an array in memory with as many entries as horses in the population at any given time. The nine state variables are: horse number, sex, age in days, age group, number of

days away from the yard, infection status, number of days spent on that status, number of days left for vaccination and number of days resting or laid off. The program starts by reading the initial (seed) population and parameter values, performing 1 complete set of calculations. It then resets the population status and repeats the calculations 364 times. Each iteration represents one simulated day in the life of the horse population and in each iteration the population produced by the previous iteration is used as the starting population. During the simulation process, the population exists in the memory of the program, as an array of variables containing the status of the yard population. Counter variables keep track of the numbers of animals in each age stratum and infection state, the number of horses leaving and entering the yard, the number of horses laid off due to vaccination and disease, etc.

A simulation can be run one or several times. Each simulation or run of the model consists of the outcome of a single yard over a 365-day period, from 31 March through 30 March of the following year. Being a stochastic model, no two runs with the same initial population and parameters will yield the same results. When more than one run is carried out the outcome variables from each run are averaged.

Modelling scenarios

Several scenarios simulating the effect of different vaccination policies were investigated. Only the results of the following scenarios are presented in this paper:

1. No intervention during the simulation period, on a fully susceptible yard population.
2. Mass vaccinate horses twice on 30 November and 15 March, i.e. before beginning of racing season and at the end of the season.
3. Mass vaccinate horses three times a year on 15 June, 30 November and 15 March, i.e. same as before with an additional vaccination in the middle of the racing season.
4. Mass vaccinate horses three times a year on 15 June, 1 September and 15 December, i.e. vaccination around Christmas, mid racing season and before the arrival of new yearlings.

Each simulation scenario was run 50 times on a PC, thus mimicking the performance in 50 yards with identical initial conditions. Using a Pentium 150 MHz computer with 32 megabytes of RAM all fifty realisations could be completed in about 8 minutes.

RESULTS

Fig. 2 provides the daily cumulative breakdown of the total horse population into age and sex strata as simulated by the program. The initial population consisted of 30 male yearlings, 30 female yearlings, 24 two year old males, 8 two year old females, 12 three year old males, 8 three year old females, 16 four year old and older males and no females of 4 years of age or older. The age structure of the horse population remains quite stable along the simulation period, although the proportion of yearlings increases slightly at year's end and decays afterwards. The behaviour of the population model mimics the real situation observed in typical thoroughbred yards, where yearlings are brought from the sale yards in large numbers during the second half of the year.

The model was then run 50 times to predict the effect of different vaccination strategies on the daily number of susceptible, infectious, convalescent and immune horses in the population. Only the vaccination dates were allowed to change across scenarios, while the other parameters remained the same. The simulations assume that the general horse population is being affected by an EI epidemic peaking in the beginning of August and the beginning of November. Figures 3 through to 6 show the distribution of horses in the four disease states during a 365-day simulation under each scenario. The results shown on the charts are the daily averages of the values obtained after running 50 realisations per scenario.

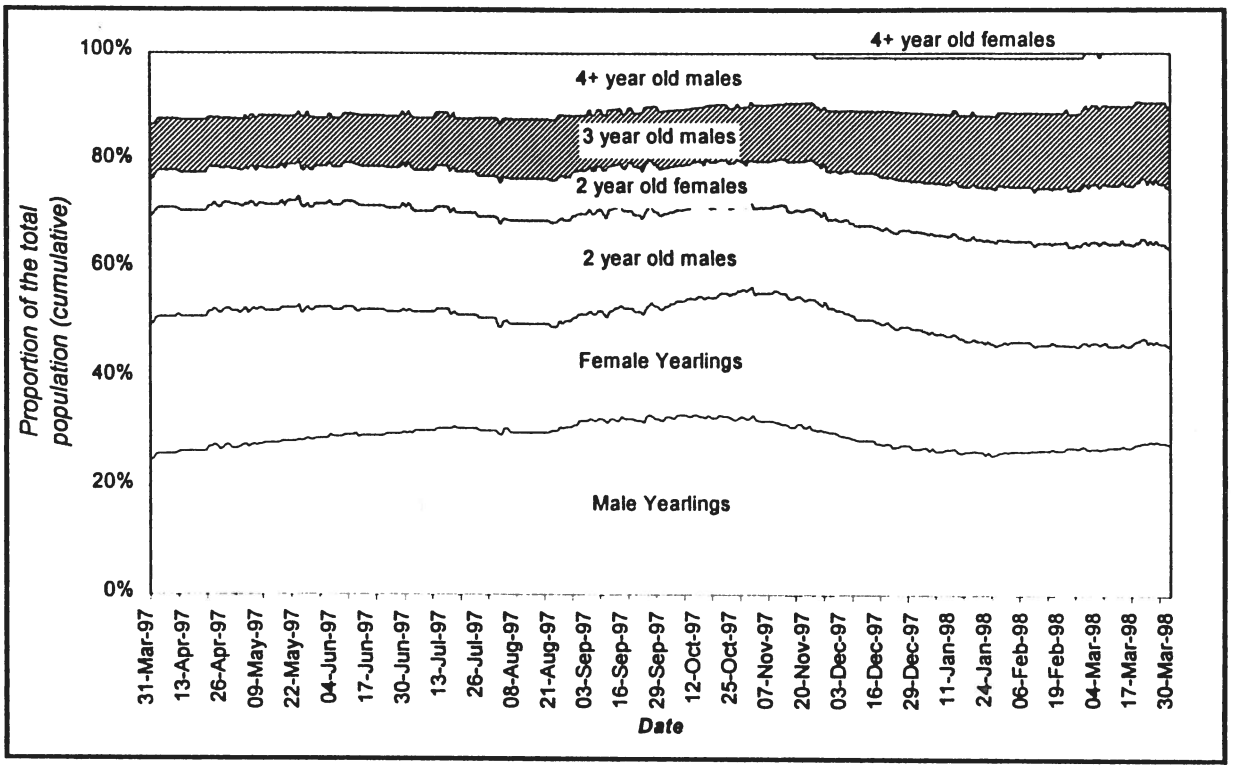

Fig. 2 Composition of the yard population over the 365-day simulation.

The hypothetical situation where every horse is initially susceptible to the EI virus (Scenario 1) and no vaccination is performed is illustrated in Fig. 3. The distribution shown in Fig. 4 is obtained when two vaccinations are given at year's end and before the onset of the flat racing season, which is the standard policy in many yards. This doesn't seem to control the spread of EI into the yard, although the daily prevalence of the infection is lower than in the no-vaccination scenario. The expected situation when the EI vaccine is administered to 99% of the population at three different times is presented in Figs. 5 and 6. Only when the horses receive three doses of vaccine in the middle of June, the 1 September and around Christmas time can vaccination control the EI outbreak. In this instance, the only cases of EI observed are the primary cases that contracted the infection outside the yard. The infection, however, does not spread to the susceptible population within the yard (Fig. 6).

DISCUSSION

With the availability of cheap computer power iterative simulation models have become more popular. On the one hand, these type of models do require some programming skills and fast computers. On the other hand, they do not depend on a set of complicated differential equations with closed-form analytical solutions to define relationships in a system, unlike analytical models (Hurd and Kaneene, 1993; Sivam, 1995). Moreover, they can deal with single individuals as modelling units within a heterogeneous population, as opposed to deterministic analytical models (Sivam, 1995). Both analytical and simulation models have been extensively used to mimic livestock systems and to study several infectious and parasitic diseases of livestock and humans (for a review on this topic see Hurd and Kaneene, 1993). However, to our knowledge, nobody until now had attempted the development of a stochastic iterative simulation model to represent the spread of an infectious disease within a horse population.

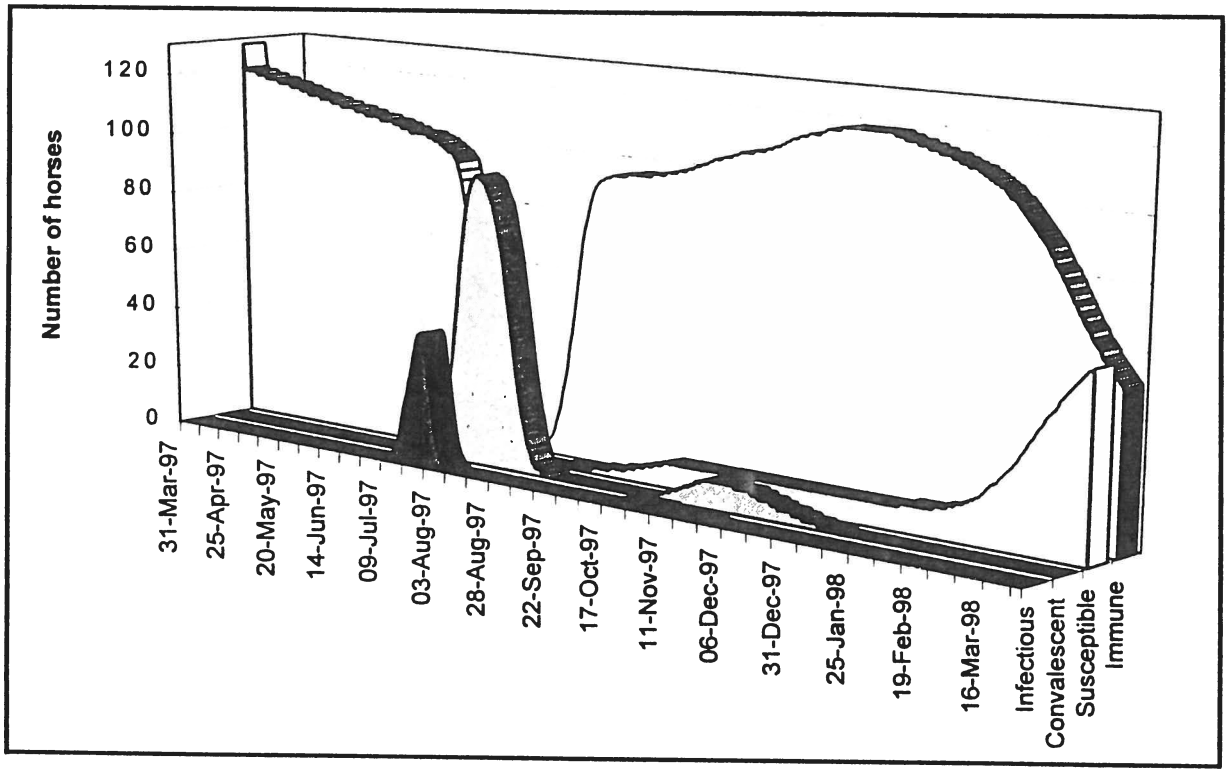

Fig. 3 Predicted daily distribution of infectious, convalescent, susceptible and immune animals in scenario 1: no intervention on a fully susceptible starting population

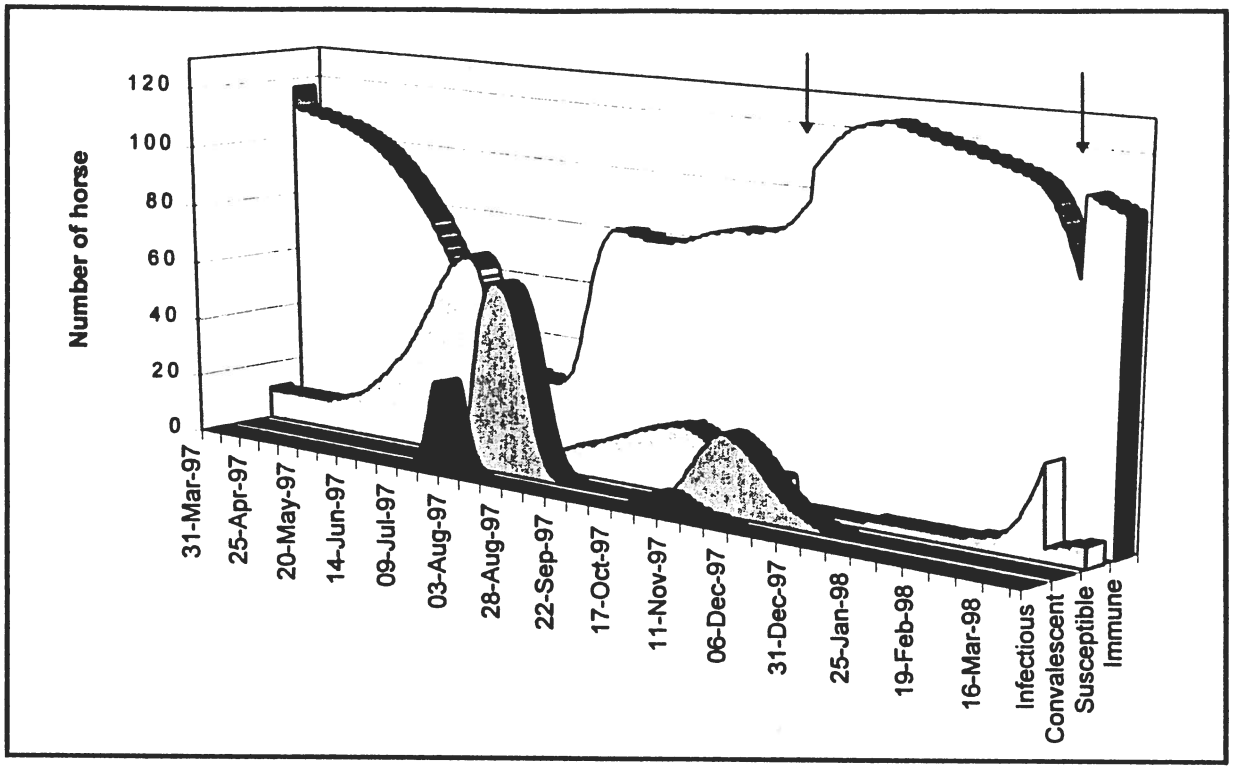

Fig. 4 Predicted daily distribution of infectious, convalescent, susceptible and immune animals in scenario 2: vaccinating 99% of the population on 30 November and 15 March (arrows)

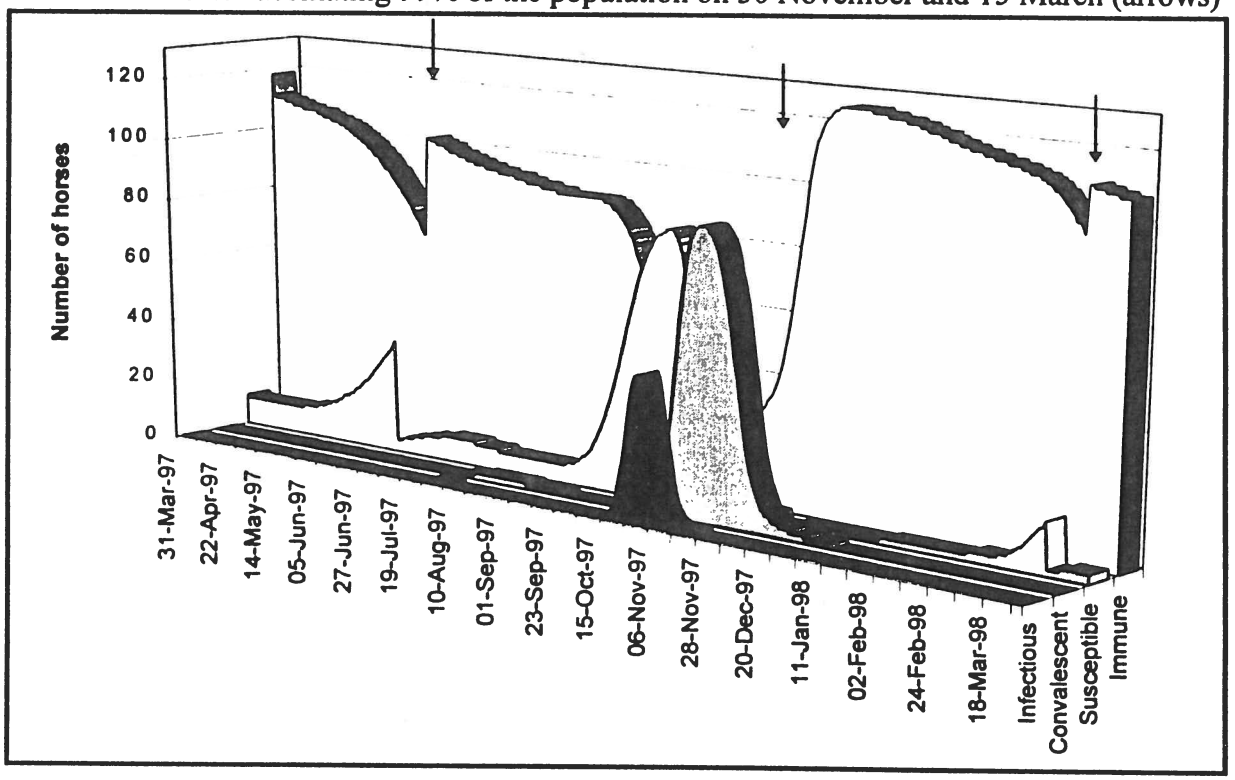

Fig. 5 Predicted daily distribution of infectious, convalescent, susceptible and immune animals in scenario 3: vaccinating 99% of the population on 15 June, 30 November and 15 March (arrows)

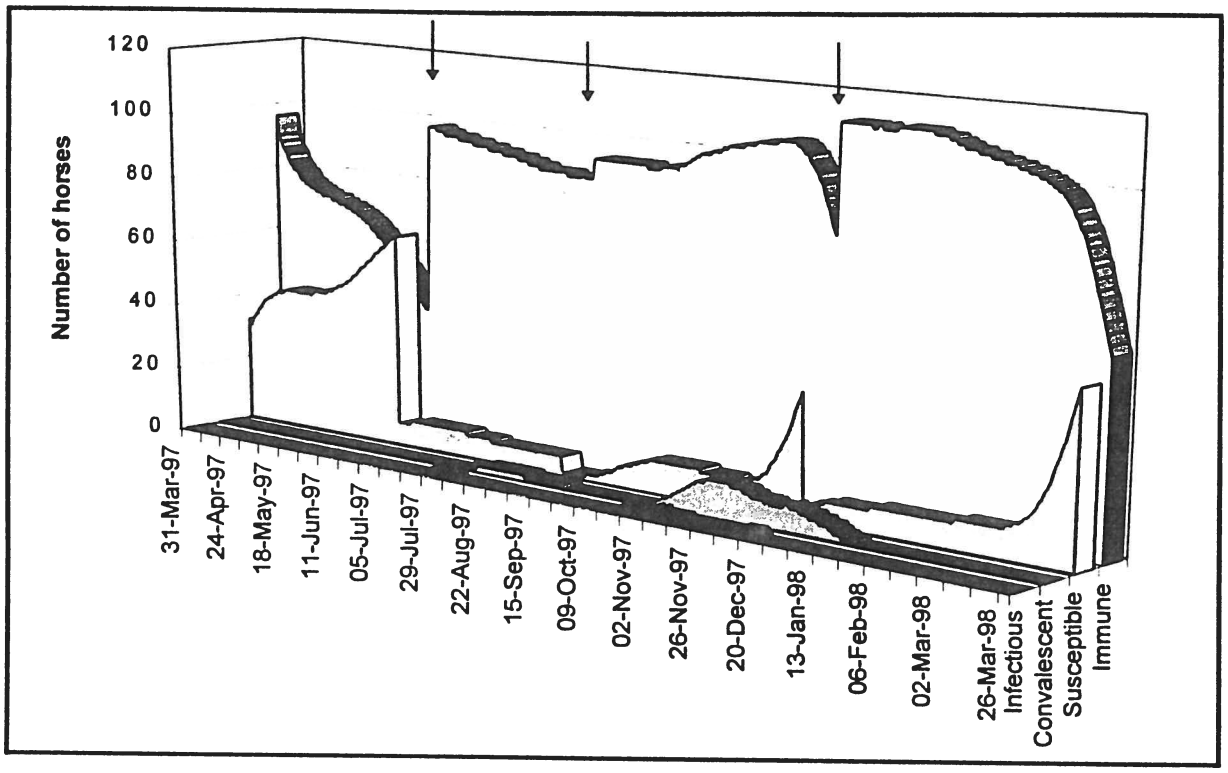

Fig. 6 Predicted daily distribution of infectious, convalescent, susceptible and immune animals in scenario 4: vaccinating 99% of the population on 15 June, 1 September and 15 December (arrows)

EQUISIM provides a tool to investigate disease dynamics within individual horse yards. There is no published scientific information on the structure of the Thoroughbred population in the UK other than the MAFF agricultural census of 1994, which only records horses on agricultural holdings, and the work of Mellor (1997). Mellor identifies over 96,000 horses in Scotland and five northern English counties, 30% of which were allegedly Thoroughbreds or Thoroughbred mix. Likewise, lists of trainers and racehorses are compiled yearly in the Horses in Training directory, but they provide only a snapshot of the composition of the training yards at the time of publication. Therefore, the population structure simulated in the model had to be based on two years' management data collected from a well-run training yard in Newmarket.

Several factors have been associated with a greater occurrence of EI in stabled horses during outbreaks in Canada and Britain (Morley, 1995). These included breed (pony horses), age (less than 4 years old), low pre-exposure antibody titres for EI, racing fewer times prior to epidemics, frequent contact among stabled horses, a history of previous exposure to the virus and vaccination outside the period comprised between 2 and 12 weeks prior to exposure. Of the above factors, the immunogenicity and the timing of EI vaccines are perhaps the most critical ones in determining the spread of the infection in horse population.

Even though vaccination of thoroughbred horses against EI has been mandatory in the UK since 1981, EI infections continue to occur among vaccinated horses due to antigenic drift,

outdated vaccinal strains and short-lived immunity (Wood and Mumford, 1992; Mumford et al., 1997). Depending on the type of adjuvant, EI vaccines can provide different durations of immunity (Mumford et al., 1994). This partly dictates the appropriate re-vaccination interval. For each horse that responded well to the vaccine challenge, the program simulated the days of protection by drawing a random number from a normal distribution, with mean duration ("Immuavg") and standard deviation ("ImmuSdv") determined by the user. Thus the disease model can take into account whether an aluminium phosphate or hydroxide, carbomer or ISCOM-adjuvant vaccine was used for vaccinating the horses simply by changing the expected duration of protection against infection.

In many yards vaccination is not a continuous event, but a mass vaccination that takes place two or three times a year. The vaccination programme recommended by manufacturers consists of a primary series of two doses given 3-6 weeks apart, a booster 6 months later and annual or biannual boosters thereafter. Most trainers, however, when a new horse is brought from abroad, give a primary course of vaccinations in November and December, followed by a booster in March, before the start of the flat racing season. They do not vaccinate until the following Christmas, so that horses become susceptible again by the time of maximum risk, i.e. when they return from the races and mix with incoming yearlings from the autumn sales. This is the situation simulated in scenario 2, which shows that two epidemics of EI would take place in July-August and October-November.

It has been suggested that a strategic vaccination policy targeting the most likely times of viral challenge could provide protective antibody levels year-round, especially during the high risk seasons of summer and autumn (Newton, pers. comm.; Newton et al. 1997). According to this, older horses should receive twice yearly boosters in March and June, and even a 3rd one in September. The results of our computer simulations, run under the assumption that the protection stimulated by the vaccine lasted 3.5 months on average, support this view. Only when a primary course of vaccination was simulated in mid-December, followed by boosters in mid-June and September the EI epidemic could be prevented (scenario 4). However, trainers may be reluctant to follow such a protocol, out of fear that horses under perform if vaccinated during the racing season. Moreover, vaccination in June and September forces racehorses to be laid off for ten days in the middle of the racing season, an unacceptable practice for many horse owners.

In the computer program, persistence of immunity against infection (not against disease) following natural infection is simulated by assuming that duration of immunity is normally distributed around a value half way between a maximum and minimum. The values used as default were those reported in EI challenge experiments (Hannant et al., 1988). The resulting period of immunity tends to be longer than that stimulated by vaccination. So, rather than assuming a rate of antibody decay following vaccination, the program at present keeps subtracting one less day of protection until this variable becomes zero and the animal becomes susceptible. This is probably not the ideal way of mimicking antibody decay, so we are considering using an array of risks of infection for different post-vaccination time intervals, rather than a mean duration.

It is intended that EQUISIM could be of service to practising veterinarians, or used as a decision support tool by a management team responsible for the welfare of the equine population in a Thoroughbred yard, or used as a teaching aid in professional development programmes for

equine clinicians and students. It is also intended to validate the model against EI case reports from real life, to obtain other estimates of the effective contact rate and to make further improvements to the model.

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MODELLING OF *NEOSPORA* SPECIES INFECTION IN DAIRY CATTLE: THE IMPORTANCE OF HORIZONTAL AND VERTICAL TRANSMISSION AND DIFFERENTIAL CULLING.

N P FRENCH[*], H C DAVISON[†], D CLANCY[‡], M BEGON[§], AJ TREES[†]

Since it was first recognised as a cause of abortion in cattle in 1989 (Thilsted & Dubey, 1989), *Neospora caninum* has become established as a major infectious cause of abortion in cattle world wide (Anderson et al., 1991, Boulton et al., 1995, Duff & Otter, 1994, Nietfeld et al., 1992, Pare et al., 1997, Thurmond et al., 1997, Trees et al., 1994). The economic loss associated with *Neospora sp.* infection is not only via abortions but also endemic foetal loss (Pare et al., 1997) and increased culling of infected animals (Thurmond & Hietala, 1996).

There is much speculation about the nature and routes of transmission of *Neospora caninum*. It has been established that *Neospora caninum* is a vertically transmitted parasite (Anderson et al., 1997) and some authors have suggested that vertical transmission alone may be responsible for maintaining the infection in populations of dairy cattle (Bjorkman et al., 1996). However, this would appear to be at odds with ecological theory; similar studies have demonstrated that if such a parasite adversely affects either reproduction or survivorship of the host, it will not persist in the population in the absence of any other route of transmission (Lipsitch et al., 1995).

This study develops and adapts mathematical models to explore the behaviour of a vertically and horizontally transmitted parasite in a population that is of a fixed size in which the birth and death rates vary between subgroups. In a herd of dairy cattle this would be consistent with the need to maintain a stable herd size, the preferential selection of certain family lines and the effect of disease on fertility and culling. It is intended to complement the empirical studies of *Neospora sp.* infection and provide a theoretical basis for our understanding of the transmission process.

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MODEL 1: Vertical and horizontal transmission and no differential culling

The simplest model assumes that the parasite has no effect on the fitness of the host (i.e. negligible virulence) and that the host population of cattle has a fixed size N. The system is represented by a system of non-linear differential equations that is an adaptation of the classical SIR model. Throughout the study X represents the population of uninfected and susceptible cattle and Y the population of infected cattle and there is no recovered or immune class.

$$dY/dt = \rho Y (\phi - 1) + \beta X Y /N \quad (1)$$

$$dX/dt = \rho Y (1 - \phi) - \beta X Y /N \quad (2)$$

or equivalently:

$$dY/dt = \rho Y (\phi - 1) + \beta (N-Y)Y /N \quad (3)$$

where $N-Y = X$

Here ρ is both the birth and death rate of the population of cattle, ϕ is the vertical transmission probability (and is always less than 1) and β is the horizontal transmission rate. The birth rate specifically refers to the rate at which females are added to the milking herd. The horizontal transmission rate, β, can be interpreted as the contact rate between cattle multiplied by the probability that the contact would result in infection, if it were with an infectious individual. When multiplied by the prevalence of infection (Y/N) the expression $\beta Y/N$ becomes the per-capita rate of infectious contact (equivalent to the force of infection or the incidence rate of horizontally transmitted infections). Although this implies direct contact between an uninfected and an infected cattle, it could apply equally to contact between uninfected cattle and material from infected cattle. In other words, the incidence rate of infection via the horizontal route is proportional to the prevalence of infection.

The equilibria are the points at which the rates of change of both X and Y are zero (i.e. $dY/dt = dX/dt = 0$). By performing a local stability analysis (May 1974), the behaviour of the system at each equilibrium point can be assessed. In this system two equilibria can be identified:

$X = N, Y=0$ (stable if $\beta < \rho (1- \phi)$ and unstable if $\beta > \rho (1- \phi)$

and if $\beta > \rho (1- \phi)$

$Y = [(\rho \phi - \rho + \beta)/\beta]N$, $X= N - [(\rho \phi - \rho + \beta)/\beta]N$ (stable node)

ie. a stable prevalence of infection at equilibrium of

$$(\rho \phi - \rho + \beta)/\beta \quad (4)$$

Including virulence in the model

The model can be extended by adding an extra parameter μ to represent the death rate (in dairy herds this would be mainly the result of culling). We can now introduce virulence by assuming that the death rate for infected cattle, μ_1, is higher than the death rate for uninfected cattle, μ_2, and birth rate for infected cattle, ρ_1, is lower than the birth rate for uninfected cattle,

ρ_2 . This would be consistent with *Neospora sp.* infection increasing the culling rate of infected cows (Thurmond & Hietala, 1996) and causing increased foetal loss (Pare et al., 1997, Thurmond et al., 1997).

The system of equations is now:

$$dY/dt = \rho_1 Y \phi - \mu_1 Y + \beta X Y /N \quad (5)$$

$$dX/dt = \rho_1 Y (1-\phi) + \rho_2 X - \mu_2 X - \beta X Y /N \quad (6)$$

The growth of the population of uninfected cattle, $\rho_2 - \mu_2$, is now allowed to vary:

$$\rho_2 - \mu_2 = [(\mu_1-\rho_1) Y]/ X . \quad (7)$$

This means that if $\mu_1 > \rho_1$ then $\rho_2 - \mu_2$ would have to be greater than zero in order to maintain herd size. In other words the birth rate in unaffected cattle (i.e. the rate of production of female replacements) would have to be higher than the culling rate in that group.

Equivalently

$$dY/dt = \rho_1 Y \phi - \mu_1 Y + \beta (N-Y)Y /N \quad (8)$$

where N-Y = X

It can be seen that the basic reproductive number Ro is

$$(\rho_1 \phi + \beta)/\mu_1 \quad (9)$$

which is the sum of the vertical and horizontal components of transmission multiplied by the average life span of an infected individual ($1/\mu_1$).

Provided $0 < \mu_1 - \rho_1 \phi < \beta$ the stable equilibrium prevalence of infection is now:

$$(\rho_1 \phi - \mu_1 + \beta)/\beta \quad (10)$$

If $\mu_1 - \rho_1 \phi > \beta$

then the equilibrium at Y = 0 is stable.

Figure 1 shows the behaviour of the above systems of equations. The vertical transmission probability, ϕ, is assumed to be high (0.8) but less than 1.0 (Dubey & Lindsay, 1996). The death and birth rate parameter values used in the simulations were based on the age distributions of a number of commercial dairy herds. Importantly, the birth rate refers to the rate at which female dairy replacements are born and is not simply the inverse of the calving interval.

In each simulation the infection is introduced into the herd in a group of 10 animals, and the prevalence of infection either rises or falls to an equilibrium prevalence according to the parameter values. From this analysis it is evident that a certain amount of horizontal transmission is necessary to result in an equilibrium prevalence of infection above zero. Below this threshold (i.e. if $\beta < \mu_1 - \rho_1 \phi$) the infection prevalence will not increase above the level at which it was introduced into the herd and will inevitably decay down towards zero prevalence.

It can also be seen that the amount of horizontal transmission needed to maintain infection is reduced as the vertical transmission probability is increased. For example, if ϕ is 0.9, $\mu_I = 0.3$ and $\rho_I = 0.2$ then the threshold value of β is 0.12. At a level of transmission slightly above this threshold, $\beta = 0.15$, the prevalence of infection in a herd at equilibrium would be 20.0 % and the incidence rate of new infections resulting from horizontal transmission would be 3.0 per 100 cow-years. This is much lower than the rate of seroconversion (8.5 per 100 cow-years) observed by Pare et al (1997) suggesting that this level of horizontal transmission can easily be achieved in commercial dairy herds.

Examination of Eqs. 9 and 10 and Fig. 1 shows that, if the horizontal transmission rate is kept constant, then increasing the virulence (i.e. increasing the death rate and/or reducing the birth rate of infected cattle) will reduce both the prevalence of infection at equilibrium and the value of Ro. This is evident when the top and middle lines in Fig. 1 are compared; in both situations the value of β is 0.25 but the middle line includes some virulence. This has important implications for the evolution of virulence. If virulence is part of an evolutionary process aimed at maximising Ro (May & Anderson 1990) then the degree of horizontal transmission must have increased as the virulence increased. This would be consistent with a reduction in birth rate due to foetal loss, in particular abortion, coinciding with an increase in horizontal contact with infected material.

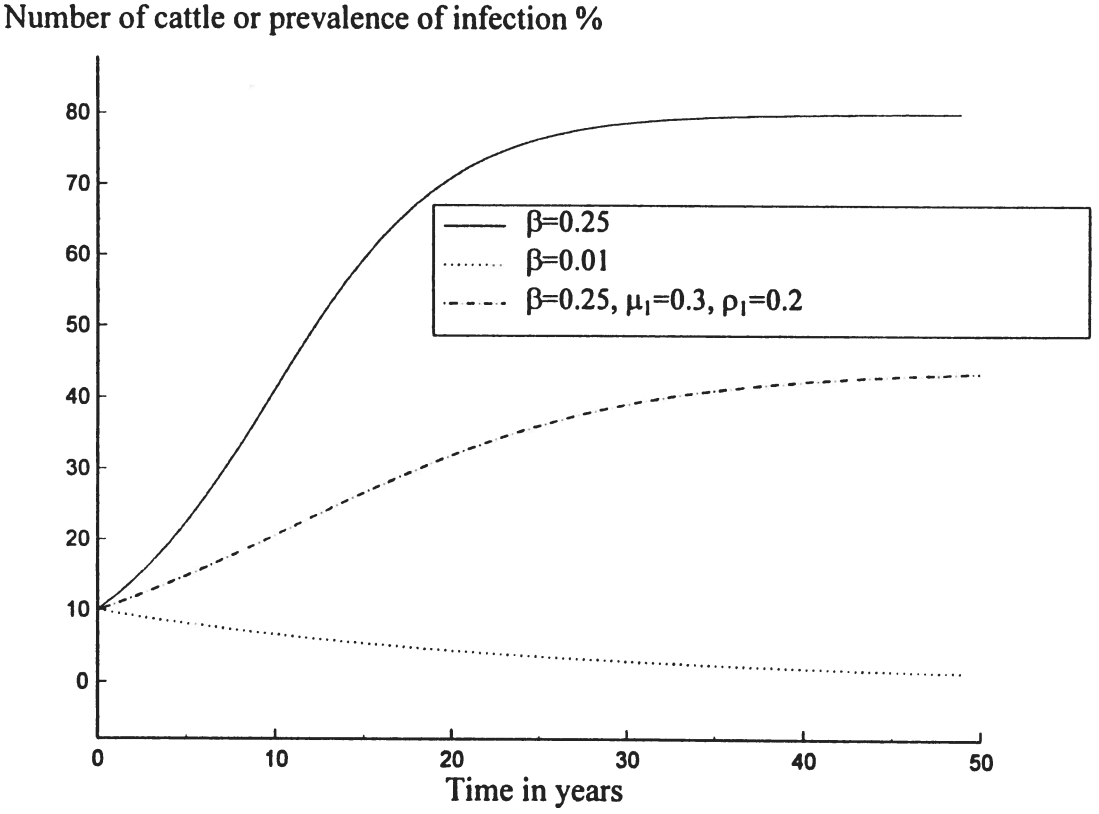

Fig. 1. Three simulations of the systems of Eqs. 1 and 2 (top and bottom lines) and 5 and 6 (the middle line). In all situations ϕ=0.8, N=100 and the starting conditions are X=90 and Y=10.

The model can be modified in order to separate out cattle infected by vertical or horizontal routes. Figure 2 shows that the number of cattle in the herd infected by vertical transmission increases if horizontal transmission alone increases. This is demonstrated by increasing the value of β, but keeping ϕ the same.

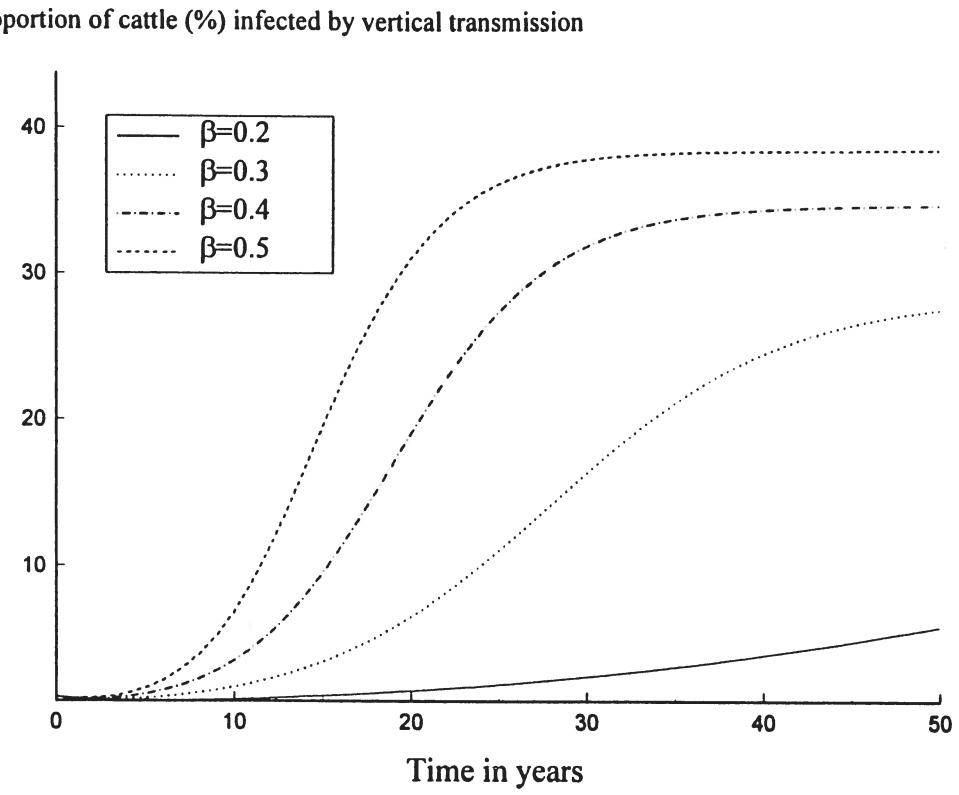

Fig. 2. The proportion (%) of cattle in the herd infected by vertical transmission for a range of horizontal transmission rates. In all situations ϕ=0.8, μ_1 = 0.3, ρ_1=0.2, ρ_2=0.25, N=100 and the starting conditions are X=99 and Y=1.

MODEL 2: Imperfect vertical transmission and differential culling.

In the above models it is assumed that there is no preferential selection of infected cattle and, if anything, there is a reduced fertility and/or survivorship of infected cattle. This model explores the behaviour of a similar system but with "differential culling". The aim of this part of the study is to explore the behaviour of the system assuming that the infection is introduced into the herd in a group of animals of superior genetic merit. This is done by preferentially selecting for animals descended from the original infected group. Instead of a net reduction in fitness of infected animals, those descended from the original group of infected cows, regardless of infection status, are more likely to enter the milking herd. It is important to distinguish this from a situation where infection status alone determines fitness. The models are still subject to the same constraint that the herd size remains the same throughout.

The model now has three categories of cattle, Y = infected of high genetic merit, X_1 = uninfected of high genetic merit (i.e. descended from group Y) and X_2 = uninfected of low

genetic merit. In the simplest system, we assume that fertility and survival are purely determined by management and not by infection, and there is no horizontal transmission.

$$dY/dt = \rho \phi Y - [(X_1 + Y) \rho Y]/N \quad (11)$$

$$dX_1/dt = \rho X_1 - [(X_1 + Y) \rho X_1]/N + \rho (1-\phi) Y \quad (12)$$

and

$$X_2 = N - (Y + X_1) \quad (13)$$

Where ρ is the birth and death rate of the herd in the absence of preferential selection. It can be seen that $[(X_1 + Y) \rho] / N$ is the culling rate for animals of high genetic merit. As the proportion of animals with high genetic merit increases so the culling rate increases to ρ thus maintaining the herd size. However, initially the culling rate for the superior animals would be very low compared to the cattle of low genetic merit.

Two equilibrium points can be identified

$Y = 0, X_1 = 0$ (unstable node)

$Y=0, X_1=N$ (stable node)

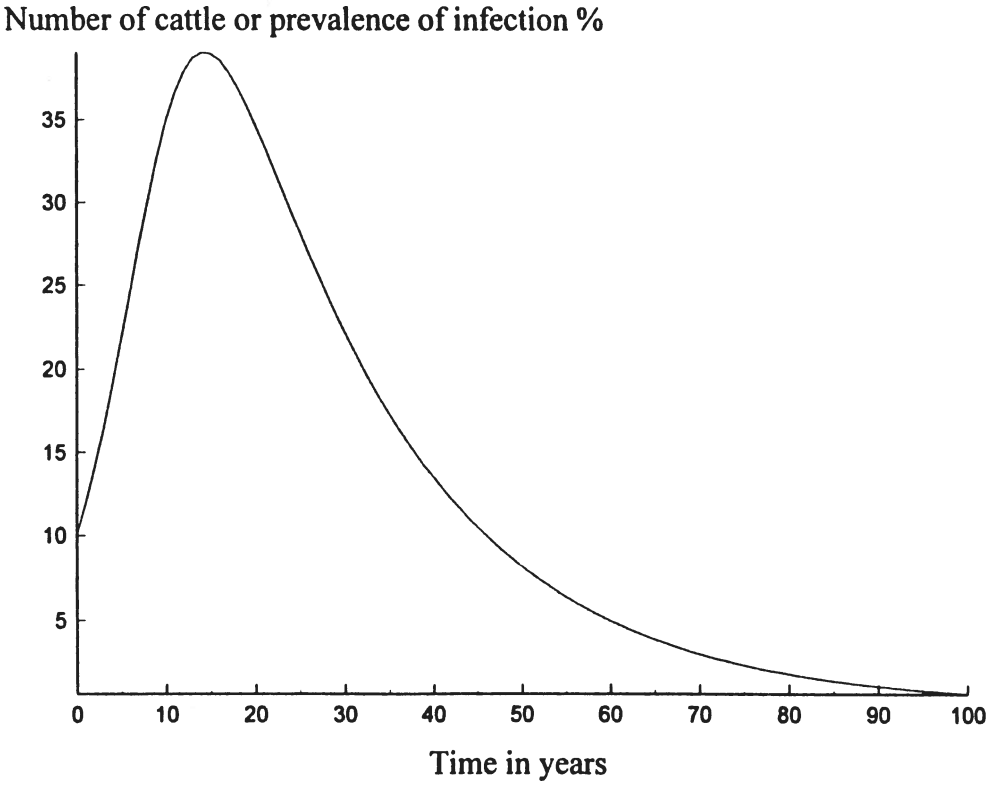

Fig. 3. The dynamic behaviour of model 2 with $\phi = 0.8$, $\rho=0.25$ and N=100. The starting conditions are $Y=10$, $X_2=90$ and $X_1=0$

The preferential selection of infected cattle results in an increase in the prevalence of infection up to 40%, but this is inevitably followed by a decline towards zero prevalence. This shows that, with vertical transmission alone, the prevalence can increase substantially above the level at which it was introduced into the herd, but over time the disease will gradually disappear.

If we extend the model to include horizontal transmission, another category of animal is introduced. The infected group is now divided into high (Y_1) and low genetic merit (Y_2) cattle where the latter were low merit animals infected by the horizontal route. The system then becomes:

$$dY_1/dt = \rho \phi Y_1 - [(Y_1+X_1) \rho Y_1]/N + [(Y_1 + Y_2) \beta X_1] / N \quad (14)$$

$$dY_2/dt = \rho \phi Y_2 - [(N+Y_1+X_1) \rho Y_2] / N + [(Y_1 + Y_2) \beta X_2] / N \quad (15)$$

$$dX_1/dt = \rho X_1 - [(Y_1+X_1) \rho X_1]/N + \rho (1-\phi) Y_1 - [(Y_1 + Y_2) \beta X_1] / N \quad (16)$$

$$dX_2/dt = \rho X_2 - [(N+Y_1+X_1) \rho X_2] / N + \rho (1-\phi) Y_2 - [(Y_1 + Y_2) \beta X_2] / N \quad (17)$$

The death rate for the animals of high genetic merit is still $[(X_1 + Y_1) \rho] / N$ and, in order to maintain a stable population size, the death rate for cattle of low merit is now $[(N + Y_1 + X_1)] \rho / N$.

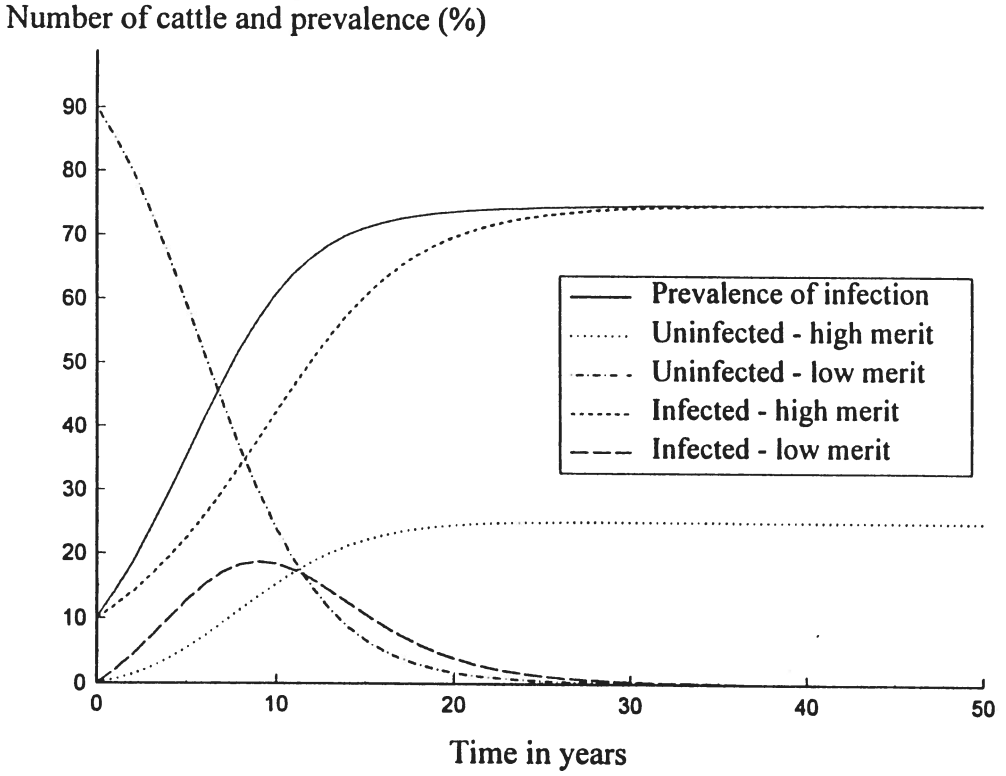

Fig. 4. Shows the behaviour of the system of Eq.s 14-17. $\phi = 0.8$, $\beta = 0.2$, $\rho = 0.25$ and $N = 100$. The starting conditions are $Y_1 = 10$, $Y_2 = 0$, $X_2 = 90$ and $X_1 = 0$

It can be seen that low merit animals are gradually replaced by superior animals by the policy of selective breeding. In the process, the prevalence of infection increases steadily to an equilibrium point above zero. The group of infected animals of low merit are all infected by horizontal transmission and, although they show an initial increase in prevalence, they inevitably decline towards zero.

DISCUSSION

The purpose of this study was to examine the behaviour of a simplified representation of *Neospora sp.* infection in cattle. The aim was not to make precise quantitative predictions of infection prevalence in dairy herds, but to provide mathematical models that could be explored analytically and their behaviour examined by numerical simulation. Similar mathematical studies of the dynamics of vertical and horizontal transmission have been conducted (Lipsitch et al., 1995, Busenberg & Cooke 1993, Fine, 1975) but rarely in an applied veterinary situation, where there are particular constraints on the population dynamics of the host.

If the models provide a reasonable representation of the host-parasite system then a number of points are evident, including:

- Imperfect vertical transmission alone will not sustain the infection in the population
- Higher levels of horizontal transmission enhance the degree of vertical transmission
- In the absence of horizontal transmission and with imperfect vertical transmission, preferential selection of infected cattle and their offspring will result in a transient rise in prevalence, but this will be followed by an inevitable decay towards zero prevalence.

These findings have a number of implications for the long term control of neosporosis. If horizontal transmission does not occur in a herd, or if measures are taken to reduce the level to below a certain threshold, then the infection will inevitably die out. However, in the absence of any other control measures this could take a considerable length of time, particularly if the infection prevalence is high at the time the measures are introduced. This threshold level can be raised, and the rate of decline in infection prevalence increased, by raising the culling rate of infected individuals and reducing their contribution to the inflow of heifer replacements. It appears that increasing the culling rate would be more effective than an equivalent decrease in the birth rate, although careful consideration of the economic implications of this would be required.

The preferential selection of infected animals, either by deliberately selecting particular lines or by chance, as reported by Bjorkman et al (1996), can result in high levels of infection even if no horizontal transmission is believed to occur. Identifying infected cattle and reducing their contribution to the herd would clearly have a major impact on preventing the infection becoming established in a herd.

Although there can be little doubt that vertical transmission or congenital infection does occur (Anderson et al., 1997) and that it is likely to be the major mode of transmission, relatively little is known about alternative sources of post natal infection. It is clear that the identification of the precise mode of horizontal transmission is a priority if control strategies are to be effective. However, there are still major gaps in our understanding of the biology of the parasite that are highly relevant to the question of horizontal transmission. For example the existence of

a definitive host would have a major impact on the dynamics of the host-parasite system but, unlike similar protozoan parasites, there is no evidence of such a host to date.

The models presented here are all simple deterministic systems of differential equations that have contributed to our understanding of the epidemiology and potential control of *Neospora sp.* infection. As more is known about the biology of the parasite, so further refinements and modifications can be made. The addition of further complexities such as stochasticity, time delays and age dependent transmission will be explored in future theoretical studies, and further comparisons will be made with data from empirical studies. It is hoped that the combination of theory and observation will hasten the development of a long term strategy for the control of neosporosis in dairy herds.

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VETERINARY PUBLIC HEALTH

FOODBORNE HUMAN DISEASE: IS IT A VETERINARY PROBLEM?

P.G. WALL*

With increasing numbers of food poisoning cases, the BSE crisis, the emergence of *Escherichia coli* O157, poor standards in abattoirs, as well as ongoing debate over who should be the regulatory agency to protect the public's health, we cannot afford to be complacent about food poisoning. A mild bout of diarrhoea for a robust young adult can be a life-threatening illness for an infant, elderly person, or person who is already frail as a result of some other disease.

In the UK over 100,000 cases of food poisoning are reported each year, with about 200 fatalities annually. *Campylobacter*, *Salmonella* and *E. coli* O157 are the bacteria of greatest concern. Controlling food poisoning involves a chain of responsibility from the farm to the consumers' tables. The bacteria should be controlled in the livestock, contained in the abattoirs, food processing industry and distribution network, and eliminated by effective cooking in the kitchen.

Food animals and poultry are the source of the three main bacterial causes of food poisoning, and if we are to reduce the number of cases we need to reduce the number of these bacteria entering the food chain. Farms are not operating theatres, and where you get animal faeces you get bacteria; however these need to be reduced to a minimum by simple hygiene measures. This can be achieved by hygienic animal husbandry and by ensuring that clean, disease-free animals and poultry leave the farm.

Campylobacter is now the commonest bacterium causing food poisoning in the UK, with 47,600 cases in 1996. Poultry are the principal source of human infections. The birds carry the bacteria, but it does not induce clinical disease in them.

Salmonella-induced food poisoning increased steadily during the 1980s, with over 30,000 cases in 1996. The main strain of over 2,200 different subtypes responsible for much of the increase is *Salmonella enteritidis* Phage type 4. The increase in human cases mirrored trends in infections in poultry in the UK, and control measures led to the slaughter of nearly 400 flocks (2 million infected birds) between 1989 and 1993. This strain is found in poultry meat and eggs, and can cause illness in infected birds. Although the level of infection in poultry flocks is falling, the number of human infections continues to increase, suggesting that people have forgotten - or are ignoring - the government's previous advice to cook poultry and eggs well and avoid cross contamination.

More recently another subtype, *Salmonella typhimurium* DT104, has emerged in both animals and humans, increasing from under 250 human cases in England and Wales in 1989, to over 4,000 in 1996. This is now the most common strain found in cattle, and is also found

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in sheep, goats, pigs, horses and poultry. Of particular concern is the fact that over 98% of the strains are resistant to five antibiotics, with a subset being resistant to seven. The overuse of antibiotics on farms can kill off all the sensitive bacteria leaving the resistant ones free to multiply without competition. 'Multiresistant' *S. typhimurium* has emerged as a global problem, with cases in many EU countries and the US. The emergence of these resistant bacteria is a cause of grave concern for both veterinary surgeons and physicians because there is not an array of new and more powerful antibiotics available to deal with these bacteria. The therapeutic options for treating sick animals is reduced, increasing the morbidity on farms and increasing the likelihood that these strains will cross into the food chain. Antibiotics should be used in animals only when absolutely necessary, and should not be used as a substitute for hygienic husbandry.

Salmonella is the most common bacterium involved in outbreaks of human foodborne disease, with poultry, eggs, egg products, red meat and meat products being among the most commonly implicated foods.

Escherichia coli O157 was virtually unknown prior to 1980, but since then overall UK rates have risen to over 1,100 cases in 1996. Although it affects far fewer people than either *Campylobacter* or *Salmonella*, it causes a more serious illness with up to 15% of cases developing renal failure, and 10% of these die. Those that survive often require renal dialysis or transplants. In the UK, Scotland shows the highest rate, 9.5 cases per 100,000 population in 1996, followed by England and Wales with 1.3 cases per 100,000 and then Northern Ireland with 0.8 cases per 100,000. As data are not collected nationally in the Republic of Ireland, the figure is unknown, but hopefully it is similar to that of Northern Ireland. Scotland has also suffered a disproportionate number of outbreaks (24 between 1987-1996) of which the worst was in November/December 1996, affecting 501 people, 20 of whom died: the largest total of deaths associated with any such outbreak world-wide. This disaster in Central Scotland precipitated intense media interest and the government has reacted with increased control measures, surveillance and research into this bacterium. In Ireland, the lessons from this outbreak must be learned without the necessity of a similar disaster here. The bacterium is found mainly in cattle where it causes no illness, being part of the normal gut flora. However, it can get onto meat which will bring it into butchers' shops. It is killed by cooking. However, if raw meat comes into contact with cooked meat, bacteria can be transferred, and human illness will be a consequence. It is essential that inspectors working in abattoirs are vigilant to ensure there is no faecal contamination of carcasses and that practices in butchers' shops are of the highest standards. Inspectors checking food premises have a duty of care to ensure that facilities and practices are such that illness will not occur. Food handlers often use, in their defence, the fact that the carcasses passed their routine inspection and so they thought that the situation was satisfactory.

The Meat Hygiene Service in the UK is currently trying to improve the standards of meat inspection in all abattoirs. We had the bizarre situation (which still exists to a degree in Ireland) where meat for export received a much more rigorous inspection than meat for home consumption. The EC Directive 64/433/EC governing meat inspection needs to be reformed because the emphasis in red meat inspection is on incising lymph nodes to identify tuberculosis lesions, and cutting muscles to look for tapeworm cysts. It is important to eradicate tuberculosis from the national herd, but nobody is acquiring tuberculosis from eating meat; moreover, tapeworms are becoming increasingly rare. The current system is not

targeting the main causes of food poisoning: *Campylobacter*, *Salmonella*, and *E. coli* O157. In the abattoirs, we need to focus on the high risk areas to reduce the level of faecal contamination of the final product. The incoming cattle and sheep should be healthy and as clean as possible, and the transport should be clean and not overloaded - "keep the farmyard out of the abattoirs." Removal of the hide and internal organs are the areas where special attention is needed. Meat from a faecally-soiled carcass can have up to 1,000 times more bacteria than meat from a clean animal.

There have been several recent outbreaks in the UK of both *Salmonella*, *Campylobacter* and *E. coli* O157, and one tragic outbreak of *Streptococcus zooepidemicus*, with two deaths, associated with the consumption of unpasteurised milk. A 1997 survey that was undertaken on 1,205 samples of unpasteurised milk at the point of retail sale showed 20% to be of unacceptable quality, with bacteria ranging from *Campylobacter*, *Salmonella*, *E. coli*, *Staphylococcus aureus* and *Streptococci* being found. You just have to see one child on renal dialysis, as a result of drinking unpasteurised milk, to be convinced that it is not worth the risk.

There have been several outbreaks of food poisoning associated with the consumption of contaminated baby milk resulting from pasteurisation failures, resulting in tragic consequences for the infants consuming these products. Two such outbreaks occurred in 1997. One involved a product manufactured in France in a factory producing 36 tons of baby food per day which was distributed internationally, including to Ireland. The other was associated with milk produced in England and distributed nationally. Effective surveillance managed to identify both contaminated products and they were withdrawn from the market. Ireland failed to pick up the problem because it does not have an effective surveillance system, and was alerted by the British authorities. Two cases were identified: one in an infant in Donegal and one in Cork.

A large outbreak with 100 cases of *Salmonella Goldcoast* occurred in the UK in December 1996 when a pasteurised Cheddar cheese became contaminated. Investigations fortunately tracked the cheese to a state-of-the-art plant producing 8,000 tons of cheese per year. The plant had all kinds of safety records, and all abnormal readings were recorded, but no action was taken. The pasteuriser, when switched on in the morning, often did not reach a high enough temperature to kill the bacteria. Additionally, on one operator-shift, the milk was allowed flow as soon as the machine was switched on. While it was heating up a slug of unpasteurised milk entered the system. *Salmonella Goldcoast* was identified in a herd of cows supplying the dairy. The plant had a HACCP plan, and thermograph-reading failures and positive phosphatase results were "dutifully recorded" but no corrective action was taken. Tons of cheese had to be destroyed, and the company nearly went out of business. This emphasises that when things go wrong in large manufacturing plants a large amount of product can be contaminated, making it so important that effective surveillance exists. Inspectors in these units need to be 'paranoid' about safety, and veterinary surgeons caring for cattle supplying any cheese-making operation must alert the cheese makers as soon as any disease is identified in the stock.

Veterinary surgeons have a role to play on the farms to assist the stockmen in producing healthy animals. Simple measures need to be reinforced continuously. It is important that the animals have access to clean water. The feed supply should be protected from rodents and wild birds, and should be transported in clean containers. Equipment for

transporting animal slurry should not be used for distributing feed. Farmers should purchase stock from reputable sources, and newly-purchased animals should be isolated for a period before introduction to the home herd to ensure that they have not brought in disease from the market. Farmers should use separate calving pens and separate pens for sick animals, and these should be disinfected after use. Antibiotics should be used responsibly under the guidance of the veterinary surgeon and should not be a substitute for hygienic husbandry. Vaccination should be used where appropriate. Finally, farmers need to be aware that animal transporters, machinery and humans can introduce infection onto their farms.

The safety of our food involves interventions at all stages. Farmers - the primary producers - are the first step in the chain, and if we start with an infected animal we will end up with a contaminated final product. Veterinary surgeons have a role to play both on the farm and in meat inspection to ensure that dangerous bacteria are kept to a minimum. Foodborne human disease is a veterinary problem!

COXIELLA BURNETII IN FARMWORKERS AND THEIR FAMILIES

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Coxiella burnetii is the aetiological agent of Q fever, a zoonotic illness of world wide distribution. Domestic animal reservoirs are mainly sheep, goats and cattle in which infection is often sub-clinical and of little economic concern, although abortion and still-birth may occur (Kazar, 1996). In humans infection may range from sub-clinical to influenza-like illness or pneumonia: however, serious long-term sequelae including endocarditis and liver complications may occur in those clinically affected (Marrie & Raoult, 1997). Although recent outbreaks of infection have been attributed to contact with ruminants and diverse exposures such as parturient cats (Marrie *et al*, 1988), straw (Salmon *et al*, 1982) and wild rabbits (Marrie *et al*, 1986), it has not always been possible to link illness with direct exposure to animals (Thomas & Palmer, 1994).

In a representative cohort of farmworkers in England and the Welsh borders, seroprevalence of *C. burnetii* was associated with exposure to the farming environment in general and more specifically dairy cattle (Thomas *et al*, 1995). To investigate further the importance of Q fever as an occupational disease in farmworkers, a representative sample was recruited in the Norfolk area, using the methods previously described by Thomas *et al* (1994). Seroprevalence of *C. burnetii* antibodies was measured and associations between positive titres and a variety of animal and other occupational exposures in the farming environment examined. The data for the Norfolk sample was combined with the data from Preston (Lancashire) and Herefordshire (the samples having been recruited by the same methods) and the larger data set analysed as a combined cohort of farmworkers to test the hypotheses generated.

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METHODS

Subjects and enrolment

A sample of 202 people was recruited from 137 farms randomly selected from Ministry of Agriculture, Fisheries and Food lists of agricultural holdings in the English local government districts of Breckland, Broadland and South Norfolk (Norfolk). The recruitment and enrolment methods were as previously described for a cohort of farmworkers in Lancashire and Herefordshire (Thomas *et al*, 1994).

Participants provided a 10 ml venous blood sample at enrolment. Questionnaires, administered by research assistants, were completed and a database of serology results and exposure data constructed using EpiInfo version 6.02 (Dean *et al*, 1994).

Description and validation of the Norfolk sample

The Norfolk sample of farmworkers was described in terms of age, sex, occupation and farm type. The sex distribution of the sample was compared by the Mantel-Haenszel version of the χ^2 test with data from the 1991 Office of Population Censuses and Surveys based on 744 persons (a 10% sample) aged 16 or over in the study area giving their occupation as agriculture. The age group distribution was compared with the age groups given for 675 persons (a 10% sample) classed by the 1991 census as working in agriculture, forestry or fishing.

Serology

Serum from the farmworkers in Norfolk was separated from blood samples and stored at -20^{O}C. Concentrations of serum IgG specific antibody to *Coxiella burnetii* phase II antigen were measured at Norwich Public Health Laboratory with an indirect immunofluorescence antibody test kit (*Coxiella burnetii* spot IF test, bioMerieux, France). Serum samples with a titre of 32 or more were taken as positive.

Analysis

Statistical associations between the presence of antibodies to *C. burnetii* and various occupational exposures (eg. farm types, contact with domestic and wild animals) were measured for the Norfolk sample (n=202) by calculating odds ratios (OR) and 95% confidence intervals (CI) using logistic regression in STATA version 5 (StataCorp., 1997). Ranked contact scores, based on the handling and frequency of animal contact, were designated as shown in Thomas *et al* (1994) and compared with Q fever serology results by the Mann Whitney test. Similar analyses were performed for the complete farmworkers cohort, comprising the Norfolk, Preston and Hereford data (n=586).

RESULTS

Description and validation of the Norfolk sample

At the first stage of recruitment 685 holdings were identified, and 600 were successfully contacted by telephone. Of these, 280 did not have anyone currently fitting the description of a farmer (Thomas *et al*, 1994), and 180 were eligible but refused to take part. The reasons given for not taking part included "too busy", "too well", or "too ill". Others did not want have blood taken, regarded the work as a waste of public money, considered themselves too old, or refused on the grounds that they were semi-retired from farming. 137 individuals agreed to take part in the study, a response rate of 43%. They nominated a further 65 subjects giving a total of 202. The sample comprised 150 men and 52 women with no significant difference in the sex distribution from the 1991 census (χ^2=0.05, df=1, p=0.83).

The age range of the Norfolk sample was 18 to 79 years (mean= 49.7, SD=12.5), and when compared with the census data had the same modal age group: the majority of male study participants and those in the census being aged between 45 and 64, and females being aged between 45 and 59.

The occupations of the Norfolk sample were principal farmers (n=149), their spouses (n=31), other family workers (n=6), regular hired workers (n=10), one salaried manager, and five in "other" (non-farming) occupations. 188 (93%) were involved in farming full time and 14 (7%) part time.

The predominant farm types of the Norfolk sample were general cropping, fruit and vegetables (26%) and pig farming (22%). Subjects had contact with 24 different animal species, including sheep (42 subjects), dairy cows (35 subjects) and beef cattle (56), broiler (6) and laying chickens (48), pigs (60), goats (7), cats (91), dogs (133), turkeys (5), horses (39), ducks (13), geese (13). Less than five subjects had contact with each of the following: bantams, guinea fowl, pheasants, cockateil, rabbits, guinea pigs, ostrich, tortoise, paraqueets, donkeys and parrots.

Analysis of seroprevalence and exposure data for the Norfolk sample

201 of the 202 subjects in the Norfolk sample provided a blood sample. Of these, 66 (33%) were seropositive for *C. burnetii* IgG antibodies. No significant difference was observed in the seroprevalence between male (33.6%) and female (30.8%) participants (χ^2=0.14, df=1, p=0.71). No age related trend was observed (Table 1).

No specific occupations were associated with being seropositive for *C. burnetii*. The prevalence varied between 30 and 40%, with the exception of the only farm manager in the sample who was seropositive. Of 187 full time workers 59 (31.6%) were seropositive, compared with 7/14 part-time farmworkers (50.0%).

Table 1. Person details and prevalence of *Coxiella burnetii* IgG antibodies in the Norfolk sample of farmworkers and their families (n=201).

Exposure		Seroprevalence	Unadjusted odds ratios OR	95% CI
Sex:	Male	50/149 (33.6%)	1.00	-
	Female	16/ 52 (30.8%)	0.88	0.45- 1.74
Age group:	<30	2/ 13 (15.4%)	1.00	-
	30-39	13/ 35 (37.1%)	3.25	0.62-17.01
	40-49	20/ 56 (35.7%)	3.06	0.62-15.18
	50-59	13/ 55 (23.6%)	1.70	0.33- 8.69
	60+	18/ 42 (42.9%)	4.13	0.81-20.97
Occupation:	Non farming	2/ 5 (40.0%)	1.00	-
	Principal farmer	46/148 (31.1%)	0.68	0.11- 4.19
	Spouse	11/ 31 (35.5%)	0.83	0.12- 5.71
	Other family workers	2/ 6 (33.3%)	0.75	0.06- 8.83
	Manager	1/ 1 (100%)	-	-
	Regular hired workers	4/ 10 (40.0%)	1.00	0.11- 8.95
Full-time employed:	Part-time	7/ 14 (50%)	1.00	-
	Full-time	59/187 (31.6%)	0.46	0.15- 1.37
Farm type	Cereals/horticulture	18/ 53 (34.0%)	1.00	-
	Specialist dairy	3/ 7 (42.9%)	1.46	0.29- 7.23
	Mainly dairy	**10/ 16 (62.5%)**	**3.24**	**1.02-10.35**
	Livestock mainly cattle	4/ 25 (16.0%)	0.37	0.11- 1.24
	Livestock mainly sheep	6/ 12 (50.0%)	1.94	0.55- 6.90
	Livestock sheep & cattle	2/ 8 (25.0%)	0.65	0.12- 3.54
	Poultry/pigs	17/ 56 (30.4%)	0.85	0.38- 1.90
	Mixed	4/ 18 (22.2%)	0.56	0.16- 1.94
	Other	2/ 6 (33.3%)	0.97	0.16- 5.82

The highest seroprevalence of *C. burnetii* antibodies was in those living or working on a mainly dairy farm (62.5%) and the lowest in farmworkers on livestock farms where mainly cattle were reared and fattened (16.0%). Compared to working on a cereal/horticultural farm (seroprevalence 34.0%) working on a "mainly dairy" farm significantly increased the odds of being seropositive for *C. burnetii* (seroprevalence 62.5%) (OR=3.24, CI= 1.02-10.35, P<0.05).

Specific animal exposures were investigated further (Table 2). The highest seroprevalence of *C. burnetii* was in farmworkers who had contact with dairy cattle (51.4%) and the lowest in those in contact with pigs (18.3%). Only having contact with dairy cattle was significantly associated with the presence of *C. burnetii* antibodies (OR=2.60, CI= 1.24-5.47, P<0.05). Contact with pigs was inversely associated with seropositivity (OR=0.35, CI=0.17-0.73, P>0.05).

Table 2. Effect of exposure to different animal groups (yes/no) on the prevalence of antibodies to *Coxiella burnetii* in the Norfolk sample of farmworkers (n=201).

Animal exposure (yes/no)	Seroprevalence (not exposed displayed above exposed)	Unadjusted odds ratios	
		OR	95% CI
Dairy cattle	48/166 (28.9%)	1.00	-
	18/ 35 (51.4%)	**2.60**	**1.24-5.47**
Beef cattle	53/146 (36.3%	1.00	-
	13/ 55 (23.6%)	0.54	0.27-1.10
Sheep	52/160 (32.5%)	1.00	-
	14/ 41 (34.2%)	1.08	0.52-2.22
Goats	64/194 (33.0%)	1.00	-
	2/ 7 (28.6%)	0.81	0.15-4.30
Pigs	55/141 (39.0%)	1.00	-
	11/ 60 (18.3%)	0.35	0.17-0.73
Horses	52/162 (32.1%)	1.00	-
	14/ 39 (35.9%)	1.18	0.57-2.46
Chickens	46/148 (31.1%)	1.00	-
	19/ 52 (36.5%)	1.28	0.66-2.48
Dogs	22/ 64 (34.4%)	1.00	-
	42/133 (31.6%)	0.88	0.47-1.66
Cats	40/107 (37.4%)	1.00	-
	25/ 91 (27.5%)	0.63	0.35-1.16

Since contact with dairy cattle was associated with having antibodies to *C. burnetii*, and remained so after adjusting for contact with all the other animals in Table 2 (OR=2.31, CI=1.01-5.22, P<0.05), dairy cattle exposures were investigated in more detail. Most participants had no dairy cows on the farm (166/201), and no contact with them. However, the 35 seropositive

subjects had contact with a mean of 45 dairy cows (range 0-380) compared with a mean of 15 (range 0-424) amongst the seronegative subjects. They also had a higher ranked contact score ($P<0.025$: Mann Whitney test).

Of the specific activities undertaken by people involved with dairy cows, milking (seroprevalence 48.4%), drinking raw milk (seroprevalence 54.3%) and handling aborted material (seroprevalence 56.0%) were all significantly associated with seropositivity for *C. burnetii* (Table 3).

Table 3. Effect of dairy cow contacts on prevalence of antibodies to *Coxiella burnetii* in the Norfolk sample of farmworkers (n=210).

Exposure (yes/no)	Seroprevalence (not exposed displayed above exposed)	Unadjusted odds ratios	
		OR	CI
Milking cows	51/170 (30.0%)	1.00	-
	15/ 31 (48.4%)	**2.19**	**1.01-4.76**
Drinking raw cows milk	47/166 (28.3%)	1.00	-
	19/ 35 (54.3%)	**3.01**	**1.43-6.34**
Assisting at the birth of calves	47/148 (31.8%)	1.00	-
	19/ 53 (35.9%)	1.20	0.62-2.32
Handling aborted material	52/176 (29.6%)	1.00	-
	14/ 25 (56.0%)	**3.03**	**1.29-7.13**
Nursing calves in the home	63/193 (32.6%)	1.00	-
	3/ 7 (42.9%)	1.55	0.34-7.12

No other exposures (handling rats; having a rat problem on the farm; having a pigeon loft on the farm; having been bitten by a tick in the last 12 months; noticing ticks on their animals; handling straw, or animal feed, or hay; having contact with a slurry lagoon; being exposed to slurry spray) showed significantly elevated odds ratios for antibodies to *C. burnetii*, either unadjusted or adjusted for age, sex and full time employment. However, the prevalence of antibodies was lower in those people who handled rats (21.6%) than those who did not (35.4%).

Seroprevalence and exposure in the complete farmworkers cohort

The mean prevalence of antibodies to *C. burnetii* was 29.2%, and there was no significant difference between the three study sites: Hereford 50/194 (25.8%), Preston 55/191 (28.8%) and

Norfolk 66/201 (32.8%) (χ^2=2.40, df=2, p=0.30). Although the Norfolk data showed that living or working on a mainly dairy farm was associated with antibodies to *C. burnetii*, in the complete cohort no particular farm type was similarly identified. Of the animal contacts investigated (Table 4) exposure only to dairy cows was associated with *C. burnetii* antibodies (18/35, 51.4%) (OR=1.48, CI=1.03-2.12, P<0.05), and remained significant when adjusted for contact with the other animals in the table (OR=1.67, CI=1.14-2.45, P<0.05).

Table 4. Effect of exposure to different animal groups (yes/no) on the prevalence of antibodies to *Coxiella burnetii* in the complete cohort (n=586).

Animal exposure (yes/no)	Seroprevalence (not exposed displayed above exposed)	Unadjusted odds ratios	
		OR	95% CI
Dairy cattle	90/348 (25.9%)	1.00	-
	81/238 (34.0%)	**1.48**	**1.03-2.12**
Beef cattle	72/248 (29.0%)	1.00	-
	99/338 (29.3%)	1.01	0.71-1.45
Sheep	67/219 (30.6%)	1.00	-
	104/367 (28.3%)	0.90	0.62-1.29
Goats	168/560 (30.0%)	1.00	-
	3/ 26 (11.5%)	0.30	0.09-1.03
Pigs	147/487 (30.2%)	1.00	-
	24/ 99 (24.2%)	0.74	0.45-1.22
Horses	132/459 (28.8%)	1.00	-
	39/127 (30.7%)	1.10	0.72-1.68
Chickens	119/421 (28.3%)	1.00	-
	51/164 (31.1%)	1.15	0.77-1.70
Dogs	27/ 88 (30.7%)	1.00	-
	142/493 (28.8%)	0.91	0.56-1.50
Cats	79/248 (31.9%)	1.00	-
	91/334 (27.3%)	0.80	0.56-1.15

Seropositive subjects had contact with a mean of 43 dairy cows compared with 30 for the seronegative subjects, and had a higher ranked contact score (P<0.05: Mann Whitney test): having handling contact with dairy cows on most days (rank 5) was associated with having antibodies to *C. burnetii* (60/172, 34.9%), increasing the risk over having no contact (rank 0) with these animals (OR=1.54, CI=1.03-2.28, P<0.05).

212 of the farmworkers milked cows and 76 (35.9%) of these were seropositive, compared with 25.4% of those who did not (unadjusted OR=1.64, CI=1.14-2.36, P<0.05) (Table 5). However, although the odds ratio was higher when adjusted, it was no longer significant (OR=1.99, CI=0.78-5.09, P>0.05). None of the other activities connected with cows were significant.

Table 5. Effect of dairy cow contacts on prevalence of antibodies to *Coxiella burnetii* in the complete cohort (n=586).

Exposure (yes/no)	Seroprevalence (not exposed displayed above exposed)	Unadjusted odds ratios		Odds ratios adjusted for age, sex, full-time employed (yes/no), study site & full dairy cow contact model*	
		OR	CI	OR	CI
Milking cows	95/374 (25.4%)	1.00	-	1.00	-
	76/212 (35.9%)	**1.64**	**1.14-2.36**	1.99	0.78-5.09
Drinking raw cows milk	87/332 (26.2%)	1.00	-	1.00	-
	84/254 (33.1%)	1.39	0.97-1.99	1.00	0.47-2.14
Assisting at birth of calves	72/278 (25.9%)	1.00	-	1.00	-
	99/308 (32.1%)	1.36	0.95-1.94	0.99	0.60-1.66
Handling aborted material	134/485 (27.6%)	1.00	-	1.00	-
	37/101 (36.6%)	1.51	0.96-2.38	1.25	0.73-2.15
Nursing calves in the home	168/575 (29.2%)	1.00	-	-	-
	3/ 9 (33.3%)	1.21	0.30-4.90	-	-

*handling level and frequency of dairy cow contact, milking cows, drinking raw cows milk, assisting at the birth of calves and handling aborted material.

None of the other occupational exposures examined were significantly associated with having antibodies to *C. burnetii*. Handling rats was negatively associated (adjusted OR=0.52, CI=0.29-0.95, P<0.05).

DISCUSSION

By recruiting 202 extra farmworkers in Norfolk and adding them to a cohort from Herefordshire and Lancashire (Preston) the range of farming practices that could be investigated was extended. In Lancashire the predominant holdings were "mainly dairy", in Hereford livestock rearing and fattening or mixed farming (Thomas *et al*, 1994) and in Norfolk cereals/horticulture or poultry/pigs. The prevalence of *C. burnetii* antibodies was not significantly different between the three regions, but is higher than previously reported for a comparison group of police and ambulance workers (Thomas *et al*, 1995). Thus the farming environment is a risk factor for *C. burnetii* infection in the UK.

Although cases of Q fever reported to the Communicable Disease Surveillance Centre (CDSC) by laboratories in England and Wales occur predominantly in males with a mean age of 46.2 years (Thomas *et al*, 1996), no similar pattern was identified serologically in the farmworkers, probably because of their defining occupational exposures.

In Norfolk, living or working on a mainly dairy farm and having contact with dairy cows was associated with being seropositive. The full cohort, although not implicating any specific farm type, also showed that exposure to dairy cows was a risk factor, as in the Preston and Hereford samples (Thomas *et al*, 1995). Cattle, along with sheep and goats, have been shown to be reservoirs of *C. burnetii* with a public health risk, the loci of infection being in the uterus and mammary glands (Marrie and Raoult, 1997). In Norfolk, milking cows, drinking raw cows milk and handling aborted material were all associated with infection. However, in the full cohort only milking cows remained significant and when adjusted for other exposures in the dairy farm environment, no single specific exposure was significantly associated with seropositivity for *C. burnetii*. There is, however, evidence that some sort of contact with dairy cows in general is a risk factor in farmworkers. However, since transmission of the organism to humans is airborne, and it is resistant to many environmental pressures such as desiccation, elucidation of the pathways involved is difficult: concurrent microbiological studies of the dairy farming environment would also be useful.

Exposure to sheep, goats, ticks, rats, straw, hay, cats, cattle slurry and pigeon lofts were not associated with seropositivity for *C. burnetii* in this study, although they have been identified as reservoirs of the organism or the sources of outbreaks of Q fever in humans (Thomas and Palmer, 1994; Marrie and Raoult, 1997).

Although exposure to *C. burnetii* results in lifelong IgG antibody and past rather than current exposure may be measured in seroepidemiological studies, this study shows that farmworkers with current dairy cow contact have a greater likelihood of antibodies than farmworkers with no contact, although no specific exposure(s) have been identified. Indeed, there may be none.

Since Q fever mainly manifests as a non specific febrile illness, the true incidence of disease is unknown, but the seroprevalence shown in this study suggests that it is probably generally underestimated. This is important because of the level of complications reported in identified

cases of Q fever. 11% of clinical cases reported to the CDSC between 1975 and 1981 progressed to endocarditis, and Q fever accounted for 3% of all endocarditis cases reported in England and Wales (Palmer and Young, 1982). In addition, significant neurological sequelae have been reported in proven Q fever infections in man (Reilly et al, 1990). It will be important to know whether similarly high rates of serious sequelae occur in subclinical cases. The farmworkers cohort provides an ideal opportunity to conduct a follow up study to asses the public health significance of occupational exposure to *C. burnetii*. This work also reaffirms the importance of clinicians considering the diagnosis of this treatable infection in farmworkers and their families.

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A VETERINARY INTERVENTION TRIAL: CAMPYLOBACTER INFECTION OF BROILER POULTRY

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Thermophilic *Campylobacter* bacteria are an important cause of human food poisoning in Great Britain, and broiler poultry are a commonly identified source of infection (Anon, 1993). Control of the infection in poultry is one approach to the control of human infection. A randomised, controlled intervention trial which assessed whether the risk of a poultry broiler flock becoming infected with *Campylobacter* could be reduced by applying certain disease security procedures was carried out. This paper discusses the methodological problems encountered, including the difficulties of achieving an adequate sample size, ensuring and measuring compliance with the intervention, and the complexities of analysis. The main findings are presented and possible biases are discussed.

MATERIALS AND METHODS

Study population

Thirty-nine flocks on company owned sites belonging to one of three integrated broiler producing companies were recruited. Inclusion criteria defined the number of houses (flocks) on site (4-12), required an all-in/all-out stocking policy and the houses to be in a good state of repair. Most flocks were allocated to either the intervention or the control group using random number tables, in the ratio 1:2; location or the time of repopulation prevented the random allocation of 12 flocks which were allocated by convenience. One intervention flock was unable to contribute to the study and a previously designated control flock was transferred to the intervention group to replace it. Ultimately 38 flocks took part in the study.

Sample Size

A previous study (Evans & Sayers, 1997) indicated that 80% of broiler flocks will be infected with *Campylobacter* by 42 days of age. In order to detect a 50% reduction in this prevalence of infection with 80% power and 95% precision, the study required 56 flocks to be recruited; this included an extra 10% to allow for losses to follow-up. The 39 flocks that were recruited would enable detection of a 50% reduction in incidence of *Campylobacter* infection in intervention flocks, compared with control flocks, with 80% power at the 10% significance level (Epi-Info 6, control to intervention ratio of 2:1[Dean et al, 1994]).

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Study Design

A single house was identified by convenience on each study site for monitoring infection and, if appropriate, application of the intervention. This was in two parts: defined cleansing and disinfection of the study house before repopulation, and special disease security measures during the study flock's life. Control flocks took no additional disease security precautions beyond those normally required by the poultry company. Relevant information was collected from all flocks using questionnaires completed during the study period. *Campylobacter* infection was monitored longitudinally over one production cycle in all flocks.

Data collection

Three questionnaires derived from those used in previous studies were completed for all flocks. These collected (1) detailed information about house size, structure, equipment, state of repair, and cleansing and disinfection method; (2) details of chick placement dates and numbers, and disease security on the site; and (3) the dates of depopulation, use of medicated feed, and weekly mortality figures.

Compliance with the intervention was monitored by inspection and questionnaire. Compliance with the initial cleaning and disinfection of each study house was pursued by personal contact with each farm manager, the provision of a dedicated protocol for each farm giving quantities and dilutions of chemicals required (determined by house size) and specifying the procedure to be followed. The farm manager was asked to supervise the contractor during the process and a questionnaire was completed by the farm manager, where possible in conjunction with the area manager, based on inspection of the house. Compliance with the disease security protocol for entering intervention poultry houses was measured by questionnaires completed by the area managers at each of their regular visits. At the end of the study area managers were asked to classify each of their intervention farm mangers according to the degree of diligence in applying the intervention procedures. Regular telephone contact was maintained with all farm managers, to remind them to take samples and to encourage them to observe the intervention.

All flocks were monitored for *Campylobacter* infection by weekly collection of 16 cloacal swabs from chickens in the 4 quarters of the study house, which allowed detection of infection at 5% prevalence (Evans, 1997). These were cultured according to conventional methods (Ayling *et al*, 1996). Each flock was classified as not infected with *Campylobacter* ('negative') if all swabs collected at each sampling time were *Campylobacter* culture-negative. If any thermophilic *Campylobacter* were detected the flock was classified as infected. Speciation of the thermophilic *Campylobacter* isolated was carried out on up to 5 cultures per flock. Flocks were monitored from 21 days of age to either 49 days of age, slaughter or becoming infected with *Campylobacter*, whichever was the sooner.

'Missing' Data: A rolling schedule was used throughout the study to maintain contact with all participants. Questionnaires were pursued if not received within 5 days of the due date for their completion; these were checked and coded before the next contact telephone call was made, when any missing data was pursued. Farm managers were telephoned regularly during the study, the day before the next samples were due to be collected, to remind them of this, and to convey the results of

the last swabs collected, which determined whether further sampling was needed. A reminder system for sample collection was set up to pursue swabs if they were not received.

Data Entry: Double data entry was employed throughout the trial and data was compared and validated using Epi-Info 6; all discrepancies were checked and corrected.

Analysis

Exploratory Stage: Questionnaire data were examined individually for association with intervention status using chi-squared analysis of 2xC tables, and for association with age of development of *Campylobacter* infection using Kaplan-Meier survival curves and the Wilcoxon rank sum test. Possible confounders and variables independently associated with outcome ($p \leq 0.2$) were identified. All other variables were discarded in the first instance.

Definition of exposure: Three definitions of exposure were used:

(1) the 'allocated status' of the flock (intervention or control);

(2) 'status group': a score was generated from the questionnaire data which reflected the efficacy of cleaning, the use of defined disease security measures and the degree of compliance with the intervention protocols. Flocks were placed in one of 4 groups according to score, reflecting good or poor compliance with the intervention and two levels of control flock status.

(3) all individually measured intervention procedures which were dictated by the intervention protocol, but excluding 'allocated status' and 'status group'.

Data sets: Flocks from the previous longitudinal study (Evans & Sayers, 1997) which met the inclusion criteria for the intervention study (n=13) were compared with the control flocks from the latter study and found to have no significant difference in survival time to infection with *Campylobacter* (Wilcoxon p=0.62). Information and results from these flocks were included in the data set, 'allocated status' being defined as control (as the previous study was purely observational), and all analyses were carried out both with and without the data from these extra flocks.

Model Building: Six models were defined, based on three definitions of exposure and two data sets. All variables identified as important in the initial examination of the data from this study were included stepwise in each model, those with the greatest association with exposure and outcome being added first. Variables were retained in the model if they had an effect at $p \leq 0.1$. All other variables were then added to each model individually; variables which had an effect on the relationship between the defined exposure and the risk of *Campylobacter* infection (ie altered the estimate of the odds/hazard ratio) were retained. Survival analysis using Cox' proportional hazards regression model was used to define the important variables and modelling was then repeated using the proportional hazards interval censored method (Collett, 1994).

The six models were rebuilt to analyse the probability of infection at 42 days of age using conventional logistic regression.

In all the models 'status group' was fitted as a trend.

The effect of partial depopulation and withdrawal of medicated feeds (if used) was examined by Mantel-Haenszel stratified analysis in those flocks which were still being sampled after the relevant event.

Statistical analyses were performed using the statistical software packages Epi-Info 6, STATISTICA 5.0, EGRET and GLIM4.

RESULTS

The odds of infection in intervention compared with control flocks at each sampling stage, are shown in Table 1A. This crude analysis shows that the risk of infection at a given age may be lower in intervention flocks than in control flocks; at 28 and 42 days of age the differences are significant at the 10% and 11% levels respectively.

Table 1A. Cumulative prevalence and odds of *Campylobacter* infection in intervention compared with control flocks at each sampling stage (number of flocks infected): Intervention study flocks.

	21 days	28 days	35 days	42 days[a]	49 days[b]
Control (n = 25)	20% (5)	32% (8)	44% (11)	65% (15)	100% (9)
Intervention (n=13)	8% (1)	12% (1)	31% (4)	38% (5)	100% (6)
Odds ratio	0.33	0.18	0.57	0.33	1.0
95% confidence intervals	0.01 - 3.61	0.00 - 1.69	0.10 - 2.79	0.06 - 1.67	n/a
p value	0.31[c]	0.10[c]	0.33	0.11	n/a

[a]2 control flocks were slaughtered before 42 days of age, one was infected at slaughter, the other was not.
[b]most flocks were slaughtered before 49 days of age. n/a: not appropriate.
χ^2 test, Yates continuity correction, 1-tailed. [c]1-tailed Fisher's exact test

Inclusion of the extra control flocks from the longitudinal study (Table 1B) improves the precision of the estimate, and at 42 days intervention flocks have one third the likelihood of infection compared with control flocks, with only a 7% possibility that this difference occurred by chance (p=0.07).

Table 1C shows the prevalence of infection compared between flocks in different categories of 'status group'; although the numbers in each category are too small to allow crude analysis, the figures suggest that the defined intervention measures adopted as routine (by chance), by flocks in

the 'good' intervention category reduced the risk of *Campylobacter* infection in the first 5 weeks of the flocks' life.

Table 1B: Cumulative prevalence and odds of *Campylobacter* infection in intervention compared with control flocks at each sampling stage (number of flocks infected): Intervention and longitudinal study flocks

	21 days	28 days	35 days	42 days[b]	49 days[c]
Control flocks (n = 38)	18%[a] (5)	34% (13)	45% (17)	63% (24)	88% (15)
Intervention flocks (n=13)	8% (1)	12% (1)	31% (4)	38% (5)	100% (6)
Odds ratio	0.38	0.16	0.55	0.31	n/a
95% confidence intervals	0.01 - 4.11	0.00 - 1.36	0.11 - 2.44	0.07 - 1.39	n/a
p value	0.37[d]	0.06[d]	0.29	0.07	n/a

[a]n=28. [b]2 control flocks slaughtered before 42 days of age, one was infected at slaughter, the other was not.
[c]most flocks were slaughtered before 49 days of age; n/a: not appropriate.
χ^2, Yates continuity correction, 1-tailed. [d]1-tailed Fisher's exact test.

Table 1C: Cumulative prevalence of *Campylobacter* infection, by status group (number of flocks infected): Intervention study flocks

	21 days	28 days	35 days	42 days[a]	49 days[b]
Control flocks (n = 10)	40% (4)	40% (4)	60% (6)	(87%) (7)	100% (4)
'Good' control flocks (n = 15)	7% (1)	27% (4)	33% (5)	57% (8)	100% (5)
Intervention flocks (n = 4)	0% (0)	0% (0)	25% (1)	50% (2)	100% (3)
'Good' intervention flocks (n = 9)	11% (1)	11% (1)	33% (3)	33% (3)	100% (3)

[a]2 control flocks were slaughtered before 42 days of age, both were infected at slaughter; 1 'good' control flock was slaughtered before 42 days of age, not infected at slaughter;
[b]most flocks were slaughtered before 49 days of age: 4 control, 3 infected at slaughter; 7 'good' control, 4 infected at slaughter; 5 'good' intervention, 2 infected at slaughter .

Examination of univariate association of measured variables with allocated status confirmed that in general the intervention and control flocks were similar in terms of house structure, type of equipment and other variables that might provide alternative explanations for the effect of the intervention.

Probability of infection throughout the life of the flock: Table 2

The results of survival analysis by the interval censored method are shown in Table 2. The use of this method had little effect on the results and conclusions drawn, compared to the more conventional proportional hazards logistic regression, however the method better reflects the data as no assumptions need be made about the *Campylobacter* infection status of flocks in between sampling times. Examination of interactions with 'time' showed that the proportional hazards assumption was not violated.

When 'allocated status' or 'status group' are used as the exposure, the hazard ratio estimate is not affected by the inclusion of any other possible risk factors in the model, and the data from this study's flocks alone (Table 2, rows 1a & 2a) gives a more precise estimate of effect of the intervention than when the additional flocks are included in the database (rows 1b & 2b).

Table 2: Efficacy of the intervention at delaying infection with *Campylobacter*: Comparison of the risk of infection over the life of the flock: proportional hazard survival analysis, interval censored method.

[a]Exposure	Data Source	Hazard Ratio	95% CI	p value[c]
1. Allocated status	a. Intervention study flocks only (n=38)	0.51	0.21 - 1.21	0.11
	b. All flocks[b] (n=51)	0.58	0.26 - 1.28	0.16
2. Status group	a. Intervention study flocks only (n=38)	0.68	0.46 - 1.00	0.04
	b. All flocks[b] (n=51)	0.79	0.57 - 1.10	0.14
3. Individual intervention procedures ('status' excluded)	a. Intervention study flocks only (n=38): Frequency boot dip changed:	0.53[d]	0.22 - 1.28	0.17
	b. All flocks[b] (n=51): Frequency boot dip changed:	0.22[e]	0.08 - 0.57	0.002

[a]See text; status group: ordered categorical: 4 categories; boot dip frequency: binary: 1=once a week, 2=more than once a week. [b]Intervention & longitudinal study flocks; CI=confidence intervals; [c]LR test; [d]adjusted for frequency of use of water sanitiser; [e]adjusted for contractor used to clean house.

The hazard ratio estimate derived when either definition of intervention status is used as the exposure suggests that the intervention had a protective effect, delaying *Campylobacter* infection. The precision of the estimate is improved by the use of 'status group' as the exposure and shows that for each group the risk of *Campylobacter* infection over the life of the flock decreased by 0.68 (p=0.04), ie the best intervention group of flocks were less than one third as likely to become infected with *Campylobacter* as the poorest control group.

Examination of individual control measures (Table 2, row 3a) found that no measures achieve significance if data from only this study is used, although the analysis does reveal an additional risk factor, namely the frequency of use of water sanitiser. Use of data from both this study and the previous study demonstrates an association between *Campylobacter* infection and frequency of boot dip replenishment; changing the boot dip more than once a week, reduced the risk of infection 5 fold compared to flocks which do not (p=0.002). The cleaning contractor becomes a confounder and the results are adjusted accordingly.

Probability of infection at 42 days of age: Table 3

The second approach to analysis looked at the probability of infection at 42 days of age and results are presented in Table 3.

Table 3: Efficacy of the intervention at preventing infection with *Campylobacter* at 42 days of age: Comparison of the risk of infection at 42 days of age: Logistic regression

[a]Exposure Variable	Data Source	Odds Ratio	95%CI	p value[c]
1. Allocated status	Intervention study flocks only (n=38)	0.42	0.21 - 0.84	0.007
	All flocks[b] (n=51)	0.52	0.29 - 0.93	0.03
2. Status group	Intervention study flocks	0.12[d]	0.02 - 0.70	0.008
	All flocks[b]	0.24	0.06 - 0.94	0.03
3. Individual intervention procedures ('status' excluded)	Intervention study flocks: Frequency boot dip changed:	0.09	0.01 - 0.80	0.008
	All flocks:[b] Frequency boot dip changed:	0.07[e]	0.01 - 0.44	<0.001
	Score for effectiveness of cleaning house:	0.28[e]	0.09 - 0.89	0.02

[a]See text. allocated status= intervention or control; status group=ordered categorical, 4 categories. boot dip frequency: binary, 1=once a week, 2= > once a week. Efficacy of cleaning house: ordered categorical, 3 categories. [b]Intervention & longitudinal study flocks; CI=confidence intervals; [c]LR test; [d]adjusted for type of fans; [e]adjusted for number of houses on site

As in the survival analyses, a more precise hazard ratio estimate is obtained when only intervention study flocks are used as the data source, if the exposure is defined as 'allocated status' or 'status group'. If individual control measures are used, precision is improved when longitudinal study flocks are included in the database. The results show that intervention flocks (as allocated) are less than half as likely to be infected at 42 days of age as control flocks (p=0.007, Table 3, row 1a). Use of 'status group' once again improves the precision of the estimate, and shows that for each

group the risk of *Campylobacter* infection at 42 days decreased almost 10 fold when adjustment is made for the type of fans in the house (p=0.008, row 2a). Similar but less precise hazard ratio estimates are obtained when longitudinal study flocks are included in the data source for this model (rows 1b & 2b). Using individual control measures as the exposure, the frequency of changing the boot dip and efficacy of cleaning and disinfection of the house before repopulation, are identified as important when all flocks are included in the database (row 3b). The relationship is confounded by the number of houses on the site. Flocks where the boot dip was changed more than once a week were more than 10 fold less likely to be infected with *Campylobacter* at 42 days of age than those which did not (p<0.001). Efficacy of house cleaning was defined by score and flocks were allocated to one of three categories: average, good or excellent. For each category the risk of *Campylobacter* infection at 42 days was decreased more than 3 fold, so flocks in the 'excellent' category of house cleanliness were almost 50 fold less likely to be infected than those in the 'average' category (p=0.02).

Effects of confounders: Side fans rather than roof fans (p=0.03), and less than 8 houses on site rather than 8 or more (p=0.002), reduce the risk of infection. The frequency of use of water sanitiser was identified as a risk factor for *Campylobacter* infection which confounded one of the survival analysis models; daily use of water sanitiser as opposed to less frequently or not at all achieved a 2 fold to 5 fold reduction in risk of infection (p=0.05).

Other results

Examination of the effect of partial depopulation (thinning) on all flocks which were not infected at the time of thinning suggested no association between the risk of infection and thinning (p=0.26), however this analysis was confounded by flock status (intervention or control). Examination of intervention and control flocks separately suggested that control flocks were more than 8 times as likely to become infected with *Campylobacter* by the time of slaughter if they were thinned (p=0.08), however no such effect could be demonstrated in intervention flocks (p=0.55). No significant effect on *Campylobacter* infection was found in relation to the withdrawal of medicated feeds.

Prevalence of infection in infected flocks was generally high, with 54% of such flocks having 13 or more swabs positive for *Campylobacter sp.*, and only 23% having less than 4 of the 16 swabs positive. The proportion of swabs found positive was not associated with intervention/control status (p>0.5).

Campylobacter jejuni was the species most commonly isolated in this study, being recovered from 25 of the 26 flocks which became infected. *C coli* was recovered from 2 flocks, being the only species isolated in one flock but present as a mixed infection with *C jejuni* in the other.

There was no missing data for the 3 main questionnaires for all the flocks in the intervention study, nor for the culture results of any of the flocks in either study. Twelve percent of the data relating to the standard of initial cleaning and disinfection of the poultry house was missing for the additional flocks from the previous longitudinal study from which information was incorporated; all other data required from these flocks was available. A single intervention study flock was lost to follow-up as it was unable to join the study due to statutory restrictions imposed as a result of notifiable disease.

Eleven of the 13 intervention houses achieved over 70% compliance with the protocol for initial house cleaning and disinfection (maximum possible score = 14, median = 12, range = 6 - 13). Fifty-six of the 75 expected weekly compliance questionnaires (75%) were returned and 9 of the 13 flocks achieved over 70% compliance (maximum possible score = 24, median = 17.2, range = 15.3 - 19.3). The area managers' judgements defined 5 intervention flock managers as 'excellent' in complying with the intervention protocol (score = 30), 5 as 'good' (score = 20) and 3 as 'average' (score = 10). Overall scores for intervention compliance ranged from 35.0 - 61.7 (median = 50.2, maximum possible = 68). Efficacy of cleansing and disinfection and use of some common intervention procedures (measured in all flocks using a common questionnaire) generated scores ranging from 14-28 which were used to define control flock status for the 'status group' variable; 14-18 = 'poor' , 19-23 = 'good'.

The data entry error rate was 0.2% for one operator and 0.4% for the other.

DISCUSSION

Study design

Randomisation: The randomisation of flocks to either the intervention or control group was largely successful with few variables becoming confounders in the analysis, despite circumstances dictating the allocation of almost a third of the flocks by convenience. This was probably because of the marked similarity which exists between most commercial broiler flocks, and the careful application of inclusion criteria to flocks which joined the study.

Sample size: The results achieved demonstrated that the intervention had an effect on the risk of a flock becoming infected with *Campylobacter*, despite the failure to achieve the intended sample size at recruitment. The number of companies and contractors recruited was limited by the high level of commitment required from both the poultry company and the individual farm managers for carrying out the intervention. More companies could have been approached however this was costly in terms of study resources as each contractor to be involved in cleaning intervention sites was observed and trained by the study co-ordinator so as to standardise the intervention. The greater number of control than intervention flocks partly compensated the shortfall in numbers and ultimately there were enough flocks to show an association between the intervention and a reduced risk of infection. However individual control measures that were effective could not be identified and so the data collected in the previous related study was considered. Flocks which met the inclusion criteria for the current (intervention) study were examined, and as they appeared to 'come from the same population' as the control flocks from the intervention study in terms of *Campylobacter* infection, original questionnaire data from these flocks was recoded and included in the intervention study data set. Difficulties were minimised as the questionnaires used in the two studies were very similar. However the reduction in precision of the estimate of effect of the intervention, when the intervention or control status of the flock defined the exposure, shows that the data was not directly transferable. The most likely cause is misclassification of answers to questions about the efficacy of cleaning and disinfection. In the intervention trial the same subjective score system was used as previously but clearer instruction, with examples, was given on how to score. The use of data from previous studies in a series to increase the sample size is potentially very useful in maximising the efficient use of resources. However in order to be used effectively much of the design for all of the studies in the

series needs to be clarified before any data collection begins. The more similar the methods and instruments of data collection between studies, the greater the opportunity to share the data. Thus the same questionnaire should be used in successive studies collecting the information for all studies envisaged in the series.

Compliance: Measurement of compliance is difficult as assessment of cleanliness can be subjective and different individuals completed the questionnaires. Within companies this was limited to a degree by asking area managers to complete the questionnaires for all of their company's farms; the scores achieved for the initial cleaning and disinfection procedures suggested most were compliant. Compliance with the daily procedure required to enter the house was difficult to measure and depended on area managers to administer the questionnaire and record observations at their regular visits, and the frankness of farm managers in their replies. The house entry procedure was fairly onerous, which increased the risk of poor compliance and consequent economy with the truth when answering the weekly questionnaire, however no alternative practical method of measuring compliance could be devised. Most of the questionnaires supplied were returned and the failure of most to achieve 100% compliance suggests the farm managers were reasonably honest in their replies. The opinion of the area managers as to the diligence of individual managers supported the questionnaire data and despite its subjectivity helped to define the relative degrees of compliance for the models. The success of the intervention in delaying *Campylobacter* infection and the interest shown by many of the managers when contacted suggest that the intervention protocol for disease security was largely followed.

Timeliness of data collection; accuracy of the data: This was achieved by constant telephone communication and resulted in no missing data for the intervention study. The very limited missing data was from the additional flocks from the previous longitudinal study from which information was incorporated. The loss of only a single flock to follow-up compensated for the failure to recruit the number of flocks originally desired.. The use of constant telephone contact ensured maximal use of all data generated and was well worth the resources used. Coding and data entry as the questionnaire and culture results were received ensured that discrepancies could be investigated and corrected soon after the event minimising the effect of recall bias. Double data entry was possible due to the small size of the study; it confirmed that our operators are accurate and minimised errors at this stage.

Analysis

Definition of exposure: Different definitions of exposure were used to reduce the effects of misclassification and to try to identify the more effective parts of the intervention. Examination of the data made it clear that simply defining farms by the allocated intervention or control status could not allow for the differences in compliance among intervention flocks, or the use of some 'intervention' procedures by control flocks. This misclassification was addressed using a score system; this could not be entirely objective for intervention flocks as the questionnaire could not measure the enthusiasm or interest of individual managers, which to a large extent determined the degree of compliance. This was taken into account by the area managers' classification of their intervention farm managers. The subjectivity of this was limited by asking for ranking rather than a clear score and further ameliorated by using it in conjunction with the more objective compliance questionnaire data. The ideal alternative of independent assessment of compliance on a regular basis

throughout the study which involved 38 farms and extended over a five month period would not be practical.

All parts of the intervention which could be measured, and which were recorded on all farms were used as independent exposures in the analysis to try to determine which had most effect. Although this was limited by the inter-relationship between many of the variables, it proved successful in demonstrating footwear as a major route for *Campylobacter* infection to enter poultry houses, demonstrating the value of approaching the analysis from several angles. The intervention status of each flock was omitted in these models to avoid possible bias which can be introduced if adjustments are made for variables correlated with exposure (Weinberg, 1993). Similarly such variables were not included in models which included the status of the flock.

The examination of univariate associations with outcome using Kaplan-Meier survival curves gave a very useful visual indication of which variables were likely to be important, and helped in the understanding of changes in infection status over time.

Definition of outcome: Defining outcome in two ways, either survival without *Campylobacter* infection, or point prevalence of infection at 42 days enabled a better grasp of the effect of the intervention. Survival takes the entire life of the flock into account and the greatly increased risk of infection in older birds could mask the effect of the intervention, particularly if there were many longer lived flocks in the study. This proved to be the case as in all models the estimated effect of the intervention was greater and more precise when comparison of the risk of infection at 42 days of age was made. Although most flocks were randomly allocated to the intervention or control groups, it is likely that there is bias introduced into results after 42 days by the chance of remaining alive: 62% of intervention flocks were still alive at 49 days compared with only 44% of control flocks.

Summary of the discussion of methodology

In summary this study has shown that in a series of studies, careful definition of the information required at the outset can enable data to be shared between the studies, so reducing the sample size required in the later ones. Ensuring compliance and timely collection of data is labour intensive but pays dividends in terms of useful results, and maximises the contribution of each study participant. The definition of both exposure and outcome require careful consideration so that appropriate analyses are performed. The use of more controls per intervention flock would have enabled a more powerful study using comparatively few extra resources.

Summary of the study findings

A paper is in preparation which includes a detailed discussion of the study findings (Gibbens *et al*, 1998). In summary analysis of infection over the life of the flock and at 42 days of age showed that the risk of thermophilic *Campylobacter* infection of broiler flocks under commercial circumstances in Great Britain was reduced by at least 50% in intervention flocks by the application of specific hygiene and disease security measures. The protective effect of the intervention was greater in birds up to 42 days of age than later in the flocks' life, suggesting a greater effect in delaying rather than preventing infection. Specific measures that were identified as important means of control of

Campylobacter infection were at least twice weekly replenishment of boot dip disinfectant, thorough cleaning of the poultry house between flocks and daily, compared to less frequent, sanitisation of the chickens' water supply. Partial depopulation of poultry houses appeared to increase the risk of *Campylobacter* infection.

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AQUACULTURE

EXPERIENCE WITH THE USE OF AQUATIC EPIDEMIOLOGY IN NORWAY

J. JARP [1]

Since the 1970's Norwegian Atlantic salmon farming has grown into a large and important export-orientated industry (Fig. 1), (Blaalid, 1996 & Anonymus, 1997). Not surprisingly, this growth was accompanied by the emergence of new disease problems, and by the amplification of previously minor disease problems into economically severe epizootics.

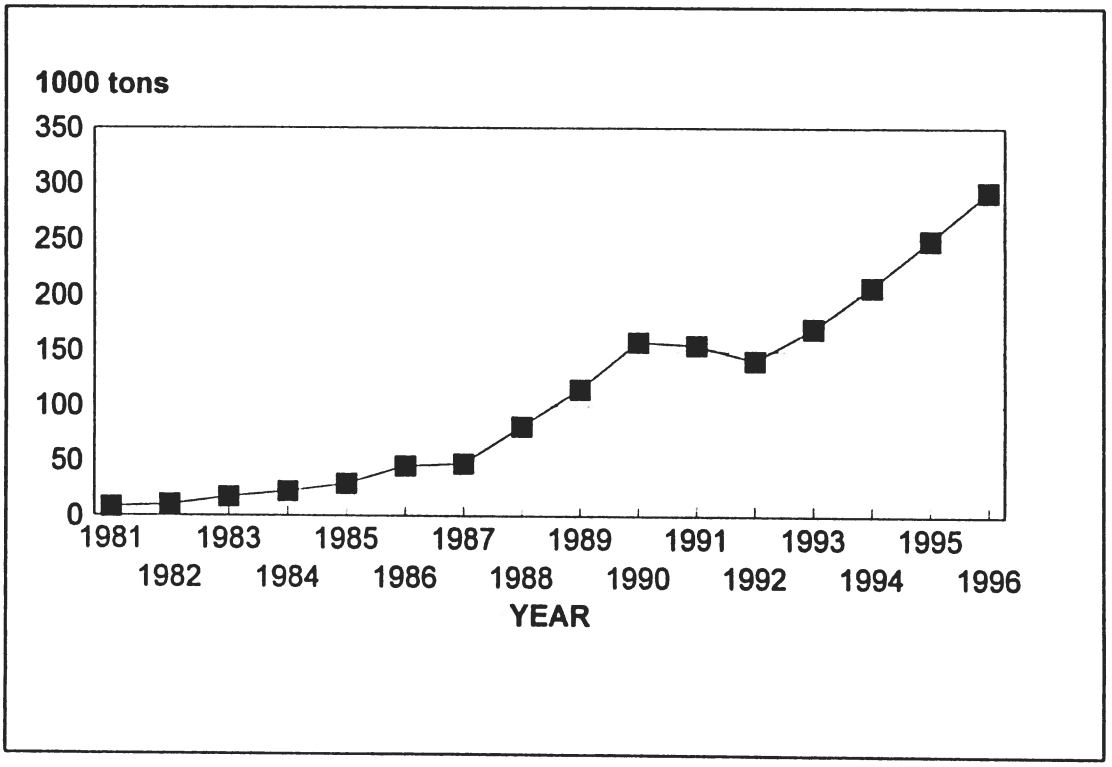

Fig. 1 The harvest (slaughtered weight) of farmed Atlantic salmon in Norway during the time period elapsing from 1981 to 1996

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During the early time period bacterial diseases such as vibriosis, caused by *Vibrio anguillarum*, cold-water vibriosis, caused by *Vibrio salmonicida*, and furunculosis, caused by *Aeromonas salmonicida* subsp. *salmonicida* were predominant. However, development and an extensive use of efficient vaccines led to a rapid decline in the incidence of these bacterial diseases. Infectious salmon anaemia (ISA), a virus disease, which up to 1997 has been diagnosed only in farmed Atlantic salmon in Norway, caused large problems in the late 1980s and early 1990s and the disease is still not eradicated from the farmed population. Infectious pancreatic necrosis (IPN), another virus disease, which can lead to huge mortality losses, has been a major problem for salmon production for several years.

The disease problems related to aquaculture have stimulated research activity in various fields. Aquatic epidemiology has been used to gain more knowledge about the incidence levels of various diseases and on the potential association between various exposure factors and disease occurrence. Previously epidemiological methods have been used in pharmacoepidemiology and to clarify risk factors for the occurrence of infectious diseases, and recently has been used to identify causal mechanisms for non-specific infectious diseases such as short-tails (deformation) and side-effects of vaccination.

The present paper discusses some aspects of the aquatic epidemiological research performed in Norway during recent years.

DESCRIPTIVE EPIDEMIOLOGY

The location and size of the farmed fish population is very dynamic compared to the traditional animal population in Norway. Today Atlantic salmon for commercial production are kept at approximately 1200 freshwater and seawater sites. One licensed fish farm may keep fish at many freshwater and seawater sites. The fish in different pens are very often slaughtered at several points in time, but within the pens the fish are also sorted and slaughtered several times during the growing period. A national data-based register containing the farm's licence number, name and volume of production is in operation. However, information on whether the farm is in business or when fish are transferred into or out of the farm is not stored in the database. Therefore, it is difficult to specify the population at risk for the various diseases at a specific point of time or during a certain time period.

Since ISA appeared in a freshwater site in 1984, the disease has caused the Norwegian salmon industry and authorities many problems. In the time period from 1988 to 1992 the incidence was very high but the mortality in the affected farms was not necessarily high. ISA has always been treated as a notifiable disease in Norway, and is listed with other significant diseases (Class B) by the OIE, whereas the European Community considers ISA a List A disease (an exotic dangerous disease). Trade partners have been concerned about the risk for the potential transmission of the ISA virus by the slaughtered fish to the various national fish populations. Therefore, it is of major interest for Norway to eradicate the disease from farmed fish. Descriptive epidemiology showed that the outbreaks of ISA had a seasonal distribution (Fig. 2), although the incidence rate was difficult to estimate due to the lack of information on the size of the population at risk. Until 1992, a higher frequency of ISA was observed in the

spring and in the fall. Although the incidence rate at present is quite low the peak in the spring still occurs.

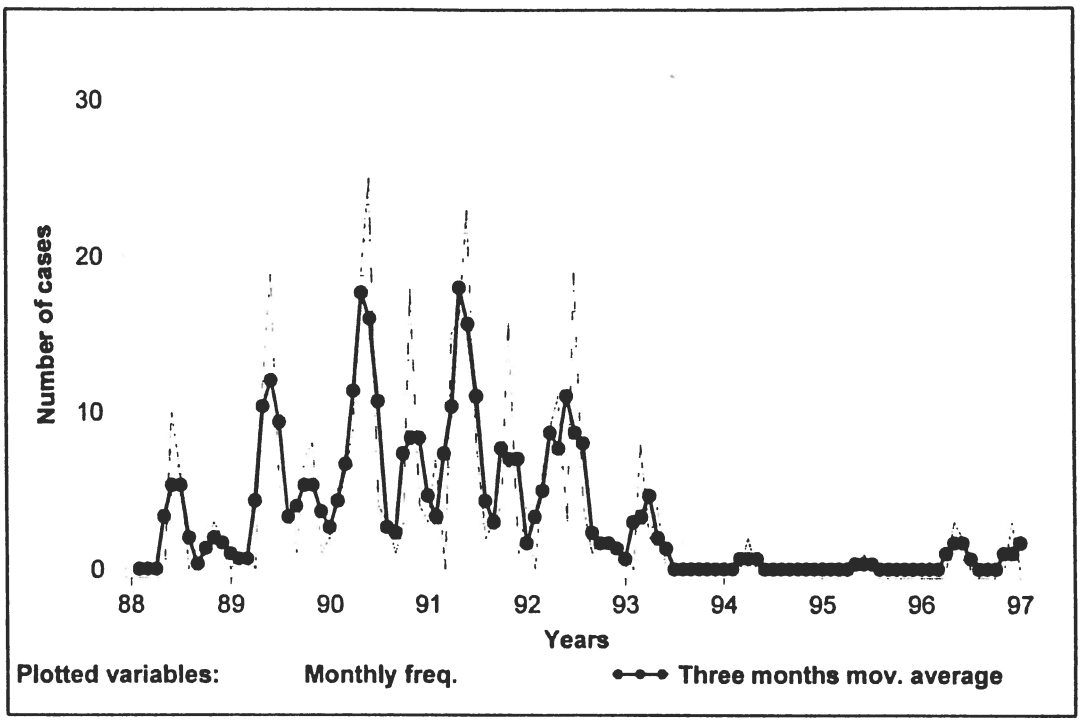

Fig. 2 The seasonal distribution of the notified cases of infectious salmon anaemia in Norway in the time period from 1988 to 1997

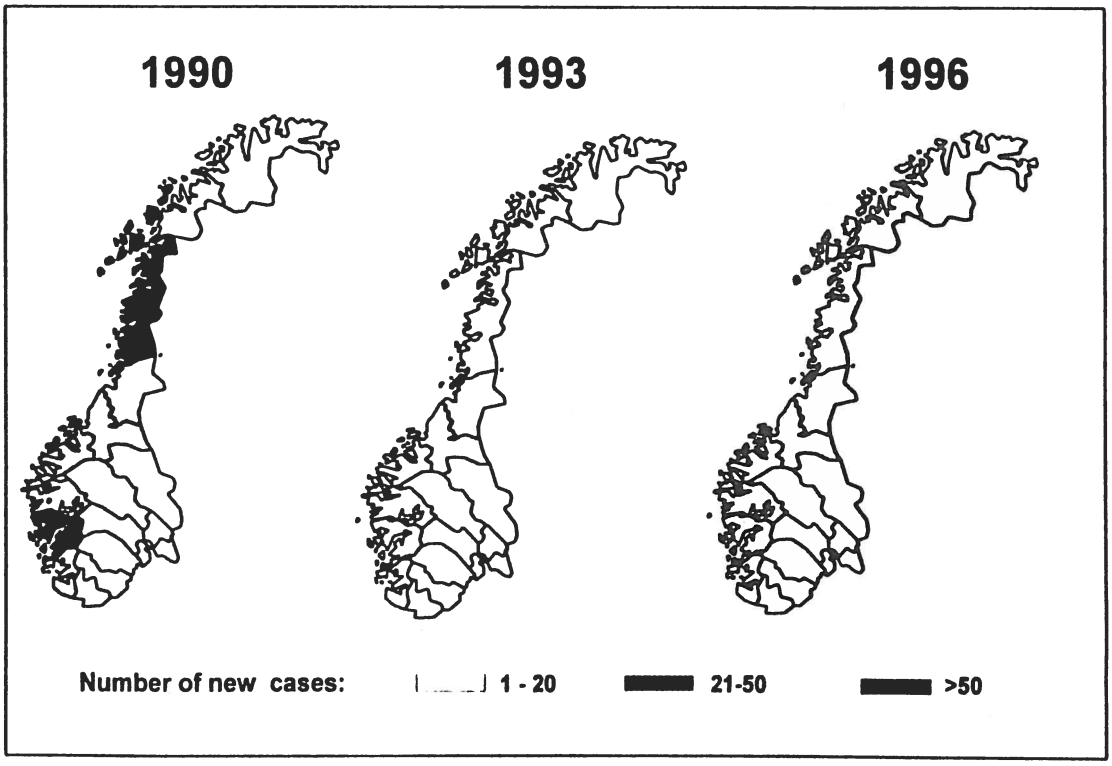

Fig. 3 The geographic distribution of the notified cases of infectious salmon anaemia at county level in Norway in 1990, 1993 and 1996

It is thought that the seasonal appearance of the disease is due to the fish being infected after seawater transfer and that the disease develops very slowly and/or that transmission and infection is associated with specific seasonal management factors in the industry such as slaughtering or transport. In the past, the geographic distribution of ISA had a clustered appearance (Fig. 3), but now, when the incidence is low, it seems to occur in several smaller clusters.

ANALYTICAL EPIDEMIOLOGY

Considerations on design

Several study designs have been used in epidemiological studies including pair-matched, frequency-matched, and unmatched case control studies, as well as retrospective and prospective longitudinal studies (Table 1). The potential sample size is not always very large, but strategies for optimising the power have been considered.

Table 1. Overview of the designs used in various epidemiological studies in aquaculture in Norway

Design	Disease	References
Case control study	Infectious salmon anaemia	Vågsholm et al., 1994, Jarp & Karlsen, 1997
	Infection with *Aeromonas salmonicida* subsp. *salmonicida*	Jarp et al., 1993a
	Infectious pancreatic necrosis in post-smolt	Jarp & Rosten, 1993
Retrospective follow-up	Sea lice and mortality	Jarp et al., 1993b
	Mortality, furunculosis and infectious pancreatic necrosis in post-smolt	Jarp et al., 1995
Prospective follow up	Sediment properties and health condition	Jarp et al., (in prep)
	Infectious pancreatic necrosis	Jarp et al.,1996, Jarp & Melby, 1997
	Mortality during and after a 6-weeks starving period	Not published
Pharmacoepidemiology	Vaccination and drug utilisation	Grave et al., 1990, Lillehaug, 1989a, Lillehaug, 1989b
Risk assessment	*Gyrodactylus salaris*	Paisley et al., 1997

The descriptive epidemiology of ISA provided important background information which was used in the planning of further epidemiological studies to clarify risk factors for disease

occurrence (Vågsholm et al., 1994, Jarp & Karlsen, 1997). Even though the transmittable nature of the disease was known, the causal virus of ISA was first isolated from cultured cells in 1995 (Dannevig et al., 1995) so the virus had not been identified when the epidemiological studies were performed. The diagnosis was based on pathological and haematological investigations in the laboratory and the clinical symptoms of the fish. In the case-control studies a case farm was defined as a farm which had been identified by the Ministry of Agriculture as having a confirmed occurrence of ISA during the study period. The control sites were selected within the same veterinary district or the municipality from sites which were not suspected to have a positive ISA case.

In some of the prospective studies repeated measurements have been used. These studies have been performed at the fish group level. Individual fish represent their group and after blood sampling or other specific investigations are sacrificed. If the follow-up period is long it may be difficult to keep the included groups separated. In the analytical studies including convenience samples potential selection bias must be considered.

In the studies of ISA and risk of infection with *Aeromonas salmonicida* subsp. *salmonicida* in freshwater hatcheries the classification of sites was based on the status of the sites according to the list of farms registered because of the diagnosis of various diseases. The registration of the diseases is based on routinely performed health controls which vary in quality from place to place and time to time. The probability of misclassification bias might have been significant since no additional testing of the status was performed.

Potential recall bias must always be considered in case control studies. In the study of the risk of infection with *Aeromonas salmonicida* subsp. *salmonicida* in freshwater hatcheries, the results showed a very high odds ratio associated with the possibility of migration of anadromous fish into the water supply. A positive diagnosis of furunculosis had dramatic consequences for the freshwater farmers as no sale of fry or smolt was allowed. As migration of anadromous fish was believed to be a very important risk factor for disease transmission, we considered that the odds ratio might be overestimated due to a differential recall bias.

Unit of concern

The unit of concern varies between the studies. Most often fish are kept in several pens or tanks at a site. The farmers start to sort the young fry in a group very soon after hatching and during the rest of their lives the fish might have new pen-mates several times. Especially in longitudinal studies the identification of the study units might be a problem. During the freshwater period fish with the same genetic background are kept together so that the seawater farmers can purchase a smolt group with homogeneous heredity. After some months in sea water the post-smolt are sorted due to weight difference. Use of fish or fish group as the observational unit may cause problems in follow-up studies.

Use of regular data recording systems is not as frequent in aquaculture as in traditional animal husbandry. This fact has lead to the frequent use of questionnaires developed for the specific projects. In both cases control studies and in retrospective longitudinal studies historic data have been used. Field veterinarians have been responsible for data recording, sampling of fish, and diagnostic work.

In most of the studies convenience samples have been used because the lack of a complete sampling frame made it difficult to obtain random samples. However, in one study on mortality on post-smolt the study sites were randomly selected. A sampling frame for all sites to which smolt had been transferred during a specific time period was obtained from the Fish Breeders Association and, from this frame, 124 seawater sites were selected in a systematic sample. In the study both the site and the smolt group were used as the units of concern.

Statistical analyses

The statistical analyses of data in aquatic epidemiological studies can be difficult due to the dependence between the various statistical units. The dependence between the study units is considered of great importance, because the outcome variable very often is related to the occurrence of an infectious disease. Development of new statistical tools, such as the multilevel analyses, which can handle dependence on several levels may be very important for the analyses of aquatic epidemiological data.

In the analyses of fish mortality, the crude mortality has been used as the outcome variable. It is not easy to calculate the disease specific mortality because the monitoring and recording in the farms is not specific.

Exposure factors

When new epidemiological studies are planned it may be difficult to decide which exposure factors to include. When the disease problems in the farms are large the farmers and others involved have their own thoughts about potential risk factors and disease occurrence. Various exposure factors have been registered and analysed in the studies. Some examples will be presented.

Location: Various aspects have to be considered before the location of new salmon sites are located. The epidemiological studies have shown that location of the salmon farms is associated with the risk of disease transmission. In the study of risk factors for infection with *Aeromonas salmonicida* subsp. *salmonicida* in freshwater hatcheries (Jarp et al., 1993) the results showed that the risk of infection at a site was closely related to the high incidence and prevalence of furunculosis in the seawater salmonid population. Location close to another furunculosis-positive farm, which most certainly was a seawater farm, increased the risk. It was also shown that risk was associated with the possible migration of anadromous fish into the water supply. This could be explained by the high prevalence of furunculosis in the seawater population and that wild fish were infected and also by the fact that infected fish escaped from several farms.

Since 1990, the Norwegian Ministry of Agriculture has issued several regulations aimed at reducing the transmission of fish infections. These include compulsory veterinary health control in smolt plants, compulsory health certification on the sale of fish, and regulations concerning the transport of live fish. The authorities also require that waste water from the salmonid processing plants be disinfected. The second case control study on risk factor for

infection with ISA in seawater sites provided results which supported the necessity of such requirements because an important risk factor for infection was location close to a slaughterhouse or processing plant without a proper system for disinfecting the waste water (Jarp & Karlsen, 1997).

Specific immunity: During the years with a very high incidence of clinical IPN in post-smolt the farmers believed that fish which had suffered from clinical IPN were at less risk of recurring disease. In a study of IPN in post-smolt (Jarp et al., 1996) we showed that the relationship between specific immunity and the risk of IPN had to be analysed by a stratified analysis. In fish groups where clinical IPN had occurred earlier during the freshwater stage no protection could be demonstrated from the presence of specific antibodies. In contrast, groups with specific antibodies where no clinical IPN had been recorded were protected against the disease. The development of efficient vaccines against virus diseases has not been as successful as the development of vaccines against the bacterial diseases. However, the protective effect of the humoral immunity against clinical IPN gained under natural conditions may be very important when considering vaccination as a control measure.

An important aspect of the use of epidemiology in the research of fish diseases is the fact that the fish farmers and the field veterinarians, as well as the authorities, are very interested in the results. This makes it important to present and interpret the results clearly.

RISK ANALYSIS

Although the export-orientated salmon industry significantly boosts the national economy, attention has been focused on the potential negative environmental impact of sea based aquaculture. Of concern are, pollution from deposited material, spreading of microbes resistant to antibacterial drugs, transmission of serious diseases and parasites to feral aquatic organisms, and genetic pollution from escaped fish. In 1996 we started to work on an analysis of the risk of transmission of *Gyrodactylus salaris,* an ecto-parasite from farmed Atlantic salmon, to the feral salmon in the Tana river located in northern Norway at the border of Finland. Today, the Tana river is reported to be Europe's best salmon river and it is thought that introduction and transmission of the parasite to local stock would be fatal.

The results from a quantitative risk assessment showed that the risk of transmission of *Gyrodactylus salaris* was very small. The mean total risk was through simulation estimated to approximately 1E-07 per year (Paisley et al., 1997). During the modelling several risk management factors with low effects on risk reduction, such as general health inspections in the smolt plant, were identified. Since many diverse environmental issues are concerned and this quantitative risk assessment included only one specific pathogen, the value of the assessment in answering all questions regarding the negative impacts of aquaculture is questioned.

CONCLUSION

The conditions in the large Norwegian farmed fish population are optimal for epidemiological studies. The fish farmers and the field veterinarians constantly raise new and interesting questions. Development of standardised procedures for variable specification and data recording systems may increase the opportunities to perform cost-efficient studies. Studies on the impact of various oceanographic parameters on the transmission of infectious agents between sites where epidemiology is linked to Geographic Information Systems (GIS) should be performed.

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EPIDEMIOLOGICAL INVESTIGATIONS OF DISEASE IN FARMED ATLANTIC SALMON (*Salmo salar*) IN IRELAND.

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Despite the economic importance of Atlantic salmon farming, attempts to understand diseases by the application of epidemiological techniques are relatively poorly developed.

A collaborative research project was established between the Irish Salmon Growers' Association and the Veterinary Sciences Division to develop a computerised management and disease information retrieval system for production of farmed Atlantic salmon. Such a database was considered essential in facilitating the development of control strategies required to minimise the losses in production encountered by the Irish salmon industry following outbreaks of various disease conditions. The initial objective of the project was to identify production, environmental, disease and management factors which are significant in determining the profitability of Atlantic salmon farming in Ireland. At the time when development of the database was proposed, pancreas disease (PD) was a condition of unknown aetiology with a relatively high prevalence, which caused losses at the marine stage of production during 1987 estimated to be in the region of 5-6 million pounds sterling (Branson, 1988).

PD was initially described in Scotland but is now known to occur in all of the major Atlantic salmon farming countries of Europe as well as in the USA (Munro et al., 1984, Kent & Elston 1987, Poppe et al., 1989, Murphy et al., 1992). This disease normally affects salmon during their first year at sea and clinically it presents itself as a rapid decline in feed intake, a tendency to congregate in the corners of cages close to the surface of the water, and the appearance of white faecal casts (Munro et al., 1984). Mortality rates of up to 45% have been reported in PD outbreaks but indirect production losses such as retarded growth are probably

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more important (Menzies et al., 1996). Earlier exploratory analysis using the database concentrated on investigating the temporal spread of PD during outbreaks on different sites; this indicated that the cause of PD was probably infectious (Menzies et al., 1996). This has since been confirmed through the isolation of a toga-like virus from salmon affected by PD and the experimental reproduction of PD using this virus (Nelson et al., 1995, McLoughlin et al., 1996).

Since 1995, cataracts have been the major impediment to profitable production in Ireland. All marine sites have been affected to a greater or lesser degree. A multi-disciplinary approach was adopted to analyse and examine epidemiological, pathological, environmental and nutritional information in an effort to determine the factors associated with the occurrence of cataracts.

The initial database, the Salmon Information System (SIS), was established in 1992 contains variables relating to disease, the environment, and various aspects of production and management in farmed Atlantic salmon (*Salmo salar*) in Ireland during the marine stage of production (Menzies et al., 1996). Analysis using SIS indicated that management practices such as single generation rearing and fallowing appeared to have a significant impact on mortality rates, and recommendations were made for changes in farm practice in future seasons (Wheatley et al., 1995).

This paper is intended to highlight the application of the SIS mainly by its use to establishing the epidemiology of PD and its central role in identifying the major factors associated with cataracts.

MATERIALS AND METHODS

Prior to construction of the database in 1992, it was necessary to survey and visit the salmon producers to assess the information which is routinely recorded and to identify those sites with adequate accurate information which could be used for intensive monitoring. The sites selected for monitoring were visited annually so that data on management and disease could be updated by completion of a smolt performance questionnaire and by interviewing the site biologist and/or the farm manager. Production information was obtained by downloading and standardising data from the various spreadsheet packages used by producers. The data were collected retrospectively for the previous season's production and analysed to provide insight into the links between disease and variables in fish farm practice. Environmental data and pathology reports were obtained from the appropriate monitoring and diagnostic services. Disease outbreaks were identified by pre-defined criteria (Wheatley et al., 1995). Clinical signs alone were not accepted as a diagnosis of PD and histological examination was required to confirm an outbreak.

Data were standardised in relation to each site and cage within a site. The variables were further categorised as annual site, annual cage, fortnightly site and fortnightly cage data. Within each of these data types, the variables were calculated for each fortnightly or annual interval. The database has been modified over the years and additional fields and tables have

been incorporated to accommodate changes in management practices and enhancements to the system such as inclusion of feed input information.

Up to 1994, the data were collected retrospectively for the previous season's production and analysed to provide insight and advice into the links between disease and other variables associated with fish farm practice. Since 1995, data have been collected during the production year allowing more rapid feedback on the findings derived from analysis of the data.

The database was established using the relational database management software package ORACLE (Oracle Corporation, Redwood City, California). The software was mounted on an ALPHA 8200 server which has 512 megabytes of main memory.

Percentage mortality was used as the measure of production due to the inability to accurately assess biomass on the majority of the monitored sites. Mortality data were subjected to the arcsine root transformation for statistical analysis. Mortality rates were compared between sites using analysis of variance. When analysis of variance resulted in a significant *F*-test value, the Student's *t*-test was used to compare sites. When variances were significantly different between groups, the Student's *t*-value was calculated using the separate variance estimate. Standard linear regression and correlation techniques were used to determine whether significant associations occurred between mortality and PD outbreaks.

Cataract study

Cataract survey: The aim was to obtain an independent assessment of the prevalence and severity of cataracts on the farms. Independent specialists were employed to visit the farms within a period of 2 weeks and examine a representative sample of approximately 100 fish. Cataracts were graded according to a standardised system. This cataract scoring system was agreed with several fish specialists in an attempt to ensure that there was consistency in recording. In the agreed system, "0" corresponds to a completely clear lens, and "4" to a lens with 100% opacity. The system determines the area of the lens which is opaque but it does not take into account the position or density of the opacity. However, in many cases, the position of the cataract in the lens was recorded. Some farms recorded sequential cataract scores over the season and some have also had independent visits and additional examinations by veterinary experts. Weight, length, feeding, sea-lice, any skin lesions and the condition of the eyes were also recorded. On each occasion at least 100 fish per site were weighed, measured and graded according to the degree of cataract (if any) in each eye. A histological examination was carried out on a sample of fish taken from several sites.

In 1995, 22 sites were visited and a total of 2,359 fish examined. In 1996, 26 sites were visited and a total of 3,408 fish examined.

Questionnaires: A detailed questionnaire was prepared by the Fish Diseases Unit, Stormont and was sent directly to all farms in Ireland, both affected and unaffected. The questionnaires covered all aspects of husbandry, relating to stock, cages, environment, feeding, treatments and disease.

Feed analysis: Representative feed samples were taken by the farmers and analysed by accredited laboratories.

Environmental Monitoring: Daily water temperatures for 1995 and 1996 were provided by the farmers on 15 sites. Daily air temperature, rainfall and sunshine hours were obtained for 1993 onwards from the Irish Meteorological Office. Environmental monitoring by independent companies included water temperature, depth, salinity, chlorophyll, oxygen, ammonia, nitrate, nitrite, phosphate, silicate, total nitrogen and total phosphates testing.

The database: The data collected in the cataracts study were entered in a specifically designed relational database (Access, Microsoft Corp.). The database consists of 47 tables of direct data, each table containing up to 20 fields at either the fish, cage or site level. The cataract database was capable of being integrated with the existing SIS database. This allowed not only current data to be analysed but also compared to historical data.

Statistical Analysis: Statistical analyses were carried out using SPSS-X (SPSS Inc., Chicago, Illinois) and GENSTAT 5 (V3.2) NAG, Oxford, UK. (Anon 1994).

Output from the database was assessed for approximation to a normal distribution. Most of the data showed a positive skew or was "J shaped", and sample variances were not equal. To avoid assumptions about the distribution when comparing samples, non-parametric tests (Mann Whitney and Spearman Rank Correlation) were most often used. Pearson Correlation Coefficient was used when the assumptions of normality appeared correct. Proportional data were arcsine root transformed.

RESULTS AND DISCUSSION

The results presented in the PD analysis relate to over 18.5 million Atlantic salmon smolts which were placed on 11 Irish marine Atlantic salmon farms located on the western coast of Ireland which cover 52 years of production at sea (from smolt input to the end of December inclusive, of the first year at sea) between 1988 and 1994. More details relating to the establishment of the database and the findings reported from preliminary analyses can be found elsewhere (Wheatley et al., 1995, Menzies et al., 1996).

During recent years (1991-1994), the number of smolts being monitored using this system represented between 35%-50% of the total smolt input into Irish marine sites. The average mortality from input to the end of December over the years monitored (1989-1994) was 23.9%. Figure 1 illustrates the major causes of mortality in the Irish industry as a percentage of the fish originally put to sea. The major causes of mortality were PD, vibriosis and those associated with transfer. The category "other causes" includes occasional (algal blooms, whale attacks) or mortalities where no cause was identified. Over this period, transfer mortalities have remained relatively constant, whilst those attributed to PD have been decreasing.

In Ireland, unlike Scotland and Norway, furunculosis has never been a major disease problem for the farmed salmon industry (only 4 sites affected and typically only 1 site per year). A mortality of 21% of input was reported in an individual cage in 1990, however the median losses for the sites affected by furunculosis were only 1.96%.

Over the period 1988-1996, SIS has collected data on over 25 million fish covering 92 years production from 23 marine sites.

Pancreas Disease

The major factor affecting the profitability of salmon farms was PD, which contributed to half (49%) of the total mortality over the years 1988-1994. There was a significant downward trend in the mortalities due to PD ($P<0.05$) during this period. Annual PD outbreaks were recorded on over 70% of the monitored sites between 1989 and 1994, indicating the ubiquitous nature of the disease.

Between 1988-1994, there were 46 outbreaks of PD reported from the monitored sites with 61% of these outbreaks starting within the period August to October inclusive.

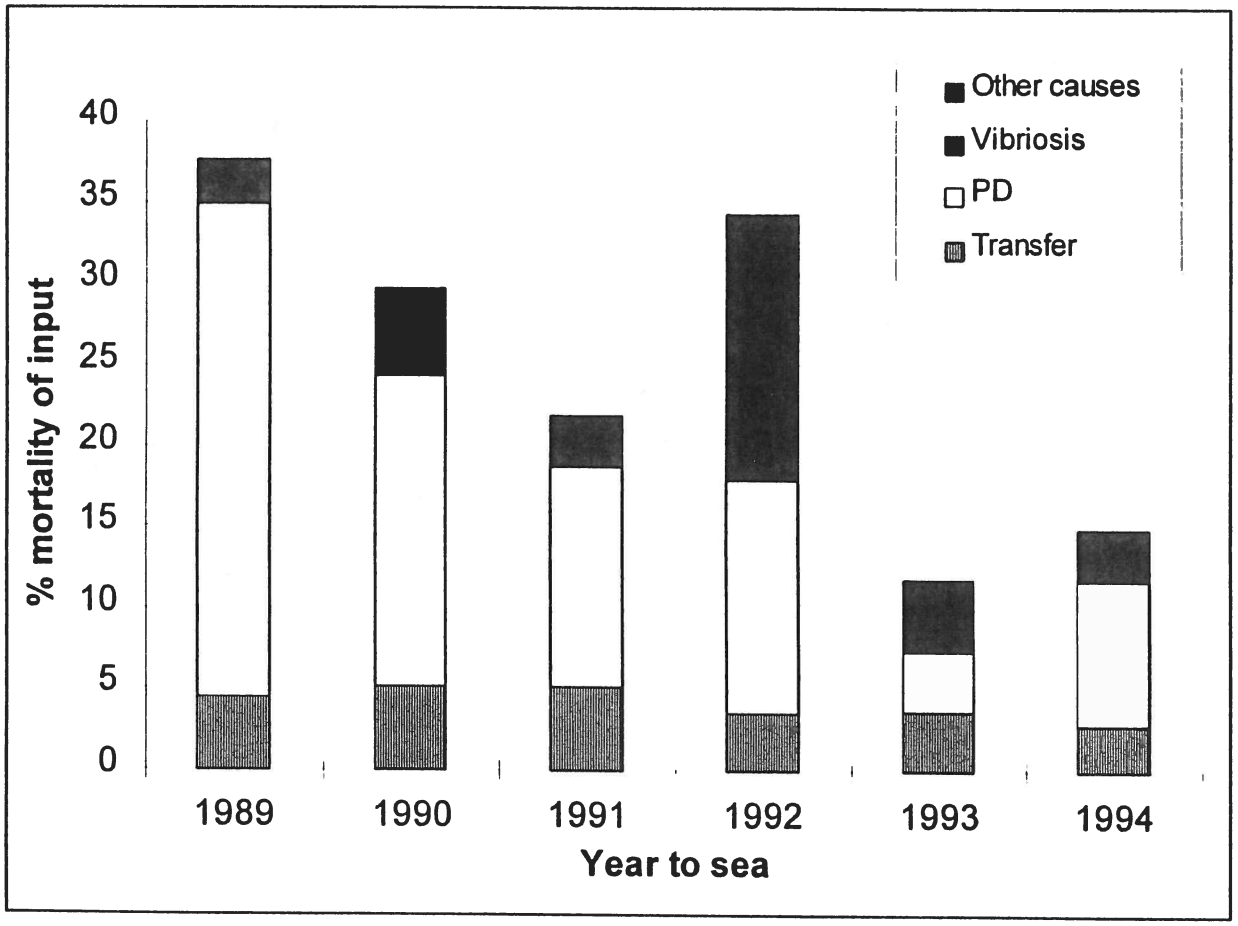

Fig. 1 Histogram to show the major attributable causes of mortality in first year at sea for Atlantic salmon smolts in Ireland between 1989 and 1994. Numbers are expressed as the percentage of smolts put to sea in that year.

Analysis of annual mortality rates during outbreaks of PD over seven sites between 1990 and 1994 showed that mortalities were statistically significantly different between sites ($P<0.05$) but were not significantly different between years. Previous analyses, in relation to mortalities generally, indicated that the site management factors which were significantly

correlated with reduced mortality were fallowing of sites, single generation rearing, slaughtering of fish away from the site and restriction of farm staff to one site (Wheatley et al., 1995). Since 1992, the vast majority of marine sites now practice single generation rearing and fallowing of sites which may have contributed to the significant reduction in overall mortality during 1993/1994 (13.6%) compared to mortality rates in the previous five years (27.6%). However, PD continues to cause 50% of the overall mortality (Fig. 1).

There was a very significant correlation obtained between the time of occurrence of PD and the percentage mortality during an outbreak in that the earlier in the year a PD outbreak occurred, the higher mortality rate ($r = -0.59$, $P<0.01$). A similar correlation was found between the time from smolt input and the percentage mortality for PD outbreaks ($r = -0.52$, $P<0.01$; Fig. 2). There was a highly statistically significant correlation between PD mortality rate and the duration of the outbreak of PD ($r = 0.60$, $P<0.01$). The duration of PD outbreaks tended to be longer when they occurred earlier in the year. One possible explanation for these findings would be that younger fish populations are more susceptible to the effects of PD.

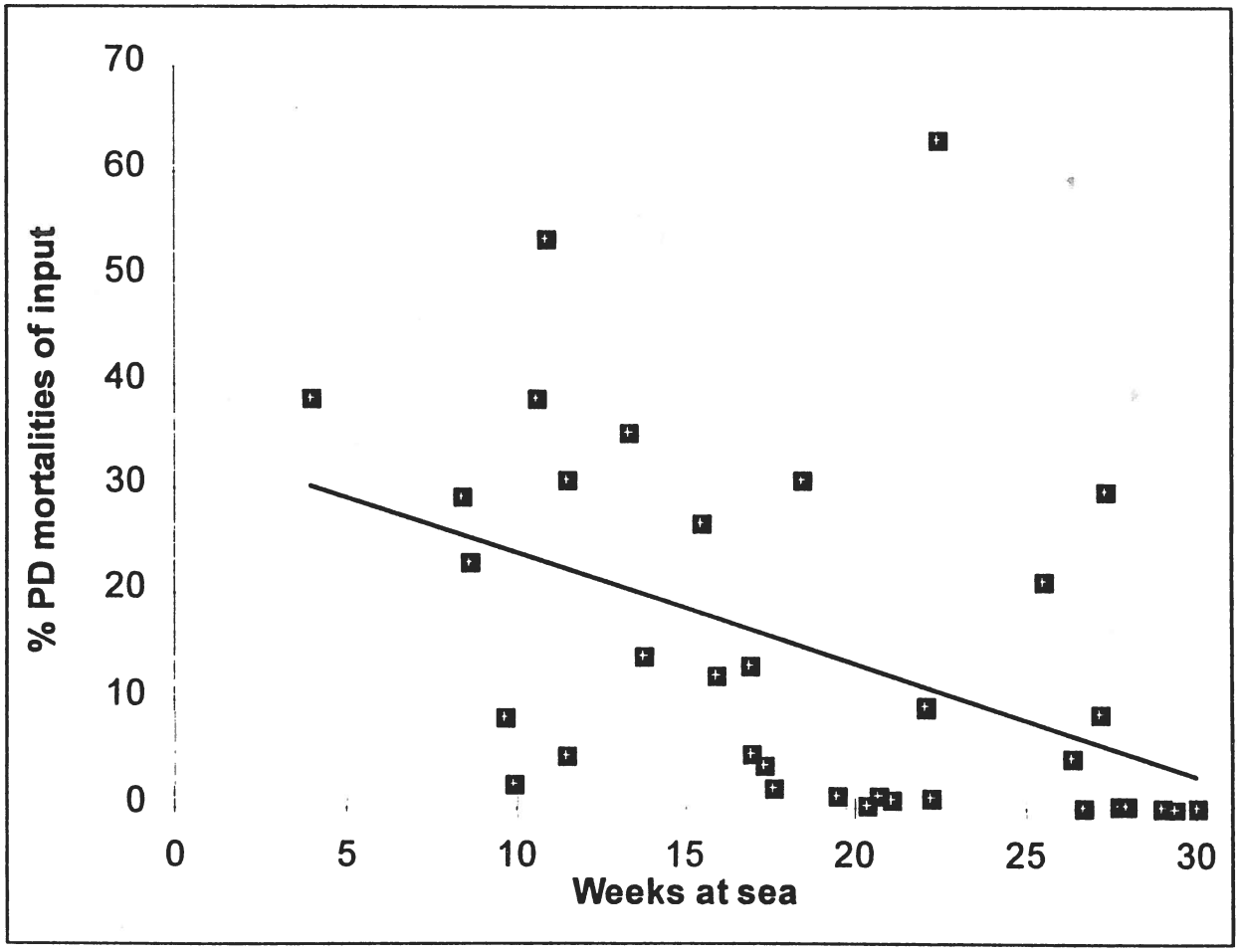

Fig. 2 Graph of the percentage of smolts put to sea where mortality has been attributed to pancreas disease against the number of weeks at sea before the PD outbreak occurs. The data refer to site means and covers the period 1989 to 1994.

There was no correlation between PD mortality and smolt input weight, initial stocking density or transfer mortality. Additionally, there appeared to be no relationship between the interval from smolt input and the start of PD outbreaks.

Cataracts

Approximately 20% of fish in the cataract survey had severe cataracts considered sufficient to affect feeding and swimming behaviour. Severe cataracts were defined as a mean cataract grade of 2.5 or more.

In 1995, questionnaires were completed for 22 sites which in total had put to sea almost 5 million smolts. In 1996, questionnaires were completed for 26 sites which in total had put to sea over 5 million smolts.

A dataset of 225 feed analyses was established which tracked the content of the major fish feeds used in Ireland over the last seven years.

Distribution of eye lesions: Bilateral cataracts were seen in 78% of fish with cataracts. Unilateral cataracts were seen in 10% of fish with cataracts. Less than 5% of fish had cataracts 2 grades worse in one eye than the other. The high prevalence of bilateral cataracts suggests that disease or diet are more likely to blame rather than physical trauma.

Of 723 eyes scored in 1996 where position of the cataract was detailed, 26% had only anterior cataracts and 44% had only posterior cataracts and 30% had both anterior and posterior cataracts. The significance of the location of the opacities in the lens is unclear, but it was felt that posterior cataracts were clinically more significant.

A cataract means lenticular opacity and is usually described in relation to its location, maturity, extent and suspected cause and opthalmoscopic appearance. However, the development of cataractous lesions in the lens appears to follow a similar pattern irrespective of cause. With the exception of post-transfer osmotic cataracts, lenticular opacities tend to be irreversible. Cataracts scored as 3 or 4 were deemed irreversible.

Histological examination: The severity of cataracts ranged from pinpoint opacity to total opacity of the lens. The majority of the fish had degeneration of the posterior and perinuclear lens cortex and variable occurrence of lens epithelial hyperplasia. The histological lesions observed in 1996 smolts were very similar to those observed in the 1995 smolts, however this does not mean that they have a common cause as the lens seems to have a limited response regardless of cause.

Environmental factors: It was determined that environmental factors were not a major direct factor in causing the cataracts.

At present, the results of the investigations into the cataract problem cannot be reported in detail. However, as a result of this epidemiological approach to the investigations of cataracts in farmed Atlantic salmon in Ireland, it is postulated that the primary determinant was a nutritional imbalance. Individual batches of feed were not identified as a problem.

The aetiology of the disease was unknown, however by use of the SIS, work has highlighted important epidemiological associations which, when they were taken into account, appear to have helped in limiting the effect of the cataract problem in 1997. As with many diseases, it would appear that the cause of cataracts is multifactorial.

CONCLUSIONS

The establishment of SIS has enabled disease, management, production and environmental variables to be compared and contrasted over spatial and temporal distributions. It has been utilised to identify site management factors which are detrimental to production as well as to help elucidate the epidemiology of PD.

As a result of this investigation, we would propose that the cause of the cataracts in Atlantic salmon smolts in 1995 and 1996 was a dietary imbalance which occurred to varying degrees in all of the major manufacturers supplying smolt feeds in Ireland.

SIS will be of on-going benefit to the Irish farmed salmon industry as it provides a unique tool in facilitating the producers ability to maximise profitable production of Atlantic salmon. This is particularly relevant as the Fisheries Operational Programme 1994-1999 seeks to increase Irish salmon production by over 50% by 1999.

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INFECTIOUS DISEASE IN TIME AND SPACE

PREDICTING THE EPIDEMIC CURVE OF BOVINE VIRUS DIARRHOEA (BVD) IN MØRE AND ROMSDAL COUNTY, NORWAY

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Bovine Virus Diarrhoea (BVD) has been diagnosed occasionally in Norway since 1967 (Loken et al., 1989). In 1992, the Ministry of Agriculture in co-operation with Norwegian Dairies and Norwegian Cattle initiated a national control and eradication program. Judging by the Norwegian National Disease Surveillance System (NNDSS) up to 1993, BVD has tended to occur as single cases in herds (i.e. sporadically).

Problems reported to have been caused by the BVD virus (BVDV) includes abortion, low non-return rates, increased problems with calf health and birth of persistently infected (PI) animals. Most of these PI animals will develop mucosal disease (MD) and die within two years of age (Houe, 1993). However, it is believed that in many cases BVDV enters a herd without causing clinical problems. Nonetheless, severe economic penalties may accumulate over long periods in affected herds (Duffell and Harkness, 1985).

The objectives of this study were 1) to investigate the trend BVD has taken since the initiation of the program and 2) to compare two different methods for statistical analysis of longitudinal data.

MATERIALS AND METHODS:

Study population

The elementary unit in this study was the cattle herd, and the study population consisted of all commercial dairy cattle herds located in Møre and Romsdal county on the north-west coast

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of Norway. The county is divided into 19 Veterinary Districts (VD) with one District Veterinary Officer (DVO) in each.

The Norwegian BVD Control and Eradication program

The BVD control and eradication program is a co-operative program with participation from the dairy and meat industry, the Ministry of Agriculture [Norwegian Animal Health Authorities (NAHA) and the Veterinary Institute (VI)]. In 1998 it is being taken over by NAHA.

All commercial cattle herds are tested once a year in January or December, with the goal of finding herds with PI animals. The initial step was the examination of a Bulk Tank Milk (BTM) sample from all dairy herds by the use of an indirect ELISA test (SVANOVA Biotech, Uppsala, Sweden). Based on increasing strength of calculated optical density (cOD) (i.e. level of antibodies) herds are classified into four BTM classes (0,1,2 and 3). Until 1998, herds in BTM class 2 or 3 had a pooled milk sample from the five first calf heifers examined. If this test was positive a pooled blood sample from five 8-12(15) months old heifers was examined. A positive sample led to the herd being put under official restrictions by the DVO. Restricted herds were offered a full or partial herd screening as the final step, but all costs for this last step had to be covered by the farmer him/herself.

A restricted farm would have its restrictions lifted if two subsequent pooled heifer blood samples taken within the BVD program(one year apart) were negative, or if a heifer blood sample taken more than 5 months after a full herd screening was negative.

Serological and virus testing

All serologic tests of herds in the county were carried out by the Mastitis Laboratory in Molde, Møre and Romsdal. Blood samples for virologic testing were forwarded to, and tested at the Veterinary Institute in Sandnes or Oslo. An indirect ELISA, applying a monoclonal antibody to bovine immunoglobulin G, was used for detection and titration of antibodies to the BVD virus for both milk and blood samples (Juntti et al., 1987, Niskanen et al., 1989). Based on the cOD the BTM samples were classified into the four BTM classes as follows: $cOD < 0.05$, $0.05 \leq cOD < 0.25$, $0.25 \leq cOD < 0.55$ and ≥ 0.55. The cut-off point for a positive pooled first calving heifer sample was set to $cOD \geq 0.1$, and pooled blood samples from heifers were considered positive if the cOD was larger than 0.25. Exact values for sensitivity and specificity of the test given the chosen cut-off points and the population specifications, such as herd size, were not available. However the ELISA method is found to have a high sensitivity and specificity when compared to a serum neutralisation test (Juntti et al., 1987).

Examination for virus was initially carried out using a virus neutralisation test (Meyling, 1983). During the period an ELISA test was developed and used in the routine virus detection. Sensitivity and specificity of this ELISA test were estimated to be 95.0% and 98.8% respectively, when compared to virus isolation (Sandvik and Krogsud, 1995).

Additional data collection: In addition to the test results information, one DVO labour cost within the BVD program was gathered from the Regional Veterinary Office, Ministry of Agriculture, for the years 1995 to 1997. The accounting system did not allow this information to be separated out for the years prior to 1995.

Data handling: Test results from the BVD program were stored in a SAS (SAS Institute Inc., 1996) database at the Veterinary Institute. The DVO labour cost information was imported into this database, and selections from the data were later exported as text files into the other software used (MLwiN).

In this study, the outcome was the number of herds that sero-converted from one year to the next in each veterinary district. A sero-converted herd was defined to be a herd that went from BTM-class 0 (cOD< 0.05) to a BTM cOD > 0.1 in the following year. This last cOD does not correspond to a BTM class definition in the program, but was chosen lower than 0.25 (BTM class 2) to increase sensitivity and > 0.1 to avoid the majority of the herds that went back to BTM class 0 or stayed in class 1 the year after sero-conversion. These herds are found in the lower cOD range (0.05<0.1) of the BTM class 1 (study not published).

Descriptive statistics were obtained using SAS 6.12 for Windows, and graphical investigation of the raw data displayed by year and grouped by VD was done in MLwiN.

Three types of trend models were built: 1) a simple regression model with time (year) as the explanatory variable, 2) non-linear trend models where alternative parameterizations of time such as fractional polynomials (Greenland, 1998) were tested and 3) non-linear trend models with information on DVO labour cost included. Before being introduced into the model, the DVO labour cost was divided by the number of BTM class 2&3 herds in the VD to yield a cost relative to the prevalence of BVD. Based on this relative cost information an indicator variable was created, separating the VD into high (H_rel_Cost = 1 when relative cost was greater than the 90'th percentile) versus low relative labour cost (H_rel_Cost = 0, reference).

The final trend model was compared to the NAHA official restriction lists for new BVD restrictions per year.

Statistical analysis: Statistical analysis of the BVD trend was performed by using two different statistical methods and software 1) the GEE method provided in the GENMOD procedure in SAS 6.12 for Windows, and 2) the multilevel method by using MLwiN developed at Multilevel Models Project, Institute of Education, University of London.

The GENMOD procedure has been extended with the ability to handle repeated measurements by fitting correlated response models using the generalised estimation equation (GEE) (SAS Institute Inc., 1996) introduced by Liang and Zeger (1986). Given the count data in this study, a log linear model using the Poisson distribution($\sigma^2(\mu)=\mu$) was chosen. The GEE method is designed to permit separate modelling of the regression of Y on X, and the association among repeated observations of Y for each unit (the correlation structure). The regression equation when using GEE can be written as

$$E(Y) = g(\alpha + \Sigma\beta_m X + \varepsilon)$$

where the expected value of Y, depends on a set of co-variates, X, through the link function, g(). The variance-covariance matrix, Σ, used by GEE can be written as

$$\Sigma = V^{1/2} R V^{1/2}$$

where V is the variance function and R is the correlation matrix of the repeated observations within the same subject. When including the R matrix the standard errors of the regression coefficients are adjusted for the correlation among measurements within the specified cluster (VD). In non normal data the correlation structure also influences the values of the regression coefficients, GEE provides an option of choosing several different correlation structures such as m-dependent, exchangeable, unstructured and auto regressive. In this study we used unstructured correlation.

MLwiN is the windows version of the DOS program, MLn. It was developed at the Institute of Education, University of London within The Multilevel Models Project which started in 1986. The hierarchical or multilevel modelling tools such as MLwiN(Prosser et al., 1991, Rasbash et al., 1995), HLM(Bryk et al., 1994) and VARCL (Longford, 1990) are all developed with the purpose of modelling hierarchical structured data. There is now considerable literature on multilevel modelling, both theoretical and applied (Bryk and Raudenbush, 1992, Longford, 1998, Goldstein, 1995 and Hox, 1995).

The regression equation for the multilevel or random effect model can be expressed as

$$E(Y|U) = g(\alpha + \Sigma\beta_r x + U + \varepsilon)$$

where U represents the random contribution of the appropriate probability distribution. The extra variation is included by U, and the β_r can be interpreted as a level(cluster) specific parameter since it is dependent on the random component specific for each level.

As in Proc GENMOD we started out by modelling the data using a Poisson model. Unfortunately, the Poisson assumption ($\sigma^2(\mu)=\mu$) is often inconsistent with empirical evidence (Diggle et al., 1995). The models were therefore investigated with regards to presence of extra-Poisson variation. In the case of over-dispersed count data (variance>mean) the negative binomial distribution, which is an option in MLwiN, can be used.

RESULTS

Descriptive statistics: Numbers of herds classified according to the BTM test are listed for the years 1993-1997 in Table 1.

Table 1. Bulk tank milk (BTM) classification of dairy herds in M&R county for 1993-1997.

BTM class	1993	1994	1995	1996	1997
0	1927	1918	1888	2005	2147
1 - cOD 0.05 - 0.1	117	105	91	128	87
1 - cOD 0.1 - 0.25	239	196	184	163	140
2	252	273	235	151	129
3	147	170	204	62	17
Herds tested	2682	2662	2602	2509	2522
Restricted herds	16	110	160	131	87

In the first year of the program a total of 2,682 dairy herds were tested, but due to a reduction of cattle herds in dairy production the number of herds declined to 2,522 in January 1997. The average herd size is about 13 cows. Further results from testing in the first year of the program showed that of the herds in BTM classes 2 & 3, 59% had a positive pooled heifer milk sample, of these 51% had positive pooled heifer blood sample. This resulted in a total of 110 herds having restrictions due to BVD at the end of the first year of the BVD program (1993). Of these herds about 58% had one or more PI animals present.

In Table 2, the count of sero-converted herds are presented as well as the number of new herds put under restrictions. Column one contains the mean count by veterinary district - % in brackets, of herds sero-converted. The corresponding ranges are given in the following column. Overall counts (%) for the county are given in the two next columns. In the first column for the entire population, and in the second for the group of herds initially free from BVD (BTM class 0). The last column lists the number of new herds being put under restrictions according to NNDSS.

Table 2. The count of sero-converted herds and the number of new herds put under restrictions.

Year	Mean count(%)	Range	No.(%) Sero-Converted	No.(%) Sero-Converted; Initially BVD free	New BVD restrictions
1993	7 (4.7)	1-28	133 (5.0)	133 (7.0)	94
1994	5.4 (3.7)	0-15	103 (4.0)	79 (4.3)	50
1995	1.6 (1.3)	0- 8	31 (1.2)	23 (1.3)	41
1996	4 (3.0)	0-12	76 (3.1)	50 (2.8)	34

A stem-and-leaf plot of the count variable indicated that the data were right skewed, and the Shapiro-Wilk statistic, W, was equal to 0.8 ($p = 0.0001$).

When we investigated the Poisson assumption (variance=mean) for the counts by VD, we found the variance to the mean ratio to be 6.7, 3.3, 2.2 and 3.2 for the years 93-96 respectively.

From the graphical display of the raw data we observed an overall declining pattern from 1993 up to 1995. From 1995 to 1996 it seemed to be a shift to an increasing trend. However some VDs had an increasing trend also in the second year of the program (1994) even though the overall trend was decreasing.

The average DVO labour cost related to the BVD program showed a decreasing trend for the years 1995 to 1997. At the same time we found that the relative cost by herds being classified into BTM classes 2 or 3 increased (see Table 3).

Table 3. The District Veterinary Officer (DVO) Total and Relative Labour Cost (LC) in Nkr. Ranges are given in brackets.

Year	Average Total DVO LC(range)	Average Relative DVO LC(range)
1995	3,002 (0 - 9,186)	124 (0 - 343)
1996	2,166 (0 - 7,108)	199 (0 - 790)
1997	1,419 (0 - 4,104)	231 (0 - 1,368)

Results from Proc GENMOD in SAS using the GEE method: Among the different non-linear models built the Sine_T = sine(3.14*1.1*Year) variable, which made the trend line oscillate around a main trend, gave the largest single change in deviance when introduced to the simple time model. A fractional polynomial model, where time, time to the power of ½ and time to the power of 3/2 were included, gave a lower deviance, but led to a non plausible prediction for 1998 and was discarded.

In the simple time model (Table 4) we found that the indicator variable for high versus low relative labour cost (Hi_rel_Cost), as well as its interaction with time (Time*Hi), significantly contributed to the model. Sine_T also was significant in this model, but did not show significant interaction with the cost variable.

Table 4. GEE models - coefficient estimates with standard errors (S.E.) in brackets: Model 1, a simple time dependent model, Model 2, a non-linear model including time and sine (time) and Model 3, a non-linear model including time, sine (time), the high relative cost variable and its interaction with time as explanatory variables.

	Model 1	Model 2	Model 3
Deviance	182.08	153.53	138,51
D.f.	74	73	71
Intercept	-2.906 (0.223)	-2.532 (0.202)	-2.684 (0.208)
Time(T)	-0.252 (0.079)	-0.419 (0.078)	-0.343 (0.077)
Sine_T		+0.506 (0.128)	+0.502 (0.132)
Hi_rel_Cost			+0.895 (0.383)
Time*Hi			-0.533 (0.140)

Results from MLwiN

The previous models from proc GENMOD were rebuilt and re-tested in MLwiN using the negative binomial distribution. The estimating procedure gave the results presented in Table 5.

Table 5. MLwiN models - coefficient estimates with S.E. in brackets: Model 1, a simple time dependent model, Model 2, a non-linear model including time and sine (time) and Model 3, a non-linear model including time, sinus (time), the high relative cost variable and its interaction with time as explanatory variables.

	Model 1	Model 2	Model 3
D.f.	74	73	71
Intercept	-2.934(0.203)	-2.653(0.191)	-2.782(0.201)
Time(T)	-0.225(0.074)	-0.389(0.074)	-0.309(0.074)
Sinus-T	-	+0.583(0.133)	+0.574(0.126)
Hi_rel_Cost		-	+0.878(0.434)
Time*Hi	-		-0.568(0.186)

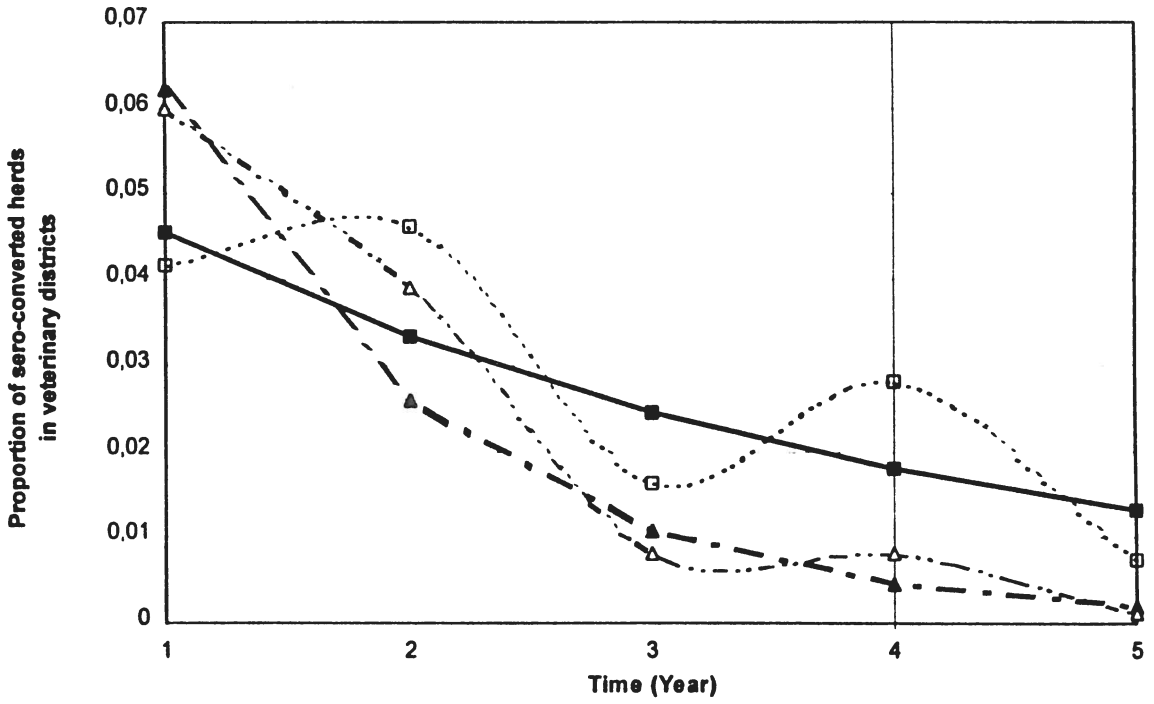

Fig. 1 shows the BVD trend based on the estimates from model 3 in MLwiN. The vertical line at time 4 indicates where the graph converts from describing the modelled data to predict the epidemic curve for the year to come (1997). —Trend when controlling for high relative cost VD and without oscillation. ..... Trend with oscillation when controlling for high relative cost VD. — - - Trend in high relative cost VD without oscillation. — -- — -- Trend in high relative cost VD with oscillation.

DISCUSSION

Comments about software

SAS provides several options for analysing repeated measurement count data such as Proc GENMOD (SAS v 6.12) and a number of macros (GEE, GLIMMIX). In this study we used the GENMOD procedure with the GEE method since one of the goals was to compare estimates between GEE, and estimates given by the multilevel modelling tool MLwiN. Both of which are methods recommended for analysis of longitudinal data.

MLwiN is a more narrow product when compared to SAS. It was developed based on the theory of hierarchical linear models (Bryk and Raudenbush, 1992), and designed for modelling of hierarchical data. A large emphasis has been put into making the interface of MLwiN assist the user in the understanding of the complexity of multilevel models.

Comments on the analytical methods

The need for analytical tools that handle correlated data has been recognised within the social sciences for decades (Lindley and Smith, 1972). More recently the problems with interdependence have come in focus within veterinary science and different methods to handle such data have been described (McDermott et al., 1994).

Ignoring clustering will generally cause standard errors of regression coefficients to be under-estimated. Thus, when correlation is not accounted for, it is more likely to infer that a relationship is present when in fact it should be ascribed to chance (Type I error).

Both the GEE and the multilevel method handles data with interdependence in the response variable and they provide valid estimates for both the coefficients and their standard errors.

The two methods gave similar coefficient estimates for the different groups of models. In general, the standard errors were slightly higher for the GEE models, but this was not consistent. However, the differences in magnitude were small except for the labour cost indicator and its interaction with time in the last group of models. Here the MLwiN had a marked increase in S.E. for the cost as a main effect as well as for the interaction term though not leading the effects to become insignificant. This observation may be related to pushing the limits for a reasonable number of parameters (random and fixed) being estimated (< 10% of n, n=sample size) in the negative binomial model in MLwiN.

The main difference between the analytical methods applied is that GEE, which provides a so called marginal model, can only be used for making inference about the population average. The multilevel model or random effects model, makes it possible to do subject specific predictions, if desirable.

Comments on the BVD trend

Notwithstanding the limitations of our data (very few years of data) the BVD trend show an overall declining pattern. There seems to be a shift from 1995 to 1996 and we have chosen to model this variation as an oscillation around an overall declining trend. Such oscillations may have several different explanations which are discussed below.

First we need to acknowledge the imperfect sensitivity of the BTM test with regards to picking newly infected herds. The sensitivity of the BTM test is related to the chosen cut-off value for further testing (cOD>0.25), and with the present cut-off several of the animals contributing to the BTM at the day of sampling must carry antibodies. Hence the infection must have spread to a certain degree within the herd in order to be detected with a high degree of certainty.

In addition there is a limit to the amount of information the BTM test conveys about the infection status in the young stock. If a PI animal is born in a dairy herd it is most likely that it will first infect its own age cohort. The rate of spread to susceptible cows in production will depend on the rate and degree of contact from young stock to the animals in production. In a worst case scenario, where there is a high degree of separation of age cohorts in the herd, the time lag from infection to a positive BTM test may be more than two years. In the mean time if the young stock are let out to common forest pasture with young stock from other herds, which is a management system many farmers follow in the county, an epidemic may be the result. In 1996, when more herds were becoming susceptible (BTM class 2&3 herds were reduced from 439 at its peak in January 1995 to 146 in January 1997) the BVD virus if introduced by risk factors such as purchasing of live animals, sending animals to common pasture and over fence contact (Valle et al., in press) would have an increasing likelihood of meeting a susceptible animal and spread BVD.

A large part of the increase in sero-converted herds in 1996 (26 of 76 herds) came from the group of herds that initially had been exposed to BVDV (2682-1927=755). Variation in cOD may arise due to different animals contributing to the BTM sample from one year to another, and one single animal does have a large contribution to the BTM antibody level given the small herd sizes in the county. Therefore one can not exclude that what we observe is simply a result of different animals being milked on the day of sampling. Thus we may be detecting earlier still infected herds.

The relative high sero-conversion count among earlier infected herds may also be related to re-infection. Farmers may still carry a high risk taking attitude or have a management system leading to a higher risk level for getting BVDV infection. Conversely, it is reported (Brownlie J., personal communication) that PI animals may not shed virus continuously. Some PI animals may act as latent carriers and at a later stage start shedding the virus; the herd will therefore appear to have been re-infected.

When comparing the trend given by using our cut-off for sero-conversion to the number of new herds being put under restrictions the patterns are not similar. The number of new restrictions is steadily decreasing over the whole period. It may be argued that the discrepancy is due to our low cut-off value, which because of the lower specificity increases the

background "noise". On the other hand it is expected that this noise would be present in all the years modelled. Further it is not likely that all infections will lead to the birth and presence of PI animals, which are the type of herds the BVD test scheme is aimed at finding and controlling. Also the number of restrictions is based on a higher cut-off point for the BTM test, and therefore a similar pattern may show up for new restrictions in a later year. There is a considerable time lag between a BTM test with cOD > 0.1 and one with cOD > 0.25, given that the spread of BVDV within a herd may take months. We do therefore believe that the decreasing trend with its oscillations do reflect the true infection trend though maybe at too high a level.

The promising part of the modelled trend is that in VDs with a high relative labour cost at a later stage in the program, the increase in BVD does not occur in 1996. The relative labour cost can be regarded as a surrogate measure for the DVO's efforts to control BVDV. Efforts such as control of animal movements by restrictions and information and following up infected herds and potential contact herds. However, it is expected that there are differences both in the efforts and the quality of the efforts not reflected in this surrogate measure. The findings related to the relative cost, which is an intervening factor for the actual work done in the VD, must therefore be handled with care.

The trend in BVD is promising since it indicates that when sufficient efforts are placed into the BVD program at the veterinary district (field) level, it may be possible to control and eradicate BVD. A combination of sufficient efforts at the local level and improved sensitivity of the testing program may force the BVD epidemic curve down to zero.

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RELATIONSHIP BETWEEN CLIMATE EVENTS AND THE FREQUENCY OF EQUINE *CORYNEBACTERIUM PSEUDOTUBERCULOSIS* INFECTIONS (1982 – 1995) – A TIME SERIES APPROACH

M.G. DOHERR[1], T.E. CARPENTER[2], W.D. WILSON[2] and I.A. GARDNER[2]

Deep intramuscular abscesses in horses caused by *Corynebacterium pseudotuberculosis* have been reported since the beginning of the century from California. Scientists at the University of California, Davis, have a long research history on *C. pseudotuberculosis*-related abscesses in horses, and the disease is still estimated to be one of the most important infectious diseases in the UC Davis horse patient population (Hall & Fisher, 1915; Hughes & Biberstein, 1959, Hughes et al., 1962; Knight, 1969; Miers & Ley, 1980; Aleman *et al.*, 1996). A marked seasonality with peak incidence in late summer and fall has been observed. Consequently several hypotheses have been advanced linking a combination of certain climate conditions, insect population density and survival of the bacterium in the environment, with the increased incidence during this time of the year (Wheat, 1961; Wisecup *et al.*, 1964; Knight, 1969a; Addo, 1983; Miers & Ley, 1980; Welsh, 1990). Interactions between climate factors, host and vectors have been described for the spread of several (predominantly viral) diseases from various regions of the world (Sellers, 1980; Sellers & Maarouf, 1988 & 1993; Ward, 1994; Ward & Thurmond, 1995; Ward & Johnson, 1996). Many infectious diseases show a seasonal behaviour and random changes of trend. Consecutive observations over time are rarely independent, therefore violating the primary assumption of classical statistical methods. For this kind of non-stationary time dependent data simple descriptive techniques as well as the ARIMA model approach suggested by Box & Jenkins in 1976 were recommended to explain the temporal variation (Helfenstein, 1986 & 1996). The methods typically decompose the variation of single series into long-term changes (trend), seasonal effects (annual), short-term cyclic changes and a remaining random or non-random fluctuation (Chatfield, 1989). In a further step, multiple models can be employed to estimate the (time dependent) association between hypothesized risk factors and the outcome of interest (Shumway, 1995).

The objective of this study was to describe the temporal pattern of equine *C. pseudotuberculosis* cases seen at the Veterinary Medical Teaching Hospital (VMTH) between January 1, 1982 and September 30, 1995 and to evaluate the association between monthly case frequency and regional climate pattern (temperature, precipitation) using time series techniques.

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MATERIALS AND METHODS

Case records

In 1992/93 a search was initiated in the previous VMTH database system (January 1982 - December 1985; not available on-line) and in the current system (January 1985 - December 1993; available on-line) to identify computerized medical records containing the keywords 'coryne*', 'pseudotuberculosis', 'pigeon fever', 'lymphangitis', 'pectoral abscess', or 'internal abscess' in the 'diagnosis' field (Aleman *et al.*, 1996). Identified case records were combined with hits from an on-line search of the current VMTH computerized medical record database (January 1, 1985 – September 30, 1995) utilizing the keywords 'coryne*' or 'pseudot*' (one search) and 'pigeon' or 'lymphangitis' (second search). The two case-databases were merged and redundant records, based on horse ID, age, breed, gender, and admission date deleted. Information from multiple admissions or visits of the same horse for the same disease or procedure (e.g. rechecks of draining abscess, microbiology or serology) was incorporated into the record on the first admission when the disease was mentioned. All second and higher order admissions on the same case were deleted from the database. Selection criteria were applied to identify clinically confirmed cases (typical pectoral abscess and/or positive microbiology or synergistic hemolysis inhibition SHI titer ≥ 1:256), strongly suspected cases (clinical diagnosis unsure, other abscess location, SHI titer between 1:80 and 1:256), and negative horses (Doherr, 1997). The admission date for each case under study was used to derive the monthly case frequency.

Climate data

A CD-ROM-based climate database was used to retrieve information on daily minimum and maximum temperature and daily precipitation recorded by the UC Davis weather station and 8 additional stations within a 20 to 60 km distance from the VMTH. Monthly summary measures for daily minimum temperature (TMINAV), daily maximum temperature (TMAXAV) and precipitation [daily average (PDAV) precipitation, maximum precipitation in one day (PDMAX) and monthly total (PMTOT) precipitation] were calculated.

Descriptive statistics and time series models

Microsoft EXCEL[3] was used to manipulate the data and plot the case series and climate variables over time. For further evaluation of the series and the modeling, the time series software package ASTSA/WIN[4] was employed. All series were checked to determine whether they were stationary (no consistent long-term change over time) and whether their variances were normally distributed (no extreme differences). The autocorrelation function (ACF) and partial autocorrelation function (PACF) were used to evaluate the within series correlation between an observation at time 0 (t_0) and its past values (at t_{-1}, t_{-2}, t_{-3} ...). Correlation between

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the case series and each of the climate factors was evaluated using lagged scatterplots of the two series and the cross correlation function (CCF) (Shumway, 1995). For univariable model identification and decomposition of the variance of each series, autoregressive integrated moving average (ARIMA) models following the Box-Jenkins approach were developed (Helfenstein, 1986 & 1996). Least square regression models relating climate factors to the case frequency were constructed in the form of a generalized regression model as follows:

$$y_t = \beta_1 z_{t1} + \beta_2 z_{t2} + \cdots + \beta_q z_{tq} + e_{ti} \qquad \text{(Formula 1)}$$

The terms with z_{t1}, z_{t2},...,z_{tq} represent a collection of known functions of time that influence the output y_t, and e_{ti} is the random error term uncorrelated over time *t*.

RESULTS

The number of cases (CSE) of *C. pseudotuberculosis* as well as the average minimum temperature (TMINAV), maximum temperature (TMAXAV) and daily precipitation (PDAV) were plotted over time (Figures 1-3). None of the series showed a statistically significant ($P < 0.05$) linear trend (increase or decrease over time). The histograms, however, indicated that the series CSE and PDAV required a logarithmic transformation to achieve normality and therefore weak stationarity. The CSE series showed consistent peaks in case frequency per month for the months August (1992 only) and October – December (all years). The amplitude of the seasonal peaks, however, varied considerably (Fig. 1)

TMINAV and TMAXAV showed a very regular sine-shaped pattern with a minimum in December or January and a maximum in July or August of each year (Fig. 2). The average precipitation per day for each month (PDAV) showed more variability in the amplitude than the temperature series, with a maximum between November and March and lows (little or no precipitation) between June and September for most of the years (Fig. 3).

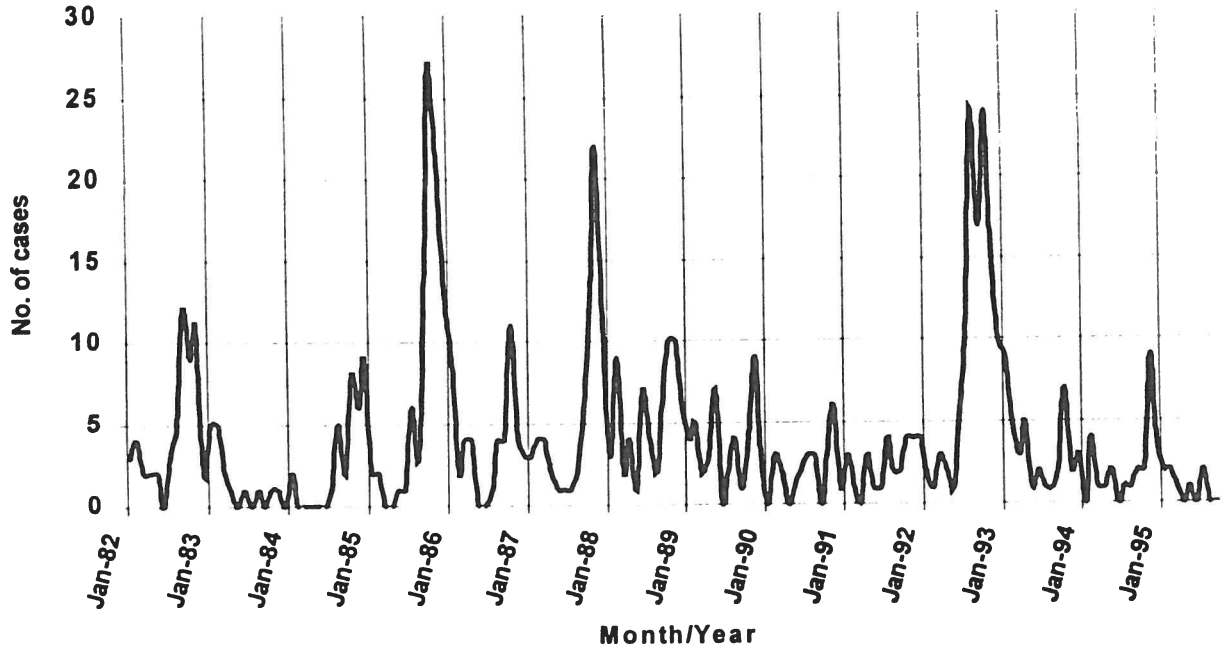

Figure 1 – Monthly frequency of equine *Corynebacterium pseudotuberculosis* cases seen at the UC Davis Veterinary Medical Teaching Hospital between Jan. 1, 1992 and Sep. 30, 1995.

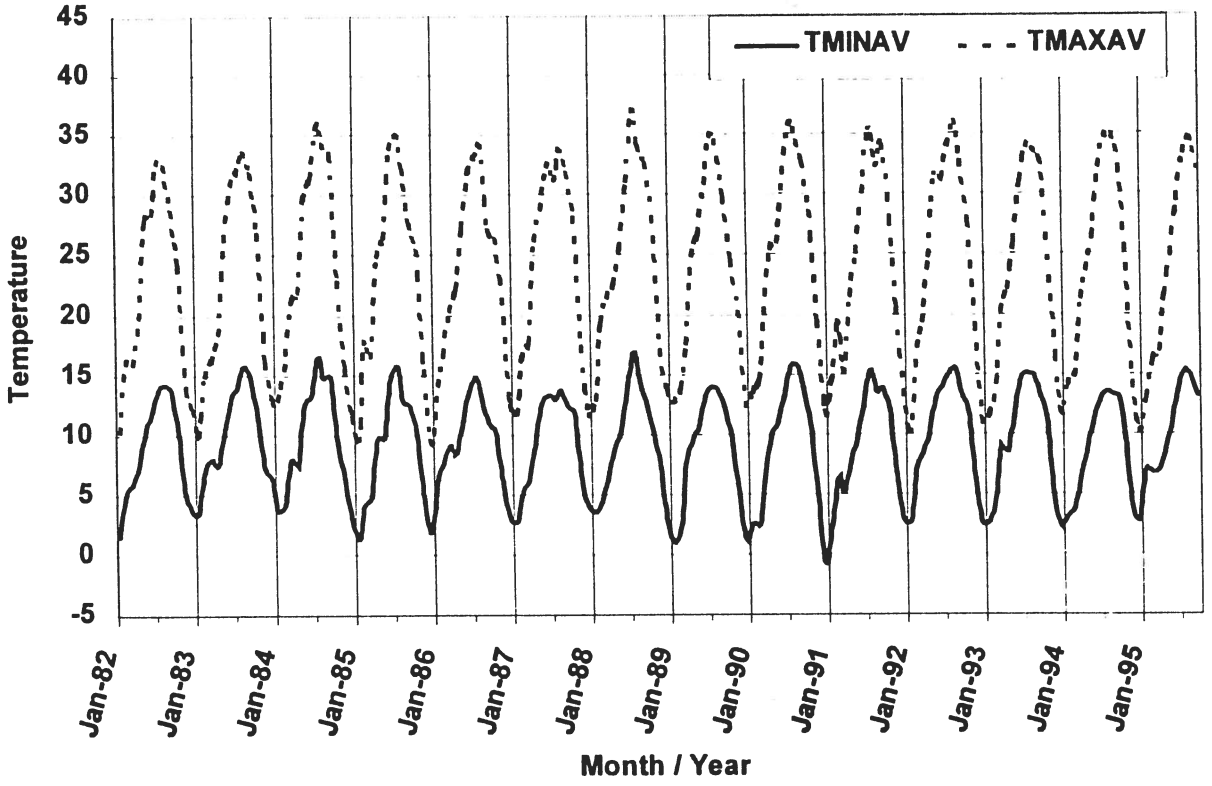

Figure 2 – Monthly average of the daily minimum (TMINAV) and maximum (TMAXAV) temperature in degrees Celsius for 9 weather stations in the northern Central Valley of California and the time interval January 1, 1982 – September 30, 1995.

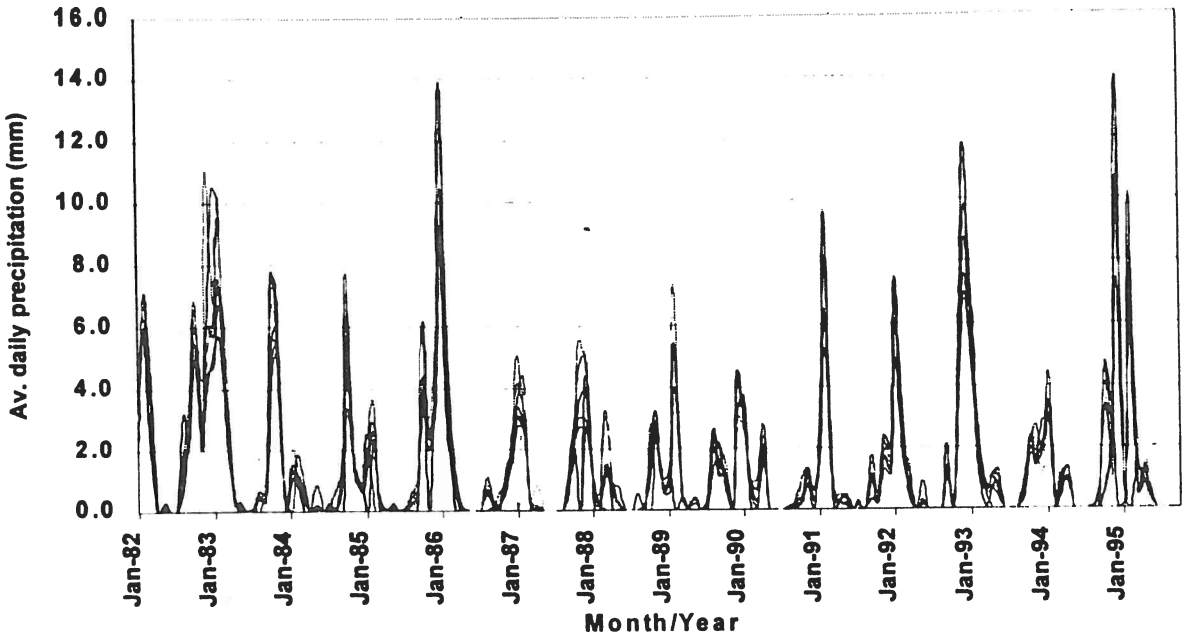

Figure 3 – Monthly average precipitation per day (in mm) for 9 weather stations in the northern Central Valley of California for the time interval January 1, 1982 – September 30, 1995.

Both temperature series showed the highest cross-correlation coefficient for a lag of –12 month, indicating a strong correlation between the average minimum and maximum temperature of the current month and the respective temperatures of the same month of the previous year. For precipitation, the strongest predictor for the current months' PDAV was the rainfall of the previous month, followed by the amount of rainfall of the previous season. For CSE we observed a fairly strong correlation (correlation coefficient r = 0.58) between the number of current cases and the number of cases seen the month before, followed by the number of cases 2 months before (r = 0.45). Cross-correlating the case series to each of the climate series resulted in the highest (negative) correlation coefficients (-0.47 and –0.51) for TMINAV (lag -2) and TMAXAV (lag -2). The precipitation series also had it's highest correlation (0.41) with CSE at lag –2. Evaluation of the autocorrelation function (ACF) and partial autocorrelation function (PACF) to build ARIMA models (Box-Jenkins approach) resulted in fairly similar models for all series until the remaining variation in each series was reasonably random (Table 1).

Based on Akaike's (corrected) Information Criterion (AICc), the best fitting multiple regression model of the form

$$\ln(CSE)_{t=0} = \beta_1 \ln(CSE)_{t=-1} + \beta_2 x_{2,t=y} + e_{t=0} \qquad \text{(Formula 2)}$$

was the model with x_2 = TMAXAV at lag –2, followed by the model with x_2 = TMINAV lagged at -2. For both models, the error terms were reasonably random (white noise). None of the precipitation variables were significant ($P \leq 0.05$) in the logistic regression model with ln(CSE) lagged at -1 as the first predictor variable (Table 2).

Table 1 – Box-Jenkins (ARIMA) models resulting in white noise of the residuals of the series of equine *Corynebacterium pseudotuberculosis* cases (CSE) and the climate parameters monthly average minimum temperature (TMINAV), average maximum temperature (TMAXAV), daily average (PDAV) and maximum precipitation (PDMAX) and monthly total precipitation (PMTOT).

Series	**ARIMA (p,d,q)x(P,D,Q)$_S$ model[a] and no. of parameters used**		**zero lag autocovariance**
ln (CSE)	$(1,1,0)x(0,1,0)_{12}$	15	0.8273
	$(2,1,0)x(0,1,0)_{12}$	16	0.7571
TMINAV	$(1,1,0)x(0,1,0)_{12}$	15	11.0258
	$(2,1,0)x(0,1,0)_{12}$	16	10.6845
TMAXAV	$(1,1,0)x(0,1,0)_{12}$	15	29.4242
	$(2,1,0)x(0,1,0)_{12}$	16	27.6200
ln (PDAV)	$(1,0,0)x(0,0,0)_0$	2	0.0055
	$(1,0,0)x(0,1,0)_{12}$	14	0.0069
	$(1,1,0)x(0,1,0)_{12}$	15	0.0098
ln (DPMAX)	$(1,0,0)x(0,0,0)_0$	2	0.1209
	$(1,0,0)x(0,1,0)_{12}$	14	0.1345
	$(1,1,0)x(0,1,0)_{12}$	15	0.2069
ln (PMTOT)	$(1,0,0)x(0,0,0)_0$	2	0.4269
	$(1,0,0)x(0,1,0)_{12}$	14	0.4761
	$(1,1,0)x(0,1,0)_{12}$	15	0.7315

[a]Monthly and seasonal autoregressive (p, P), moving average (q, Q) and difference (d, D) components and season (12 months)

Table 2 – Multiple linear regression relating the dependent series of equine *Corynebacterium pseudotuberculosis* cases (CSE) to itself (at lag = -1) and one of the climate parameters monthly average minimum temperature (TMINAV), average maximum temperature (TMAXAV), daily average (PDAV) and maximum precipitation (PDMAX) and monthly total precipitation (PMTOT).

X1: ln(CSE) t-1	**X2:**	
β-coefficient (p-value)	**Climate variable**	**β-coefficient (p-value)**
0.5410 (0.001)	TMINAV t-2	0.0122 (0.001)
0.5363 (0.001)	TMAXAV t-2	0.0080 (0.001)
0.8851 (0.001)	ln (PDAV) t-2	-0.3123 (0.636)
0.8890 (0.001)	ln (PDMAX) t-2	-0.0597 (0.638)
0.8856 (0.001)	ln (PMTOT) t-2	-0.0257 (0.705)

DISCUSSION

The time series approach helped to formally describe the case and the climate series under study. It was of no surprise that all climate variables showed a strong seasonal (annual cycle) in addition to the autoregressive component (highest correlation between current and the most recent observation). This is typical for all series over time that have an annual cycle and gradual changes over time. We were able to almost completely explain the case frequency series using a similar ARIMA model containing an autoregressive and a seasonal component. This again pointed towards the seasonality of the event and the fact that the number of cases seen in the current month is correlated with the number of cases seen the previous month. Any disease with a low endemic baseline level and seasonal (risk) factors that increase the disease frequency above baseline for certain periods of the year would show this pattern over time, and could be explained with a seasonal autoregressive model.

Associating the case series with itself and with either of the climate events was of little help in supporting the hypotheses regarding the association between precipitation and other weather patterns and the case frequency that had been postulated. In a regression model that already contained the case series lagged at t-1 as a predictor, none of the precipitation variables lagged at t-2 were significant. The highly significant negative coefficients of the temperature series (average daily minimum and average daily maximum temperature) lagged at t-2 in the same model were not surprising when looking at the plot of the case and the temperature series. The average daily temperature (with its minimum and maximum) peaks were in July or August and then decreased to reach a minimum in December or January of each year. During these periods of temperature decrease, however, the number of cases typically increased to a peak between October and December. A numerical association is therefore to be expected; whether this association has an underlying biological explanation or is spurious remains unknown. One could speculate that the decrease in daily temperature gradually increases the chance for prolonged survival of the bacterium in the environment and therefore increases the risk of transmission. The onset of the rain season would additionally support the development of environmental conditions (humid and not too hot) that would foster bacterial survival. This combination of climate events (temperature and precipitation) could also explain the lower number of cases seen between May and August, which is the hottest and driest period of each year and the least suited for survival of the agent. This hypothesis, however, is contradicted by the fact that in some years (including 1992) the fall epidemic started as early as July, at the same time as we typically observe the (hypothesized) least favorable conditions for bacterial survival.

Even if the observed associations between climate conditions and case frequency could partly explain the seasonal variation of the incidence, there is still no explanation for the considerable variation in the number of cases seen from year to year. The high disease seasons (1985, 1987 and 1992) were not preceded by significantly different conditions.

In conclusion, the available data did not enable us to confirm a relationship between precipitation and case frequency. We also could not support the hypothesis that the observed association between temperature and case frequency is causal. The hypotheses developed during the last decades associating climate patterns (most often rainfall) with epidemics of the disease were based on single events, and our study shows no evidence of regularity that would be needed to support these assumptions. Further research, potentially incorporating the

microenvironment of each horse (with local climate and environmental parameters before and at time of disease), will be necessary to explore, and hopefully determine, the role of these factors in equine *Corynebacterium pseudotuberculosis* infections.

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SPATIAL ASPECTS OF BROILER FOOT-PAD DERMATITIS

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Foot-pad dermatitis, also known as plantar pododermatitis or 'ammonia burns', is a condition which is characterised by lesions on the foot pads of poultry (Wise, 1978; Schulze Kersting, 1996). The lesions start with degeneration and discoloration of the epithelial cells, leading to erosions and, in severe cases, ulceration and inflammation (Nairn & Watson, 1972).

RISK FACTORS FOR FOOT-PAD DERMATITIS

Foot-pad dermatitis in broilers is related to moisture and chemical irritants in the litter (Nairn & Watson, 1972; Harms et al., 1977; Greene et al., 1985; Martland, 1985; McIlroy et al., 1987; Schulze Kersting, 1996). There is a correlation between foot-pad dermatitis and other types of contact dermatitis in broilers, such as breast blisters and hock burns (Harms & Simpson, 1975; Greene et al., 1985; Martland, 1985).

Wet litter is a common problem in the broiler industry world wide, and quite substantial research has been carried out to investigate different possible risk factors. There is, for example, an association between litter depth and material and the prevalence of foot-pad dermatitis (Smith, 1956; Shanawany, 1992; Ekstrand et al., 1997a). Stocking density (McIlroy et al., 1987; Cravener et al., 1992; Tucker & Walker, 1992; Gaardbo Thomsen, 1993), type of water equipment (Elson, 1989; Lynn & Elson, 1990; Tucker & Walker, 1992) and climatic conditions (Payne, 1967; McIlroy et al., 1987) have also been found to influence litter quality.

REGIONAL AND SEASONAL ASPECTS

Several of the risk factors listed above are likely to vary both among countries and regions within a country. Litter condition is correlated with indoor relative humidity which is linked to the outdoor relative humidity (Payne, 1967). In studies carried out in Northern Ireland, McIlroy and co-workers (1987) and Bruce and co-workers (1990) showed that broiler hock lesions and breast blisters are significantly more common during the winter months when the air humidity is

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high. A similar pattern of seasonality in the prevalence of foot-pad dermatitis in broilers in Sweden has been demonstrated by Ekstrand & Carpenter (submitted). Regional differences in the prevalence of broiler foot-pad dermatitis could thus be expected as outdoor relative humidity will vary between different regions.

McIlroy and co-workers (1987) also found an effect of feed manufacturer on the prevalence of contact dermatitis in broilers, which might be of relevance when analysing spatial information as, in a commercial situation, different feed companies are likely to deliver feed to different regions.

AIMS OF THE STUDY

This cross-sectional observational study is based on a Swedish surveillance programme which was developed to improve the knowledge about the prevalence of the disease and its distribution in the population. The programme also contained an advisory system which aimed at decreasing the incidence of the lesions. The analyses presented here are based on the first two years of the programme, from July 1994 through June 1996. Results considering some other variables recorded in this programme have been reported previously (Ekstrand et al., 1997b).

The aim of the part of the study presented at this conference was to further describe and analyse spatial aspects of foot-pad dermatitis in Swedish broilers.

MATERIALS AND METHODS

The foot-health surveillance programme and the data collection methods have been described at a previous SVEPM conference (Ekstrand, 1997). In summary, the programme included classifying foot-pad lesions and recording flock prevalence at slaughter. For each flock, information on producer, breed, feed manufacturer, region, abattoir, date of slaughter, age at slaughter, planned and actual stocking density was recorded.

A total of 6988 flocks, representing approximately 110 million broilers, were examined. A total of 175 broiler producers from 15 counties are represented. From every slaughtered flock 100 single feet were systematically taken out for gross examination at the abattoir. The foot-pad lesions were scored by the veterinary inspectors or assistants at each slaughterhouse. A flock-specific value, called the 'foot-pad score', was calculated as a weighted average of the lesion severity scores and their relative frequency.

Data on location of broiler farms, origin of flocks slaughtered and the mean foot-pad score in different regions were mapped (MapInfo Professional, 1995) using geographic data from the Swedish Central Bureau of Statistics (SCB). This way, maps showing the geographical distribution of areas with high mean values for foot-pad dermatitis were created. The level of clustering in space, i.e. similarity of location, was analysed on 2-digit zip code level using Moran's I test (Moran, 1948; 1950), which measures similarity of location or adjacency. The level of clustering in space was also analysed using the I_{pop} test (CAST, 1993), which takes into

consideration not only the degree of disease in different areas but also the number of flocks at risk, i.e. the number of flocks delivered from each region.

RESULTS AND DISCUSSION

We found a significant ($p<0.001$) clustering of regions with respect to foot-pad dermatitis score using Moran's I test, and a significant ($p<0.0001$) clustering in space when related to the number of flocks delivered from each region.

The regions with high mean values for foot-pad dermatitis score were predominantly located in the south-western part of the country. The flocks with very high prevalence of foot-pad dermatitis were significantly ($p<0.05$) clustered in both time and space. The clustered case pairs were mainly found during the autumn and winter months, and several originated from the same farm.

The Moran's I test showed marginally significant ($p=0.08$) clustering of regions with respect to outdoor relative humidity. The spatial clustering of high mean values of foot-pad dermatitis score in the south-western region could also be a result of differences in feed composition between feed suppliers delivering feed to different parts of the country, as the feed composition can affect the bird's droppings (Schulze Kersting, 1996) and thereby litter moisture. Spatial clustering could also be linked to other local factors such as management advice given by local advisors at the slaughterhouses, which are consistently receiving birds from specific areas.

This information about the spatial distribution of broiler flocks with high prevalence of foot-pad dermatitis will permit us to focus the control efforts within the foot-health surveillance programme on specific regions in specific time periods, thus making the programme more effective.

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RISK ASSESSMENT

THE USE OF MONTE CARLO SIMULATIONS TO EVALUATE THE RISK OF INTRODUCTION A DISEASE EXOTIC TO NORWAY VIA IMPORTATION OF LIVE SWINE.

LARRY G. PAISLEY *

This report will compare the safeguards provided by the European Union (EU) and Norwegian importation rules and regulations with the importation protocol proposed by NORSVIN, the major swine importer, with regards to the risk of introduction of diseases exotic to Norway via the importation of live pigs from the EU. Norway is in the enviable position of being "free" of all Class A and Class B swine diseases (with the exception of Atrophic Rhinitis), as defined by the Office Internationale des Épizooties (OIE), as well as many other important porcine diseases not specifically reported by the OIE.

Although not a EU member state, Norway is a member of the European Economic Area Agreement (EEA). The advantages of membership include tariff-free access to markets within Europe, as well as the assurance that all imported animals meet certain basic requirements. The EEA provides general health requirements for swine traded between members of the EU and specifically addresses the diseases Foot and Mouth Disease (FMD), African Swine Fever (ASF), Classical Swine Fever (CSF), Swine Vesicular Disease (SVD), Aujeszky's Disease (AD) and Brucellosis. The general protections provided by the rules and regulations include:

- All farms or holdings must be registered.
- All animals must be identified by their holding number.
- Producers and dealers must maintain a record of all animals moving onto or off their premises.
- All markets and assembly points must be under the control of the veterinary authorities.
- The European computer network (ANIMO) will provide advance notification of any imports.

The EU and EEA rules for the importation of livestock present some challenges to the animal health status of Norway. These challenges include:

- Correctly certified animals may not be excluded from the country, nor may they be required to be quarantined.
- In relation to any disease, import restrictions may only be imposed if national disease freedom has been proven.

At present, the EU does not recognize that Norway is free of Transmissible Gastroenteritis (TGE), Swine Influenza (SI), Porcine Respiratory and Reproductive Syndrome (PRRS), Porcine Epidemic Diarrhea (PED) and other reportable diseases even though the diseases have never been reported there. As a result, freedom from these diseases can not be made a requirement for importation. In effect, the

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EU trade regulations provide little protection against the importation of these diseases. This deficiency was recognized by NORSVIN, a major importer of swine in Norway. In response, they developed a protocol for the importation of live pigs. It asks both the Norwegian importer and the exporter (in the country from which the pigs will travel) to provide additional guarantees that the pigs have undergone specific tests and periods of isolation (Homo, 1997, personal communication). However, compliance with the code is voluntary and has no legislative basis. The additional guarantees include:

- Serological testing for Swine Influenza (H1N2 & H3N2), TGE, Porcine Respiratory Corona Virus (PRCV), *Actinobacillus pleuropneumoniae* (Serotypes 1,5,11& 12) and PRRS at the farm of origin. All animals tested must be seronegative for these diseases in order for any animal to be selected for importation.
- Isolation for 30 days and serological testing after 21 days in the country of origin. In addition to those diseases tested for at the farm of origin all animals are serologically tested for Leptospirosis (6 serotypes), Porcine Epidemic Diarrhea (PED), Avian and bovine tuberculosis and Salmonella, as well as cultured for Salmonella spp. and treated for internal and external parasites within 7 days of export.
- Additional isolation and testing for the above diseases after importation to Norway and before release to the importer has been proposed.

GOALS

The overall goal of this project was to develop general simulation models that could be used for the analysis of the risk of the introduction of any disease exotic to Norway via the importation of live swine.

These models were designed specifically to compare the risk of introduction of a disease under the safeguards provided by the EU and EEA rules and regulations and those provided by the protocol proposed by NORSVIN. In addition, the models provided information about the risk in regard to:

- The effect of the prevalence of the disease in the exporting country.
- The effect of the number of animals and herds screened prior to selection of consignments and placement in isolation in the exporting country. (Selective screening in the herd of origin or isolation in the exporting country will not be officially allowed or required under the future EU and EEA rules. *However, it can be demanded by the importer*).
- The effect of the number of animals per consignment.
- The need for testing in isolation in Norway.

THE MODELS

Two stochastic simulation models were developed to compare the EU and EEA import safeguards with those of the NORSVIN protocol (Vose, 1996; Winston;1996). The following criteria for the two protocols were compared:

- The probability that 1 or more infected pigs would enter Norway.
- The distribution of the number of infected pigs that would enter Norway.
- The probability that 1or more infected pigs would be missed by testing in isolation in Norway.
- The distribution of the number of infected pigs that would be missed by testing in isolation in Norway.
- The probability that 1or more infected pigs would be released to the importer in Norway
- The distribution of the number of infected pigs that would be released to the importer in Norway.

The models were developed on Excel spreadsheets (Microsoft Corporation, Seattle, Washington) with the @Risk, risk analysis add-in (Palisade Corporation, Newfield, NY) which allows Excel to generate Monte Carlo sampling from probability distributions. The sampling method was latin hypercube and the standard recalculation was Monte Carlo. The true value for some of the parameters in the models were unknown or uncertain. These included the herd prevalence, with-in herd prevalence, herd size, number of animals tested and test sensitivity. These variables were represented in the models by a range of values with known probabilities in the form of probability distribution functions (PDF). Parameters for PDF's can be based on values obtained from the literature, experimental data and expert opinion (Vose, 1996). Since these were models for a generic disease the PDF's were based on hypothetical data. The input variables and their PDF's are shown in Table 1.

Table 1. Input variables and probability distribution functions

	PDF	
Input variables	EU/EEA model	NORSVIN model
Herd prevalence (hp)	beta(126,376)	beta (126,376)
Probability a herd was infected (hi)	binomial(1,hp)	binomial(1,hp)
Within herd prevalence (wp)	betapert(0.05, 0.65,0.85)	betapert(0.05, 0.65,0.85)
Test sensitivity (se)	betapert(0.85,0.9,0.95)	betapert(0.85,0.9,0.95)
Number screened (ns)	0	30
Number in consignment (nc)	betapert(10,12,25)	betapert(10,12,25)
Number of infected (ni)	binomial(nc,wp)	binomial(ns,wp)
Number detected (nd)	binomial(ni,se)	binomial(ni,se)

The EU/EEA model

The first model quantified the risk of introduction of an exotic disease by a single consignment from an EU country. The following assumptions were used to develop the model.

- All of the official EU/EEA and Norwegian rules and regulations regarding importation of live swine from the EU were followed.
- The exporting country had not been declared free of the disease and the disease was, in fact, present. The herd prevalence estimate was considered unreliable but was reported to be about 25% based on testing 500 breeding herds and finding 125 positive for the disease.
- The source herd was randomly selected from the population of breeding herds.
- The consignment size ranged from 10 to 25 pigs. The most likely number was 12.
- The within herd prevalence in infected herds ranged between 5% and 85%. The most likely prevalence was 65%.
- The consignment would be selected from a herd and then transported to an isolation facility in Norway where it would be tested for the disease for the first time.
- The mean sensitivity of the diagnostic test was 90%, range 85% to 95%, and the specificity was 100%.
- If an infected pig was detected the entire consignment would be rejected or destroyed.
- If an infected pig was released from isolation in Norway the probability of transmission to other pigs in Norway was 100%.

Results of the EU/EEA model: The results of the EU/EEA simulation with 30,000 iterations are shown in Table 2. With random selection of a potential source herd there was about a 25% probability that it would be an infected herd. Based on the estimated within herd prevalence, a consignment would contain from 0 to 20 infected pigs. All of the pigs in a consignment would be tested for the first time in the isolation facility in Norway where most of the time at least 1 of the infected pigs would be detected and the consignment would be rejected or destroyed. However, a consignment containing 1 or 2 infected pigs would be missed by the test in the isolation facility16 times out of 10,000 (0.0167%). These consignments, along with the infected pigs, would be released from the isolation facility and potentially infect the importing herd if additional precautions were not taken.

Table 2. Results of the EU/EEA model

Name	Minimum	Mean	Maximum
Herd Prevalence	18.2%	25.1%	33.0%
Number of pigs in consignment	10	13.83	24
Percentage infected consignments sent to isolation	0%	25.05%	100%
Number of infected pigs placed in isolation	0	8.6	20
Percentage of infected consignments. not detected in isolation	0%	0.163%	100%
Number of infected pigs released from isolation	0	0.00167	2

The NORSVIN model

A second model was developed to quantify the risk of introduction the same disease with a single consignment from an EU country where the NORSVIN import protocol was followed. In addition to the assumptions in the EU/EEA model, the following assumptions were used to develop the NORSVIN model:

- Serological screening of 30 pigs would be done in the prospective source herd prior to the selection of specific animals for export. If all tested pigs were seronegative a consignment would be selected from this group.
- Consignments, ranging in size from 10 to 25 pigs, were selected from the previously tested, sero-negative group.
- The consignments would be sent to an isolation facility within the exporting country for a minimum of 30 days. After a minimum of 21 days in isolation the animals would be re-tested for the disease.
- If all pigs were sero-negative on the second test they would be released from isolation in the exporting country and transported to an isolation facility in Norway where they would be held for a minimum of 30 days.
- After a minimum of 21 days in the isolation facility in Norway the pigs would be tested for a third time with the same serological test.
- If all pigs were sero-negative on the third test they would be released to the importer in Norway.

- If 1 or more pigs were seropositive on any of the tests the entire consignment would be rejected.

Results of the Norsvin model: The results of this simulation of 30,000 iterations of the NORSVIN model are shown in Table 3. The mean probability of screening an infected herd was the same as the probability of selecting an infected consignment from an unscreened herd, i.e. about 25%. The mean probability that the group of pigs that was screened contained 1 or more infected individuals that were not detected by the test was 0.01%. In other words screening 30 pigs prior to selection of the consignment would reduce the risk of importing an infected pig from about 25% in unscreened consignments to 0.01% in screened consignments. The second test, done in isolation in the exporting country, reduced the risk to 0.0% for 30,000 iterations. The third test done in the isolation facility in Norway would be expected to detect any remaining infected consignments resulting in a final risk of introducing the disease, of virtually 0. This means that under the conditions described for this model one not expect to import a pig infected with this specific disease if the NORSVIN importation protocol were followed.

Table 3. Results of the NORSVIN model.

Name	Minimum	Mean	Maximum
Herd Prevalence	17.9%	25.1%	33.4%
Prevalence in an infected herd	0.7%	58.3%	84.9%
Number screened in herd of origin	30	30	30
Number of infected pigs in screening sample	0	4.4	29
Percentage of herds seropositive in screening test	0%	25.1%	100%
Percentage of herds seronegative in screening test	0%	74.9%	100%
Percentage of infected screen samples missed	0%	0.00067%	100%
Number in consignment	10	13.18	23
Number of infected in consignment	0	0.00033	1
Infected consignments missed in isolation #1	0%	0%	0%
Number of infected allowed into Norway	0	0	0
Infected consignments missed in isolation # 2	0%	0.0%	0%
Number infected released from isolation	0	0	0
Percentage of consignments released to importer	0%	99.99%	0%

Table 4. shows a comparison of the risks associated with the two import protocols. Under the EU/EEA protocol, which requires no testing in the exporting country, any consignment, infected or not, if selected, would be transported to the isolation facility in Norway. Under the EU/EEA rules about 25% of the consignments would be rejected because 1 or more infected pigs were detected when tested in isolation in Norway. However, 0.163% of the consignments would be infected and missed by the single test and would be released from the isolation facility. With the NORSVIN protocol most of the infected herds would be identified prior to selection of the consignment leaving just 0.0067% infected

consignments containing a maximum of 1 infected pig each to potentially introduce the disease to an isolation facility in Norway. Testing in isolation would further reduce the risk of actually releasing an infected pig into the Norwegian swine population.

The percentage of consignments that would actually be released in Norway under the NORSVIN protocol would be approximately 99.99% whereas, the comparable percentage under the EU/EEA rules would be approximately 75%.

Table 4. Comparison of the NORSVIN and EU models.

Variable	NORSVIN Mean	EU Mean
Herd Prevalence	25.1%	25.1%
Number tested in herd	30	0
Prevalence in untested infected herd	58.3	58.3
Number in consignment	15.8	15.8
Percentage of herds with consignments sent to isolation	74. 9%	100%
Percentage of infected consignments sent to isolation	0.0067%	25.05%
Number infected pigs in consignment	0.0003	8.06
Infected consignments missed in isolation	0%[2]	0.163%
Number of infected allowed into Norway	0	8.06
Percentage infected consignments released	0%	0.163%
Number infected released from isolation	0	0.0017
Percentage of consignments released to importer	99.99%	75.14%

DISCUSSION

Monte Carlo simulation modeling allows one to account for the uncertainty and variability inherently present in natural systems to create models that produce results more likely to be similar to the situation in nature than do deterministic models with point estimates for values of variables. Variables that had associated uncertainty or variability were represented in the present models by probability distribution functions (PDF). For each iteration that the simulation was calculated a different value for each variable was generated based on the probability that the value would occur (Vose, 1996). The final results, based on 30,000 iterations for each model, were probability distribution functions of the total risk from which the mean, minimum, maximum and percentile rank of the risk could be determined. Had only point estimates had been used in the models the PDF's of the results would have been straight lines because there would have been no variability or uncertainty to the results. Because many of the

[2] 0.0% in 30,000 and 60,000 iterations.

PDF's in risk models are skewed, the PDF of the total risk is also skewed such that the point estimate of the total risk may lie in one of the extreme tails of the PDF and thus represent an risk of extremely high or low probability of occurrence (Thompson, et.al., 1992).

Because these are generalized models, the risk for any specific disease might vary considerably from the results reported here. However, once developed the models can easily be modified such that the inputs reflect the specific disease characteristics. Given the inputs used in these models, the overall risk of actually introducing a diseased animal under either protocol is quite low. However, it is not the purpose of risk analysis to determine whether either risk is acceptable or not. That decision must be made by the decision or policy makers.

One of the advantages of modeling as a method of risk analysis is the ability to identify areas where risks can be reduced or eliminated and, as such, it can be a very valuable tool for risk managers. These models demonstrate several points where risk management procedures could greatly reduce, if not completely eliminate, the risk of introduction of a disease through importation of live swine.

The greatest reduction in the risk could be obtained through the screening of potential source herds prior to selection of consignments before they enter Norway. This procedure would also eliminate the costs of transportation and the isolation facility. Under the assumptions for herd prevalence, within herd prevalence, consignment size and test characteristics in the 2 models it would be about 3800 times more likely that a diseased animal would enter Norway under the EU/EEA rules than with the NORSVIN protocol. The major factors that make the NORSVIN protocol more effective are the larger number of pigs tested and that the pigs are tested more than one time. Another effective risk reduction measure would be the isolation and testing of the animals in the exporting country before they were allowed to enter Norway. In this case transportation and isolation costs would be incurred but few if any infected pigs would ever reach Norway. Under the EU/EEA regulations about 25% of the consignments selected would contain at least 1 infected pig that would be not be detected until it was in the isolation facility in the exporting country under the present rules or in the isolation facility in Norway under future rules. Although not specifically addressed as an outcome in the models, it is logical that it would be much easier to prevent introduction of a disease by infected animals if infected animals were never allowed to enter Norway in the first place, rather than to allow them to enter and then discover that they are infected.

It could be argued that the second and third tests in the NORSVIN protocol do not reduce the risk enough to justify the extra time and expense required to complete them. However, the goal of the importation protocol is total exclusion of infected pigs and a single test will not insure that. The second test done in isolation in the exporting country appeared to reduce the risk remaining after the first test to 0%. However, if the disease were introduced to the herd after the screening test the prevalence in the consignment may be low and thus more likely to be missed by the second test. The third test would further reduce the risk remaining after the second test. At present, the small numbers of pigs per consignment and the small number of consignments imported each year are such that the costs of the extra tests and isolations would be insignificant compared to the economic consequences that would be expected if an exotic disease were introduced to the NORSVIN facilities. The risk and consequences of this possibility will be the topic of a future study.

Under the current EU/EEA rules, Norway is allowed to require isolation and testing for PRRS, SI , PRCV, and PED in the exporting country, prior to importation. However, this will not be required in the future. If one were to assume these were the disease modeled in this study this change in requirements would increase the risk of introducing each of the diseases to Norway by up to 3800 times depending on the probability that the source herd was infected.

In summary, the results of these simulations show that the NORSVIN importation protocol is clearly superior to the existing EU/EEA regulations in terms of the reduced risk of introduction of a disease exotic to Norway via the importation of live swine. It has the greatest advantage in that infected pigs would rarely ever enter Norway in the first place and even more rarely be missed by the final serological test conducted in Norway. The current EU/EEA rules which allow for isolation and testing for some diseases in the exporting country provide less protection against infected pigs entering Norway and the changes in the rules that will take effect in the future will virtually eliminate that protection. Although the total risk under either protocol might seem small, compared to the consequences (Meredith, 1995) that would be expected if an exotic disease such as PRRS were introduced to Norway, the extra inconvenience, time and expense required by the NORSVIN protocol appear to be well justified.

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RISK ASSESSMENT OF IMPORTING CLASSICAL SWINE FEVER TO DENMARK

RUGBJERG, H., CHRIÉL, M., THOMSEN, L.K. & WILLEBERG, P.[1]

Classical Swine Fever (CSF) is placed on list A on OIE's list of notifiable diseases and is a highly contagious disease mainly transmitted through live pigs and their products. The infection has not occurred in Denmark since 1933. The introduction in 1993 of the free movement of goods including live animals between the European countries allowed the pig producers to trade without national legal requirements of the use of quarantine facilities and veterinary inspection when live pigs or pig products are imported. Epidemics of CSF have frequently occurred in Germany and in 1997 a large epidemic emerged in the Netherlands - countries close to the Danish border and with relatively large trade turnover. Introduction of the infection into Denmark would result in severe losses in the gross national product due to lost export markets as well as individual losses for the farmers having their herds stamped out.

It was therefore decided to evaluate the influence of the different trading patterns and other contacts on the risk of introducing CSF to Denmark.

MATERIAL AND METHODS

Probabilistic risk analysis models as described by Vose (1997) and Van der Logt et al. (1997) were used to quantify the risks associated with the various trade activities with Germany. Monte Carlo simulation was used to model uncertainty and variability (@Risk, Palisade Corp.).

Three potential risk factors for introducing CFS from Germany into the Danish pig population have been evaluated:

a) Returning empty animal transport vehicles,
b) Import of live pigs,
c) Import of slaughter offal to a Danish incinerating plant

Reports from Germany revealed that 28% the outbreaks were associated with the movement of live pigs, 3% of the outbreaks occurred after swill feeding and 3% were reported to be associated with indirect contact between herds due to live animal transports. One third of the cases were never cleared up.

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Prevalence of sub-clinically CSF infected herds in Germany

The number of herd days at risk was calculated using data from Eurostat concerning the number of herds in Germany. Based on extrapolation of the decline in the number of herds between 1993 and 1995 the number of herds in 1997 was estimated. The annual number of outbreaks is given by the Animal Disease Notification System (ADNS) in Brussels, and it was shown to occur reasonably steadily using a four-year moving average (Fig.1).

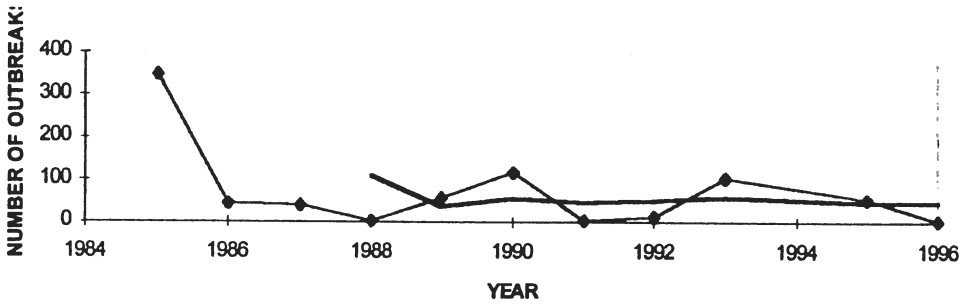

Fig. 1. Annual number of CSF outbreaks in Germany. A four-year moving average is indicated.

The number of outbreaks was modelled using a log-normal distribution. The time between infection of a herd until clinical symptoms are observed was estimated using a mean and standard deviation for this period calculated by Dr. M. Meredith from the recent Dutch CSF epidemic.

Empty live animal transport vehicles returning from Germany

A survey was established in order to determine the extent of live animal transport vehicles crossing the Danish/German border. During a one-year period, beginning at the autumn 1996, 455 hours of inspection were performed in collaboration with the Danish police. The days were selected at random. All animal transport vehicles were inspected and the drivers questioned regarding the type of animals transported and their destination.

A total of 123 vehicles were carrying live animals and 223 empty transports crossed the border. The vehicles crossed the border on average 7.2 times per month, all passing Germany but approximately 20% of the lorries had visited eastern European countries and another 20% had been in the Netherlands. Three out of four lorries were transporting live pigs for export and 60% of these were exported to Germany and 20% to the Netherlands. Out of the returning vehicles 30% were destined to a reloading place and 11% directly to a Danish farm.

The Danish legislation states that returning empty vehicles should be properly cleaned and disinfected before crossing the border and, if they return from abroad, they are not allowed to come in direct contact with a Danish pig producing unit.

The inspection revealed that 70% of the trucks were acceptably cleaned and disinfected, but especially lorries returning from pig farms were unacceptable. The washing place for lorries, situated close to the Danish border, is frequently used especially by the Dutch drivers, but large differences were found between the companies involved in live animal transports.

A scenario tree for the risk related to empty transport vehicles returning from Germany is shown in Fig. 2. The estimated number of infected transports was assumed to be proportional to the prevalence in Germany. The probability of disinfection and of going directly to a farm or to a reloading place was estimated from the questionnaire, and the distribution of the uncertainty around these parameters was modelled using a beta distribution. The risk of cross-contamination occurring at the washing place and at the reloading place, the risk of infected transports despite disinfecting and the risk that the vehicles would actually infect the visited herd were sampled randomly from uniform distributions due to lack of knowledge.

Import of live pigs

In Denmark are 56 importers of live pigs registered at the Danish Veterinary Services in 1997. Two of the importers receive porkers from UK for production of piglets. The other imports were all left in the Danish Pig Federations quarantine station in the vicinity of Copenhagen. The total number of annual imports was four in 1996 and 7 in 1997. The pigs are left for 4 weeks in the quarantine and, irrespective of the number of imported animals, 48 are serologically evaluated for the presence of a wide number of contagious diseases including CSF, before the consignment is released.

A scenario tree for the risk related to import of live pigs from Germany is shown in Fig. 3. The proportion of infected animals in a consignment is given by the with-in herd prevalence. Based on data from German outbreaks the average with-in herd prevalence is estimated to 21%. It is assumed that the import originates from only one herd. The sensitivity of the serologic test is set to 0.8.

Importation of slaughter offal to Danish incinerating plant:

The risk of importing slaughter offal from Germany consist of both the risk associated with the transportation of the offal, and the feeding of pigs with meat and bone meal originating from the imported offal.

Cutting factory offal from German slaughterhouses consists primarily of fat and bones. There is no exact knowledge regarding the slaughterhouse from which the offal is originating. In average is around 100-150 tons of offal imported per week to Danish incinerating plants corresponding to offal from approximately 4000 pigs.

Only one lorry is involved in transportation of the offal. Based on data from the Danish Road service the rate of accidents per km and the number of km driven by the loaded transport vehicle was calculated. The risk of infected transports was given by the estimated prevalence, and the rate of accidents for infected transports could then be estimated. A Poisson distribution gave the distribution of the number of events. Considering the number of road accidents on the route no risk could be estimated from transporting offal.

The manufacturing procedures of the offal in the Danish incinerating plant secures that there is practically no risk is associated with recontamination of the final product of meat and bone meal. One of the critical control points in the production is stated to be the temperature of the meat and bone meal when the product leaves the roast. The sterilisation process lasts 2 hours and the goods reaches a temperature above 110C° - sufficient to inactivate CSF-virus (Radostits et al., 1994).

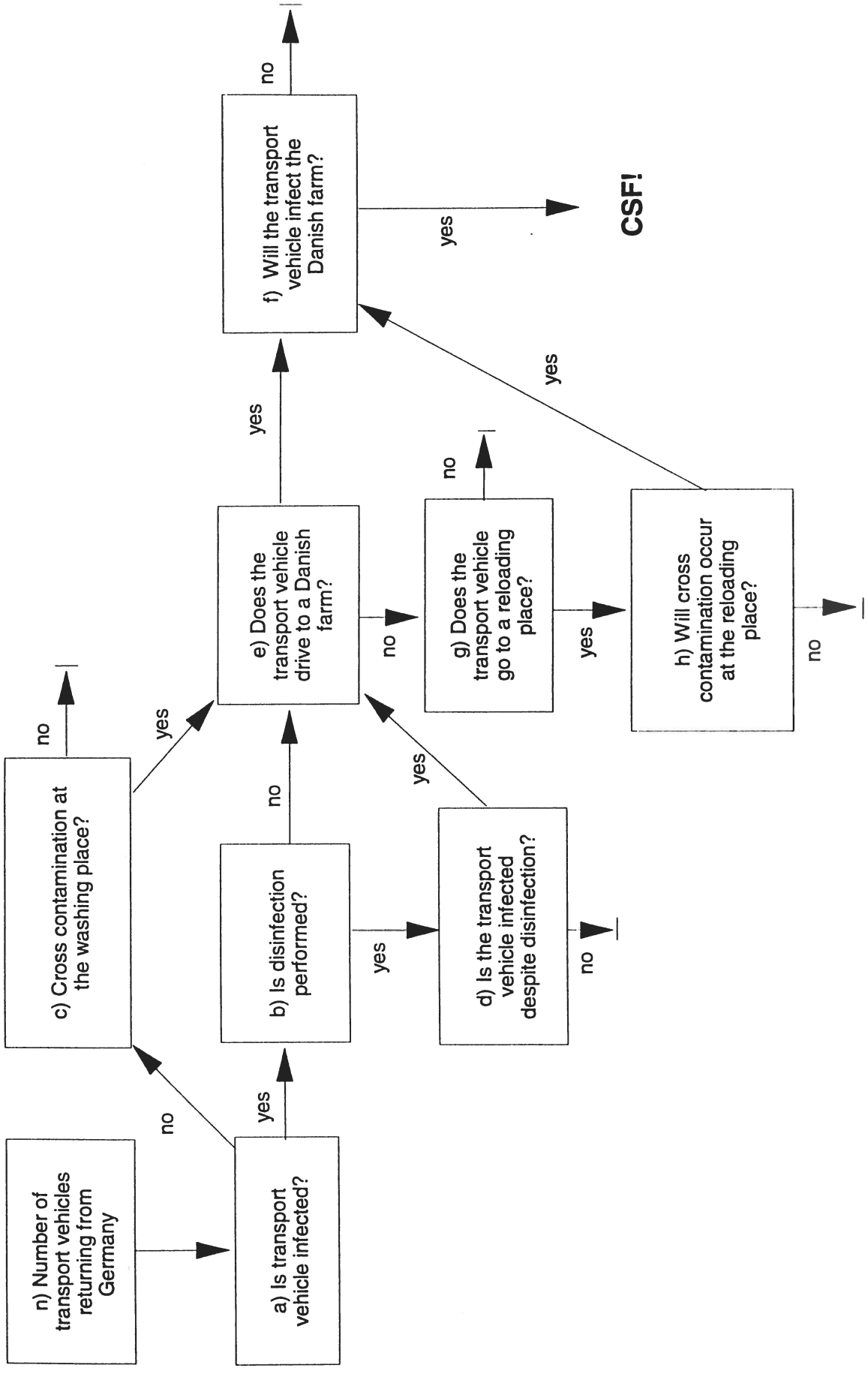

Figure 2. Scenario tree for introducing CSF with empty transport vehicles returning from Germany

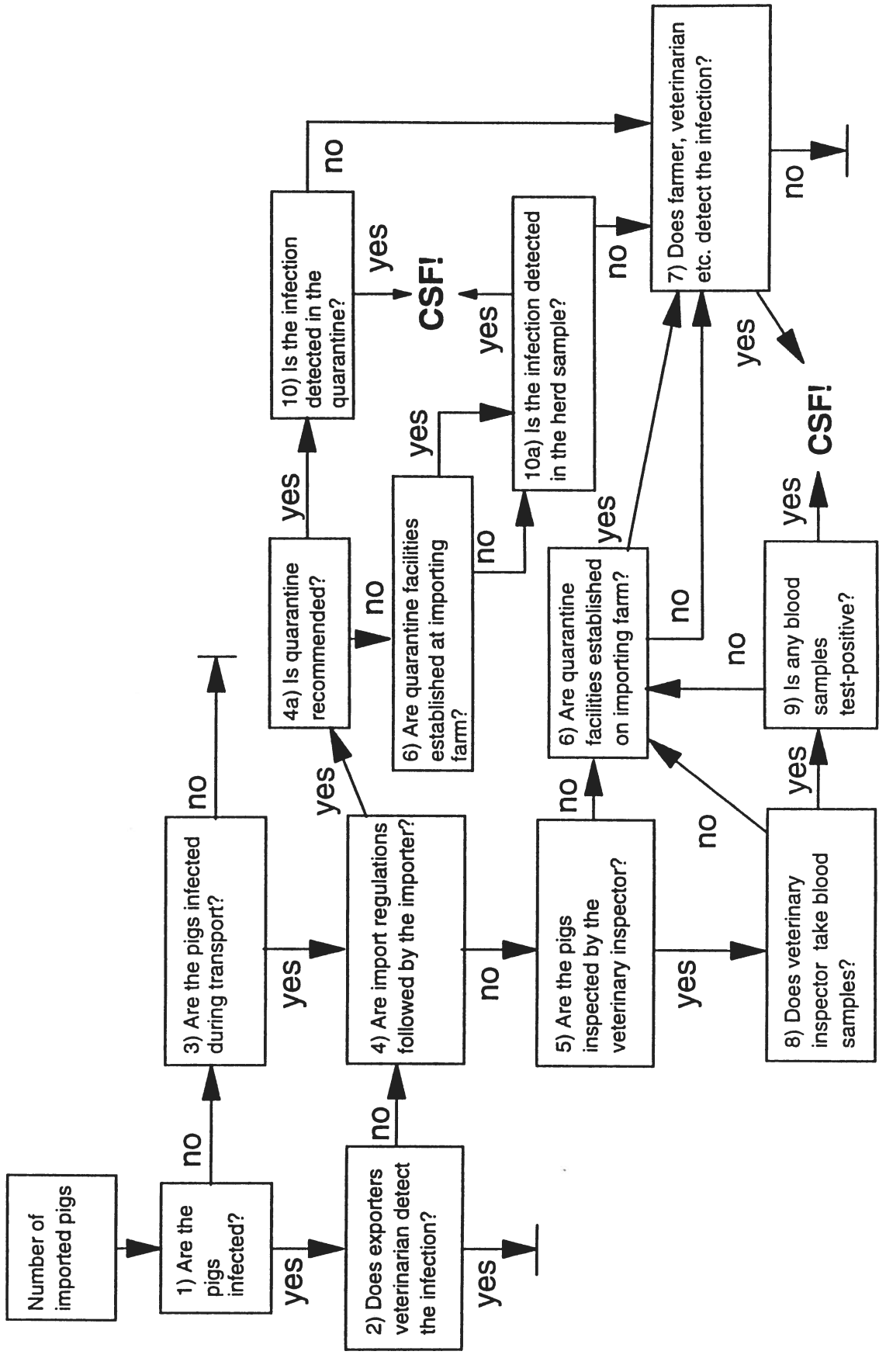

Figure 3. Scenario tree for introducing CSF when live pigs are imported from Germany

The probability of infected pig meat in offal was modelled as $1-(1-p)^n$ and the offal was assumed to originate from pigs all from different farms allowing the model to evaluate the worst case scenario.

Probability of infective doses surviving heat treatment was arbitrarily set to 1%, 0,1% and 0,01%, respectively.

RESULTS

The prevalence was estimated as the number of subclinically infected herd days divided by the total number of herd days at risk with an average value of $3*10^{-5}$ (95%-CI $1*10^{-6}$-$1*10^{-4}$).

In the scenario of empty live animal transport vehicles going directly to farms as well as to reloading places, the mean number of annual herd outbreaks was estimated to $5*10^{-3}$. In a scenario where transports were only allowed to pick up animals at a reloading place, the mean annual number of herd outbreaks was reduced with a factor ten.

The probability that infected pigs are imported has been calculated to $3*10^{-5}$. Around 4% of infected imports will not be found at post-import testing. The simulation model for these very small probabilities of infection results in a number of infected animals close to zero. Import of subclinically infected pigs depends additionally on the with-in herd prevalence in the source herd.

The risk of releasing infected meat-and-bone meal given that 0.01% of virus survives the heat treatment have been estimated to $7.2*10^{-5}$ (95%CI: $1.5*10^{-5}$ - $1.0*10^{-4}$). The risk was directly proportional to the effectivity of the heat treatment.

DISCUSSION

The use of expert knowledge revealed the importance of verifying the knowledge before using it in the models. The experts, normally involved in experimental infections, assumed a with-in herd prevalence of 99%, whereas a with-in herd prevalence of 21% was found in natural occurring outbreaks in Germany.

The results revealed that returning empty animal transport vehicles are associated with a relatively high risk, due to the large number of herd contacts. The contact rate across the borders is strongly affected by the monetary value of both the piglets and the slaughter pigs on the international markets. The pig producers are maximising herd profit and take very little account of the risk involved in the trade abroad. As a consequence of the recent Dutch CSF epidemic, a shortage of piglets occurred in Germany. Therefore, growing trade with Danish piglets gave rise to an increasing number of contacts. Information directly to the farmers as well as to the drivers has been given in order to emphasise the control measures needed to reduce the risk of between-herd contacts and to perform proper cleaning and disinfection of the transport vehicles.

Due to the present large production in Denmark no imports of piglets or slaughter pigs from Germany are taking place. The only imports made are of new breeding stock and the control measures at the quarantine station reduce the risk involved to a very low level. However, the results

can be used to make future importers aware of the relatively large risk involved in importation.

The risk associated with the import of slaughter offal was shown to be negligible, due to the strict control measures set up at the incinerating plant to control the temperature during the heat treatment of the meat and bone meal.

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- ☐ 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