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The Gareth Davies Lecture

SURVEILLANCE – PAST, PRESENT AND FUTURE

J.M. SCUDAMORE*

Gareth Davies had and still has a major input into development of veterinary epidemiology and surveillance in the UK and Europe. I have worked with Gareth for many years and remember the 1970s when I started my career as a veterinary epidemiologist working for the Kenya Government. Gareth at that time was working on Contagious Bovine Pleuropneumonia, a topic which has been one of his and my interests ever since. In view of our close association over the years it is a privilege for me to be invited to present this lecture. I would at the outset like to thank colleagues at the Veterinary Laboratories Agency (VLA) and in Ministry of Agriculture Fisheries and Food (MAFF) for their advice and assistance in dealing with surveillance not only in the preparation of this paper but also for all the work involved in the past three years.

Gareth has produced many papers on a variety of topics including epidemiology, economics, surveillance and the relationships between them. Since they began in 1998 the Gareth Davies lectures have reviewed much of his work. Martin (1998) referred to the paper by Davies (1985) entitled "Arts, science and mathematics: New approaches to animal health problems in the agricultural industry." The paper concentrated on principles and concepts and as Martin remarked the word epidemiology was only mentioned once. Davies included in the paper sections on, measuring disease not infections, building herd health programmes, environmental controls, the use of economic analysis, setting targets for disease production and using operational research. Much of which is very relevant today

Dijkhuizen (1999) dealt with the 1997/98 outbreak of classical swine fever in the Netherlands and the lessons to be learned from the economic perspective. Again the author was influenced by his contact with Gareth Davies particularly in the areas of animal health and the related problems. The close relationships between epidemiology and economic modelling with the formulation of practical disease control policies was explored. This work reflected Gareth's support for the use of the qualitative and integrated epidemiology and economic modelling approach to help improve policy making and contagious disease control. Indeed in his fourth career working in the EU Commission he has been instrumental in developing epidemological investigations, contingency planning and disease control programmes on an EU wide basis.

In this lecture I will consider another important contribution by Gareth Davies to epidemiology which was not included in the previous two lectures. He has played an important role in the development of veterinary surveillance in Great Britain and

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Europe since the mid 70s. However as Chief Veterinary Officer, I would like to consider surveillance from a slightly different perspective. It is perhaps not the academic and intellectual aspects of surveillance which are important but the more mundane and practical questions which need to be asked. Who needs surveillance, more importantly, who pays for the surveillance and ultimately what do we get for our investment. Is there value for money in surveillance and why do we need it, a question that is constantly asked these days by those who provide the financial and other resources.

I propose to limit my paper to the G.B. situation.

THE PAST

Surveillance in one form or another has been around for many years. The Committee of Inquiry on Rinderpest set up in the 1700s made a number far sighted recommendations which have been the basis for much of our animal health legislation. One of the main recommendations was for compulsory notification of disease which is perhaps one of the most effective methods for surveillance provided the disease is recognisable and the population are prepared to report their suspicions of disease. Over the years a range of diseases in many species have become notifiable. It was not until the 1900s that surveillance for non notifiable diseases became increasingly important. The first Veterinary Investigation Centre (VIC) was opened in 1922 and the numbers of diagnostic laboratories increased over the next 60 years providing countrywide coverage in England and Wales. A similar process occurred in Scotland.

Collecting and collating the information available from this network of government laboratories was a challenge until the development of the Veterinary Investigation Diagnostic Analysis system (VIDA) during the early 1970s. This unfortunately was not a success for a number of reasons in particular its complexity and the failure to provide feedback. Lessons were learnt and the new VIDA II introduced in 1975 was effective and remains the basis of the information collection system for non notifiable disease throughout GB (Hall 1980). Many modifications have been made over the years with perhaps the most significant during the past two years.

The Veterinary Investigation Centres in England and Wales have amalgamated with the Central Veterinary Laboratory to form an executive agency of MAFF, the Veterinary Laboratories Agency (VLA). The VLA carries out a large proportion of MAFFs surveillance activity

Information on all specimens submitted to the regional laboratories of the VLA is now recorded on a new central Sample Management System database - "FarmFile " which replaced an earlier standalone computerised data recording system also known as "Farmfile" The new system delivers a millennium-compliant single database of submissions to VLA laboratories that is up-dated in real time. Additional fields have been added to the new system for surveillance information on samples submitted by veterinary surgeons or received as part of planned surveys. This information is then used for updating the VIDA II database.

Until the Farmfile redevelopment in 1998, VIDA II was the only centralised database of VLA regional laboratory data. The new networked "FarmFile " system provides an accurate record of the submissions received by the regional laboratories and the recent changes allow for much improved epidemiological analysis and reporting. Of particular importance is the ability to record presenting signs which in turn will assist in identifying new or changing diseases

In 1976 a MAFF working party chaired by Gareth Davies completed a review of animal disease surveillance in Great Britain. The report concluded that no single surveillance system could, by itself, provide the wide range and variety of information that was required. The most appropriate strategy was to ensure that all existing information was drawn together in order to provide for five basic needs. These have been documented by Davies and were to provide:

- a comprehensive body of data which is readily available;
- the ability to undertake in depth investigations;
- facilities for tracing the course of outbreaks;
- an early warning system for new events;
- systems that record details on certain farms over long periods;

This report which was presented to the Chief Veterinary Officer in 1976 was the basis for disease surveillance in GB for the next 20 or so years. For the succeeding 10 years there was little activity although in the early 1990s there was increasing interest in surveillance. Davies (1993) discussed the need for Europe wide surveillance and the importance of ensuring systems were in place throughout the Member States of the EEC. On the international level the OIE has taken an interest in surveillance and has published a number of review articles. At the same time there has been an increasing interest in risk analysis particularly in the context of the WTO with the need to ensure that non tarriff trade barriers do not inhibit international trade.

THE PRESENT

The rationale for Government expenditure on non notifiable disease surveillance and the appropriateness of the levels of expenditure has been under close scrutiny in the GB for the past three years. Various reports have looked at the position of disease surveillance in relation to the overall objectives of the Government and more importantly the nature of future Government involvement. In 1997 an internal MAFF group concluded that surveillance of non notifiable animal disease was a legitimate area for Government involvement and that with rising incidence of food borne zoonoses in man increased involvement might be needed.

However, the last comprehensive review of surveillance occurred in 1976 since when a number of piecemeal reviews had looked at various aspects of disease surveillance. None had taken an overall view and as a result in 1999 MAFF concluded that whilst the Department spent significant sums of money on surveillance the precise nature and justification of these expenditures and activities was not clearly documented.

As a consequence an internal MAFF review embracing all aspects of notifiable, non notifiable and reportable diseases commenced in 1999 to consider:-

- the justification for veterinary surveillance;
- whether this surveillance is comprehensive in view of the regional spread of the laboratories;
- whether there are other sources that can be drawn on to carry out these activities;
- what diseases are monitored;
- how much was being spent.
- the outputs from MAFF surveillance and how they are used

The review, conducted by Dr N Meah and Mr G Lewis concluded that the primary reason for Government to carry out surveillance was to meet basic information needs in order to assess and manage risks effectively. That is to minimise as far as practical the probability of adverse effects on public health, trade in animals and animal products and animal health and welfare. The report included a range of topics relevant to this presentation but I will concentrate on the definitions, the objectives and the purpose of surveillance.

Definition of surveillance

There are many definitions of surveillance but until recently none clearly explained the importance or purpose of surveillance. In the 1975 MAFF review the WHO definition "*Surveillance implies the collation and interpretation of data (collected during monitoring programmes) with a view to the detection of changes in the*

health status of populations was used initially. However even then it was considered that changes in the definition were needed. Surveillance was broadly defined to include" the use of any information necessary for the control of disease."

Schwabe et al (1977) defined surveillance and listed its components one of which included prompt dissemination of disease intelligence information to all those that need to know. Whilst these definitions describe the process they still did not explain the reasons for undertaking surveillance.

Twenty years later the situation had not changed and during the 1997 UK expenditure review it was apparent that a comprehensive definition explaining and justifying the basic reasons for undertaking surveillance was needed. This was resolved during the 1999 Review of Veterinary Surveillance in England and Wales when surveillance was defined as " *the ongoing systematic collection, collation, analysis and interpretation of accurate information about a defined animal population with respect to disease and or infection, closely integrated with timely dissemination of that information to those responsible for control and prevention measures.* "

This may appear longwinded but does describes clearly the reasons for national surveillance and is relevant to veterinary authorities with responsibility in this field. Whilst it might not appeal to the purest it does reflect to those who are paying for surveillance the purpose and activities undertaken, and also the reasons why it is important to know the disease status of animal population.

Objectives of Surveillance

In the 1975 MAFF review 5 reasons for collecting surveillance information were identified but none provided a comprehensive justification as to why the information was being collected and its cost benefit. The definition of surveillance is important as it clarifies the activities involved, however there is also a need to have clear objectives which identify the information needed and clarify how it could improve policy formulation and subsequent action. Surveillance should then be appropriate to the agreed objectives. The overall objectives of surveillance are clearly defined by Meah and Lewis in the recent MAFF review and are to:-

(i) Detect rapidly outbreaks of disease /infections in animals
(ii) Provide an early warning system for new animal diseases/infections.
(iii) Allow the early identification of known diseases/infections currently not found in the country.
(iv) Estimate the level of occurrence and identify emerging trends among disease/infections currently present in the country both temporally and geographically.
(v) Confirm the absence of specific disease/infection from the country.

The recent problems associated with E coli O157 in particular the increasing incidence of disease in the human population, demonstrates the importance of surveillance in animal populations, which may act as a reservoir of infection for man without disease in animals. Surveillance therefore not only has to encompass disease but also infections, particularly zoonoses.

Information management

Information provided by surveillance systems in meeting the 5 objectives listed above will enable decision makers to take a wide range of actions including:- the identification of potential hazards, assessing whether those hazards pose a risk and if so the risk management measures which could be introduced. Furthermore surveillance information will allow the effectiveness of risk reduction measures to be evaluated. Such information will:-

(i) enable prompt recognition and appropriate response to disease outbreaks;
(ii) enable the identification of new potential hazards;
(iii) enable the early recognition of important trends to inform risk management policies;
(iv) enable the effectiveness of control measures for diseases or infections to be assessed;
(v) enable the relative importance of different diseases and infections to be assessed in relation to public health, animal health and welfare;
(vi) support the planning, development and implementation of policies for protecting public health, animal health and welfare;
(vii) enable the need for research or further surveillance to be identified and identified
(viii) generate hypotheses as to the cause of new diseases or of changing patterns of existing diseases;
(ix) allow communication of quality information on prevalence, incidence and/or trends of diseases/infections to those with an interest at local, national or international level;
(x) facilitate international trade in live animals and animal products by meeting international obligations including the prompt reporting of disease and confirmation of disease freedom to international organisations and trading partners;
(xi) allow the efficient allocation of resources for the control of, and research and surveillance into, animal diseases and infections.

Delivery of Surveillance information

Having clearly identified the objectives of surveillance and the use to be made of the information gained from the system, the next stage is to assess whether existing systems provide the information which is needed. The information can be gained

by either *passive* or *active* surveillance. It is this terminology which needs further discussion.

A definition suggested by VLA for passive surveillance is: "*...the continuous monitoring of the existing disease 'status' of the populations surveyed, using routinely collected data to produce outputs which can feed into policy decisions...*" The term "passive" is used as often no action is taken to initiate the collection of the information. Sources of passive surveillance information include:

- reports of laboratory diagnoses of disease;
- statutory notification of disease (when not supported by active effort to encourage notification);
- reports of conditions detected during routine meat inspections;
- sentinel surveillance systems.

Meah and Lewis (2000) consider the terminology in some detail. "Passive" surveillance is contrasted in conventional terminology with "active" surveillance in which the veterinary authorities make an active effort to collect the required information. This information may be direct measures of the occurrence of disease and/or infections in an animal population in a statistically designed survey, or it may be to determine the exposure of an animal population to an environmental contaminant.

There is increasing concern in both the veterinary and medical professions dealing with national surveillance about the adequacy of the conventional term "passive" to describe the kind of surveillance activity set out in the preceding paragraphs. There is a perceived failure in the term "passive" to do justice to all that is entailed in the process. The term gives the impression of being "unscientific" and dependant on chance with little or no action required by the authorities responsible for surveillance. In addition, it was felt that criticisms of passive surveillance were based on a misunderstanding of what the approach could and could not deliver As a consequence the use of definition itself could result in increased vulnerability to funding reductions.

Passive surveillance arising from laboratory diagnoses reports is currently an important component of the GB national surveillance but has limitations and biases inherent in the approach. Nevertheless, passive surveillance was considered essential to provide a clear idea of the situation regarding animal health and welfare in the field and identify particularly diseases as being absent in the country (but not prove that other diseases are absent from the country) if diseases are correctly diagnosed;

Meah considered the strengths and weaknesses of active and passive surveillance. The latter could not give direct information on disease prevalence. However, the

submission of samples to the regional laboratories for post mortem examination and the feedback of results was considered an important mechanism for establishing good relationships with farmers and private veterinarians. Without a good relationship with private veterinarians and farmers, access to "informal" intelligence on animal husbandry, health, diseases and welfare would not be possible.

Both active and passive surveillance programmes are necessary components for a national animal disease surveillance system. Passive surveillance is the first stage of a process, which identifies problems, which merit more detailed investigation. The alternative of a wholly "active" surveillance programme was considered unable to meet the objectives of surveillance. First, because it would be too expensive to carry out statistically designed surveys on more than a handful of diseases and infections. Second, an active system cannot deal with diseases and infections, which are new, or of very low prevalence. Third, a statistically designed active surveillance system is not able to pick up outbreaks of diseases and infections.

Effective early warning mechanisms for new or exotic diseases are essential. Local laboratory diagnostic services receiving a high throughput of submissions from private practitioners is one of the mechanisms for achieving this. The consequences of the failure to detect an OIE List A disease such as Classical Swine Fever in time can be catastrophic.

THE FUTURE

The current terminology used in national veterinary surveillance needs to be re-considered to better reflect the importance of the different methods. Passive and Active are no longer appropriate. Alternative definitions include *"continuous baseline surveillance"* to establish the existing disease status of the animal populations monitored and to detect changes in diseases which merit more detailed investigation. This might include defining *"norms"* for the morbidity or mortality in a given population, or production system, so that conditions resulting in unexpected disease patterns can be investigated at an early stage. *"Continuous targeted surveillance"* to monitor specific diseases of interest in defined populations. Both of these terms could be incorporated into an overall "Core *Surveillance"*, the objective of which is to provide baselines on prevalence, incidence etc using both active and passive methods

Where specific diseases or infections are the subjects of intervention or control measures, surveillance enables the risks to be quantified and the efficacy of any measures to be monitored. This category could be either *"strategic surveillance"* or *targeted surveillance*, which is targeted but time-limited to address a precise

issue. There needs to be a new consensus on appropriate terminology to describe the various kinds of veterinary surveillance.

A key issue is deciding on the balance between "active" and "passive" surveillance in an overall animal surveillance programme. Perhaps it is more appropriate to determine the balance between core and strategic surveillance The balance must increasingly lie towards more statistically designed surveys. Indeed, the number of structured surveys in MAFF's veterinary surveillance portfolio has increased substantially in the last two years. A wider debate on the future structure of veterinary surveillance would be appropriate at this time.

Surveillance is an integral part of risk analysis with its overall processes of hazard identification, risk assessment, risk management and risk communication. This is an integrated process which is being developed by organisations such as the OIE to ensure that animal health measures are not used as a non tariff barrier to trade. Only by introducing formal methodologies standardised by international organisations can member countries be assured that control measures and import rules are justified.

Similarly general principles and methodologies are being developed by the European Union, the World Health Organisation and others to give greater transparency in risk management and communication both within and between countries.

Surveillance is increasingly impacting in all these areas. Effective risk management can be achieved if hazard identification such as detection of new diseases or changing disease patterns occurs at an early stage. This allows rapid evaluation to assess whether the disease or infection poses a risk to public or animal health, the former being the priority. The characterisation of the hazard, exposure assessment and risk characterisation are all components of the risk assessment process

Risk management involves a number of stages beginning with an evaluation of the risk. This is followed, where necessary, by an assessment of the options to reduce or eliminate the risk with the implementation of the most appropriate measures in the circumstances. Monitoring and review of the impact and success of the measures is the final stage. In all these stages surveillance plays a crucial role in providing the information on which to base decisions.

Finally risk communication which is the interactive exchange of information and opinions. There is no doubt that surveillance provides the information which can and must be communicated to all those with an interest in animal disease or infection in order to protect public and animal health.

There are other key issues which need to be debated but have not been considered in this paper. Issues such as roles and responsibilities, sources of suveillance information, the integration of human, food, and environmental surveillance, Infrastructure, Data protection and confidentiality, IT issues and the use of Geographic Information Systems.

CONCLUSIONS

Surveillance is a resource issue. Clear statements of the objectives and the adoption of terminology which correctly describes the activities should lead to a better understanding of surveillance and a better use of limited resources. A clear stategy is needed for surveillance with a transparent and open system for prioritising surveillance activities. Animal health surveillance is a major information and management system supporting all aspects of risk analysis. As such it is an important national resource and must be defended and developed to meet national needs but also international requirements.

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FOOD SAFETY/
ANTIMICROBIAL RESISTANCE

MILKING PRACTICES ASSOCIATED WITH THE PRESENCE OF *LISTERIA MONOCYTOGENES* IN NEW YORK STATE DAIRY FARMS

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Listeria monocytogenes is one of the most significant food-borne pathogens because of the potential detrimental hazard it poses to certain groups in the population (Ryser & Marth, 1991). This bacterium is the cause of several major food-borne outbreaks within the last two decades (Centers of Disease Control, 1999). Because of its potential deleterious effect, it has brought much anxiety to the public and has alerted regulatory bodies to develop measures to reduce the risk of exposure to this pathogen. Consequently, the zero-tolerance level for ready-to-eat food mandated by the USDA/FSIS have burdened food manufacturers with financial losses due to massive recalls. In the United States, the annual human illness costs due to food-borne listeriosis is estimated at $1.3 - 2.4 billion. This estimate does not include the costs to producers and food manufacturers.

Listeria monocytogenes is ubiquitous in the environment, and therefore it is important that all measures should be taken to prevent its contamination of food along the production chain. Currently, much emphasis has been placed on the reduction of pathogen load from farm level to manage the final burden on food products. This approach is stated explicitly in the USDA/FSIS 'Farm to Table' hazard analysis recommendation to food producers (Food Safety and Inspection Service, 1998).

Stringent compliance with good and hygienic milking practices has been attributed to the reduction of many milk-borne pathogens (Barkema et al., 1998). Poor milking procedures have been incriminated in propagating diseases and introducing contamination in the milk (Husu et al., 1990; Pankey, 1989a). Several indices were used to measure the impact of good farm practices on pathogen reduction. In this study, we investigated the association between factors that were hypothesized to represent good milking practices and the likelihood of *L. monocytogenes* in dairy farms in New York State.

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MATERIALS AND METHODS

The target and study populations were described in detail in a previous study (Hassan et al., 1999). Briefly, the study population consisted of herds selected from farms enrolled in the Quality Milk Promotion Services (QMPS) in New York State between April 1998 to March 1999. Four hundred and four herds were included in this cross-sectional, epidemiological study. The sampled farms were stratified by region and season. Milk filters were collected once from each of the farms enrolled in the study by the QMPS's staff. Data on putative risk factors were collected at the same time by personal interviews with the farmers using a set of questionnaires. The putative factors included demographic, farm management, milking practices and health related variables.

The milk-filters were cultured for *Listeria* spp. and confirmation for the serotype of interest was made using an established PCR protocol (Bassler et al., 1995). Data collected were coded and managed in Microsoft Access and analyzed using SAS statistical software (SAS Institute Inc, Cary, NC).

DATA ANALYSIS

For the purpose of this study, eight factors describing milking practices that have a potential of either increasing or decreasing the likelihood of *L. monocytogenes* in milk were examined. These factors included: 1) examination of milk at milking; 2) preparation of udder prior to milking 3) pre-dipping of teats; 4) observing the milk for abnormal excretion 5) sanitation of equipment between milking of cows; 6) milking of problem/diseased cows last; 7) milking of problem cows using separate unit; and 8) dipping of the teats after milking. These practices were measured as dichotomous variables reflecting their presence or absence, regardless of the method used.

A systematic approach was adopted in the data analysis. First, the significance of association between each of the hypothesized factors and the likelihood of *L. monocytogenes* was evaluated using the unconditional logistic regression analysis (SAS Institute Inc, Cary, NC) at $\alpha = 0.05$. Second, factors that were found to be significantly associated with the likelihood of *L. monocytogenes* in the bivariate analysis were further considered in the multivariate approach. The multivariate analysis was carried out using the logistic regression analysis to evaluate the significance of each factor while simultaneously controlling for the effect of other factors in the likelihood of *L. monocytogenes*. Thirdly, two-way interactions between the putative factors of biological importance were also evaluated in the multivariate analysis. In the final logistic regression analysis, factors that were identified previously to associate with the likelihood of *L. monocytogenes* were forced in the model.

RESULTS

Listeria monocytogenes was isolated from fifty-one out of 404 farms that were sampled (12.6% prevalence). The median herd size in the study population was 55 cows. A summary of the distribution of the putative factors in the study population is shown in Table 1. A total of 259 farmers (64%) reported that they observe milk in the milking

process. Ninety-four percent of farms prepared the udder of milking cows (i.e. dry massage or individual paper towel) before milking.

Table 1. Frequencies distribution of factors investigated and the prevalence of *L. monocytogenes* in New York dairy herd

Variables	*No of farms*	*% L. monocytogenes +ve*
Milking observation		
Yes	255	11
No	144	15
Udder Preparation		
Yes	376	12
No	23	17
* Examine abnormal milk		
Yes	219	9
No	177	17
* Teats pre-dip		
Yes	324	10
No	75	24
Teats post-dip		
Yes	335	12
No	63	14
Unit sanitized between cows		
Yes	25	12
No	374	13
Problem cows milk last		
Yes	174	11
No	224	14
Problem cows milk in separate unit		
Yes	100	14
No	298	12

* Chi-square $p \geq 0.05$

Half of the farmers (55%) reported that they examined the milk before hooking the cows to milking machines. The majority of the farmers (81%) in the study population practised

pre-dipping of the teats using different methods (i.e., dipper cup, spray etc.). A high percentage (84%) of the farmers disinfected the udder post-milking. Only 6% of the farmers cleaned the milking units between cows. A noticeable proportion of the farmers (44%) processed cows with problems at the end of each milking session and 25% of these farmers milked these cows in separate units than the ones used for normal healthy cows.

Pre-dipping and routine examination of abnormal milk procedure were the only two factors found to be significantly associated with the presence of *L. monocytogenes* (P = 0.013 and P=0.001; respectively). Farms that practised pre-dipping of teats before milking were about 3 times less likely to have *L. monocytogenes* in the milk filters. Examination of abnormal milk excretion was associated with the reduction of likelihood in isolating *L. monocytogenes* by 2 times.

In the final multivariate analysis, farms that practiced pre-dipping were twice less likely to be positive for *L. monocytogenes* compared with those farms that did not employ this practice. Examining cows' milk for abnormal appearance was associated with a decreased likelihood of isolating *L. monocytogenes* (Table 2).

Table 2. Results of logistic regression for milking practices associated with the likelihood of *L. monocytogenes*

Variable	*Estimate (B)*	*Standard Error (SE)*	*P value*	*Odds Ratio (OR)*	*95% OR Confidence Interval*
Intercept	2.0571	0.6169	-	-	-
Season					
Winter	0.0	-	-	-	1.0
Spring	0.5579	0.4600	0.2252	1.75	0.71-4.30
Summer	0.2125	0.4826	0.6598	1.24	0.48-3.19
Fall	0.1462	0.4858	0.7635	1.16	0.45-4.00
Region					
Northern	0.0	-	-	-	-
Central	1.2307	0.5011	0.014	3.42	1.28-3.14
Western	1.2209	0.5147	0.0177	3.39	1.24-3.30
Eastern	0.4246	0.5690	0.4555	1.60	0.50-4.66
Predip[a]	-0.8421	0.3660	0.0216	0.43	0.21-0.89
Exabnmi[b]	-0.6921	0.3240	0.327	0.50	0.27-0.94

Predip[a] = teats pre-dipping
Exabnmi[b] = examination of abnormal milk

Diagnostic procedures for model validation did not reveal any multicollinearity problems between the variables (VIF≥1) and Pearson residuals and deviance chi-square proved that the model fits well because almost all points were within ≈ 0.

Geographical regions where farm were located (central, east, north or west) and season when the herd was sampled (spring, summer, fall or winter) were included in the final logistic regression model. Region remained significantly associated with the likelihood of *L. monocytogenes* in milk filters (Table 2). No effect modification, as evaluated by the significance of the interaction terms, was observed.

DISCUSSION

The purpose of this study was to identify milking practices that are associated with the occurrence of *L. monocytogenes* in milk filters as a proxy for milk contamination. It represents a partial effort towards our long-term objective of carrying out a risk assessment for this organism in dairy herds in the hope of making recommendations regarding the practices that reduce the likelihood of this organism in milk. This study investigated eight factors in the milking procedure that were perceived to be crucial for the risk management at this level in the production chain.

Our previous study demonstrated regional and seasonal variation in the likelihood of *L. monocytogenes*. Central region of New York State was found to have a significantly higher likelihood of *L. monocytogenes* recovery. It was twice more likely to isolate this organism from herds in the central region in comparison to other regions. On the other hand, herds in the northern region were less likely to be positive for the pathogen. It was three times more likely to isolate *L. monocytogenes* from the central region compared to northern (Hassan et al., 1999). The observed regional association with the likelihood of the organism was not attributed to the milking practices, herd size in our data, or the seasonal variation in sampling. Also, we do not believe that it is a reflection of climatic variation since the weather in New York State is relatively similar. It is reasonable to assume that the geographical variation could be due to other management factors we did not account for.

Farms that were sampled in the spring had the highest prevalence of *L. monocytogenes*. This declined as the season changed to summer, autumn and winter with winter having the lowest prevalence rate of the year (Hassan et al., 1999). Also, the seasonal variation in the likelihood of the organism could not be explained by the geographical location of the farm, the herd size, or the management practices.

The overall hygienic milking practices were reported in other studies to be associated with the prevalence of sub-clinical and clinical mastitis in dairy farms (Pankey, 1989a; Pankey, 1989b). Furthermore, it was suggested that these practices were associated with the level of bulk milk somatic cell counts found in dairies, which is an indicator for the quality of milk (Fenlon et al., 1995). Stringent milking practices and hygienic milking procedures are recommended as preventive measures for not only mastitis, but also to decrease the microbial load of milk-borne pathogens. Only two of the eight factors that we investigated were found to associate significantly with the likelihood of *L. monocytogenes*. This finding should not be interpreted as undermining the role of other sanitary milking measures.

Our finding is consistent with the current biological understanding of the epidemiology of *L. monocytogenes*. The observed association between the recovery of the pathogen and the pre-dipping practices reflects that cleanliness of the teats before milking is vital in reducing microbiological contamination from farm environment. As *L. monocytogenes* is ubiquitously found in nature, and has been recovered from faeces of dairy animals (Skovgaard & Morgen, 1988), sanitizing the teats apparently prevents, reduces or eliminates the pathogen effectively.

The significant finding of careful examination of the abnormal appearance of milk was not a surprise. Although infrequent, *L. monocytogenes* was reported to associate with sub-clinical or clinical mastitis (Ryser & Marth, 1991). Furthermore, the pathogen excretion may last several months after an overt case of listeriosis (Donker-Voet, 1963). These findings imply that observing the milk for abnormality before milking and intervening has the potential of reducing the likelihood of listerial contamination.

In our study population, the risk of a herd to be positive for *L. monocytogenes* seemed to increase with poor compliance to sanitary milking procedure and stringent observation of milk excreted before milking. Prevention of potential milk contamination in the farm could be achieved through effective compliance to all suggested milking procedures and hence reduction of the potential risk of human exposure to *L. monocytogenes* associated with dairy products.

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U.S. ACTIVITIES TO ADDRESS ANTIMICROBIAL RESISTANCE

M.E. TORRENCE*

Antimicrobials have played an important role in livestock production as well as the treatment and prevention of infections in humans and animals. Subtherapeutic doses of certain antimicrobials have been used as growth promotants for over 40 years. The subject of antimicrobial resistance and its development is not new nor surprising; however, over the last few years, it has gained attention because of the potential public health impact. Antimicrobial resistance remains unclear. The development of antimicrobial resistance, its persistence and transmission, and even the public health impact are multifactorial. The role of risk factors such as duration, type, and dosage of antimicrobial use is also unclear. Data are limited on the prevalence of antimicrobial-resistant zoonotic bacteria in foods of animal origin and on the use of antimicrobials in agriculture and human medicine. There are problems with study designs involving the testing of representative sampling, the determination of sources, and tracing of animals.

CURRENT EFFORTS

Current programmes in the U.S. can be divided into surveillance, education, and research.

Surveillance

The Center for Veterinary Medicine (CVM) at the Food and Drug Administration (FDA), Animal Plant and Health Inspection (APHIS) and the Food Safety Inspection Service (FSIS) at the United States Department of Agriculture (USDA), and the Centers for Disease Prevention and Control (CDC), joined forces in 1996 to establish the National Antimicrobial Susceptibility Monitoring System, now referred to as the National Antimicrobial Resistance Monitoring System- Enteric Bacteria or NARMS-EB. This surveillance system proposed to monitor changes in susceptibilities of zoonotic pathogens from human and animal clinical specimens, from healthy farm animals, and from carcasses of food-producing animals at slaughter. The first organism selected was non-typhoid *Salmonella*. This system has two parallel arms: veterinary and human. Veterinary testing is conducted at USDA's Agricultural Research Service's Russell Research Center in Athens, GA while human samples are sent by 16 State Health Departments for testing at the National Center for Infectious Diseases, CDC. The 17 antimicrobials that are monitored were selected as representative antimicrobials or classes of antimicrobials used in animal and human medicine. In 1997, *Campylobacter* was added to the human surveillance; in 1998, *Campylobacter* and *E.coli O157:H7* were added to the veterinary surveillance.

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The goals and objectives of this surveillance system are to: 1) provide descriptive data on temporal trends of antimicrobial susceptibility in *Salmonella* from animal and human populations; 2)facilitate the identification of resistance in humans and animals as it occurs; 3) provide timely information to veterinarians, physicians, health officials; 4) prolong the life span of approved drugs by promoting prudent and judicious use of antimicrobials; 5) identify areas for more detailed investigations.

In 1998, CDC reported that there were 1476 *Salmonella* isolates, 315 *E.coli O157:H7* isolates, and 382 *Campylobacter.* Of the *Salmonella* isolates, 27% were resistant to one or more antimicrobials. Of the *Salmonella typhimurium* isolates, 53% were resistant to one or more; 32% had multi-drug resistant patterns characteristic of *DT 104.* Of *E.coli* isolates, 7.3% were resistant to one or more antimicrobials. Of *Campylobacter* isolates, 55% were resistant to one or more antimicrobials; 13.3 % were resistant to ciprofloxacin.

In 1998, CVM reported that 3318 *Salmonella* and 209 *Campylobacter* were tested. The amount of resistance varied depending on species and antimicrobial. For example, 38% of the *Salmonella* isolates were resistant to tetracycline and 35% were resistant to streptomycin. *Campylobacter* isolates from raw chilled broiler carcasses were tested against 8 antimicrobials. All isolates were susceptible to gentamicin and chloramphenicol. However, 60% of the isolates were resistant to tetracycline and 11% were resistant to ciprofloxacin.

Education

There are efforts by public health officials and veterinary and human medicine to educate consumers, physicians, veterinarians, and producers on the judicious use of antimicrobials. CDC currently has a programme aimed at patients, pediatricians, and infectious disease physicians on the prudent use of antimicrobials. This includes public health training seminars, brochures, and even an innovative approach of having prescription pads for physicians to prescribe care and help tips rather than antimicrobials. The American Veterinary Medical Association (AVMA) has established a Steering Committee on the Judicious Therapeutic Antimicrobial Use. This committee is comprised of federal consultants and experts that can review various issues, provide strategic planning, and direct future efforts to address antimicrobial resistance. The purpose of the Committee is to advise the Executive Board on ways to develop the guidelines for judicious therapeutic use of antimicrobials by veterinarians and continuing education programmes to raise the awareness of the profession. The Center for Veterinary Medicine has contracted with AVMA to have continuing educational materials created. The committee has encouraged and is currently advising on drafted guidelines for antimicrobial use for each species. For example, the National Pork Producer's Council is in the final stages of getting their guidelines approved.

Research

Research is ongoing and may consist of basic or applied research. General categories of research that were funded by USDA's Cooperative State Research Education and Extension

Service (CSREES) are: surveillance of antimicrobial resistance on farms; mechanisms of resistance development and resistance transfer; risk factors of resistance; alternatives to antimicrobials; and antimicrobial usage. Specific examples include: a project to demonstrate whether or not phenotypic and genotypic associations among antimicrobial resistant pathogens in humans and livestock exist; a project to measure antibiotic usage and development resistance of *Salmonella* in cattle; a project to look at vaccination in swine as an alternative; and projects to determine the levels of antimicrobial resistance in *Salmonella* in feed. An exciting new grant programme was initiated in 1999 by CSREES's National Research Initiative (NRI) division entitled, Epidemiologic approaches for food safety. This programme enables the awarding of larger-sized grants for population/field studies. Of the 9 grants awarded in 1999, at least 4 involved some research in antimicrobial resistance. There is other research being funded by federal agencies such as CVM, USDA's FSIS and Agricultural Research Service (ARS). ARS has a large programme in developing alternatives to antimicrobials such as competitive exclusion products and examining the development of antimicrobial resistance in *Salmonella DT104.*

U.S. Public Health Action Plan

The U.S. Public Health Action plan to combat antimicrobial resistance, when completed, is supposed to provide a blueprint for specific, coordinated federal actions to address the threat of antimicrobial resistance. It has been developed by an interagency task force on antimicrobial resistance that was organized in 1999. The task force is co-chaired by CDC, FDA, and the National Institutes of Health (NIH). But it also includes other federal agencies such as USDA, the Agency for Health Care and Policy Research, Department of Defense, the Veteran's Administration, the Environmental Protection Agency, and the Health Care Financing Agency. This plan, when finished, will reflect a broad-based consensus of federal agencies on actions needed to address antimicrobial resistance and solicited input from consultants from state and local agencies, universities, professional societies, pharmaceutical companies, health care delivery organizations, agricultural producers, consumer groups, and the public. A 3 day public meeting was held in July 1998 to solicit input from these consultants. The plan is divided into 4 sections: surveillance, prevention and control, research, and product development. The following are a sample of the major points within each section.

Surveillance: The greatest need is for a national antimicrobial resistance surveillance strategy. Currently there are existing surveillance networks that monitor some specific infectious diseases (e.g. tuberculosis, foodborne organisms, and NARMS), but there is a need to unify and coordinate the data and the resources. Increased emphasis and funding is being used to enhance epidemiologic and laboratory capacity and communication. There is a need to define roles and activities among personnel, facilities, and agencies. With the move to a unified system, it is essential that methods and data elements be standardized. This surveillance system has to be sensitive and flexible and must provide information to be disseminated back to public health officials, physicians, veterinarians, and federal, state, and local officials. Available and accurate drug susceptibility data are essential for the national resistance system. This data will be dependent on the standardization of methods and data elements. A part of

this system will include the improved surveillance for antimicrobial resistance in agricultural settings (animals and plants).

Secondly, monitoring is an essential part of surveillance. Monitoring of antimicrobial drug use both in humans and non-human settings is essential. Innovative methods for collecting such data will be required. For example, is it possible to conduct periodic surveys of food animal producers and veterinarians? Confidentiality of information that is gathered is critical. Currently, on-farm information is collected by USDA's APHIS through their National Animal Health Monitoring System on selected species. This effort is dependent on federal funding and the collaboration of producers. Monitoring of antimicrobial drug use in fruit and vegetable production, in food processing, and even in the pet population is also needed.

Prevention and control: Prudent drug-use policies for antimicrobials are needed in agricultural (animal and plant) and human settings. It is essential that there is a method by which the effectiveness of these policies can be evaluated. Better diagnostic tests, particularly at point-of-care, are required to allow for less prescribing of antimicrobials. Another method of decreasing the use of antimicrobials is better infection control and reduction of infection transmission. Although hospitals have had existing programmes in hospital infections, more efforts are needed.

Prevention and control of antimicrobial resistance must include a comprehensive, multi-faceted programme. This will involve educational and behavioral interventions; the improvement of our understanding of potential risks and benefits of antimicrobial use; and the study of animal and human waste and possible soil and environmental contamination. Prevention and control in agricultural settings will involve a look at animal husbandry and food production practices and the development of the new framework document for new antimicrobials by CVM. Additional research on the risk of development and transfer of resistance related to the use of antimicrobial drugs in food and non-food is needed to help develop and implement prevention and control programmes.

Research: It is essential to identify and fill the gaps and needs in the field of antimicrobial resistance research. Some prime examples for study are the epidemiology of resistance genes; mechanisms of antimicrobial resistance emergence, acquisition, spread and persistence; effects of antibiotics used as agricultural growth promotants on microbes; and the variations in drug use regimens. For research to continue, it is important to augment the research infrastructure. This involves increased funding, an awareness about the sources of funding, and an increased number of experts. Finally, it is essential that there is translation of research findings into innovative clinical products.

Product development: It is important to identify and fill current and projected gaps in our arsenals of drugs. There needs to be development of urgently needed drugs, vaccines, and diagnostics. Most importantly, this involves some type of incentives for this development. For example, the approval process needs to be simpler. Likewise, there is a need for the production of veterinary drugs. The financial incentive for new veterinary antimicrobials is

not high. Alternatives to antimicrobials such as vaccines, immune stimulators, and competitive exclusion products are important areas for development.

FUTURE DIRECTIONS

Surveillance

There is an existing infrastructure for surveillance, and the NARMS-EB system has made great strides in the surveillance of antimicrobial resistance. Currently there is momentum for the enhancement of epidemiologic and laboratory capacity. This funding could be available for veterinary diagnostic laboratories. Veterinary diagnostic laboratories could be sentinel sites for antimicrobial resistance and even food borne pathogens (similar to FoodNet). In fact, veterinary diagnostic laboratories as sentinels have been mentioned in national strategic planning efforts for food safety. How do we make NARMS-EB better? How can we better utilize the National Animal Health Monitoring System? Standardization of methodologies and data elements between veterinary laboratories and surveillance systems are needed. Once this effort is coordinated, the ability to merge with human surveillance networks may be possible.

Research

Research is needed in many areas. Although evidence demonstrates that antimicrobial resistance can be transferred from animals to humans, little is known about the true public health impact. Data are needed on the prevalence of antimicrobial resistance on the farm and how this prevalence relates to antimicrobial resistance in humans. Research on the mechanisms of development, persistence, and transference of antimicrobial resistance is urgent. Information on drug usage, dose, and duration could provide insight into possible prevention and control programmes. For example, is it high doses of antimicrobials given short term or low doses of antimicrobials given long term that cause the most antimicrobial resistance? Finally, research determining risk factors is essential. Risk factor data are required for risk assessments and, most importantly, to develop intervention strategies. As intervention strategies and management practices are implemented, the impact of these on antimicrobial resistance must be evaluated. What are the outcome measures?

Education

There is an urgent need for educational efforts on judicious use of antimicrobials by health care professionals and producers. Aggressive educational programmes targeted at veterinary students are appropriate. Veterinary students must learn to educate clients on the risks and benefits of antimicrobials, even in small animal medicine. Why not follow the direction of human medicine and use the media as a way to educate consumers? For example, instead of advertising the newest arthritis medicine for dogs and cats, why not advertise the prudence in not asking for antimicrobials for both the health of the pet and the owner?

Prevention/Control

A major effort is underway by CVM in the development of the framework document for new antimicrobials. The framework document sets out a conceptual risk-based process for evaluating the microbial safety of antimicrobial drugs intended for food-producing animals. Categorization of newly proposed antimicrobials will be based on their importance to human medicine. For example, category 1 will result in little or no resistance transfer to humans. Category 2 would require a predefined level of maximum resistance transfer to be established. Pre-approval data will be required to show that the level of resistance transfer from animals to humans will be safe. Depending on some of the categories, there may be post-approval studies required. Finally, new and innovative management practices or quality assurance programmes must be implemented at the farm level. The impact of these strategies must be evaluated.

FIELD INVESTIGATIONS TO EXAMINE ANTIMICROBIAL RESISTANCE OF *ENTEROBACTERIACEAE* FROM CATTLE

G.J. GUNN[1], V.L. EDGE[1], R.W. HUMPHRY[1], S.A. SCANLAN[1], F.J. MURRAY[1] & J.C. LOW[2]

Does the administration of antimicrobials to animals result in the selection of resistant bacteria? Can those resistant bacteria be transferred to humans and then cause infections that are difficult to treat? These questions have been the subject of debate for more than 40 years (Anon, 1969; Anon, 1998). The UK Government's recent report "Microbial Antibiotic Resistance in Relation to Food Safety" (Anon, 1999a) indicated a need for a coherent strategy aimed at reducing the veterinary usage of antibiotics. Yet, to develop effective management policies, which might limit the frequency of antimicrobial resistance acquisition in bacterial populations, more fundamental field information is required. Researchers from SAC Veterinary Science Division (SAC VSD) have carried out a series of hypothesis-generating field studies to (i) estimate the prevalence of antimicrobial resistance in bovine derived *Enterobacteriaceae* and (ii) determine which procedures on the farm are associated with increasing prevalence of resistance. This paper describes the studies and provides preliminary findings. Analysis is ongoing and more results will be available by the time of presentation.

MATERIALS & METHODS

Three independent studies were undertaken (Table 1). Study A involved a survey of potential risk factors for the occurrence of antimicrobial resistance in *Enterobacteriaceae* isolates from calves. Data were derived from information supplied passively with 407 calf (age < 6 weeks) scour samples submitted for routine diagnosis to SAC VSD Inverness between 1990 and 1995. Only one sample was retained per outbreak. Faecal material was cultured directly onto blood agar media and MacConkey media (Oxoid w/o salt). Single selected colonies were subcultured to Isosensitest agar (Oxoid) and antibiotic sensitivity testing performed by disc diffusion assay using a range of commonly prescribed antibiotics. The analyses examined statistical associations between the occurrence of resistance to seven antibiotics: ampicillin (amp), clavulanic acid potentiated amoxycillin (clav), apramycin (apr), chloramphenicol (chlor), furazolidone (fur), sulphamethoxazole potentiated trimethoprim (sxt), and tetracycline (tet) with the variables of: age, farm type, season, year, and veterinary practice.

Study B measured the occurrence of antimicrobial resistance in *Escherichia coli* isolates collected from groups of diarrhoeic and non-diarrhoeic calves during 1996. In total 101 faecal

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samples were collected from 15 enteritis outbreak farms and from 9 control farms where no diarrhoea occurred. Faeces were plated directly on to four MacConkey agar plates (Oxoid no.3) containing: zero additives, apramycin (32μg/ml); ampicillin (16μg/ml), or nalidixic acid (15μg/ml). Isolates were confirmed as *E. coli* by indole testing and growth at 44°C. Resistance was examined by rotary plating. Farm histories and antimicrobial usage were compared.

Study C involved active surveillance for antimicrobial resistance of *Escherichia coli* from farmed cattle sampled during 1998. Faecal samples (1131) were collected from three different groups of cattle (cow, fattening and calves) on each of 100 randomly selected farms. The bacteriological methods for detection and testing were identical to those used for study B. Antimicrobial resistance patterns and management histories were compared.

RESULTS

A total of 1639 sensitivity patterns were examined (Table1). Analysis is ongoing and more results will be available by the time of presentation.

Table 1 Summary of samples

Study	Type	No. Farms	No. Groups	No. Samples
A	Diagnostic	-	407	407
B	Case-Control	24	24	101
C	Random Cross-sectional	100	246	1131

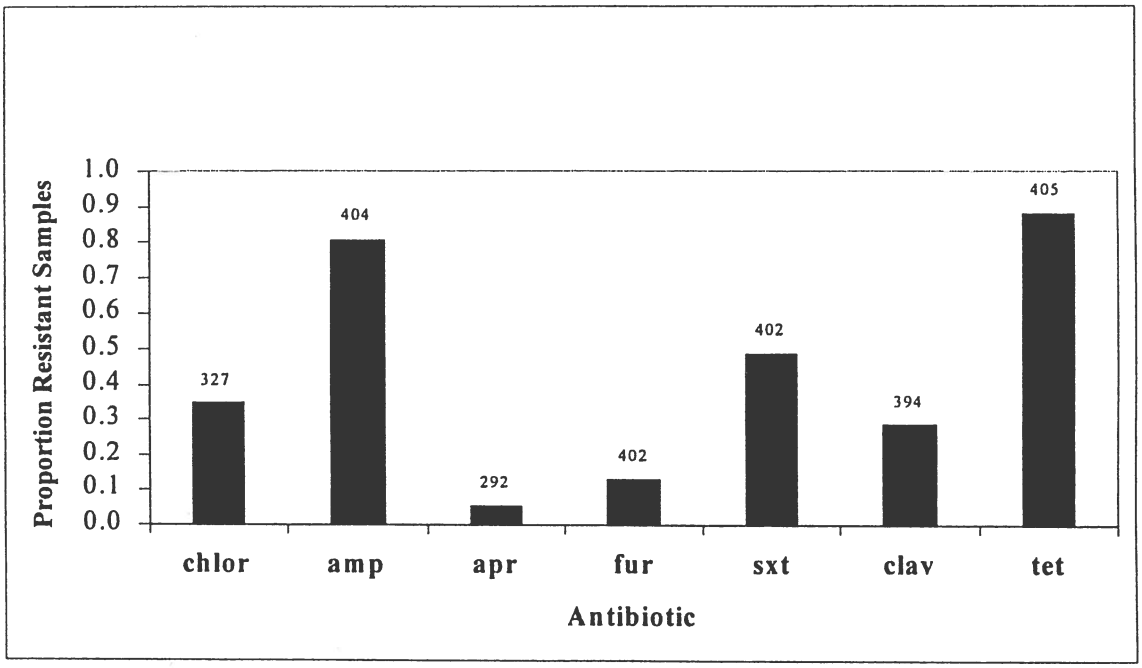

Fig. 1 Diagnostic sample study (A); proportion of resistant samples 1990-1995
(Abbreviations given in materials and methods section. No. above bar = total no. of samples tested).

Study A: The proportion of samples resistant to each of the antimicrobials differed widely. There were high levels of resistance to tetracycline and ampicillin; intermediate levels to chloramphenicol, potentiated amoxycillin and potentiated sulphamethoxazole and low levels to apramycin and furazolidone as illustrated in Fig 1. There were significant differences between years ($p<0.1$) and seasonal differences ($p=0.1$). Some veterinary practices were associated with significantly higher levels of resistance than others ($p<0.01$) (Table 2). There were, however, no clear differences between age groups and no differences between beef and dairy farms.

Table 2 Diagnostic sample study (A); percentage resistant to antimicrobial
(Abbreviations given in materials and methods section)

Vet Practice	Chlor %	Amp %	Apr %	Fur %	Sxt %	Clav %	Tet %
A	51	85	9	15	56	28	90
B	28	80	10	14	48	35	93
C	24	71	0	13	41	31	85
D	17	78	0	4	44	19	89

Study B: There was an increase in resistant isolates from outbreak animals compared with control animals for all three test antibiotics and major differences in the level of resistance between the antibiotics as illustrated in Table 3.

Table 3 Case-control study (B); percentage resistant to antibiotic

	n	Ampicillin %	Apramycin %	Nalidixic acid %
Control Samples	44	64	2	0
Outbreak Samples	57	93	23	11
Control Farms	9	78	11	0
Outbreak Farms	15	100	40	13

Study C: Resistant isolates were recovered most frequently from calves and least frequently from finishing cattle (Table 4).

Table 4 Random cross-sectional study (C); percentage samples resistant to antibiotic

Antibiotic	Cows	Fattening	Calves
Ampicillin	48	49	88
Apramycin	4	3	16
Nalidixic acid	3	0	20

DISCUSSION

Frequency of antimicrobial resistance was found to be very high in the examination of diagnostic samples (Figure 1). Though this study of diagnostic samples is inherently biased, the percentage resistance results are similar, in broad terms, to the results reported by Wray *et. al.* (1993) and higher than the levels reported by Jackson (1981). The high level of bacterial resistance detected for both tetracycline and ampicillin and the moderate levels to trimethoprim potentiated sulphamethoxazole and chloramphenicol are in accord with the results of Wray *et. al.* (1993). Interestingly, all the studies identify marked differences in the proportion of resistant isolates for the different antimicrobials. Though antimicrobial usage is presumably part of the explanation for these findings fundamental molecular differences in resistance mechanisms presumably also have major impacts on the development and persistence of resistance.

Our study did produce very important findings in identifying trends to differences in resistance patterns associated with the serving veterinary practices. The significant difference detected was for the prevalence of chloramphenicol resistance. Though this antibiotic should not have been used widely during the period of the study it is tempting to hypothesize that veterinary practice A used it more frequently than the others. This was later confirmed by discussion with the practitioners though it should be noted that Aalbæk *et. al.* (1991) reported the persistence of resistance to chloramphenicol 10 years after it was withdrawn in Denmark. The results indicate that there is potential to develop prudent usage strategies to combat the perceived problem of the occurrence of antimicrobial resistance as have been recommended (Anon, 1999a; Anon 1999b).

The case-control study demonstrated that high levels of antimicrobial resistance in calves were associated with outbreaks of enteritis. These results support the findings of Wray (1986) and Blanco *et. al.* (1993) and suggest the selection pressure that arises from antimicrobial use. The absence of nalidixic acid resistance on control farms was reassuring, but the increase in percentage resistance between the controls (1996) and the randomly selected calves examined in 1998 suggests that resistance to nalidixic acid is increasing as the use of the related fluoroquinolones becomes more widespread in veterinary practice. The higher level of apramycin resistance compared with the earlier diagnostic studies is also of concern.

We are not able to explain the possible significant difference due to season found in Study A. Additionally we were not expecting to detect a difference due to age in young calves but we were concerned to identify high levels of resistance in animals aged between birth and three days old. We expected a difference in prevalence between beef and dairy cattle and, though this has not been identified this area, it will be explored in more depth in the ongoing analysis.

Where we collected random samples, the cow and fattening animal groups had much lower rates of resistance than calf groups. These studies suggest that concentrating on calf diagnostic samples can lead to an overestimate of the level of resistance compared to studies of normal or older cattle. This finding is of fundamental importance when one considers the introduction of accurate surveillance methods and extension of monitoring to normal populations as has been recommended (Anon, 1999a). It should be noted that older cattle probably represent a greater risk to the human population through the food chain than calves yet this group in general seems less likely to be a source of resistant bacteria. Though we believe that some of the higher rate of resistance in calves is due to the higher numbers of bacteria present in their faeces relative to older cattle (Williams Smith & Crabb, 1961) and this will be examined in more depth in our ongoing studies.

Though a number of studies of antimicrobial resistance were conducted in the 1970's and 80's (Walton, 1972; Hinton, 1986) more recent comparative studies have been lacking. Further analysis of our data will undoubtedly provide greater insight into this important problem but we support the view of Wray (1986) that there is a need to establish standard protocols that will allow the monitoring and epidemiological study of antimicrobial resistance.

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QUALITY ASSURANCE

THE DEVELOPMENT OF A CONTROL PROGRAMME FOR CLINICAL MASTITIS FOLLOWING HACCP PRINCIPLES

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European legislation on food production requires that food plants have to follow Hazard Analysis and Critical Control Point (HACCP) procedures. However, this is not compulsory at farm level - at least, in Italy. On the other hand, dairies often require that farmers implement a quality-assurance programme for the milk delivered to dairy plants. The application of HACCP procedures at farm level is not easy, mainly because the identification and monitoring of critical control points is a difficult exercise.

Clinical mastitis is one of the major sources of economic losses for the farmer (Hoblet *et al.*, 1991; Hortet & Seegers, 1998) and also represents a risk for the consumer. Indeed, the antibiotic treatments to cure clinical mastitis increase the risk of residues in milk (McEwen *et al.*, 1991). There are many studies on the epidemiology of clinical mastitis in dairy herds showing its multifactorial pathogenesis (Sargeant *et al.*, 1998; McClary *et al.*, 1991; Hogan *et al.*, 1989; Wilesmith *et al.*, 1986).

The multifactorial aspect of clinical mastitis, the influence of its pathology on milk quality and safety, the quality control requirements from the dairy industry and, finally, the legal requirements for milk and milk products, make a programme to control clinical mastitis one of the most suitable candidates to follow HACCP principles. However, the development of such a programme under the HACCP system is not an easy task. Indeed, if theoretically most of the requirements can be easily fulfilled, putting them in practice faces a number of problems. The seven principles of HAACP, as described by Mortimore & Wallace (1994), are reported in Table 1 with a general comment on their possible practical application at herd level. The milk production process at herd level has been described and the main production phases and related critical control points (CCPs) identified. However, the identification of CCPs in the different phases and the establishment of critical limits is much more difficult to achieve. Moreover, the major production tool is the cow, a biological organism that introduces a large variability in the measurements as a result of its individuality.

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Table 1. Principles of HACCP and their possible practical application at herd level for mastitis control (Mortimore and Wallace, 1994, modified)

Principle	Description	Feasibility and possible application
1	Conduct a hazard analysis. Prepare a list of steps in the process where significant hazards occur and describe the preventive measures	Milk production process at herd level is well-know and there are plenty of studies on possible hazards and risk factors
2	Identify the Critical Control Points (CCPs) to manage the safety of the production	The practical identification of CCPs at herd level is not easy, many (risk) factors are involved in most of the possible CCPs
3	Establish Critical Limits for the preventive measures associated with each identified CCP	When CCPs are identified critical limits can be proposed based on epidemiological studies
4	Establish CCPs monitoring requirements. Establish procedures from the results of monitoring to adjust the process and maintain control	Monitoring of CCPs is possible for many of them. However the monitoring of some of them could be impracticable or too expensive. Some "on line" measurements could be used as monitoring systems
5	Establish corrective actions to be taken when monitoring indicates a deviation from an established critical limit	Once that CCPs are identified corrective actions can be proposed based on current knowledge on the epidemiology of mastitis
6	Establish procedures for verification that the HACCP system is working correctly	The incidence of clinical mastitis and other health and hygienic parameters could represent a suitable verification systems
7	Establish effective record-keeping procedure that document the HACCP system	There are different useful record-keeping procedures, however farmers should be convinced and trained to use them.

The development of a HACCP protocol requires that the seven principles should be covered from first to last. However, when this project was started, we realised that there was no study specifically describing the epidemiology of clinical mastitis in Italy. The scientific data available allow only general management of the problem: the information on the specific risk factors and the aetiology of the clinical cases is completely lacking. Therefore, we decided to reverse the process, starting from the bottom and moving up to the top. The project was designed to apply the seven principles following the steps described in Table 2.

Table 2. Practical development of a mastitis control programme based on HACCP principles in Italian dairy herds

Principle	1st Target	2nd and further targets
7	Collect data on clinical mastitis occurrence, characteristics, aetiology and distributions within the different cows. Collection of data on therapeutical protocols currently applied in the different herds	To define clinical mastitis incidence at herd level and to identify cows at risk.
6	The assessment of the most sensible indicators at herd level to verify the fulfilling of HACCP procedures	The development of further procedures based on easily-obtained parameters to verify HACCP procedures
5	The definition of practical hygienic procedures to be put in place when the critical limits are reached	The definition of safe and effective therapeutical protocols to cure clinical mastitis
4	Define the parameters to be monitored and, particularly, the suitability of "on line" as monitoring variables	The identification of further parameters that can be easily monitored
3	The definition of critical limits for "on line" and herd-based parameters	The validation of the critical limits in the different herds and, if the case, their fitting to the specific herd
2	The identification of the most suitable and practical parameters related to monitor the different CCPs at herd level	The identification of further CCPs at herd level that can be monitored
1	Establish a procedure to collect data routinely on different production process phases and to define their role as CCP	The identification of further sub-phases in dairy herd production process to be monitored

The project is still in progress and this paper describes the preliminary results regarding mainly principles 1 and 4. The distribution and characteristics of clinical cases collected during the first 10 months of this study are described and discussed. Moreover, the dynamics of three "on line" parameters, recorded during milking, are also described. These parameters were considered as possible candidates for monitoring clinical mastitis occurrence.

MATERIAL AND METHODS

Definition of clinical mastitis

A quarter was defined as clinically affected when changes in the milk (i.e. flecks, clots) were found or when the quarter was inflammed (presence of one of the following signs: swelling, heat, pain, redness, changes in consistency of udder tissues).

Clinical signs were scored by the milker following this scheme: (1) healthy quarter (no alteration), (2) milk alterations; (3) clinical signs at quarter level, with or without milk changes and without systemic signs; (4) udder inflammation with systemic signs.

Samplings

The samplings were taken of each clinical case by the milker following NMC procedures (NMC, 1990) before any treatment was started. Ten ml of milk were collected in sterile plastic tubes for each clinically affected quarter, immediately frozen (-20°C), and sent to the laboratory weekly.

Clinical case data collection

When a clinical case occurred, the milker completed a simple form to report the following information: date of the occurrence, cow identification, number of lactation; days in milk; quarter affected; clinical score; if the case was recurrent; cow group within the herd; treatment applied. This form was sent together with milk samples to the laboratory.

Microbiological assay

One hundred and fifty ml of milk were seeded on blood agar containing 5% calf blood without aesculin. After incubation for 18 h at 37°C, the number and type of colonies were evaluated; if no growth was observed, the plate was further incubated for 24 h.

In positive samples, a representative colony of the single or two different species present was seeded again on a 5% blood agar plate, in order to have a single strain growth for the further tests, and incubated for 18h at 37°C. The following tests were performed on the isolates:

- *catalase*
- *Gram staining*
- *If catalase-negative and Gram+, the isolates were assessed with CAMP test and, if the case, confirmed by SLIDEX STREPTO B (Biomerieux, Florence, Italy) to evaluate the presence of* Str.agalactiae .
- *If haemolytic, catalase positive and Gram+, the isolate were assessed with a coagulase test to evaluate the presence of* Staph.aureus.
- *If Gram negative, the colonies were seeded on McConckey agar to evaluate the presence of coliforms, and then were identified by the appropriate API set of tests (Biomerieux, Florence, Italy).*
- *In all the other cases, the micro-organisms were identified by the means of the appropriate API set of tests (Biomerieux, Florence, Italy) or an equivalent method.*

Definitions

Definition of bacteriologically-positive quarter : A quarter was defined as infected when no more than 2 types of micro-organism were found, in relation to the following criteria:

- *a contagious pathogen (*Str.agalactiae *or* Staph.aureus *) was found;*
- *an environmental (*Streptococci *spp. other than* Str.agalactiae *or coliforms) were found in pure culture or with more than 30 UFC/ml;*
- *Coagulase-negative* Staphylococci *were found in pure culture or with more than 60 UFC/ml.*

Definition of contaminated sample : a quarter was defined as contaminated when more than 2 types of colonies were found.
Definition of bacteriologically negative quarters: a quarter was be defined as not-infected when negative cultures were found.

"On line" measurements

Three herds (B D F) have a system connected with milking machines for "on line" measurements of different parameters during milking (Afimilk, TDM Italy). This device records milk yield, conductivity and milking duration for every cow at every milking, in addition to other parameters not considered in this study. The system is totally automated and an electronic device, that recognises every cow when the cow enters the milking pen, activates it by an electronic transponder. The system then starts to collect the data coming from the milking device and to record it on a computer.

Data handling

Clinical and bacteriological data: clinical and bacteriological data were recorded in a database specifically developed and analysed by S.A.S software (SAS Institute, 1996) using the procedures FREQ, TABULATE,

"On line" measurements: the weekly backup of each farm database was collected from the computers in each farm and transferred to a specifically designed database. Data regarding cows with clinical cases were extracted starting 14 days before the onset of the clinical case and then collated in the database. The same procedure was performed for the healthy cows milked in the same period defined for the clinical case. A database was thus built up containing all the "on line" measures for clinical and healthy cows, standardised to the clinical case occurrence and covering the period from - 14d to day 0. These data were analysed by the SAS software procedures FREQ and GLM for repeated measurements.

RESULTS

Herds with a known history of clinical mastitis, free from *Str.agalactiae* infections and with a prevalence of *Staph.aureus* infection below 1%, were included in the field study. Up to the end of November 1999, 8 herds supplied a sufficient number of samples and data to be considered in the study; 633 clinical cases were recorded by this date. The distribution of samples within the 8 herds is reported in the Table 3. The 8 herds are located in the Northern part of Italy, which is the area with the highest density of dairy herds, having between 120 and 600 lactating cows. Most of them (5/8) have cows in cubicles, while the remaining 3 herds have cows in loose houses. Grazing is not practised in any of the herds, and seasonal calving is not applied. All of them feed cows with

TMR, and the nutrition status, bedding hygiene and milking hygiene is equal to, or higher than, the average for the area.

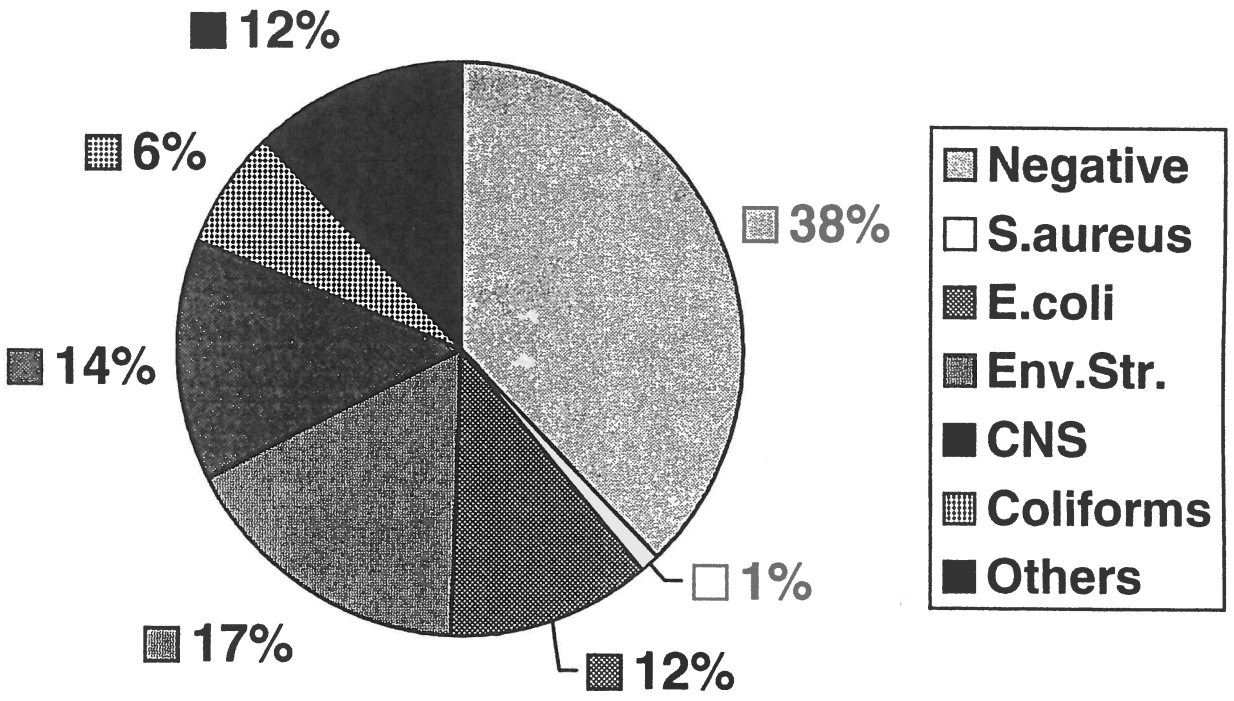

Figure 1. Distribution of aetiological agents isolated in milk samples from clinical cases

Table 3. Distribution of clinical cases within the 8 herds considered

Herd	Clinical Cases N (%)	Herd	Clinical Cases N (%)
A	80 (12.6)	E	104 (16.4)
B	180 (28.4)	F	44 (7.0)
C	15 (2.4)	G	119 (18.8)
D	71 (11.2)	H	20 (3.2)

The aetiology of the clinical cases in described in Fig. 1. Even if a specific technique to improve the recovery of bacteria was applied, 37.9% of the samples were bacteriologically negative. Within the bacteriologically positive samples, environmental *Streptococci* and coliforms represent the most frequently isolated bacteria, while coagulase-negative *Staphylococci* (CNS) were recovered in 13.7% of samples. Table 4 reports the distribution of the different species of *Streptococcus* and *Staphylococcus* isolated. Of the clinical cases collected, 41.2% were classified as mild (only milk alterations), 49.4% as moderate (milk and quarter alterations) and only 9.4% as severe (with systemic signs).

Table 4. Distribution of streptococcal and staphylococcal species

Streptococcal species	Frequency on total *Streptococci* (%)	Staphylococcal species	Frequency on total *Staphylococci* (%)
Aerococcus vir.	0.9	*Micrococcus spp*	14.9
Lact.lactis	7.5	*S.chromogenes*	29.9
Str.dysgalactie	17.8	*S.epidermidis*	13.8
Str.faecalis	10.3	*S.haemolyticus*	5.6
Str.mitis	4.7	*S.sciuri*	8.0
Str.uberis	45.8	*S.simulans*	4.6
Str.species	13.1	*S.warneri*	1.1
		S.xylosus	8.0
		Staph.spp	14.9

The number of lactations influenced the frequency of clinical cases. Indeed, 1st lactation cows showed a frequency of 35.9%, 2nd lactation cows, 25,3%; 3rd lactation cows, 15.5% and cows with more than 3 lactations, 23.3%. This distribution is biased by the cases supplied by herd G which is mainly a 1st lactation-cow herd. If these samples are withdrawn, the relative distribution among lactation is as follows: 1st lactation, 24.5%; 2nd lactation, 28.6%; 3rd lactation, 18.7% and higher than 3 lactations, 28.2%.

The analysis of the distribution of clinical cases by days in milk (DIM) showed that this factor had some influence on occurrence (Fig. 2). The data showed that clinical cases had the highest frequency in the first 15 days after calving (22%); a second smaller peak was observed between 30 and 60 DIM. These results were expected because the risk for clinical mastitis is the highest in the post-calving period (Smith *et al.*, 1985). Less obvious is the peak observed at 150-210 DIM, far from both post-calving period and peak-yield.

The distribution of clinical cases by month of the year comprises only the period February-November because the study started in February, and data for November were the last that were available for analysis. The distribution is reported in Figure 3 and showed that the period with the highest frequency of clinical cases is between April (10,1%) and July (8.6%) with a peak in May (18.95). This is different from other studies, indicating that the summer period had the highest frequency of clinical cases. However, the increasing temperature and humidity normally occurring in the period April-June in the area can easily explain the highest frequency of clinical cases during these months. Moreover, this season requires extra labour in the fields, taking workers and attention away from herd management.

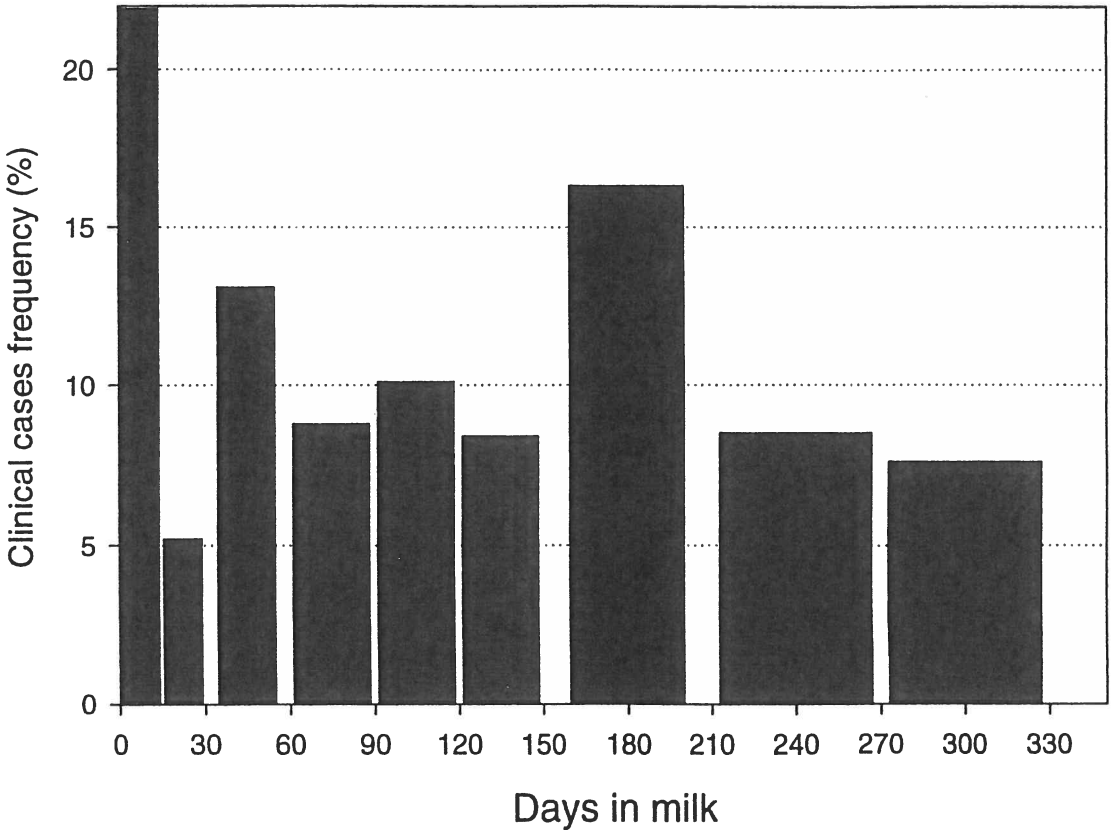

Figure 2. Distribution of clinical cases by days in milk

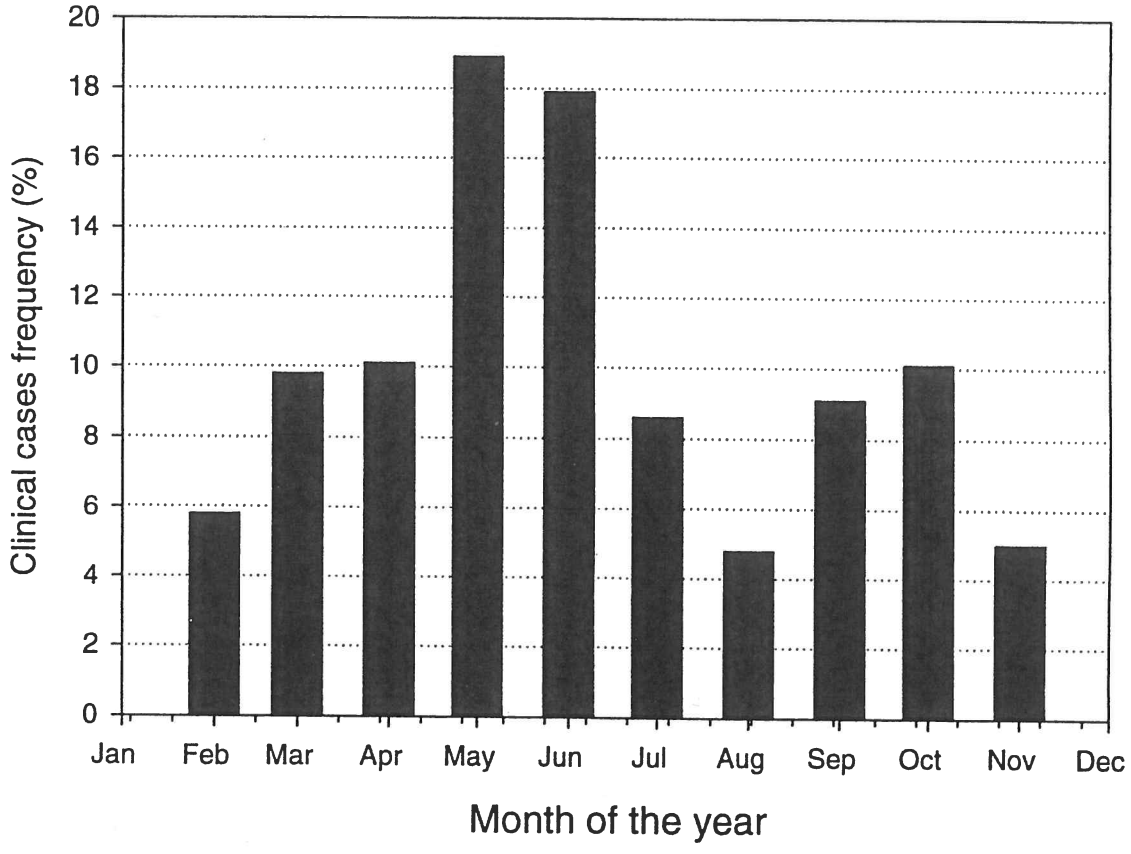

Figure 3. Distribution of clinical cases by the month of the year

The different bacteria caused clinical mastitis with different characteristics. Nearly half of streptococcal cases were mild, while the other half were moderate. On the contrary, *E.coli* cases were mainly moderate (57.14%) or severe (21.43%). Most of coliform and CNS cases were mild, (51.28% and 50.62%, respectively) while bacteriologically-negative cases were mainly moderate (49.77%).

The aetiology of clinical mastitis was also associated with the number of lactations. Coagulase-negative *Staphylococci* were strongly associated with 1st lactation cows (59.52%), bacteriologically-negative and *E.coli* cases also had the highest frequency in young cows: 36.21% and 34.25%, respectively. For all other bacteria the cases were more evenly distributed within lactation, even if environmental *Streptococci* cases had the highest frequency in older cows (31.07%). The results of bacteriological analysis were stratified by DIM (Fig.4). Coagulase negative *Staphylococci* were the most frequently isolated pathogens in the first 15 d of lactation and in the period immediately after the peak of lactation (60-90 d). Environmental *Streptococci* were prevalent at 15-30 DIM and at 90-120 DIM. *E.coli*-affected and bacteriologically-negative animals reached their peak at 15-30 DIM and in the middle of lactation (150-210 DIM). Nearly 40% of the cases observed immediately after calving were classified as severe, independent of the aetiological agent.

The distribution of clinical cases within herds showed some differences (Fig.5). Bacteriologically-negative cases were the most frequently reported cases in herd A (57.5%) in herd D (nearly 50%) . *E.coli* is the aetiological agent with the highest frequency in herd C (26.7%), while environmental *Streptococci* were the most frequently isolates in herd E (21.1%) and in herd F (29.6%). In this last herd, *E.coli* reached a frequency close to the one observed for environmental *Streptococci* (25%). Finally, CNS were the most frequently isolated pathogens in herd G (27.7%).

Clinical cases' frequency during lactation showed some differences when data were stratified by herd. Indeed, herd C had most of the cases in the first 15 d after calving (53.3%). All the other herds, out of herd B and H, had a relative high frequency of cases in the first 15 d after calving (range 15.8-43.7%). Instead, herd B showed the highest frequency (17.8%) in the period 150-210 DIM. Herd H showed the highest frequency in the period 90-120 DIM. Moreover, in 4 herds (A, D, E, H) the lactation period between 150 and 210 DIM had the 2nd highest frequency of cases.

The distribution of clinical cases within lactations in the different herds showed a clear pattern: 4 herds having the highest frequency of cases in the 1st lactation (C,D,F,G); 3 herds having it in the 2nd lactation (A,B,H) and only herd E showing the highest frequency of clinical cases in the cows with more than 3 lactations (Fig. 6).

Monitoring and "on line" measurements

The sensitivity of the "on line" measurements during milking of 3 parameters; milk yield, conductivity and milking duration, were assessed by collecting and analysing all the data regarding these parameters, starting 14 days before the occurrence of the clinical case. For each measurement, the average of 20 milkings (from day 14 to day 4) and the average of 8 milkings (from day –8 to day-4) were calculated. Then, values recorded in the following milkings for each milked cow were compared with these averages and the changes [100* (actual value /average)] computed.

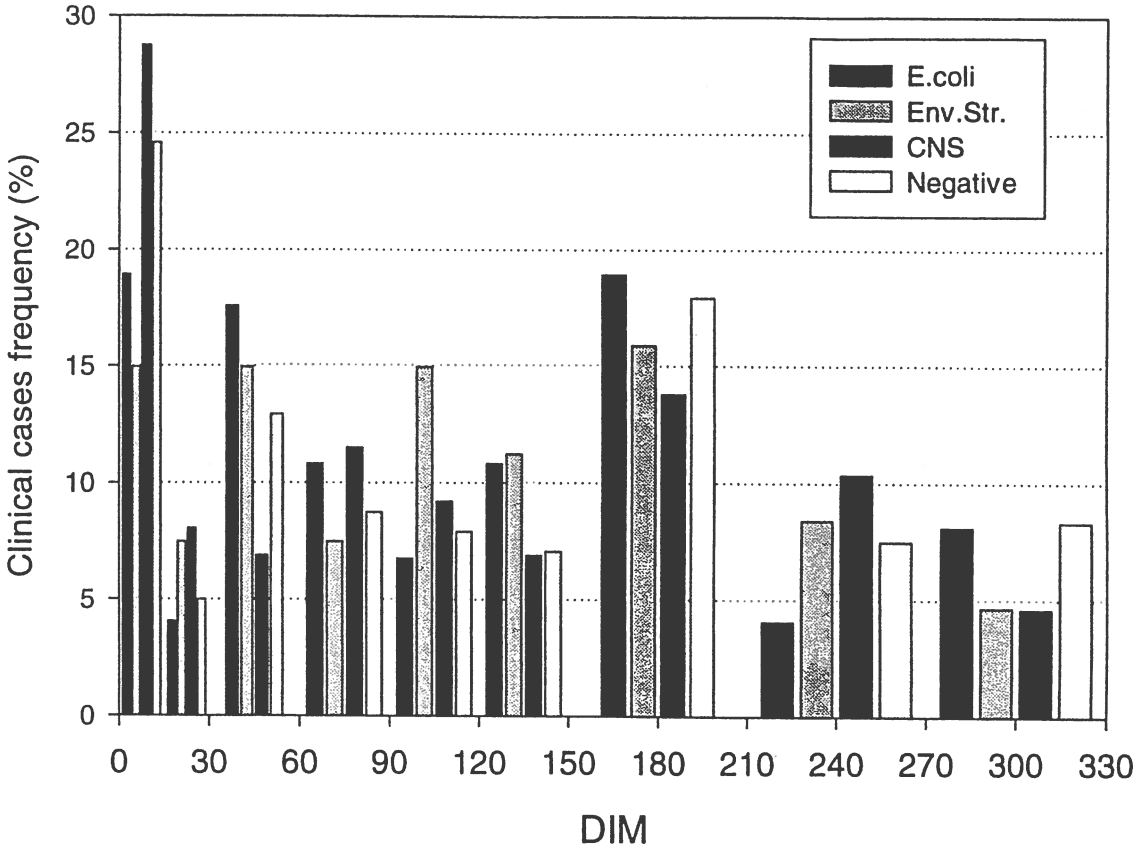

Figure 4. Distribution of aetiological agents by days in milk

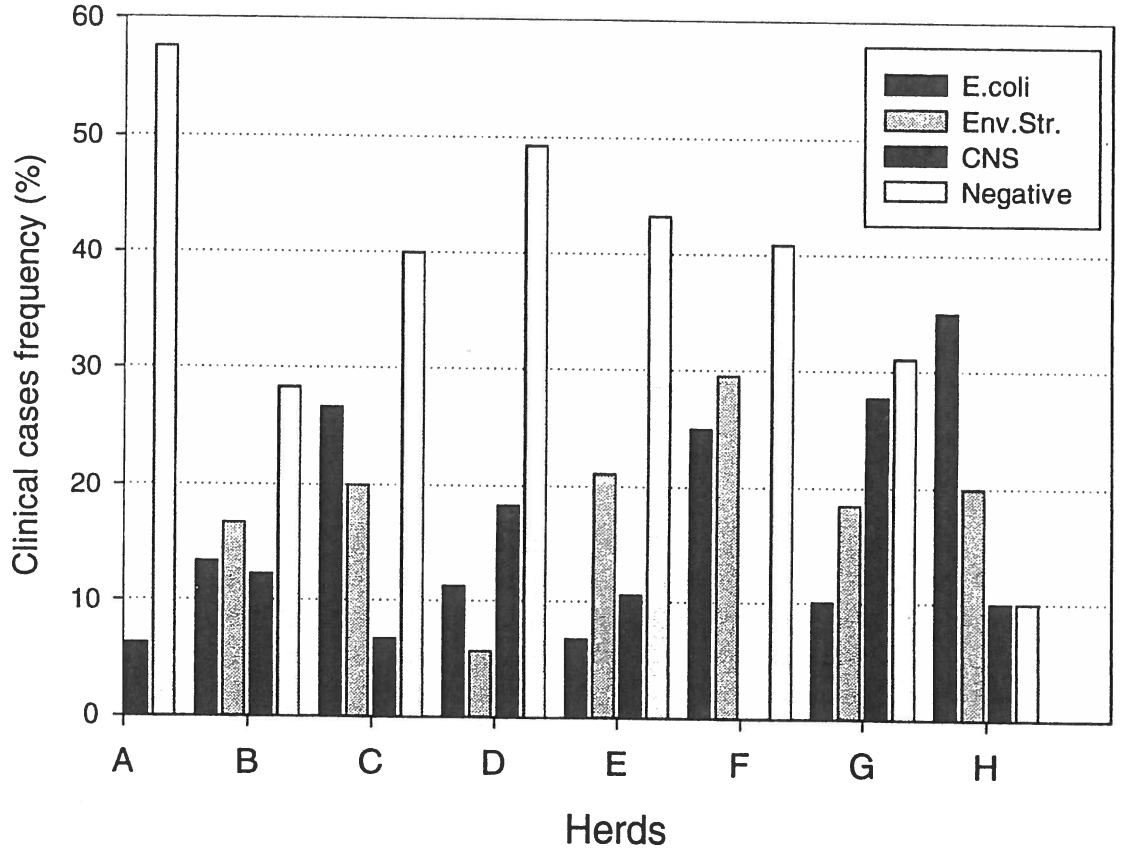

Figure 5. Distribution of aetiological agents by herd

A preliminary analysis showed that 10 days-average had a low sensitivity (data not shown), while the comparisons with 4 days average were more promising. The data were further stratified by severity of clinical cases in 2 groups (mild cases and moderate-severe cases). The dynamics of the changes of the 3 parameters considered in the two groups are reported in Figures. 7 and 8.

Data were analysed by the GLM procedure for repeated measurements and the results showed that the changes in the measurements observed starting at day 1 from the clinical case occurrence were significantly different from the 4-days average. Therefore 3 different threshold values were selected to define cow at risk. Practically, cows showing a change in one of the parameters higher then the threshold (for conductivity and milking duration) and below the threshold for milk yield were considered at risk. The odds ratio of a cow that will have clinical mastitis in the next 1 and 2 days was then calculated for the whole batch of data. The results are reported in Table 4..

Table 4. Odds ratio for clinical mastitis occurrence when the thresholds for "on line" measurements are exceeded.

On line Parameter	Threshold	-2 days			-1 day		
		Odds ratio	Conf.lim. 95%	P<	Odds ratio	Conf.lim. 95%	P<
Milk Yield	<95%	1.32	0.87-1.97	0.17	2.07	1.37-3.14	0.01
Condu-ctivity	>105%	1.53	0.99-2.37	0.05	2.38	1.59-3.56	0.01
Milking duration	>110%	1.17	0.76-1.82	0.47	1.29	0.84-1.98	0.24

DISCUSSION

This paper reports the preliminary results of a field study aimed at developing a control programme for clinical mastitis following HACCP principles. The goal of the study implied a knowledge of the epidemiology of clinical mastitis in Italian dairy herds. This knowledge was missing because no study had been undettaken hitherto. It could be argued that information from other countries could be used, but this would assume that most of risk factors and their distribution were similar in Italy. Therefore, to avoid building a programme on a weak foundation, we decided to start by collecting the basic epidemiological information on clinical mastitis in Italian dairy herds. This step was fundamental to properly identifying the most important critical control points and risk factors involved.

The preliminary results of this study revealed that the epidemiological pattern of clinical mastitis in Italy shows some differences with analogous studies undertaken in other countries. Indeed, the number of bacteriologically-negative samples was larger than in many other studies (Keefe & Leslie, 1997), even if an augmented technique was applied. *E.coli*, coliforms and environmental *Streptococci* were the most frequently isolated pathogens, with a frequency of about 18% each; minor pathogens such as CNS were isolated from 14% of clinical cases. Of the different bacteria, *E.coli* alone represented 6% of the isolates, but the most frequently isolated pathogen was *Str.uberis*, recovered in about 8% of samples, while one third of the CNS were *S.chromogenes*. Coagulase-negative *Staphylococci* were mainly recovered from primiparous cows, suggesting that

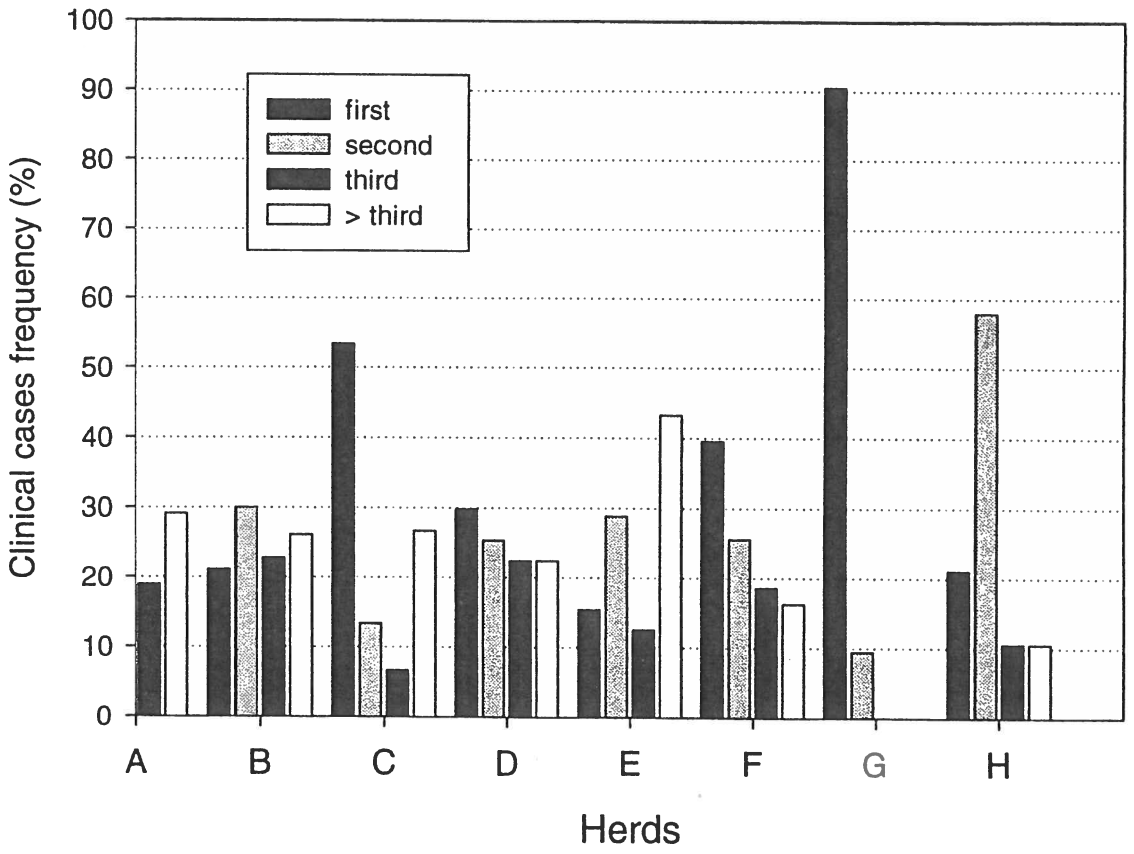

Figure 6. Distribution of clinical cases by herd and by number of lactations.

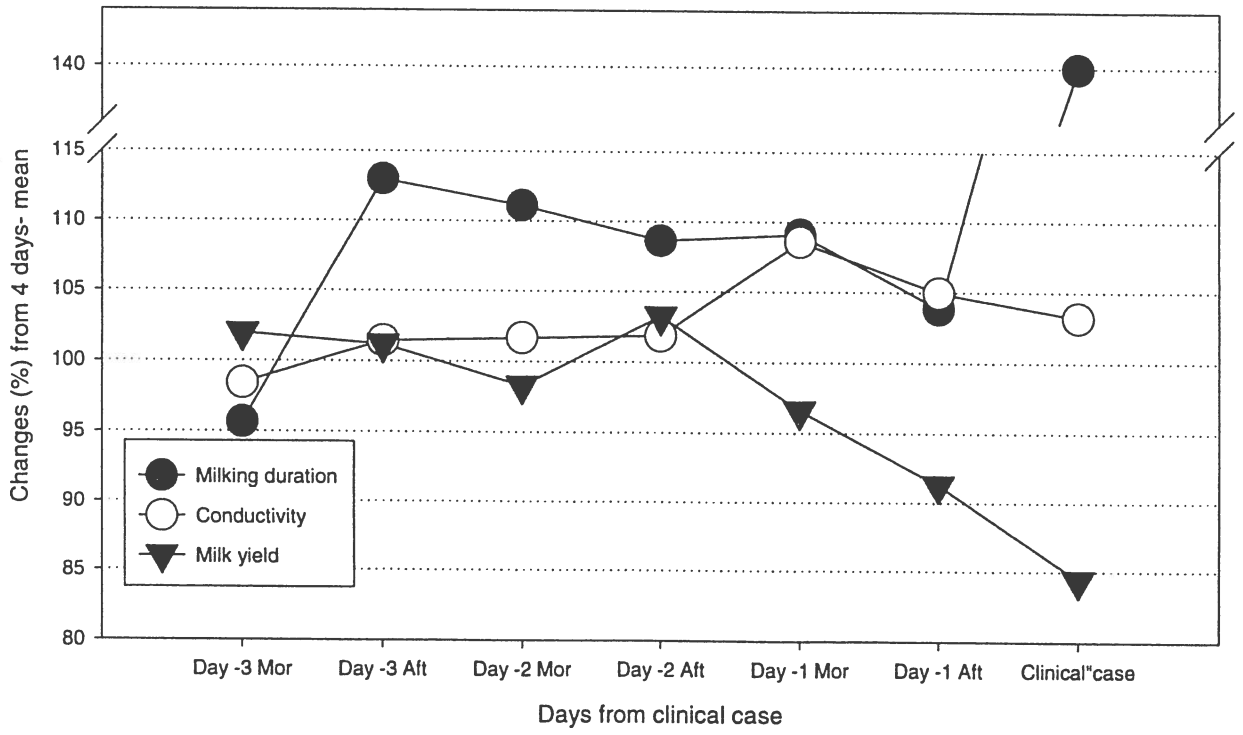

Figure 7. Dynamics of the changes of three "on line" parameters (milking duration, conductivity and milk yield) from the respective 4 days-means in mild clinical cases

milking procedures and the milking machine could play a major role as risk factors for those animals (Zecconi *et al.*, 1996) and therefore represent critical control points. The importance of this CCP was confirmed by the highest frequency of CNS recovered in the first days after calving, when cows return to milk, after drying-off.

Clinical mastitis had a similar distribution throughout lactations; the lowest rate was observed in cows with 3 lactations (19%). Within the lactation period, two periods showed the highest frequency: immediately after calving, and between 150 and 210 DIM. If the first result was expected, the second one was more surprising. This suggests that factors, other than calving and peak yield, could influence clinical mastitis risk within the lactation period.

The distribution of clinical cases within the months of the year also showed some differences with analogous studies. The highest frequency of clinical mastitis was observed during the period May-June, and not during summer, as generally reported (Smith, *et al.*, 1985; Hogan, *et al.*, 1989). This result suggests that one of the most important critical control points will be hygiene management during spring.

Coliforms and *E.coli*, unexpectedly, were isolated more in the median part of lactation than after calving. The large number of bacteriologically-negative samples could bias this result, if we assume that most of those samples came from clinical cases caused by Gram negative bacteria. However, the increased recovery rate of coliforms in the median part of lactation is close to the prevalence of environmental *Streptococci* during the same lactational period and could have the same origin.

When the analysis focused on herds, more differences emerged. A different aetiological pattern could be identified in each herd. The distribution of cases within the lactation showed also differences, when the herd was considered. These results suggest that a control programme had to include procedures to identify herd-related critical control points.

One of the most critical points in the development of a control programme based on HACCP is the need for a monitoring system. Microbiological testing of quarter milk samples probably is one of the best monitoring systems that we can apply for this aim. However, sampling and analysis of milk on an individual basis is a costly and time-consuming procedure that makes it unfeasible in field conditions. To try to solve this problem, "on line" measurements as a tool to monitor clinical mastitis occurrence were evaluated. These automated systems give "on line" data at each milking for every milked cow. When a reference mean can be calculated for a short period of time, this study showed that cows at risk (of developing clinical mastitis) can be identified and then the appropriate veterinary procedures applied.

The missing information on the epidemiology of clinical mastitis in Italy obliged us to reverse the development of a HACCP-related control programme, starting from the end. When the study is completed, and will include a complete year's data, a more detailed epidemiological analysis will be performed and the different CCPs and related risk factors will be identified. This will allow us to develop and apply the programme, this time starting from the beginning and fulfilling the requirements of the HACCP principles.

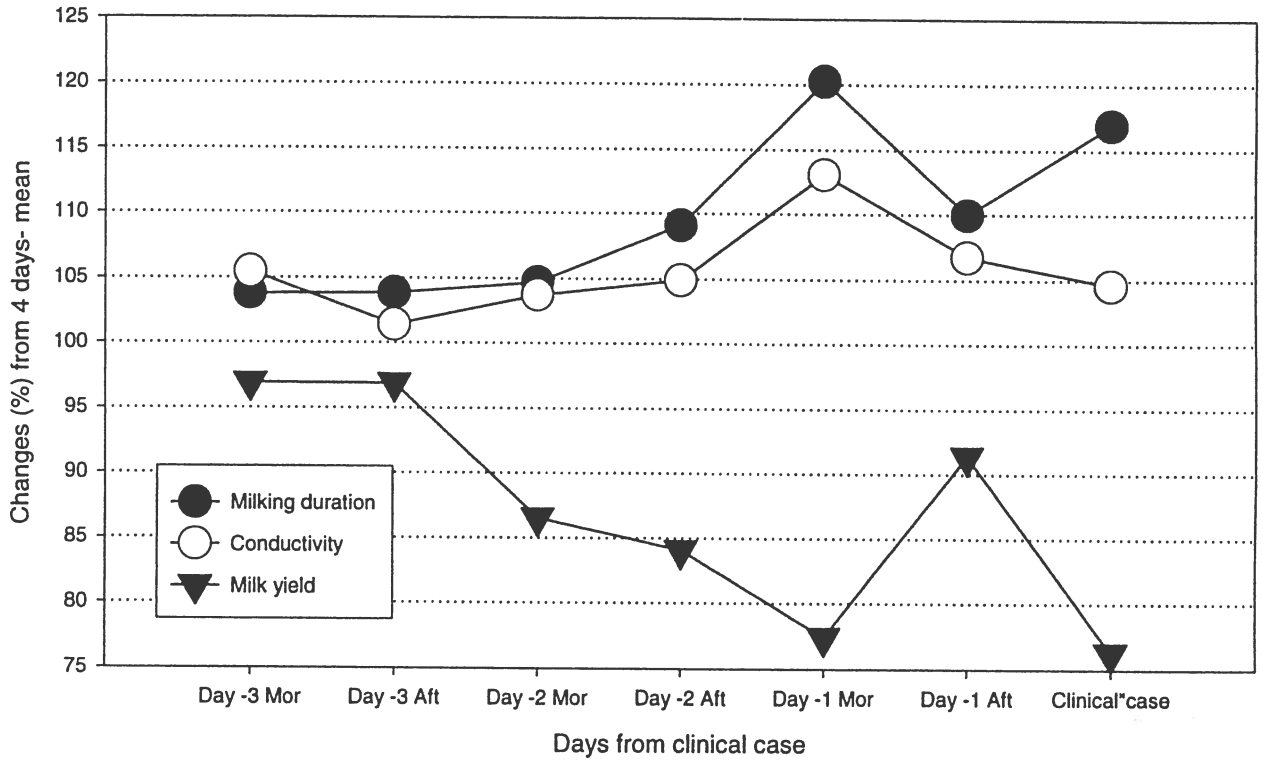

Figure 8. Dynamics of the changes of three "on line" parameters (milking duration, conductivity and milk yield) from the respective 4 days-means in moderate-severe clinical cases

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PREVENTIVE MEDICINE PRACTICE AND QUALITY ASSURANCE THROUGH HACCP

J.P.T.M. NOORDHUIZEN[1]

Veterinary herd health and production management (HHPM) programmes for dairy farms have been developed in Europe over the past two decades, starting off with herd fertility schemes in the 70's and developing into integrated multidisciplinary management support programmes in the 80's (Esslemont, 1975; de Kruif, 1975; Brand et al., 1996). The latter were largely focussed on the reduction of operational costs and/or an increase in farm productivity in order to optimize farm income. Since diseases, such as mastitis and lameness, and reproductive disorders, such as failure to conceive, cause economic losses on the one hand, and the basis for optimizing farm profits is in health care and through adequate nutrition and production monitoring on the other hand, these were in particular the areas where the veterinary HHPM-consultant became active. Such activities have proven to be economically profitable for the average dairy farmer (Sol et al., 1984).

In recent years, attitudes of the general public have had an increasing impact on the animal production sectors. The first element regards food safety issues and public health in general – exacerbated by several disasters such as those caused by *S.enteritidis*, *E.coli* $O_{157}H_7$ *and BSE.* The general public, as represented by the consumer and public opinion - or rather the large retailers - requires high-quality food products of animal origin. Quality, moreover, is then defined in its broadest sense: it refers not only to the products but also to the production methods applied on the farm. Issues such as animal welfare and environmental aspects then become important.

In The Netherlands, dairy farms have had to participate in a dairy quality assurance programme (KKM) on a compulsory basis since January 1st 2000. This quality assurance programme consists of 6 modules: antimicrobial medicine use, cleaning and disinfection, health and welfare, milk harvesting and storage, environmental issues, and feed and water management. Practising veterinarians, too, have to comply with the regulations set by this programme in order to gain access to these farms. The question can be raised whether the veterinarian has a further role to play in this context (if any), and if so, how this role should be played.

In this contribution, the emphasis is on integrating a conventional HHPM with a quality control concept, in order to provide the veterinary dairy practitioner with the tools to play a role as veterinary quality consultant.

HERD HEALTH AND PRODUCTION MANAGEMENT (HHPM) PROGRAMME

A core element in the HHPM programme is the so-called *protocol*, a standard operational procedure to conduct the HHPM activities along given pathways (see Fig.1, Brand et al., 1996/1997).

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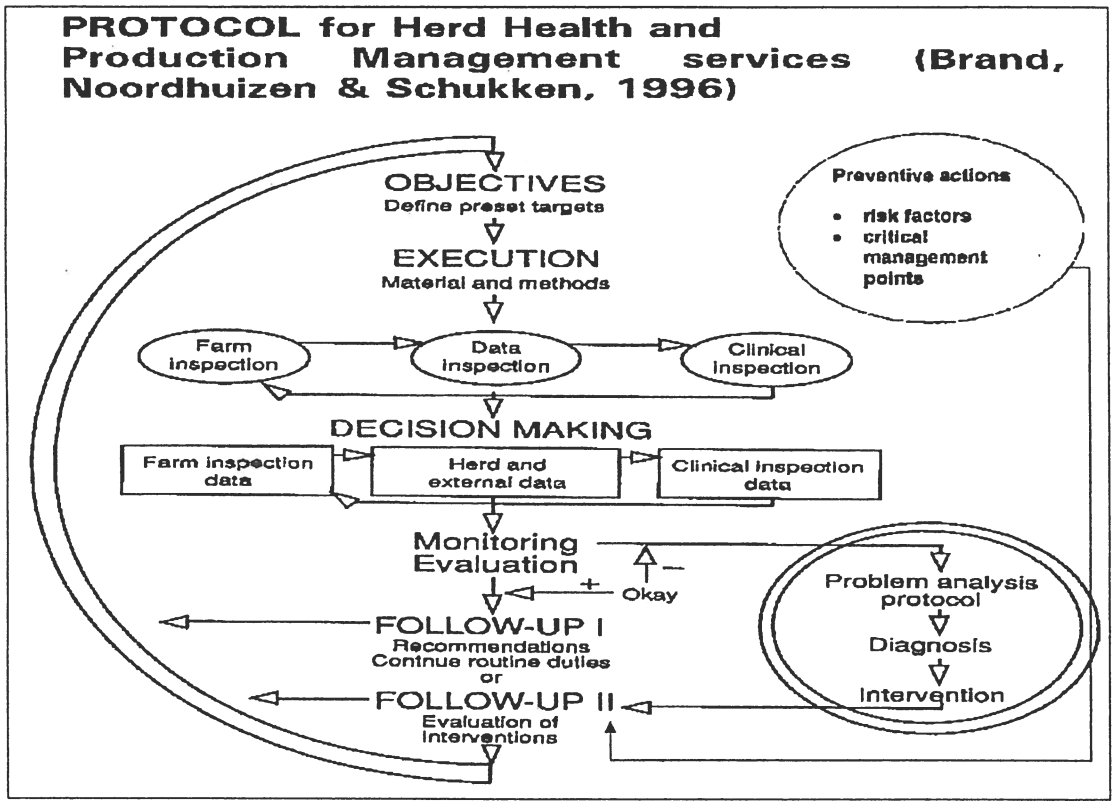

Fig. 1 Standard operational procedure to conduct the HHPM activities: routine monitoring, analysis and prevention (" the protocol")

Crucial elements in a HHPM programme are the *routine procedures*, the *analysis procedures* and the *preventive procedures*. Each of these procedures covers the areas of clinical diagnostic and laboratory examination (animal/herd related issues), farm management and animal environment (namely risk indicators), and a cluster of external factors or conditions.

Clinical examination refers to the routine monitoring of reproduction of cows through, for example,. rectal palpation, and to the monitoring of body condition, teat-end callosity, and weight assessment in young stock. These activities are meant to detect deviations from reference values in time, and hence, to prevent impending problems at an early stage. Laboratory investigations are used to aid of such monitoring.

The area of farm management and animal environment refers to the identification of potential risk factors at the farm, contributing to the occurrence of disorders in the animals. Examples are housing, feeding, climate, milking conditions. The simplest form is farm inspection: routine monitoring of the respective management and farm conditions which – generally speaking – are

known to contribute to disease occurrence. An example of routine monitoring elements is given in Table 1.

Table 1. An example of routine monitoring elements in a HHPM programme

Clinical elements	Environmental elements	Data inspection
Body condition scoring	Housing of animals	Milk recording
Claw scoring	Climatic conditions	Milk quality
Teat-end condition scoring	Feed bunk management	Performance data
Rumen fill scoring	Hygiene procedures	Roughage analysis
Faeces quality scoring	Pasture management	
Reproductive tract	Surface water quality	AI data
Young stock grows	Soil analysis	List of sires

In its broadest sense, monitoring refers to the outcome of, for example,. a veterinary practice survey, conducted according to epidemiological rules and yielding identified risk indicators and quantified contributions (e.g. odds ratios, attributable fractions). With such a "risk profile" the farm can be screened for specific risks, and risk management plans can be drawn. Examples of risk profiles can be found in Frankena et al. (1993), Willeberg (1980), Beaudeau et al. (1994), van Dorp et al. (1999) for different disorders.

The last step in the routine application of the HHPM programme is to re-allocate the respective animal-, herd- and environment-related risk factors to the place on the farm where they exert their effect. These factors provide both the veterinarian and the farmer with priorities and specifications for further action such as intervention and management adjustment. In case of problems, the analysis procedure in the respective farm area will be invoked. Herd performance figures will be broken down into group and animal figures to identify the problem, while conditions on the farm are screened for a possible causal relationship with the problem. A preventive action plan can be drawn when specific risk conditions are known; prevention then is largely based on disease risk management.

HHPM programmes, as described briefly above, are centered around frequent and routinely planned farm visits (e.g. once every fortnight or month for a farm of 8o cows). Each visit concludes with a farm visit report, stating the current herd status, positive/negative trends detected, advice given and follow-up of previous advice. Analysis reports are handed over at a later stage.

THE QUALITY CONTROL CONCEPT OF "HACCP"

HACCP, *Hazard Analysis Critical Control Points*, refers to a quality control concept originating from the food industry and specifically focussing on microbiological hazards potentially occurring during the production process. It deals with the risks associated with the manufacturing, distribution and use of a particular foodstuff, and the definition of means for risk control. HACCP is recognized by the EU as standard for the prevention of microbiological risks

(EU food hygiene directive 93/43/EEC). Publications about application of HACCP at farm level (Bender, 1994; Cullor, 1995) are scarce and often only qualitative in nature.

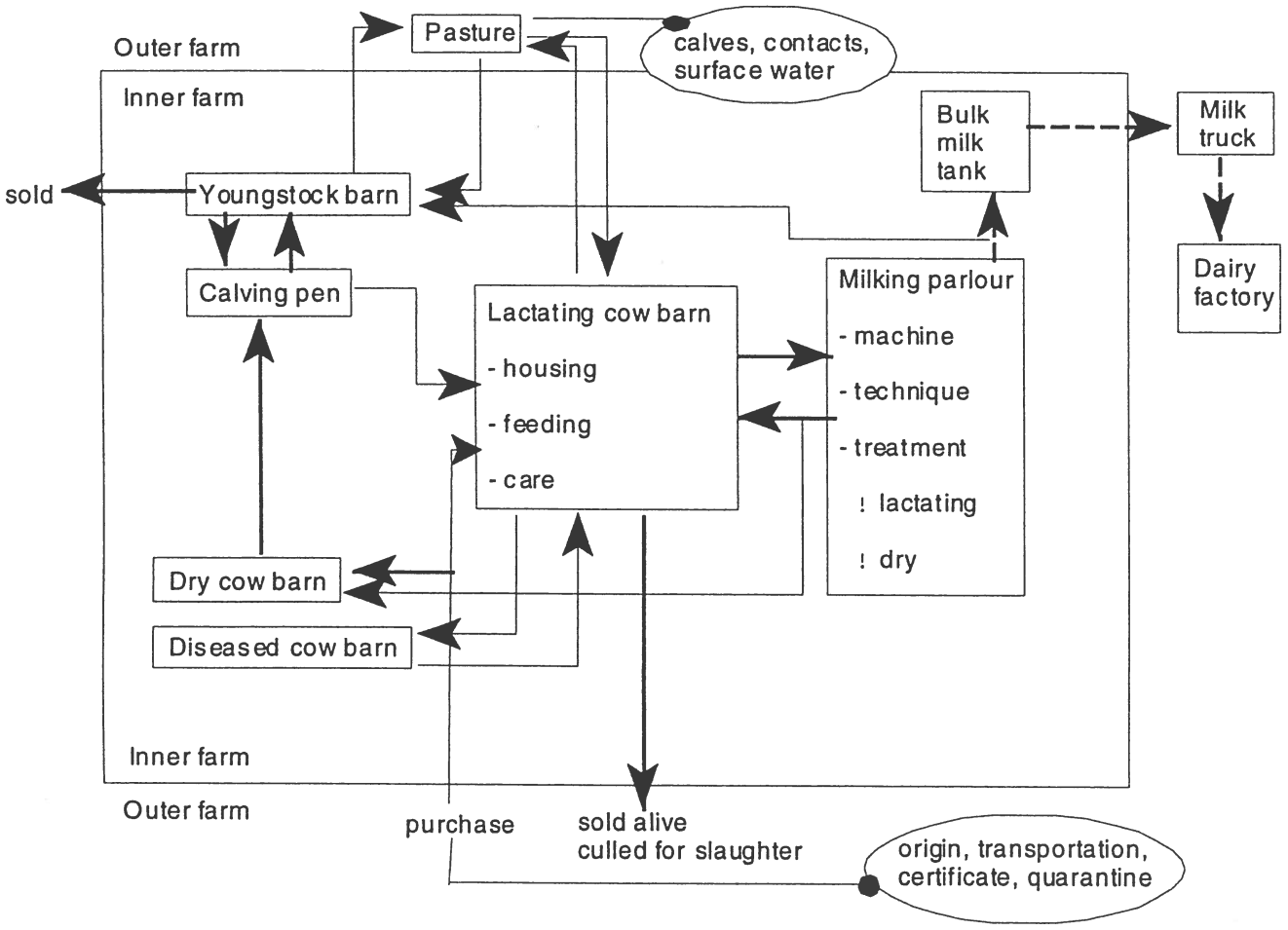

Fig. 2 Example of a decompositional diagram of the dairy production process.
Some risk conditions are mentioned in the ovals.

It has been stated earlier that HACCP could be worthwhile for managing and controlling risks of animal diseases at farm level (Noordhuizen and Welpelo, 1996). These authors have listed the characteristics of this concept in comparison to features of Good Manufacturing Practice codes and ISO-9000 specifications. The farm specificity, low labour requirements, limited documentation, simplicity, monitoring of critical control points and the demonstrable nature of health status are core elements in the HACCP concept. One of the first steps in risk management planning is the drawing of a process decomposition diagram: the dairy farm is "broken down" into different "production steps" (Fig.2).

The basis for applying the concept is hazard identification and the determination of disease risk conditions; this is exactly the area where (quantitative) epidemiology is of direct relevance: risk

identification and quantification of the risk contribution (Thrusfield, 1995). The latter parameters can be derived not only from epidemiological studies but also from methods such as adaptive conjoint analysis (Horst et al., 1995; Noordhuizen et al., 1997; Vos et al., 1999).

A core element in HACCP plans is the network of *critical control points* (CCP): sites in the farming process associated with the hazard, where deviations may occur, where measuring can take place with respect to standards and tolerance levels, and where measures are available to adjust such deviations for re-establishing control.

An example is the water temperature for cleaning the milking machine, usually set at 80 °C (standard) with 2 °C tolerance. On the dairy farm, there also will be *critical management points,* (CMP) because there are biological components (i.e. the cows) for which standards or tolerance levels are not always known or defined. A true critical control point, as defined in HACCP, then cannot be determined and a management point or attention point is given. Quality assurance is the art and science of establishing a network of CCPs and CMPs on the dairy farm to provide the farmer – and his veterinarian – with appropriate means for disease risk management and control.

HHPM AND HACCP INTEGRATED INTO ONE CONCEPT

Looking at HACCP, it seems most appropriate and promising to integrate it with HHPM. First, there is an area of common interest: disease risk management; secondly, it provides the practitioner with a further means of focussing on herd monitoring, analysis and prevention, instead of veterinary-technical procedures alone. The latter has been an issue of criticism by Dutch dairy farmers about HHPM support: there is currently too much emphasis by veterinarians on rectal palpations, disease cure, vaccination etc (Lievaart and Noordhuizen, 1999). Moreover, analysis of problems after they have occurred is, by its very nature, retrospective.

In order to illustrate that HACCP can be integrated into a HHPM programme, we give the example of Johne's disease (*M. paratuberculosis*). HACCP in this case refers to both the prevention of introduction of the disease onto the farm, as well as spread of the disease within and between farms.

By means of adaptive conjoint analysis (data unpublished) the following lists of prime risk factors was identified and ordered according to importance of expected impact for both introduction (Table 2) and spread of the disease (Table 3).

Tables 2 and 3 comprise critical management and control points which need to be addressed every time they become relevant. This means that cattle should not be purchased at all, or only from herds with a disease-free certificate. Every time that young stock are put to pasture, it should be clear beforehand that no other cattle have just left that plot (CMP). If there are other farms around, drinking water in pasture plots should be provided with tanks to prevent calves from drinking surface water (CMP). Cattle returning from the market should not join the herd unless testing has proved freedom from disease(*CCP*).

Young stock should not be put to pasture together with sick cows (CMP). Separation in housing should be done appropriately, that is, by full physical separation and not by fences only (CMP). Culling should be regarded as a proper management tool to lower the disease level in the herd (CMP). Drinking troughs should be kept free from contamination and checked routinely (*CCP*).

Cows sent to the market or an export collection site should be tested beforehand (*CCP*). Visitors should change clothes and boots at the entrance of the premises; the farmer should check on the procedure (CMP). If contact with cattle from neighbouring farms is to be prevented, either these farms should be tested or fences should be made such that contact is not possible.

Table 2. Important risk conditions for introducing *M. paratuberculosis* onto a dairy farm (Vos et al., 1999 unpublished)
The median utility only represents the relative qualitative importance in this sequence.

Risk indicator	Median utility
1. Purchase of cattle from a farm with status unknown	113
2. Young stock with access to grazed pasture	91
3. Supply of manure from farm + young stock grazing	89
4. Cattle of all ages with contacts to feral ruminants	46
5. Cattle returning from markets or collection sites	45
6. Young stock drinking from rural surface water	44
7. Own cattle transported with cattle from other herds	31
8. Cattle returning from export collection site	23
9. Visitors without proper hygiene measures	22
10. Young stock drinking surface water, no farms around	7

Table 3. Important risk indicators for the transmission of *M.paratuberculosis* on and between dairy farms (Vos et al., 1999 unpublished)
The median utility only represents the relative qualitative importance in this sequence

Risk indicator	Median utility
1. Cattle and young stock housed in one group	64
2. Culling is not common	62
3. Young stock grazing with sick cows	62
4. Calves are fed fresh cow milk or penicillin milk	58
5. Calving takes place without hygienic measures	53
6. Clinical cases are not tested nor culled	48
7. Calving pen is also used as sick cow pen	48
8. Young stock grazes with sheep or goats	47
9. No calving pen available	44
10. Cows in overcrowded loose house with manure slide	44
11. Calves are fed colostrum of own mother	42
12. Faecal contamination of drinking troughs not prevented	39
13. Young stock grazing on plots of farms with unknown status	37
14. Cattle is moved between pens on the farm	33
15. Housing calves in groups not truly separated	26
16. Cows in loose house	26
17. Cows in loose house, on slatted floor, overcrowded	26
18. Same tools are or equipment is used all over the farm	20

Most of these aspects are *critical management points, CMPs,* while some are *critical control points, CCPs.* Within the HACCP concept, the different critical points are assigned to the respective steps in the production process where they particularly apply (see Fig.2). Hence, they form a monitoring network over the various production process steps on the farm.

For other diseases the same CMPs (e.g. purchase policy, hygienic procedures, animal separation, manure handling) are applicable as found here. This means that a dairy farmer must obtain a proper awareness of what is happening on his farm and who is doing what. The farmer's day-to-day activities must be made transparent.

Looking to the elements listed in Tables 2 and 3, it is obvious that these fit well in a herd health and production management programme. The main reason is that in HHPM the routine monitoring of many farming aspects is a basic characteristic. Monitoring the CCPs or CMPs of a quality assurance programme on the farm and the monitoring of the animal-related and farm-environment related aspects in a HHPM should therefore be integrated for the benefit of the farmer.

DISCUSSION

It has been stated by Ryan (1997) that "applying HACCP may seem unwieldy but is nothing else than the formalization and structuring of what a truly good farmer is doing anyway". That is why this concept fits so well in veterinary herd health and production management advisory work. Integrating the HHPM and the HACCP concept in situations where quality assurance is required from the dairy farmer, such as currently in The Netherlands, seems therefore quite appropriate for the benefit of both the farmer and the veterinarian. In that situation, the veterinarian has to adopt "quality-focussed thinking" and has to be trained in quality control concepts and methodologies. The Dutch dairy farmers have recently reported that they expect vets this type of approach from their veterinarians (Lievaart and Noordhuizen, 1999).

The number of CCPs should be limited in order to retain the overview on the farm; the CMPs are more numerous but can be spread over time to make monitoring easier. The whole network of CCPs and CMPs depends on the diseases considered to be of relevance on the farm, or, in other words, the level of health promotion the farmer desires to attain.

Next to diseases such as *M. paratuberculosis*, disorders like mastitis and lameness are prominent. Epidemiological studies of such disorders yielding risk factors may prove helpful in determining critical points in the production process on the dairy farm. Most critical control points, however, have still to be developed and validated.

Implementation of HACCP on dairy farms requires a "quality attitude" from the farmers, meaning that they are already addressing quality as a farming issue and have made it part of their farming style. On that sound basis, further quality control concepts can be founded. Dairy farmers already participating in a HHPM programme could possibly be better prepared for that action because they know how to keep records, they are used to receiving technical advice, and are regularly confronted with results of farm analysis.

Ultimately, by applying the proposed HACCP concept, the farmer will be able to demonstrate to third parties the herd health status on his farm and the activities undertaken to optimise this status. The latter could be beneficial when, for example, risk insurance becomes relevant and when the public image of the dairy sector is addressed.

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VACCINATION

SIMULATION OF TWO DIFFERENT EMERGENCY VACCINATION CAMPAIGNS WITH MARKER VACCINE IN THE CONTROL OF THE DUTCH CSF EPIDEMIC IN 1997/98

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Outbreaks of animal diseases can have severe consequences for animal welfare, livestock production, export of animal products and in some cases, public health and the environment (Murray and McCutcheon, 1999). For the more serious diseases, such as classical swine fever (CSF), outbreaks may also have a significant impact on the national economy of a country in regard to control and eradication of the disease and the possibility of export bans set by other countries. When an outbreak occurs, the need for rapid identification and response mechanisms is of great importance.

In case all other measures to eradicate an outbreak do not succeed, paragraph 14 from the directive 80/217/EEC of the European legislation foresees an emergency vaccination (CEC, 1980). In case of an emergency vaccination, the same directive forces the member state to exclude all meat originating from vaccinated pigs from the regular pork market, except when heat treated. In the last decade some severe outbreaks occurred in the EU, but no emergency vaccination was applied. In 1994 the German proposal for an emergency vaccination was refused by the European Commission (Blaha, 1994). Also during the 1997/98 epidemic in the Netherlands, emergency vaccination was not implemented, despite the fact that a marker vaccine was close to market introduction. Some people required vaccination as an ethic necessity, instead of the killing and destroying of so many healthy piglets and hogs (Jorna, 1997).

The use of a marker vaccine during a CSF outbreak in a high pig density population, seems to be technically feasible given the close market introduction and is requested by different people (Jorna, 1997; Van Oirschot, 1994; Vagsholm, 1996: Leopold, 1994). Of course there should be a companion diagnostic test to serologically distinguish infected pigs from vaccinated pigs. Furthermore, changes in the swine herd structures and the degree of some high-density pig populated areas raise logistic and organisational problems, next to high costs, when applying a stamping-out policy to eradicate an epidemic. Public objection against the destruction of healthy animals increases as well. Those are all factors in favour of an emergency vaccination. On the other hand, there is the aim of the European community policy to have a high health status regarding animal health and public health. To reach this goal, the preventive non-vaccination policy has been chosen for the control and eradication of animal diseases of major importance for international trade. In brief, the use of vaccine "means" the presence of disease (Westergaard, 1996).

With the nearby introduction of marker vaccine against CSF and so the possibility to serologically distinguish infected pigs from vaccinated pigs, emergency vaccination should be discussed again as a possible supplement measure to control an epidemic and overcome some of the above mentioned problems. Epidemiological, political and economic advantages and disadvantages have to be analysed and clarified before being able to decide if marker vaccine is a realistic option. Computer

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simulation can be an adequate tool for the analysis of the epidemiological and economic consequences of different control options, either before an epidemic to be better prepared, or during and after an epidemic to evaluate the situation (Dijkhuizen and Morris, 1997). This paper will use the simulation model InterCSF, which was developed in order to simulate the Dutch CSF epidemic of 1997/98 as close as possible (for details see Jalvingh et al., 1999). InterCSF is a spatial, temporal and stochastic simulation model, such that the outcomes of the various replications give an indication of the variation of size and duration of possible CSF-epidemics. InterCSF was specifically developed to answer what-if questions on the Dutch 1997/98 epidemic. Emergency vaccination was recently added as another disease control mechanisms and is described in this paper. Output parameters of InterCSF were used in EpiLoss (Meuwissen et al., 1999) to calculate the cost and losses of the epidemic.

In this paper two emergency vaccinations strategies with a marker vaccine that could have been applied in the 1997/98 Dutch epidemic are discussed. Those are compared with earlier simulated preventive slaughter strategies, described in Nielen et al. (1999). The impact of small changes in the emergency strategies, the effectiveness of the marker vaccine are further shown and discussed.

MATERIALS AND METHODS

General outline

InterCSF simulates disease spread from day to day from an infected farm through 3 contact types, animals, vehicles and persons, and through local spread up to 1000 m. All Dutch pig farms are known by their coordinates. The main disease control mechanisms that influence the disease spread in InterCSF are, diagnosis of the infected farms, depopulation of infected farms, movement control areas, tracing and preventive culling. Details can be found in Table 1. Emergency vaccination, as another control measure was added and will be described in the following pages.

Emergency vaccination was incorporated in the basis scenario such that it reflected the situation in the real epidemic as closely as possible. This involved that a total of 37 farms with fixed infection date before 4-2-97, - date of first detection -, and fixed suspect, detection and depopulation dates later, were incorporated as historical data. New infections were only simulated after this date. The first outbreak happened in a highly densely populated area and 9 outbreaks were already notified in the first week after the first outbreak. In our simulations, we assumed that after 5 outbreaks in the first week emergency vaccination would start. This means that vaccination was always initiated at the end of the first week. When deciding to start with the emergency vaccination campaign, all earlier detected farms from the last week were looked up. In the basis vaccination scenarios, a vaccination zone with a radius of 3 km was defined and installed for each detected farm. To mimic restricted vaccination capacities, all defined vaccination zones were put on a vaccination list and all further defined vaccination zones were lined up as well. If more than one new vaccination zone was defined on the same day, they were sorted depending on their pig farm density, starting with the highest pig farm density. We further assumed that emergency vaccination would be stopped based on certain criteria, whereby the stop criteria could depend on the vaccination strategy.

For all basis scenarios, we assumed 5 days of preparation before the vaccination could actually start. Vaccination zones are vaccinated one by one, not parallel, by 150 vaccination groups. Each vaccination group (1 veterinarian and 4 helpers) was supposed to handle about 2000 pig places (hogs and young stock) or 465[1] sow places per day or a combination of both.

[1] Assuming 21.5 piglets/sow/year, which is equal to 0.0059 piglets born/sow/day. We assume, that a piglet will stay for 70 day on a sow farm. As all pigs need to be 14 days old when being vaccinated, we will have (0.0059 * 56 days =) 3.30 piglets/sow place and 1 sow/sow place to vaccinate.

Table 1. Chronological order of all control measures, except vaccination related control measures, in case of a new detected CSF outbreak in InterCSF (Jalvingh et al., 1999)

Time	Measure
Day of detection	- Infected farm will be put on slaughter list and destroyed as soon as capacities are available (highest priority) - Movement standstill is imposed on the protection (3 km) and on the surveillance zone (10 km), only a reduced number of person contacts (50%) is allowed. Animal contacts and vehicles contacts are forbidden. - Farms within a certain radius could be subjected to pre-emptive slaughter, limited by rendering capacity. Preventive slaughter could lead to an earlier detection.
Start on 2nd day	- Surveillance (clinical inspection) of all farms in the protection zone (3 km), which may lead to earlier detection. - Tracing farms that had contact with the infected farm. Traced farms are put on surveillance (clinical inspection), which may lead to earlier detection.
Start on day 28th	- Start welfare slaughtering of hogs and piglets in the surveillance zone (3-10 km) until the movement control zone is lifted up. This may lead to earlier detection.
Earliest on day 35th	- Start of serological end-screening of all farms lying in the restricted zone (10 km), if no additional farm was detected during those last 35 days.
Earliest on day 49th a)	- If there is no new detection in a restricted zone, the movement standstill in the surveillance and the protection zone will be lifted up

a) We assumed 49 days instead of 42 days, to mimic a waiting period for the laboratory results

Vaccination effects

With respect to the effect of vaccination, two kinds of infected farms were distinguished in the simulation model. The first category consisted of infected farms that were never vaccinated and of farms, which were first infected and later vaccinated. The second category consisted of farms, that were vaccinated before they became infected.

For the first category of infected farms we assumed no reduction in virus spread. All parameters remained as described in Jalvingh et al. (1999). In short, the infectious period started between 5 and 10 days after infection, based on the assumption that some virus replication has to take place on the farm before it becomes infectious. The infectivity of the farm remained the same for the total infectious period, which ended on the day that the farm was depopulated. The interval between infection and detection was modelled with a single probability distribution, based on observation of the real Dutch CSF epidemic. The selected interval could be influenced downward by certain events, such as welfare slaughter, preventive slaughter, traced contacts, surveillance and end-screening. The time interval between entrance of infection and the event influences the probability of an earlier detection (see table 2). Those detection probabilities of non-vaccinated farms were used as base to estimate the detection probabilities for all vaccinated farms (table 2 and 4). Vaccination as such could also influence detection (table 3).

Farms of the first category could be detected earlier due to clinical inspection at the vaccination day. The detection probability depended on the time since infection and the source of infection (table 3). If the source of infection was a direct infectious animal contact, we assumed a higher probability of detection for the first weeks after infection, than for all other contacts. In case that an infected farm was not detected during vaccination, we assumed that during vaccination the virus was mechanically and massively spread all over the farm. After the incubation time of a week, the large amount of sick

animals could again lead to a possible earlier detection (Tielen, 1999). In both cases we assumed that 2 days after suspicion the diagnosis was established. For all other events, time consuming tests are necessary, so we assumed 7 days after suspicion before diagnosis would be given.

Table 2. Probability of detection based on a control event relative to the time since infection on an (undetected) infected and non-vaccinated (Non) or afterwards vaccinated (Vacc) farm.

Time since infection	Probability of detection by control event (Diagnosis date = + 7 days)									
	Traced contacts		3 km radius		Pre-emptive slaughter		End-screening		Welfare slaughter	
	Non[a]	Vacc[b]	Non	Vacc	Non	Vacc	Non	Vacc	Non	Vacc
0 - 2 weeks	0	.9	0	.9	0	.9	0	.9	0	.9
2 - 4 weeks	1	1	0	1	1	1	.25	1	0	1
4 - 6 weeks	1	1	.25	1	1	1	.50	1	0	1
> 6 weeks	1	1	1	1	1	1	1	1	1	1

[a] As in Jalvingh et al. (1999).
[b] In this case the farms were first infected and afterwards vaccinated.

Table 3. Probability of detection due to vaccination, relative to the time since infection on an (undetected) infected farm and the source of infection.

Time between infection entrance and vaccination	Probability of detection by control event (Diagnosis date = + 2 days)		
	Vaccination day (direct animal contact)	Vaccination day (no direct pig contact)	1 week after vaccination
0 - 2 weeks	.25	.05	.90
2 - 4 weeks	.90	.50	.95
4 - 6 weeks	.99	.90	1
> 6 weeks	.99	.99	1

Table 4. Probability of detection based on a control event relative to the time since infection on an (undetected) vaccinated and afterwards infected farm.

Time since infection	Probability of detection by control event (Diagnosis date = + 7 days)				
	Traced contacts	3 km radius	Pre-emptive slaughter	End-screening	Welfare slaughter
0 - 3 weeks	0	0	0	0	0
3 - 4 weeks	0	0	.5	.5	.5
4 - 6 weeks	0	0	1	1	1
> 6 weeks	0	0	1	1	1

For vaccinated and afterwards infected farms, the second category, we assumed a reduction in virus spread, expressed in a reduction factor. This reduction factor depended on the time interval between vaccination and infection and was modelled with a single probability distribution, based on EU field experiments from spring 1999 (see Annex 1 for more details). The reduction factor was multiplied with the probability of transmission for a simulated contact, respectively for local spread.

For the farms in the second category we assumed no change in the latent period, but the infectious period was reduced to maximal 1 month. On vaccinated farms only small outbreaks are expected, and

as a consequence such farms will only be infectious during a short period. Further we assumed that vaccinated pigs showed none or few clinical signs when infected, so detection could only happen due to serological screening. This results in detection only when a control measure such as welfare slaughter, preventive slaughter and end-screening was applied, whereby the probability of detection increased with the time since infection (table 4).

Susceptible farms were also classified in two categories: non-vaccinated and vaccinated farms. We assumed that a non-vaccinated susceptible farm had no protection against a possible infection, whereas a vaccinated susceptible farm was partly protected. The degree of protection depended on the time interval between vaccination and a possible infection and was expressed as a protection factor, modelled with a single probability distribution (see Annex 1 for more details). Similar to the reduction factor, the protection factor was multiplied with the probability of transmission for a simulated contact, respectively for local spread. However if an infectious pig was moved to a susceptible vaccinated farm, the protection factor was not considered. We assumed that this farm always became infected.

In the EU field experiments horizontal transmission was significantly reduced 3 weeks after vaccination for both marker vaccines. In our basis emergency vaccination scenarios, we assumed that maximal immunity was reached after those 21 days. For sensitivity analysis this time interval was reduced, respectively increased by 5 days to 16 days respectively 26 days (see Annex 1 for the details). In an additional analysis only 1 week was assumed to be needed to build up the maximal protection level.

Delayed destruction alternatives

The first emergency vaccination strategy, called "delayed destruction" (I), assumed no political acceptation of vaccinated pig meat as fresh meat, which is the current EU policy. Vaccination would only be applied to overcome a shortage in destruction capacity, created by regularly applied control measures. With vaccination, the risk of virus spread will be reduced and the destruction of the vaccinated farms can be postponed until destruction capacity is available. In contrast to all earlier simulated scenarios and also to the second emergency vaccination strategy, all pigs on all vaccinated farms needed to be destroyed before the end-screening could be applied to declare the region free again of CSF. All pigs older than 14 days were vaccinated once. We assumed that vaccinated pigs were maximally protected for at least 6 months. If the vaccinated pigs were not slaughtered out within those 6 months, the pigs would be re-vaccinated after 6 months to keep maximal protection in place.

As soon as a vaccination zone was defined, all farms inside this vaccination zone were put on the preventive slaughter list. Priorities were set to deal with the insufficient capacities. First all farms lying in the preventive slaughter radius (1000 m in the basis) and contact farms were slaughtered out. This group was further split up. Farms not predestined for vaccination had the highest priority, followed by farms where vaccination was not yet applied. The lowest priority inside this group was given to already vaccinated farms. The second category consisted of farms lying in the vaccination zone (0-3 km), but outside the preventive slaughter zone (0-1 km). Here again the highest priority was given to non-vaccinated farms, followed by vaccinated farms. As the preventive slaughter is applied from the beginning till the end of the epidemic, the decision criteria to stop with emergency vaccination depended on the delay caused by destruction capacities. The number of farms notified for preventive slaughter, including the vaccinated farms was divided by the daily destruction capacity to receive the number of days needed for destruction. When the delay was ≤ 3 days during a period of 14 days, no new vaccination zones were installed.

In the basis scenario of delayed destruction, we assumed that maximal protection was reached in 21 days, preventive slaughter was applied in a radius of 1000 m, a vaccination radius of 3 km was installed for each detected farm and 5 days of preparation were needed before the start of the

emergency vaccination campaign. In alternative scenarios, we assumed that time to build maximal protection was reached in 1 week, 16 days or 26 days. In further sensitivity analysis various input parameters were changed to analyse their effect on the outcomes of the simulation. The time needed to prepare the emergency vaccination campaign was such an input parameter. In alternative scenarios the time needed to prepare the emergency vaccination campaign was reduced, respectively increased from 5 days to 2 days, respectively to 8 days.

Intra community trade alternatives

For strategy II, the intra-community trade strategy, we assumed that after lifting up the surveillance zone, pig meat originating from vaccinated pigs could be sold on the intra community market. After the movement standstill was lifted, a so-called post-vaccination zone was installed on a vaccination zone. This post-vaccination zone was imposed for 4 months. All movements were allowed, except that live pigs could only leave this zone to be directly slaughtered in specific slaughterhouses. This supplement measure should convince all trading partners that no live carrier piglets could leave the vaccination zone alive to spread the disease. Emergency vaccination was stopped when there were less than 2 new outbreaks during the past 4 weeks.

In strategy II, all pigs older than 2 weeks in a vaccination zone (0-3 km) were vaccinated similar to strategy I. In addition we assumed for the duration of the surveillance in a vaccination zone that all newborn piglets and also all farrowing sows would be re-vaccinated. These measures would assure a maximally protected pig population. A further assumption was that maximal protection of vaccinated farms would last for the total duration of a post-vaccination zone. After the lifting up of the post-vaccination zone all farms lost their protection.

In case of intra-community trade strategy, vaccination will reduce the susceptible pig population, so preventive slaughter is optional and not applied in the basis scenario. If preventive slaughter is applied, only non-vaccinated farms will be destroyed.

For the basis scenario, we assumed that maximal protection was reached in 21 days, no preventive slaughter was applied, a vaccination radius of 3 km was installed for each detected farm and 5 days of preparation were needed before the start of the emergency vaccination campaign. Alternative scenarios were simulated for sensitivity analysis, similar to strategy I.

SIMULATION RESULTS

Nielen et al (1999) compared the real 1997/98 CSF epidemic with various alternative eradication strategies, all simulated with the InterCSF model (Jalvingh et al., 1999). All simulated strategies, including the emergency vaccination strategies were performed 100 times, whereby the simulation time was set at maximal 1 year. The most effective scenario according to Nielen et al. (1999) was with preventive slaughter in a radius of 1 km from the day of the first detection. Complete results were shown in Nielen et al. (1999) and are partly shown in tables 5 and 6. In the real epidemic preventive slaughter of the neighbouring farms was only applied for the first 2 detected farms, stopped and applied again from 10-4-97 onwards for all newly detected farms (LNV, 1998). Table 5 recalls the most important key features of the real epidemic, the simulated epidemic and the preventive slaughter scenario. We chose the preventive slaughter scenario as basis to compare all emergency vaccination strategies. In a future epidemic preventive slaughter would most likely be applied from the beginning, making this scenario the best comparison for our simulated emergency vaccination scenarios.

Table 5. Some key features of the real 1997/98 Dutch CSF epidemic, the median of the simulated epidemic and the median of the preventive slaughter scenario (Jalvingh et al., Nielen et al., 1999).

Real epidemic or simulated scenario	Key features (median for simulations)			
	# Detected farms	# Infected farms	# Preventive slaughtered farms	Duration of the epidemic (in days)
Real epidemic	429	?	1247	>365
Simulated epidemic	381	465	741	306
Preventive slaughter scenario	70	99	451	164

Delayed destruction alternatives

Compared to the preventive slaughter scenario, delay destruction strategies (table 6) reduced both, the number of infected and detected farms. Furthermore, the duration of the epidemic was decreased, compared to the preventive slaughter scenario. All vaccinated farms are destroyed with delayed destruction and not only those located within a preventive slaughter zone (0-1 km). So the number of destroyed farms increased nearly fourfold compared with the preventive slaughter scenario. There was no difference on the size of the epidemic when maximal protection was changed from 21 days to 16 or 26. Only a vaccine with maximal protection in 7 days was on average slightly more effective in controlling the epidemic.

By means of sensitivity analysis, we calculated the following scenarios. When reducing the preventive slaughter radius from 1000 m to 500 m, no large effect on the size of the epidemic was found. The same was true, if the installed vaccination areas from the first day had a vaccination radius of 10 km instead of 3 km. In case more (8 days), respectively less days (2 days) are needed to prepare for the emergency vaccination campaign, the number of infected and detected farms was increased, respectively decreased.

Intra-community trade alternatives

Also for the intra-community trade alternatives, the number of infected as well as the number of detected farms was smaller than in the scenario of preventive slaughter (table 6). Furthermore, as preventive slaughter was not applied in the basis intra-community trade scenario, the number of preventive slaughtered farms was equal to null. However, in the intra-community trade basis, an epidemic lasted longer than in the preventive slaughter scenario.

Table 6. Comparison of preventive slaughter with the two basis emergency vaccination strategies, delayed destruction and intra-community trade (maximal protection is reached on day 21).

	Preventive slaughter			Emergency vaccination strategies					
				Delayed destruction			Intra-community trade		
	5%	50 %	95 %	5%	50 %	95 %	5%	50 %	95 %
# Detected farms	47	70	232	48	58	92	57	68	133
# Infected farms	69	99	349	54	64	113	57	68	133
# Prev slaughtered farms[a]	342	451	1211	1056	1181	1915	0	0	0
# Vaccinated farms	-	-	-	958	1037	1601	1043	1135	1961
Duration in days	114	164	344	99	108	177	236	258	322
Vaccination stopped[b]	-	-	-	96	102	154	78	101	164

[a] In case of delayed destruction, preventive slaughter includes all vaccinated farms.
[b] The criteria to stop installing new vaccination areas was fulfilled.

There were only slight differences in size of the epidemic, when the maximal protection level was varied from 21 days to 16 or 26 days. The number of infected, detected and vaccinated farms was lower for all iterations if maximal protection was reached in 7 days.

By means of sensitivity analysis, we calculated the effect of a vaccination radius of 10 km instead of 3 km for vaccination areas installed on the first day. No effect was found. In case more (8 days), respectively less days (2 days) were needed to prepare for the emergency vaccination campaign, the number of infected and detected farms was also increased, respectively decreased.

Assuming that preventive slaughter was also applied in the intra-community trade strategy, whereby only non-vaccinated farms would be destroyed, the number of detected and infected farms was slightly below the basis intra-community trade scenarios. The number of vaccinated farms was lower, because some of those farms, designated to be vaccinated, were destroyed before being vaccinated. The duration of the epidemic did not change.

DISCUSSION

Although the EU regulations currently prohibit vaccination against CSF, it is expected that the development of a so called marker-vaccine might lead to a reassessment of the non-vaccination principle (Laddomada and Westergaard, 1999). Therefore the main goal of the shown simulations was to analyse different possible emergency vaccination campaigns, which could have been applied in the Dutch CSF 1997-98 epidemic. In the current paper alternatives were compared with the preventive slaughter scenario discussed in Nielen et al. (1999). This scenario was considered to be the most effective that could have been reached with the normal and available control measures.

Model constraints

If a marker vaccine will become available, a good diagnostic test distinguish serologically infected pigs from vaccinated pigs, is a necessity. A high sensitivity, as well as a high specificity of the test is required. A specificity of less than 1 would lead to the detection of false positive farms. As a consequence new movement control zones would be installed and healthy pig farms would be slaughtered out. So low specificity could lead to some severe economic consequences. A high sensitivity is required, as we want to detect all infected farms. In the current simulations we assumed a specificity of 1 and a sensitivity of nearly 1. So in our simulation, we detected nearly all infected farms, but we would never find false positive farms.

Only horizontal transmission was incorporated in the simulation model, using 3 levels of contacts (high, medium, low). The addition of vertical transmission (sow conveys the infection to its unborn offspring) would have meant a complete overhaul of the contact structure in the model (Jalvingh et al. 1999). This could lead, in case of the intra-community strategy to an underestimation of the epidemic. We assumed for vaccinated and afterwards infected farms an infectious period of only 1 month, which does not take into consideration the birth of carrier piglets afterwards. In case of delayed destruction, newborn piglets, including carrier piglets will be killed. For all other infected farms, we assumed that they stayed infectious until being slaughtered out after detection.

We assumed that only vaccinated and later infected farms would show a reduction in virus spread, whereas infected and afterwards vaccinated farms were assumed to keep the same level of virus spread as non-vaccinated and infected farms. Here further information is needed. Infected and afterwards vaccinated farms were supposed to stay infectious until detection, without any reduction in infectivity. As infected and afterwards vaccinated farms had a high probability to be detected earlier due to vaccination, their effect on the outcomes is rather small. So the effect of vaccination consisted not only of reduction of virus spread and protection of susceptible herds, but vaccination

could also lead to earlier detection of infected farms, which of course is not the main goal of vaccination.

During the 4 months of the installment of the post-vaccination zone, we assumed maximal protection of all vaccinated farms. This will lead towards an overestimation of the vaccination effect. The number of not vaccinated or not protected pigs will increase over time. If maternal immunity will protect against transmission only during a short period, the overestimation can be quite large, whereas if the maternal immunity will protect against transmission over a longer period of time, there will be a negligible overestimation. The maternal immunity needs further research which can than be integrated in a more generic model. With lifting up a post-vaccination zone the protection level of all vaccinated farms was set to null. This will lead to an underestimation of the vaccination effect, as there will be still some vaccinated and protected pigs kept on those farms.

Maternal immunity can also be a problem, when it interferes with the immune reaction to a vaccine. We assumed no reduction in the effectiveness of the vaccine in case of maternal immunity of the piglets. What will be the effect of maternal immunity against virus transmission but also if maternal immunity can protect against clinical signs are further questions, which need to be answered.

It has to be remembered that vaccination has not only advantages, but also some disadvantages and possible hazards. Vaccination may engender a false sense of security, leading to relaxation of other control measures and/or less strict sanitary behaviour of people involved (Anonymous, 1997). In our simulations we assumed the same strict measures as without vaccination.

A further constraint in an emergency vaccination campaign could be the diagnostic capacities needed for the serological differentiation between infected and vaccinated pigs. The high number of diagnostic tests could lead to a delay in diagnosis. This effect was not considered as such in our simulation model. We assumed minimum 49 days instead of 42 days before lifting up the standstill, to mimic a waiting period for the laboratory results.

Comparison of vaccination strategies

In both emergency vaccination strategies, the main goal was to reduce virus spread and to reduce the number of susceptible animals. The delayed destruction strategy seemed to be the more effective one and both were better than the preventive slaughter scenario. If the delayed destruction strategy would have been applied in the 1997/98 Dutch CSF epidemic, the number of detected respectively infected farms would have been reduced to an average of about 68 (median =58) respectively 76 (median 64), compared to 120 (median 70) respectively 166 (median 99) in case of preventive slaughter. Applying the delayed destruction strategy as a control measure would have lead to a shorter epidemic. Compared with the preventive slaughter scenario, vaccination costs for on average 1240 farms are made, and four times as many farms are preventively slaughtered. Economic analysis will need to demonstrate if the extra costs for vaccination and additional preventive slaughter will compensate the benefits of a shorter outbreak and smaller epidemic.

If the intra-community trade strategy would have been applied in the 1997/98 Dutch CSF epidemic, the epidemic would have lasted longer compared with the preventive slaughter scenario. The epidemic lasted longer, but the number of infected and detected farms was on average smaller, about 75 versus 120 detected farms. It has to be remarked, that in case of intra-community trade the worst case scenario were never as bad as for the preventive slaughter scenarios. The big advantage of this strategy compared to the preventive slaughter scenario and also to the delayed destruction is, that except for welfare slaughter in the surveillance zone, no healthy pigs need to be destroyed. This is an advantage from an ethic, as well as from an economic viewpoint. Pig meat originating from vaccinated pigs, we assumed, would be sold on the intra-community market. This strategy, more than the delayed destruction strategy, therefore entirely depends on marker vaccine with a good, reliable

and easy to handle companion diagnostic test. Such a test should convince the trading partners that only not infected pigs will enter the market. Again, economic analysis will be needed to appreciate the potential benefits of this strategy.

The sensitivity analysis pointed to some interesting aspects of the vaccination campaigns. For both strategies, the effect of the size of the vaccination area installed on the first day (3 km versus 10 km radius) was very small. This was probably due to the high-density pig area, with many infected farms close together, so large vaccination areas would most likely overlap.

Also for both strategies, only a very effective vaccine (maximal protection in 7 days), showed any effect on the outcomes of the simulations. The range 16-21-26 days to maximal protection showed very little effect on the outcomes. It should be noted that the 7 days refers more or less to the effectiveness of the conventional non-maker CSF vaccine, as has been used historically in Dutch outbreaks.

A last interesting point is the effect of the actual start of the vaccination after the decision has been made. The default delay of 5 days seemed realistic, but sensitivity analysis showed that the total size and duration of the simulated epidemic was very sensitive to this parameter. If this is correct, it shows again that effective measures are only effective when applied as early as possible in an epidemic.

CONCLUSION AND FUTURE WORK

The comparison of different emergency vaccination alternatives to the basis scenario may lead to some general conclusions on the Dutch 1997/98 CSF-epidemic. A first obvious conclusion was that emergency vaccination, assuming a reliable diagnostic test and no relaxation of other control measures, seemed to be an effective strategy to reduce the size of an epidemic. In comparison with preventive slaughter, all emergency vaccination alternatives were at least as effective as the preventive slaughter scenario. The average number of infected farms was even smaller, which was due to a lower variation around the median compared to preventive slaughter. Comparing the delayed destruction strategy with the preventive slaughter strategy, the large number of preventively slaughtered farms is a negative aspect of the delayed destruction strategy. If we compare the intra-community trade strategy with preventive slaughter strategy, emergency vaccination is certainly the tool to chose. Vaccination costs, assuming no extra cost for the post-vaccination zone, are mainly in competition with the cost for preventive slaughter. Furthermore a large number of healthy pigs do not need to be destroyed, but can be used for human consumption.

In general InterCSF was considered a useful tool to compare the effects of different emergency vaccination strategies of the Dutch 1997/98 CSF-epidemic. In the near future the emergency vaccination scenarios should be economically analysed, using EpiLoss. Further the InterCSF model will be improved towards a more generic CSF model. The improvements should include adaptation and addition of mechanisms, as well as adaptation of parameter settings, based as much as possible on new results that will come available.

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APPENDIX - THE CALCULATED REDUCTION AND PROTECTION FACTORS

In horizontal transmission experiments co-ordinated by the EU, animals in groups of 10 were vaccinated and 5 of them were inoculated with CSF 7, 10, 14 or 21 days after vaccination. From the data generated by these experiments, parameters of the standard SIR model were estimated (Klinkenberg et al., in prep.). These parameters were used to calculate reduction in total infectivity of a herd depending on the time between vaccination and infection. Results showed that for both vaccines 21 days was the time from vaccination until maximal protection of the individual animal. The relative decline of the estimated transmission parameter between animals is used as protection factor. To see what a faster or slower working vaccine would do, time scales of protection and reduction curves have been changed. Reference point of these changes have been the maximal protection date of the individual animals, 21 days, which has been changed to 7, 16 or 26 days. As both vaccines do not differ in their ability to reduce horizontal transmission, we used the average of the estimated parameters of both vaccines. The applied reduction and protection factors are shown below in figure 1 and 2.

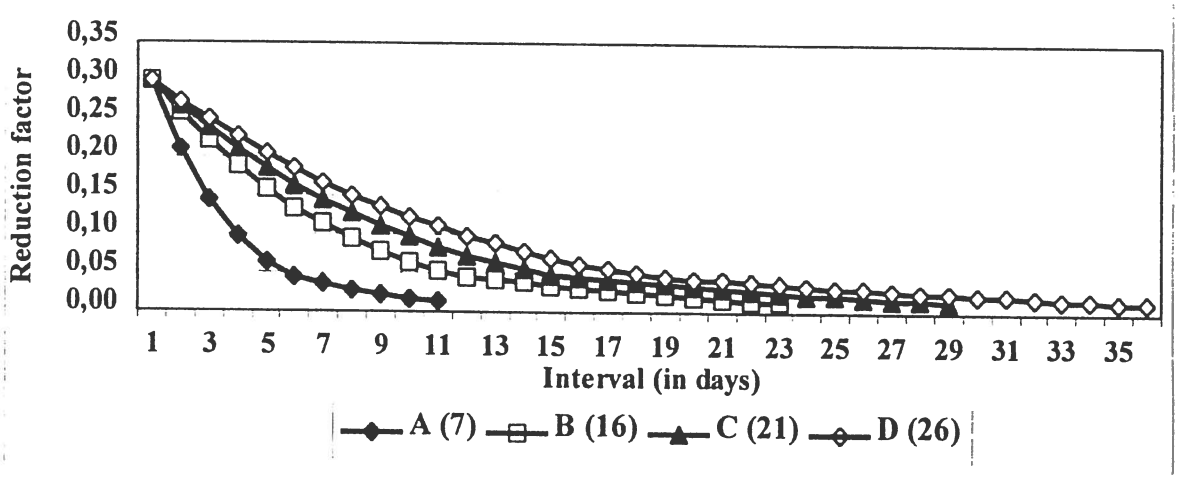

Figure 1. Reduction factors for the different reference points, 7 (A), 16 (B), 21 (C) or 26 (D), depending on the time interval (days) between vaccination and infection

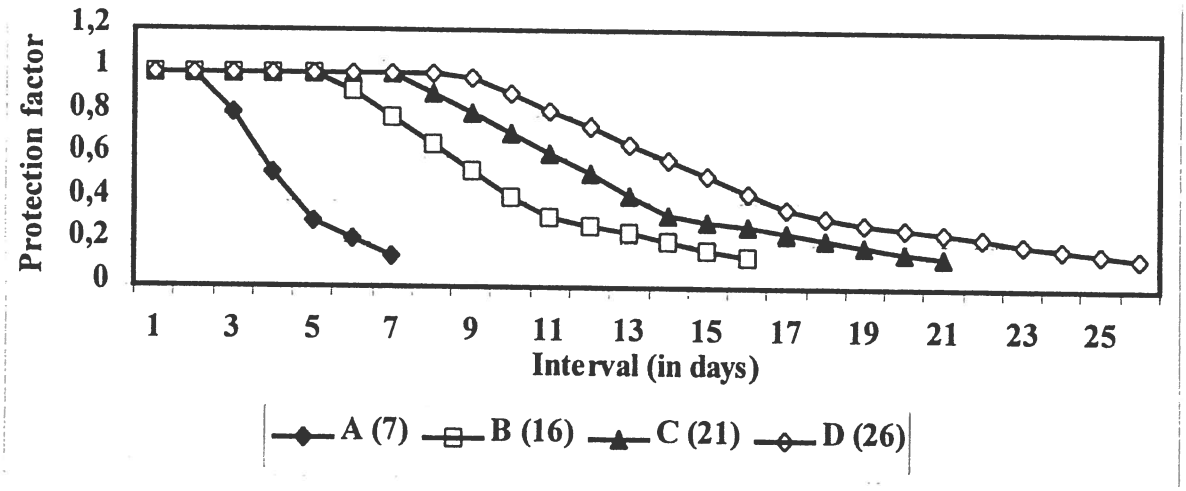

Figure 2. Production factors for the different reference points, 7 (A), 16 (B), 21 (C) or 26 (D), depending on the time interval (days) between vaccination and infection.

ANALYSIS OF RISK FACTORS FOR INFECTION OF SOW HERDS WITH PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) VIRUS

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Porcine Reproductive and Respiratory Syndrome (PRRS) was first recorded in Denmark in 1992 (Bøtner et al., 1994). The infection spread among Danish pig herds, first in Southern and Eastern Jutland and later throughout the rest of the country reaching an annual incidence of 5-10%. In 1996, it was estimated that 25% of sow herds and 33% of finishing pig herds were infected with PRRS (Mortensen, 1996; Mortensen & Strandbygaard, 1996). The industry represented by the Danish Bacon & Meat Council (DBMC) decided to launch a 3-year national control programme, which was voluntary and free of charge for the individual pig producers. The purpose of the programme was to stop the PRRS dissemination among the herds and to reduce the herd prevalence of PRRS. One of the tools of the programme was to determine the PRRS status of the Danish pig herds (starting 1. February 1996) in order to reduce the dissemination through trade with infected animals. A second tool was vaccination with a modified live vaccine[4] based on a US strain of PRRS virus. After the authorities had approved the vaccine, vaccination started in approximately 1,100 herds during the period 1 July 1996 – 31 October 1996. Before then, following a legal exemption, the vaccine had already been used on boars in the Danish artificial insemination (AI) system such that as of 26 December 1995, most replacement boars were vaccinated before introduction into AI centres.

It is possible to distinguish herds infected with the PRRS field virus (PRRS-EU) from herds infected with US strains of PRRSV (PRRS-US) by serological investigations of blood samples (Wensvoort et al., 1992;

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[4] INGELVAC® PRRS (Boehringer Ingelheim).

Sørensen et al., 1998). In July-August 1996, the Danish Veterinary Institute for Virus Research (DVIVR) reported the first isolations of PRRS-US from field material. The PRRS-US strain was shown to have disseminated through semen from AI-boars to PRRS-free, non-vaccinated herds, as it was found in samples collected before the vaccine was freely marketed (Bøtner et al., 1997). A screening in November 1995 showed no evidence of presence of antibodies against US subtypes of PRRSV in the Danish sow population (Mortensen, 1996). Until March 1997, DVIVR had documented PRRS-US dissemination to 132 non-vaccinated herds that were probably otherwise free of the PRRS field virus (Bøtner et al., 1997). Many of the PRRS-US-infected herds had experienced clinical outbreaks of PRRS-like disease (Bøtner et al., 1997). The first documented case of infection with PRRS-US came from a herd in Funen based on samples collected on 23 June 1996.

The objective of the investigation was to evaluate and quantify the likely routes of transmission of PRRS-US among Danish pig herds during the period June 1996 - October 1997. PRRS-US may in principle be introduced into a herd by the following pathways: a) vaccination, b) purchase of PRRS-US infected or vaccinated animals, c) use of semen from AI-stations with PRRS vaccinated boars, d) airborne transmission of infection from PRRS-US infected/PRRS vaccinated neighbouring herds or e) use of contaminated equipment, exposure from contaminated environment of the herd or vectors. A cohort of Danish sow herds was investigated in the period from 23 June 1996 to 1 October 1997. The design was a nested case-control study in which the cases were the herds that became infected with PRRS-US during the study period. A random sample of time-matched controls was used instead of all non-infected herds in the cohort.

MATERIALS AND METHODS

The primary data source for the study was the Danish PRRS register at DBMC. It comprised all diagnostic PRRS investigations in Denmark until the end of the study period. A "herd test" was used to denote a set of blood samples collected on the same day. The number of samples in a herd test varied but the typical number was 10 or 20 samples. In addition, the register comprised dates and number of vaccine doses obtained through the Danish PRRS programme (1 July 1996 - 31 December 1996) in PRRS-EU positive herds. In the register, about 40-50% of the Danish pig herds were represented. However, these farms housed 80% of the sows and 50% of finishing pigs in Denmark. On average, each herd was represented by 3 PRRS sampling rounds between 1994 and 1997.

The data from the PRRS register were linked with information from DBMC's Geographical Information System (GIS) on the location of herds and their health status. Furthermore, herd management data were collected by telephone interviews. In particular, herd managers were asked to provide the names of AI

company(-ies) that supplied semen to the herd in the study period. For each herd, dated lists of purchases of potentially PRRS-US contaminated semen were then collected through the AI companies. Herd managers were also asked to provide the names of the farms that supplied replacement breeding animals during the study period. For animals supplied by the SPF company (SPF: Specific Pathogen Free), their origins were tracked in the SPF trade database. The PRRS-US status of supplying herds was then extracted from the PRRS register.

Data on neighbouring herds, such as the number of animals, were obtained from the public Zoonosis Register (ZOOR) and linked to DBMC's PRRS register and GIS system. Additional information of PRRS-US status in selected neighbouring herds was collected by telephone interviews.

Serology

The serologic examinations involved at least one of five different tests. 1) An indirect ELISA with a Danish PRRS virus isolate as antigen (Albina et al., 1992). This test reports an OD-ratio, which is interpreted as positive if it exceeds 0.4. 2) Two immunoperoxidase monolayer assays (IPMAs) with a Danish PRRS virus isolate and the PRRS vaccine strain as antigens, respectively (Bøtner et al., 1994). The IPMA test results are reported as <50 (negative), or as the titre values 50, 250, 1250 or >=6250. By comparison of titre values it is possible to distinguish between antibodies against European PRRSV and US PRRSV strains (Wensvoort et al., 1992). 3) Two blocking-ELISAs with a Danish PRRS virus isolate and the PRRS vaccine strain as antigens, respectively (Sørensen et al., 1997 ; Sørensen et al., 1998). Samples were run in parallel in the tests. A sample was reported negative in the ELISAs if the sample reacted negatively in both tests, and positive if the sample reacted positively in one of the two tests. For positive samples the ratio between the blocking percentages between the tests was also reported. This ratio allowed to distinguish between antibodies against European PRRSV strains and US PRRSV strains (Sørensen et al., 1998).

PRRS status of herds over time

Based on at least 10 individual samples collected on the same day, a herd test was defined with a total of 9 possible outcomes. This test simultaneously designated a herd infection status of either infected, non-infected or unknown with respect to both PRRSV strains. Herd tests were often repeated at regular intervals in herds. We defined that a herd was considered PRRS-EU+ or PRRS-US+ subsequent to the date of the first positive herd test for the particular sub-type of PRRSV unless specific measures were taken to eliminate the infection from the herd and the change in disease status was documented by herd tests. The PRRSV infection is known to persist in herds over long periods (Albina, 1997; Mortensen & Søgaard,

1998). As a consequence of persistence we concluded that a negative herd test for one particular subtype of PRRSV at some point in time indicated that the herd was free of that subtype until the negative herd test.

Study period and study cohort

The study period was from 23 June 1996 to 1 October 1997 (inclusive). The beginning of the study period was defined by the first detection of herd infection with PRRS-US. Vaccination of swine herds started later except for boars of AI centres. Therefore, no local exposure or exposure from purchased animals was present before 23 June 1996 but exposure from semen purchase was possible. The end of the study period was defined by the date when the programme for PRRS testing without charge terminated.

The study cohort consisted of 1,071 non-vaccinated sow herds[5]. Information on PRRS status of neighbouring swine herds in the surrounding areas was used to estimate exposure on study herds. Study herds satisfied all the following criteria:

a) A PRRS-negative (and no PRRS-positive) herd test before 23 June 1996,
b) At least one further PRRS herd test no later than 1 October 1997,
c) Located in the western parts of Denmark (small and remote islands were excluded),
d) The herd was housed in a single-site system[6].
e) Introduction of pigs from non-genetic herds (or the herds' own off-site finishing facilities) was not practised, whereas purchase of replacement breeding animals from genetic herds was possible.

Censoring and case definition

A herd belonged to the risk set (herds that were at risk in the study cohort at a particular point in time by being non-infected) from the beginning of the study period until one of the following events (censoring),
a) Detection of PRRS-US+ (a case herd).
b) Detection of PRRS-EU+.
c) Last confirmation of PRRS-negative status before 1 October 1997.
Formally, a case herd is a herd in which PRRS-US is detected at a point in time when the herd belongs to the risk set. As stated in b), herds that were infected with PRRS-EU were censored even if they subsequently were infected with PRRS-US. Our reason for doing so was that herd management may be affected

[5] The definition encompassed farrow-to-feeder and farrow-to-finish herds with one or more sows for every 15 finishing pig (25-100 kg).
[6] Single-site system: all buildings within a 100 m radius of the sow farm. Off-site quarantine facilities for replacement breeding animals purchased from breeding and multiplying herds were allowed.

by PRRS infection of a herd. Finally, the proportion of PRRS-US cases, which were previously infected with PRRS-EU during the study period, was low (maximum 10%). As stated in c), herds were censored at the last PRRS-negative herd test in the study period because after that the PRRS status became unknown: herds may have stayed uninfected or have become infected.

Reference dates and periods

In a case herd, the "reference date" was the date when the PRRS-US positive samples were collected. A herd selected as a control for a particular case herd was assigned the same reference date and reference period (se below) as the corresponding case herd (time matching). The importance of the reference date was that the case and control(s) were compared with respect to the risk factors present on and before this date. Since more than two weeks are required before antibodies develop in a sufficiently large number of animals to be detected in a serological herd test, we concluded that infection of the case herd occurred between (reference date - 2 weeks) and 2 weeks before the last date on which the herd was tested PRRS-negative. We denote this interval the reference period; it was not truncated at the beginning of the study period.

Explanatory variables

Table 1 lists the variables used in the analysis. Further details on some of the variables are given below. In the study herds, we used the number of sows and pigs obtained in the interviews because they were considered more precise than the database records. We also calculated the number of heat producing units (HPUs) in a herd in order to obtain a herd size measure comparable between farrow-to-feeder and farrow-to-finish herds. SPF herds are required to design their production system to facilitate sound biosecurity practices and maintain them. However, most conventional sow herds also have biosafety programmes. Based on interview information on three biosafety practices[7], we created a variable assessing the biosafety level as high (all three biosafety practices) or otherwise low. We also created variables to denote whether the farm personnel worked in other herds and whether the study herd had an official biosecurity status[8].

In Denmark, genetic herds have been serologically monitored for PRRS infection on a monthly basis since 1994, and PRRS negative production herds (like the study herds) would purchase replacements from PRRS negative herds only. However, some genetic herds became infected with PRRS-US during the study

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[7] 1) Personnel access to the herd area only through a facility with change of clothes and boots, 2) no direct access of trucks/vehicles and drivers to the herd area when picking up slaughter animals, and 3) no direct access of trucks/vehicles and drivers to the herd area when picking up feeder pigs.

[8] SPF or MS herd health declaration. MS is a former SPF herd that is infected with *Mycoplasma hyopneumoniae.*

period. We defined that animals purchased from such a herd could have been potentially infected with PRRS-US up to two months before the PRRS-US infection was detected in the herd.

We estimated the study herds' exposure to PRRS-US from PRRS-US+ neighbouring herds within 3 km. Our biological assumption was that exposure from a PRRS-US-infected herd should be weighted with the inverse square of the distance from the study herd. Thus, estimated exposure decreased rapidly with distance.

Finally, we calculated the swine herd density within a radius of 5 km in terms of herds as well of HPUs. As the background exposure included unidentified aereal exposure, e.g. from herds with unknown PRRS status, and vertical transmission by machines, humans or animals, it may be unrealistic to assume equal background exposure in areas with low and high herd density. Moreover, herd density may become a confounder of neighbourhood exposure, as herds in highly dense areas would typically be subject to higher neighbourhood exposure.

The variables directly related to disease transmission (purchase of semen and animals, and neighbourhood exposure) were also defined in dichotomous versions (values 0 or >0).

Statistical methods

The design was a nested case-control design (Clayton & Hills, 1993, Chapter 33) where a matched sample of controls was used in place of all non-infected herds in the cohort. More specifically, for each case with a particular failure time (time until infection was detected), a number of controls were selected randomly from the herds in the study population at risk at that time (reference date). Herds at risk were evidenced by a negative herd test at or later than the reference date). In this study, 2 controls were sampled per case. Note that herds may be selected as controls more than once, and furthermore that a case may be selected as a control before its failure time. In total, the study included 205 different herds, out of which 74 were cases and the remaining 131 herds were used as 148 controls.

In this paper we present simple descriptive statistics and results of univariate analyses for the risk factors. For dichotomous variables we calculated the Mantel-Haenzel odds ratio estimate for data stratified in matched sets, and the associated chi-square test for the hypothesis of no association between risk and infection. The standard conditions for the validity of the approximative chi-square distribution were met (Kleinbaum et al., 1982; section 17.3.1). For continuous variables we obtained P-values for the similar hypothesis by an analysis-of-variance randomisation test procedure based on 999 replications (Manly, 1997; Section 7.4).

RESULTS

The response rate of herd owners was very high with only one control herd refusing to participate in the study. A new control replaced the herd. We were able to retrieve complete information for the variables in the study herds, except for the number of potential PRRS-US contaminated semen doses. It was only possible to identify 85% of case herds and 75% of control herds in the files from AI companies. In 74% of the case herds, farmers reported clinical signs of PRRS prior to the detection of PRRS-US infection. During the herd interviews it was verified that none of the selected study herds (cases and controls) had vaccinated against PRRS.

The results of the univariate analysis are presented in Table 1. The mean number of sows in case and control herds was 210 and 166 sows, respectively. The proportion of SPF/MS herds was just below 50% for both cases and controls. The biosecurity level was assessed as high in 74% of case herds and 70% of control herds, respectively. Further, the biosecurity level was assessed as high in 98% of SPF/MS herds and 48% in of non-SPF/MS herds, respectively (data not shown). The proportion of herds among cases and controls that had used semen from PRRS-US infected IA centres was slightly above 80%, reflecting that the remaining herds never used semen from AI centres.

Based on the univariate analyses, risk of infection was not associated with biosecurity level at the farm, labour shared with other herds, herd or pig density in the area. On the other hand, risk of infection was associated with herd size, purchase of infected animals and exposure from PRRS-US infected neighbouring herds. The proportions of case and control herds that used at least one dose of potentially PRRS-US contaminated semen were similar. However, more doses of potentially PRRS-US contaminated semen were used in case herds.

The location of study herds (cases and controls) and the remaining herds of the study cohort is shown in Figure 1. The dot representing a case or control herd has a size equivalent to a 3 km circle around the herd. By visual inspection it seems that study herds are uniformly distributed in the study cohort. Variables significant at the univariate level will be examined further by a multivariate analysis.

DISCUSSION

The reason for herd infection with the modified live vaccine strain was only obvious in few of the 74 case herds. All farmers were aware of the PRRS status of their herd as they monitored the status by herd tests at regular intervals and it must be presumed that they tried to prevent infection. Vaccination or pur-

Table 1. A nested case-control study of risk factors for herd infection with the US subtype of PRRSV in Danish sow herds. Descriptive statistics of risk factors in cases (n=74) and matched controls (n=148).

Description	Cases	Controls		
Variables based on the reference date:	*mean*	*mean*		*P-value*
Number of sows	210	166		**0.008**
Number of finishing pigs	518	352		**0.012**
Heat producing units (HPU)[A]	183	134		**0.001**
Herd density per km^2	0.64	0.62		0.63
Pig density (HPUs) per km^2	40.2	35.6		0.13
	Frequency	*Frequency*	*OR*[D]	*P-value*
SPF/MS biosecurity level (yes/no)	46.0%	47.3%	0.95	0.85
High biosecurity level (yes/no)	74.3%	70.3%	1.30	0.50
Labour shared with other herds (yes/no)	13.5%	8.8%	1.64	0.28
Variables based on the reference period:				
Semen from PRRS-US+ AI stations (yes/no)	82.4%	81.8%	1.06	0.89
Animals from PRRS-US+ suppliers (yes/no)	16.2%	2.7%	6.00	**0.001**
At least one PRRS-US+ neighbour herd (yes/no)	67.6%	43.9%	2.46	**0.001**
	mean	*mean*		*P-value*
Number of doses from US+ AI stations[B]	306	165		**0.046**
Number of animals from US+ suppliers	3.81	0.05		**0.002**
Estimated exposure from US+ neighbour herds[C]	0.16	0.006		**0.001**

[A] Heat producing units calculated as Nsows*0.45 + Npigs*0.17.
[B] Missing information in 11 case herds (15%) and 37 control herds (25%)
[C] Sum of terms: (days infected within the reference period)*(size in HPU)/(distance^2), for all PRRS-US+ neighbour herds within a radius of 3 km
[D] Mantel-Haenzel Odds-ratio (across all matched sets)

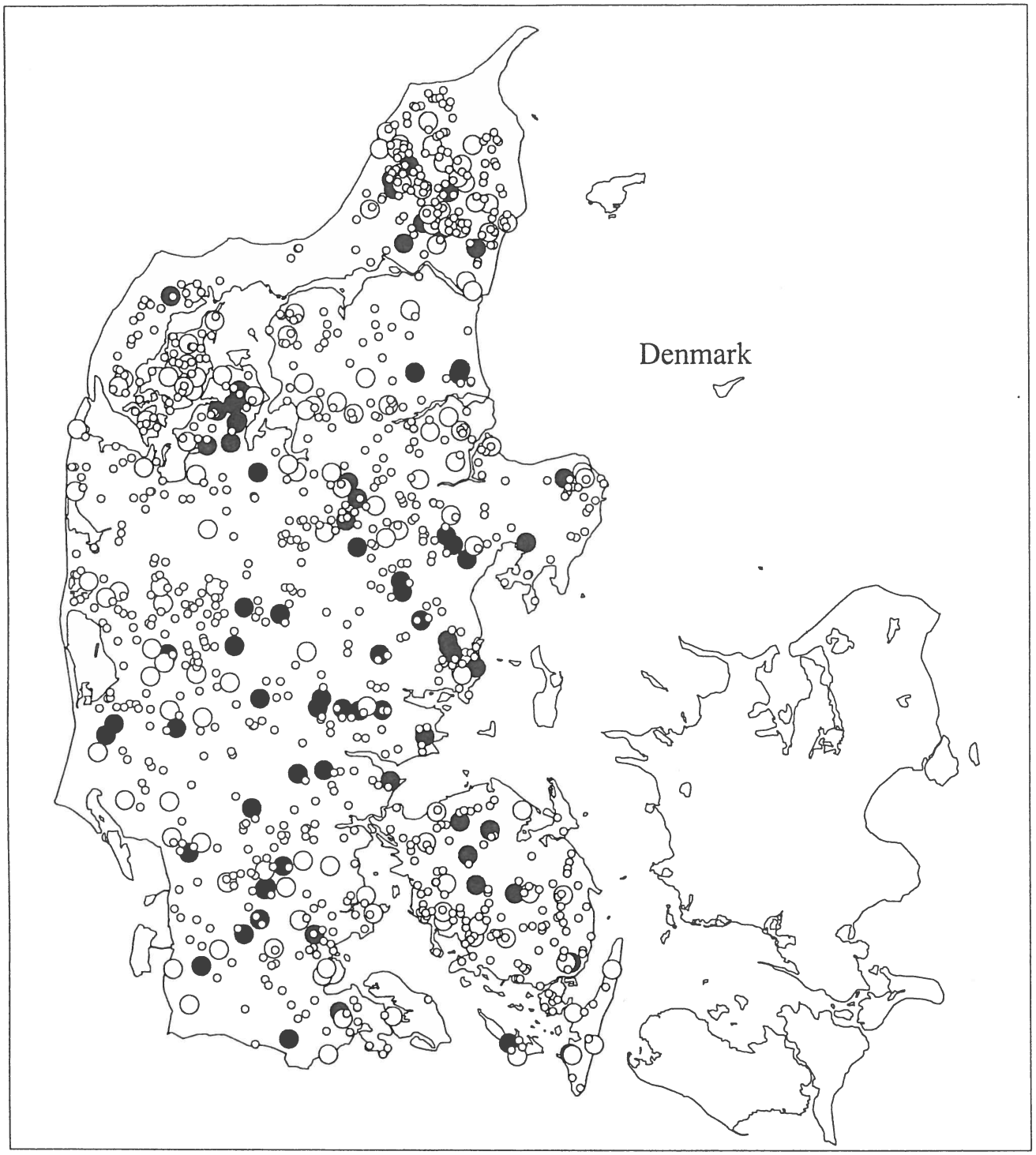

Figure 1. Location of case herds (large dark dots), control herds (large light dots) and the remaining herds of the study cohort (small light dots) in the study area. The size of a dot representing a case or control herd is equivalent to a 3 km radius around the herd.

chase of animals from a herd known at the time of the trade event to be infected did not occur. The level of biosecurity in these herds as assessed by both a contract with the SPF company and information on key biosecurity management practices was high in most herds and did not seem to alter the risk of infection. The only risk factors associated with risk of infection in the univariate analysis were herd size, estimated exposure from neighbouring PRRS-US infected herds and purchase of animals from genetic herds recently infected with PRRS-US. The number of semen doses from PRRS-US infected AI centres were moderately higher in case herds compared to control herds. However, this effect could be confounded by larger herd size in case herds. Multivariate analysis is required to determine the adjusted effects of the factors.

Twelve case herds purchased animals in genetic herds recently infected with PRRS-US. The PRRS-US infection was in all cases detected in the genetic herds within one month after the trade event. Also four control herds purchased animals from genetic herds recently infected with PRRS-US. In control herds, the PRRS-US infection in genetic herds was detected between one month and 2 months after the trade event. Based on this observation, it appears likely that the 12 case herds could have avoided infection if they had quarantined the animals in a separate facility for a one-month-period.

The study might be limited in power, primarily due to the small number of case herds (n=74). We were not able to detect effects of biosecurity level, herd density or pig density. If there was a frequent transmission of this PRRSV strain by rodents, visitors or trucks, we would have expected a protective effect of biosecurity practices studied here. At this point the preliminary evidence indicates that risk of infection with the modified live vaccine strain is indeed related to area and is spread by mechanisms apparently unaffected by biosecurity practices, e.g. airborne transmission. This suggests that the modified live vaccine strain does not behave differently from PRRS field virus regarding transmission pathways.

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THRESHOLD ANALYSIS OF COST-EFFICIENT ORAL VACCINATION STRATEGIES AGAINST RABIES IN FOX (*VULPES VULPES*) POPULATIONS

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A combination of mathematical models, optimisation algorithms and Threshold analyses is capable of supporting veterinary authorities in making urgent epidemiological decisions in uncertain situations. In the following paper the example of an oral vaccination of foxes (OVF) is to demonstrate this capability.

Oral vaccination of foxes against rabies has been practised in Europe since 1978 (Steck et al., 1978,1982) and various strategies have been implemented in order to control this disease (Artois et al., 1997). The vaccines are either modified live rabies vaccines or vaccinia-recombinant rabies vaccines. They have been orally administered by vaccine-laced baits (Müller, 1997). The baits contained Oxytetracycline (OTC) which deposits at certain parts of the body after the bait intake. The detection of OTC, and the determination of the proportion of OTC-marked foxes is used to determine the proportion of foxes which had contact with the baits.

The aim of oral vaccination programs is to induce an effective formation of antibodies in the fox populations, and to assure that the proportion of individuals, protected against an infection with rabies, achieves a size, that is capable of breaking the chain of infection.

The actual proportion of OTC-marked foxes, that has been achieved by a vaccination campaign, is an indicator of successful attempts toward the aim of control. Amongst other factors this success depends on the

- type of baits,
- density of baits, distributed in the field,
- size of the area chosen, for the bait distribution,
- timing of the bait distribution,
- perishability of the baits, and on the
- densities and the dynamics of the target (fox population) and the non-target (competitors for the baits) populations.

There are at least 4 possibilities of designing successful OVF campaigns. The first one is to continuously provide huge numbers of vaccine baits to a fox population. But, because public funds for OVF are running shorter, this "strategy" has to be ruled out. The second one is to turn to account experts' practical knowledge. This is sometimes difficult, because expert knowledge is often implicit and hence difficult to collect and to assess. The third possibility is to conduct field experiments. Their results are sometimes hard to interpret, because the above mentioned influential factors act simultaneously and change continu-

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ously in time and space. The forth possibility is the development of mathematical models. These models are based upon experts' experience and upon the results of field experiments. Thus they unfortunately inherit the disadvantages of both. But however, the numerous pieces of knowledge collected by experts and field experiments accumulate in mathematical models. Mathematical models turn experts' implicit knowledge into explicit rules. Mathematical models can be used to conduct numerous controlled "simulation" experiments, with which the partial influence of the above mentioned factors can be assessed at very short notice. Furthermore mathematical models can be linked with optimisation algorithms in order to develop optimal, i.e. successful and cost-efficient, strategies. Finally threshold analysis can tell us how changes in those model parameters, which are uncertain, would alter the optimal strategy.

In this paper the results of our research into the effect of

- the timing of OVF campaigns,
- the number of baits distributed per campaign, and
- the perishability of the baits in the field,

upon the proportion of OTC-marked foxes within an analytical horizon of 3 years is presented. Mathematical models, optimisation algorithms and Threshold analysis will be applied.

The paper is subdivided into three sections. In the first section a description of the mathematical models used to predict the time course of the proportion of OTC-marked foxes is given. This time course depends on the timing of vaccination campaigns and on the number of baits distributed per campaign. In the second section an evolutionary algorithm is presented. This optimisation algorithm is used to perform a Cost-Utility analysis in order to determine cost-efficient vaccination strategies. A particular vaccination strategy is considered to be an efficient one, if its application keeps the associated costs in line and, nevertheless, achieves a continuously high proportion of OTC-marked foxes. In the last section the results of the Cost-Utility analyses is presented. The effect of a reduced durability of the baits upon the timing of vaccination campaigns, and upon the number of baits distributed per campaign, is analysed with the help of a Threshold analysis.

THE DESCRIBTION OF THE MODEL

A deterministic Leslie matrix model (Leslie, 1945, 1948; Lewis, 1942) describing the population dynamic of foxes, has been proposed by Selhorst and Müller (1999). This model is now used in order to predict the dynamics of non-OTC marked foxes and OTC-marked foxes. Bait intake induces a flow from the first to the second group. A reflow from the OTC-marked group into the non-OTC marked group is assumed to be impossible.

The Leslie matrix

In this paper the population dynamics of the red fox *Vulpes vulpes* is simulated for a time period of three years. Discrete time steps of one week are used. The population of the red fox is divided into OTC marked and non-OTC marked foxes. The individuals of both groups are subdivided into age-groups. The indices j ($j = 0,\ldots,n$; $n = 3$ [years]) and w ($w = 1,\ldots,m$; $m = 52$ [weeks]) are to indicate the age-groups which are specified in years and weeks, respectively. Variable $x_{j,w}(t)$ represents the number of foxes in age group j,w at time t (week within year). These foxes are able to consume baits. Variable $x_{0,w}(t)$ represents fox cubs (of age class 0) that are not able to consume baits.

The links between the age-groups are given by the survival process $S(\cdot)$, the reproduction $F(\cdot)$ and the development $U(\cdot)$. The dynamics of a fox population is modelled by help of the linear difference equations (1) to (5).

$$x_{j,w+1}(t+1) = x_{j,w}(t)S(t) \quad \text{with} \quad 0 < j \leq n, \tag{1}$$

$$x_{j+1,1}(t+1) = x_{j,m}(t)S(t) \quad \text{with} \quad j < n, \tag{2}$$

$$x'_{0,w+1}(t+1) = x'_{0,w}(t)S'(1-U(w)), \tag{3}$$

$$x_{0,w+1}(t+1) = S(t)x_{0,w}(t) + S'U(w)x'_{0,w}(t), \tag{4}$$

$$x'_{0,1}(t+1) = Z(t)\sum_{j=1}^{n}\sum_{w=1}^{m}(1-c_j)F(j)x_{j,w}(t). \tag{5}$$

The realisations of the survival process $S(t)$ represent conditional survival probabilities, which mainly depend on shooting and trapping. Because hunting intensity depends on time (i.e. season), the conditional survival probabilities also depend on season (i.e. time). In the equations (3) and (4), however, the survival probability (S') is time independent ($S' = 1.0$). This assumption, is only tenable, if the mortality of newborn foxes is included into the reproduction process $F(\cdot)$. This is achieved by the reduction of the number of newborn animals to the number of those, which survive their babyhood. If we further suppose that the fox cubs stay inside the den after their births, we can assume that they are protected against hunting for a defined period.

The realisations $F(j)$ represent the age-of-vixen (i.e. year) dependent litter sizes, and parameters $Z(t)$ in equation (5) describe the variation of times, when vixens start to reproduce. The parameters $c(j)$ denote the proportion of females in the age class j, which do not reproduce (helper vixens).

The parameters $U(w)$ stand for the age- (weeks elapsed after birth) dependent probability of a fox cub developing from the state "not able to consume baits" to the state "able to consume baits".

The equations (1) to (5) form a time- and age- dependent transformation matrix $\mathbf{A}(t,w,j)$, and by repeated transformation of the OTC-marked ($\mathbf{X}^*$) and the non OTC-marked ($\mathbf{X}$) fox subgroups with the matrix $\mathbf{A}$, the population dynamics of both can be predicted, i.e.

$$\begin{aligned} \mathbf{X}^*(t+1) &= \mathbf{A}(t,w,j)\mathbf{X}^*(t), \\ \mathbf{X}(t+1) &= \mathbf{A}(t,w,j)\mathbf{X}(t). \end{aligned} \tag{6}$$

In equations (6), $\mathbf{X}(t)$ and $\mathbf{X}^*(t)$ represent vectors of the age-group distribution of OTC-marked and non OTC-marked foxes at the time t respectively.

The matrix elements, which describe the survival, the fecundity and the development are modelled by secondary functions (for details see Selhorst and Müller, 1999). The parameters of these functions are estimated by help of the data described below.

The conditional survival probabilities

The model describing the conditional survival probabilities is based on information about the time-dependent mortality rates. These mortality rates can be derived from the available data on the mean life time and the relative, monthly distribution of the hunting bag (Fig. 1) of juvenile and adult foxes.

Because the simulation program needs the weekly input of the conditional survival probabilities, the monthly mortality rates had to be transformed. When operating this transformation we assumed (on account of the missing information), that the mortality rates does not vary within a month.

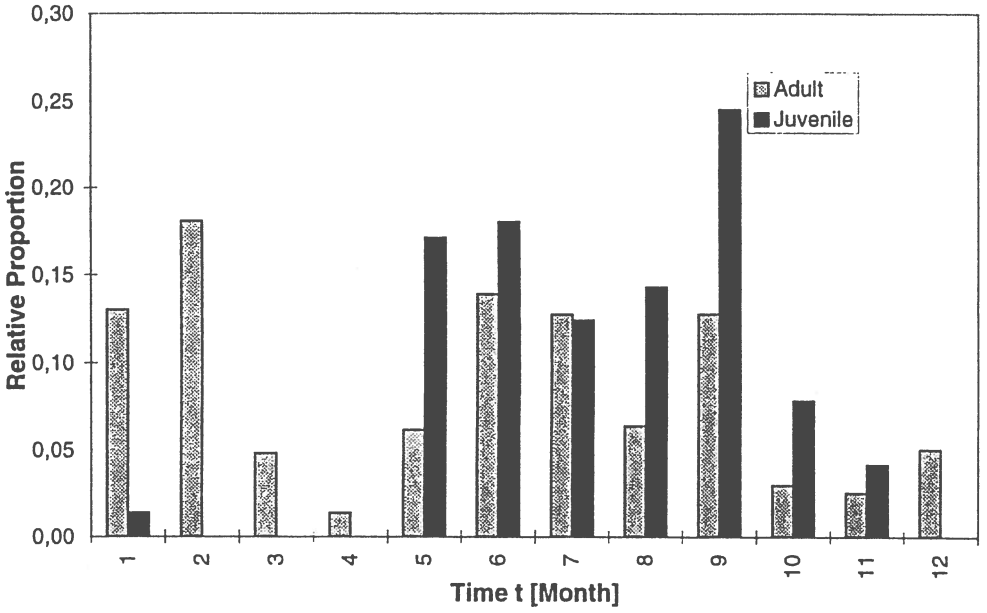

Fig. 1 Relative distribution of the hunting bag of juvenile and adult foxes in a small area of Brandenburg (Data from Tackmann, pers. comm.)

The reproduction

Because vixens reproduce only once – in spring – the population dynamics of foxes resemble a birth pulse population.

The model describing reproduction uses data with respect to a) the variation of times when vixens start to reproduce ($Z(t)$) (Paustian and Goretzki, 1982); b) the litter size distribution ($F(j)$) (Goretzki and Paustian, 1982; Smith and Harris, 1991); c) the proportion of non-breeding vixens (c_j) (Smith and Harris, 1991); d) the mortality of newborn foxes (Stubbe and Stubbe, 1977; Smith and Harris, 1991) and e) the sex ratio of newborn foxes (Goretzki and Paustian, 1982).

The day of the year when a vixen gives birth to the cubs is expected to be a different one between vixens and for each vixen between the years. In order to account for the individual variation, model $Z(t)$ is used. Its parameters are estimated by using data from Goretzki and Paustian (1982).

The figure for the mean litter size $F(\cdot)$ as reported by Goretzki and Paustian (1982) is 5.5 newborn foxes. Litter sizes reported by Smith and Harris (1991) are smaller and depend on the age (j) of the vixens (Table 1).

According to Smith and Harris (1991) the relative number of non-breeding vixens (see c_j in Table 1) also depends on the age of these vixens. The mortality of fox cubs in their first four weeks varies between

16% (Stubbe and Stubbe, 1997) and 16.8% (Smith and Harris, 1991) and the sex ratio (males:females) of the newborn foxes is about 1.36:1 (Goretzki and Paustian, 1982).

Table 1 Data for litter size and proportion of non reproducing females (Smith and Harris, 1991)

Age class j (Years)	c_j	Litter size ± s.d. [l]	$l \times (1 - \text{mortality})$ [ls]	$F(j)$ $ls \times$ prop. females
1	0.244	4.53 ± 1.54	3.78	1.60
2	0.171	4.90 ± 1.47	4.10	1.73
3	0.191	4.75 ± 1.73	3.97	1.68
4	0.029	4.73 ± 1.66	3.95	1.67
5+	0.280	4.94 ± 1.70	4.12	1.75

The development

During the first weeks of their lives, the fox cubs mainly stay within their den. If no direct den baiting is provided and the vixens do not carry any vaccine baits into the den, the newborn foxes will not consume any vaccine baits. As they get older, the fox cubs leave their den, may find vaccine baits and even may consume them. Field observations (Labhardt, 1990) indicate that fox cubs start to explore the neighbourhood of their den from the end of May to the beginning of June. This is about 8 ± 2 weeks after their births.

The model describing bait intake

The baits distributed in the field will be consumed by either foxes as the target species or by non-target species, i.e. by the competitors. The next model addresses the proportion in both bait consumer populations, that are OTC-marked, depending on the bait intake efficiency, the population density, the number of baits distributed, and the time elapsed since bait distribution.

Bait intake, i.e. predation, causes a decline in the number of baits (B) with time t (=days elapsed since the bait distribution). If we initially do not distinguish between foxes and competitors for baits the decline in the number of baits can be described with the predator-prey-model proposed by Lotka (1925) and Volterra (1926):

$$\frac{d}{dt}B = -BgP, \qquad (7)$$

P: density of predators,
B: density of baits,
g: efficiency of predation.

Equation (7) originally was proposed in order to describe the interaction between predators and their natural prey. Vaccine baits are artificial prey loosing their attractiveness with time until time t_c. From time t_c onwards, baits are no longer attractive to the predators, thus

$$\frac{d}{dt}B = -Bg(1 - t/t_c)P. \qquad (8)$$

In equation (8) it is assumed that the predator density (P) does not considerably change with time.

In order to distinguish between non-OTC marked foxes $X = \sum_{w,j} x_{j,w}$ and non-OTC marked competitors (C), equation (8) has to be modified:

$$\frac{d}{dt}B = -B(1-t/t_c)(fX + cC), \qquad (9)$$

with

$fX + cC = gP$,

and

f efficiency of predation of foxes,

c efficiency of predation of competitors.

The change in the number of non-OTC marked foxes and competitors is described according to

$$\frac{d}{dt}X = -B(1-t/t_c)(fX + cC)\frac{fX}{(fX + cC)}q_X,$$

$$= -B(1-t/t_c)fXq_X, \qquad (10)$$

$$\frac{d}{dt}C = -B(1-t/t_c)cCq_C. \qquad (11)$$

In equations (10) and (11), q_X and q_C resemble the product of the probability of OTC-formation and the probability of OTC-detection with respect to the foxes and the competitors.

Data from literature (Wachendörfer et al., 1986; Frisch et al., 1987; Schmid, 1987; Brochier et al., 1987; Schneider, 1989; Stöhr and Müller, 1990 [unpubl.]) about the cumulative proportion of baits, that have already disappeared in the course of a certain time span, is used to estimate the parameters g and t_c in equation (8).

The parameters f, X, q_X and q_C are adjusted so that the proportions of predicted OTC-marked foxes and competitors are the same as the observed proportions after the first OVF. Observed proportions are taken from a database maintained at the Institut für Epidemiologie der BFAV. The database contains all results of the diagnostic tests on an individual basis, accompanying OVF in Germany. Parameters f and X, given the parameters c and C have to meet the constrain $gP = fX + cC$.

The parameters used in the models (1) to (11) are given in Table 2.

OPTIMISATION

Evolutionary Strategies (ES) are used for optimisation (Selhorst, 1998, 1999). They imitate the principles of the organic evolution, i.e. reproduction, mutation and selection and they belong to a class of algorithms, which are considered to be analogous to nature, because they make use of natural paradigms (Schwefel, 1995; Heistermann, 1994; Nissen, 1997). Examples are: Simulated Annealing, Threshold Accepting, Sintflut Algorithms, Record-to-Record-Travel. According to Heistermann (1994), ES are especially suited to solve complex optimisation problems, which are either characterised by a large number of parameters or by a non-convex objective function. The optimisation problem considered here, is characterised by a large number of parameters: baits are allowed to be distributed at the beginning of each week within the analytical time span of three years. The course of an ES is explained in the following subsections.

Table 2 Parameters used in the models (1) to (12)

Parameter	Description	Value
	Survival	
	mean life time [years]	1.50
	hunting bag	see Fig. 1
	Reproduction	
	mean time to birth (MTB [days]	78.80
	Variation in MTB [days]	9.32
$F(j)$	No. Female offspring / vixen	see Table 1
$c(j)$	Proportion of helper vixens	see Table 1
	Development	
	mean time to development (MTD) [weeks]	8.76
	variation in MTD [weeks]	4.54
	Bait intake	
g	Efficiency of predation (predators)	0.0058
f	Efficiency of predation (foxes)	0.0105
c	Efficiency of predation (competitors)	0.0057
qX	Probability of OTC marking and detection (foxes)	0.9025
qC	Probability of OTC marking and detection (competitors)	0.6854
tc	Perishability of baits [days]	40.4
C	density of competitors [indiv. per sqkm]	18.5

Initialisation

During the initialisation a population of i ES-individuals, representing possible vaccination strategies, is generated at random. Each individual i equals a vector $\mathbf{A}_{i,t=0} = (\mathbf{B}_{i,t=0}, \mathbf{S}_{i,t=0})$. The vector elements b_j of vector $\mathbf{B}_{i,t=0}$ represent the object or decision variables. Within the optimisation problem we are aiming at, the vector $\mathbf{B}_{i,t=0}$ represents one strategy of oral vaccination of foxes. Vector elements b represent the times [weeks] when OVF strategies start and the number of baits used per sqkm and per campaign. In this paper the maximum number of campaigns, that can be conducted each year, is restricted to two campaigns.

In addition to the object variables ($\mathbf{B}_{i,t}$), so-called strategy parameters are used. They control the extent of the mutation (see Mutation below). The strategy parameters can be regarded as an internal model of the ES-environment and the ES-laws, generated by the actual optimisation problem. The development of the internal model is integrated into the ES-algorithm.

After the fitness of the i ES-individuals has been determined (see Selection below), they are ranked according to their fitness. The μ best of the ranked ES-individuals are chosen as ES-parents for the first generation ($t = 1$).

Reproduction

During one ES-generation the μ parents reproduce λ descendants. Two individuals are randomly drawn with replacement from the parent subpopulation. They reproduce the descendant $\mathbf{D}_{i,t}$.

The recombination operator ω_r is used to mix the vector elements of two parents $\mathbf{A}_{E1,t}$ and $\mathbf{A}_{E2,t}$:

$$\mathbf{D}_{k,t} = \omega_r(\mathbf{A}_{E1,t}, \mathbf{A}_{E2,t}) = ((b_1', b_2', \ldots, b_m'), (s_1', s_2', \ldots, s_m')), \quad k = 1, \ldots, \lambda \tag{12}$$

The new vector elements b_j' and s_j' are obtained from the connection of the objective variables and the strategy parameters using the recombination functions ρ_b, and ρ_s.

With respect to the objective variables and the strategy parameters, intermediate recombination functions are used:

$$b_{D,j}' = \rho_b(b_{E1,j}, b_{E2,j}) = b_{E1,j} + \alpha_j(b_{E2,j} - b_{E1,j}), \quad \forall\, j \tag{13}$$

α_j uniformly distributed random number over interval $[-d, 1+d]$, $d = 0.25$.

$$s_{D,j}' = \rho_s(s_{E1,j}, s_{E2,j}) = \frac{s_{E1,j} + s_{E2,j}}{2}, \quad \forall\, j. \tag{14}$$

Mutation

Mutation varies the vector elements of each descendant. The object variables b_j' are being mutated by using the strategy parameters s_j' and the mutation function ψ_b. The strategy parameters themselves are modified by the mutation function ψ_s:

$$\psi_b(b_j') = b_j' + s_j' N_j(0, 1), \quad \forall\, j \tag{15}$$

$N(0, 1)$ normal distributed random number, with 0 mean and standard deviation 1,

$$\psi_s(s_j') = s_j' \exp\left(\tau_1 N(0, 1) + \tau_2 N_j(0, 1)\right), \quad \forall\, j \tag{16}$$

$\tau_1 = 0.15$,

$\tau_2 = 0.20$.

The s_j' will always remain positive, because lognormal random numbers are used in equation (16). The values of τ_1 and τ_2 are chosen according to Nissen (1997).

Selection

The selection comprises the assessment of the ES-individuals' fitness $\phi(\mathbf{D}_{k,t})$, the ranking of the ES-individuals according to their fitness, and the selection of the μ best of the λ descendants as parents for the next generation.

The model $\Xi()$ predicting the proportion of OTC-marked foxes with time, defines the ES-environment wherein the ES-individuals' worth is proven. The model $\Xi()$ takes a certain vaccination strategy ($\mathbf{B}'_{k,t}$) on its input $\Xi(\mathbf{B}'_{k,t})$. The time course of the proportion of OTC-marked foxes depends on this vaccination strategy. The fitness of descendant k represents is determined according to

$$\phi(\mathbf{D}_{k,t}) = \Xi(\mathbf{B}'_{k,t}). \tag{17}$$

Actually, the ES-individual's fitness is determined by using the individual's utility ($U(\cdot)$) cost ($C(\cdot)$) ratio

$$\Xi(\mathbf{B}'_{k,t}) = U(\mathbf{B}'_{k,t}) / C(\mathbf{B}'_{k,t}). \tag{18}$$

An individual's utility depends upon the proportion of OTC-marked foxes in the course of three years, and the time (D) between the beginning of the analytical horizon and the time, when the proportion of OTC-marked foxes is above 0.70 for the first time:

$$U(\mathbf{B}'_{k,t}) = \sum_{T} OTC_T(\mathbf{B}'_{k,t})\,(1 - D/m) \mid OTC_T(\mathbf{B}'_{k,t}) \geq 0.7 \tag{19}$$

T simulation time [weeks],
OTC_T proportion of OTC marked foxes in week T,
k ES-individual,
t generation.

In eq. (19) the minimum proportion of OTC-marked foxes, which contributes to the utility, is 0.70.

The costs depend on the number of baits, used per campaign, and the number of campaigns, conducted within the analytical horizon:

$$C(\mathbf{B}'_{k,t}) = \sum (B_T C_B + C_d) \mid B_T > 0, \tag{20}$$

B_T number of baits used in week T,
C_B costs per bait,
C_d costs for distribution.

The ES-algorithm determines vaccination strategies, so that

$$\Xi(\mathbf{B}'_{k,t}) = \max_{b_j \in \Theta} \Xi(\mathbf{B}'_{k,t}), \tag{21}$$

Θ set of admissible parameter values.

When the ES-descendants in generation t are ranked according to their fitness, the μ best ES-descendants are selected to be the new ES-parents in generation $t+1$. This kind of selection resembles an artificial, extinctive mass selection, which is applicable for large populations. It is called extinctive selection, because only those ES-individuals with a high fitness value are allowed to reproduce. All the other

ES-individuals, including the adults, are being "extinct". The lifetime of each ES-individual is limited to one generation.

Termination criterion

Diverse criteria can be defined in order to terminate the ES-algorithm: The difference in the mean fitness of the ES-descendants in generation (t) and the mean fitness of those ES-descendants which are selected as parents for generation $(t+1)$ $(s(t))$ is one criterion. Its basis is the equation for the determination of the realised selection success $R(t)$ (Falconer, 1984):

$$R(t) = bs(t), \qquad (22)$$
$$R(t) = \bar{\phi}_{t+1} - \bar{\phi}_t,$$
$$s(t) = \bar{\phi}_t(\mu) - \bar{\phi}_t(\lambda),$$

b Heritability.

The second criterion is the mean Euclidean distance $(MED(t))$ between the parents in generation t with respect to their objective variables. This criterion was chosen for this analysis.

RESULTS

First of all a standard oral vaccination of foxes (OVFs) is carried out. The OVFs is used as a baseline comparator. Applying OVFs, 18 baits per square kilometre [sqkm] are distributed twice a year at the beginning of week 16 (mid April) and 40 (beginning of October) respectively. In Fig. 2 the simulated population dynamic of the Red fox (No. of individuals per sqkm) is presented along with the proportion of OTC-marked foxes.

At the beginning of the simulation (week zero), 1.5 adult (one year old) foxes inhabit one sqkm. By the end of the third year the number of foxes has been increased to 1.744 individuals per sqkm. Applying OVFs leads to OTC-marked foxes. In the first year, the vaccination of the foxes in April raised the proportion of OTC-marked foxes to approximately 40%. This proportion remains almost constant until the second vaccination campaign takes place at the beginning of October.

The autumn campaign increases the proportion of OTC-marked foxes to approximately 80%. In the following year, during the reproduction phase, the proportion of OTC-marked foxes drops below 40% and the vaccination carried out in spring only increases this proportion to approximately 55%. The next vaccination in October again leads to a proportion of OTC-marked foxes of about 90%. Within the third year, the time course of the proportion of OTC-marked foxes is similar to that of the second year.

Carrying out OVFs requires 108 baits per sqkm within the analytical horizon of 3 years. Two campaigns per year are conducted. The OVFs cost 100.62 Euro. The utility of the OVFs is only 8.32, especially because D (eq. 19) is 42 weeks.

Applying an improved strategy of oral vaccination of foxes (OVFi), which has been determined by the optimisation algorithm, now baits are to be distributed in the field at the beginning of the week 1, 22, 72 and 126. Two campaigns are carried out in the first year and one campaign is carried out in the second and the third year respectively (Fig. 3). For OVFi, the numbers of baits used per sqkm during the first, second, third and forth campaign are 21, 17, 14 and 13 baits respectively. A total number of 65 baits per sqkm is used. Applying OVFi decreases the costs to 61.54 Euro, which is nearly half of the costs of the OVFs. On the other hand, OVFi increases the utility to 82.15, which is about ten times higher than the utility of the OVFs. The fitness of the OVPi is 1.33.

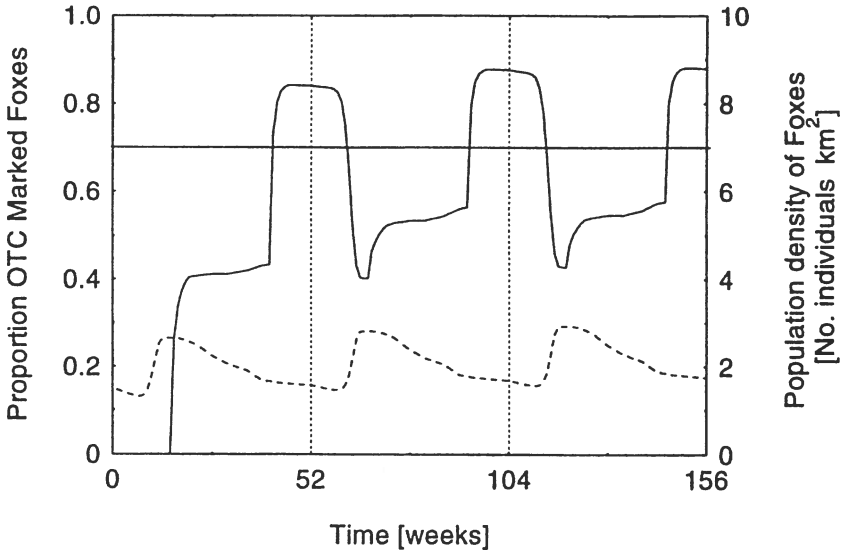

Fig. 2 Population dynamic of the red fox Vulpes vulpes (dashed line) and the proportion of OTC-marked foxes achieved with the standard strategy of oral vaccination of foxes within the time span of three years (for details see text)

If the improved strategy OVFi is applied, baits are to be distributed at the beginning of June. At this time weather conditions – especially temperature – might decrease the baits' durability. The influence of reduced durability of the baits during the weeks 21 (begin of June) to 34 (end of August) each year on the optimal vaccination strategy is analysed by using Threshold analysis.

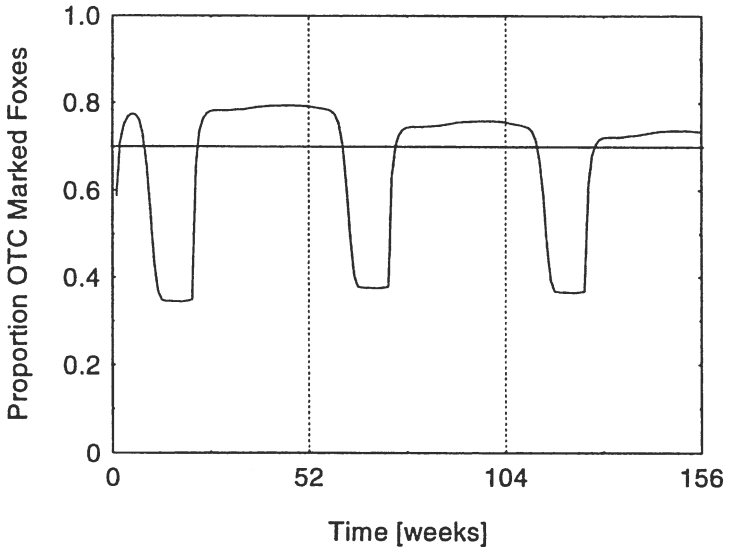

Fig. 3 Proportion of OTC-marked foxes achieved with the improved strategy of oral vaccination of foxes

The results of the Threshold analysis are presented in Fig. 4. On the y-axis, parameter t_c is given, describing the durability of the baits (see equation 10). This parameter has been reduced from 40.4 days (i.e.

used for OVFi) to 7 days in steps of one week. On the x-axis the optimal times [weeks] of bait distribution are marked. The bars represent the number of baits to be distributed per sqkm during that campaign. On the right hand side of Fig. 4 the costs and the utility associated with a particular strategy are given.

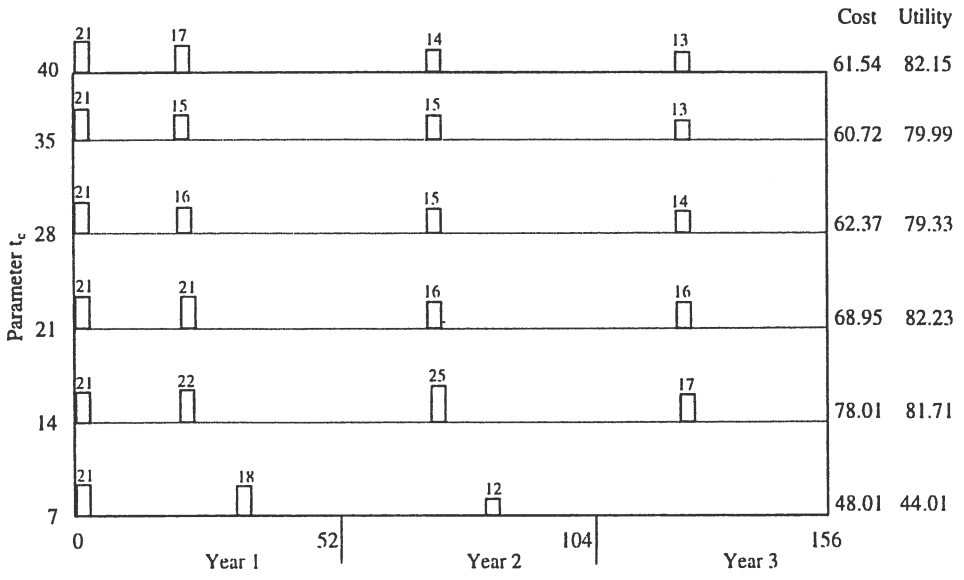

Fig. 4 Influence of reduced durability of baits on the arrangement of vaccination strategies (for details see text)

The Threshold analysis indicates that decreasing the durability of the baits to 14 days does not significantly alter the time of bait distribution in the field. The decrease of the durability is compensated by increasing the number of baits per sqkm. This kind of compensation leads to increasing costs, while the utility almost remains unchanged.

If the durability of the baits is further reduced to 7 days, then the times of bait distribution as well as the number of baits used per campaign alter noticeable. The number of campaigns that should be carried out is reduced to three campaigns and the optimum strategy is to skip all campaigns within the third year of the analytical horizon. As one would expect, the timing of the campaigns within the first and the second year excludes the time period when the durability of the baits is low (week 21 to 34).

CONCLUSIONS

This improvement of the fox-vaccination strategies is to give an example for a circumstance, in which it is only a combination of mathematical models, optimisation algorithms and Threshold analysis, that is capable of increasing our knowledge with regard to the management of biological systems.

Animal disease control, such as oral vaccination of foxes are forced to be cost efficient. That is, the funds spent must not be lowest in an absolute manner but in relation to the utilities gained. For the oral vaccination of foxes costs per square kilometre mainly depend on the number of vaccine baits distributed, and on the number of vaccination campaigns conducted. These costs should be low but the number of OTC-marked foxes should be high as this measure gives clues for the proportion of foxes actually vaccinated.

The relation between costs and the proportion of OTC-marked foxes changes according to the timing of the vaccination campaigns, and to the number of baits used per campaign. It is difficult to determine practicable solutions for this non-linear optimisation problem, because there are infinite potential strategies, i.e. combinations of the timing and the bait density. Conducting field experiments, only a small number of the potential combinations could be assessed. If we want to raise this number, mathematical models should be used. If we additionally link mathematical models and optimisation algorithms, we do not only generate the effects of numerous different strategies, but deliberately search for efficient strategies.

With respect to the oral vaccination of foxes, the combination of mathematical models and optimisation algorithms indicated, that conducting oral vaccination in summer is cost efficient. Experts, however, emphasise that summer vaccination might be impossible because of the reduced durability of the vaccine baits during summer. The impossibility of designing planned field experiments, which enable us to quantify the interaction between the durability of baits and the proportion of OTC-marked foxes, is obvious: We cannot either keep the durability of the baits in the field at a certain value, or observe the actual durability. But a combination of mathematical models, optimisation algorithms and Threshold analysis enables us to explore the effect of a reduced bait durability on the timing of vaccination campaigns and the number of baits used per campaign.

According to the OVF, a summer vaccination is the most efficient strategy on condition of the durability of the baits being higher than the threshold of 14 days. If the durability is lower, a different efficient strategy is available. All the veterinarian authorities have to do now, is to find out whether it is possible, that the durability of vaccine baits used could drop below the given threshold or not.

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SPATIAL ANALYSIS

SPATIAL ANALYSIS – A NEW CHALLENGE FOR VETERINARY EPIDEMIOLOGISTS

D.U. PFEIFFER*

The classic epidemiological triad includes space in addition to person (animal) and time as its components. Largely due to computational difficulties spatial analysis is an area that only recently has become more easily accessible for epidemiologists. Spatial epidemiological analysis includes hypothesis-driven as well as non-hypothesis driven investigations. The latter fits well within the new field of data mining, which has emerged as a result of the availability of large databases particularly in business. The complexity of spatial analysis is mainly the result of proximity inter-relationships between the observations. This causes a problem, because standard epidemiological analysis typically focuses on the attributes of observations, and makes the assumption that they are independent. Temporal analysis introduces consideration of one-dimensional dependence between repeated observations on the same subject. Spatial analysis extends this interdependence into two- or even three- dimensional space. The last 20 years have seen a significant development in the statistical methods that can deal with analysis of inter-dependent observations. Spatial analysis is a very challenging field as it introduces a whole new set of technical terms and techniques in the context of data storage as well as statistical methodology, which are different from what epidemiologists have used traditionally.

SPATIAL DATA

Any data with a spatial reference, either relative or absolute, should be considered spatial. In the simplest case, it can represent the geographic coordinates of a location where an outbreak of a disease has occurred. More general, the spatial reference associated with a single observation defines a spatial feature such as a point or a polygon. As an example, a point could represent the location where an animal was found dead, and a polygon might describe the boundaries of a farm. If the whole dataset represents spatially contiguous information it may describe either a continuous surface or a lattice structure reflecting for example the risk of disease outbreaks given specific environmental risk factors. In addition to the spatial reference, each observation can also have attribute information associated with it. For example, in the case of the location where a rabid animal had been found, attributes could be the animal species, its age as well as any diagnostic examination results. If the geographical data represents farm boundaries, its associated attribute data may for example record the name of the farmer and the tuberculin test history of the herd. Spatially referenced data can be obtained through direct data entry, digitising or remote sensing.

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Particularly the latter has over the last years become a more cost-effective source for example for digital vegetation data.

Coordinate point locations can be easily stored using standard databases and displayed using scatterplot graphs. But particularly in the case of lattice structures, geographical information systems (GIS) provide much more effective tools for input, storage, manipulation and presentation of spatial data.

One of the most important decisions, which has to be made during collection of spatial data, relates to the choice of the appropriate level of spatial aggregation. It has a strong impact on the cost of data collection, the power of the analysis and the level at which inferences can be drawn. Farms are often presented as point locations as this is the quickest and cheapest method for obtaining spatial reference data, and this may often be sufficient. But it becomes difficult to analyse neighbourhood relationships with this kind of data, as it does not take into account the shape and the size of the properties. Polygon (i.e. area) data could be presented at different levels of aggregation in that the number of infected animals is summarised per herd, district or country. Inappropriate aggregation can lead to effects such as the ecological fallacy and the modifiable areal unit problem (MAUP). The first relates to the difference between estimates at the aggregate and the individual level. The second, the MAUP, stems from the fact that areal units typically do not represent 'natural' but rather arbitrary constructs (Haining, 1998). This may result in a fixed number of areal units of the same spatial extent, but varying numbers of animals per unit. Alternatively, the number of areal units can be reduced, resulting in increased spatial extent of each unit. When analysing for presence of association, variability typically is underestimated, and therefore measures of association may increase. Ideally one would choose the scale revealing most detail, but this is likely to result in substantial costs, loss in data quality and in data quantities which cannot be processed. Therefore, a sensible compromise has to be found which still allows meaningful observations to be made and sensible inferential conclusions to be drawn.

The accuracy and precision of spatial data is an issue, which deserves special consideration. GIS often combines data from many different sources, which may well be of different accuracy and collected at different scales or precision. Often the error associated with the base maps is not documented. Combining such maps to generate new maps, one of the fundamental functions of GIS, can produce unpredictable results. This can result in error propagation particularly when these output maps are used as input for other operations.

SPATIAL DATA ANALYSIS

The analysis of spatial data can focus on the relationships between attribute variables, or on the spatial and space-time dimensions or a combination of attribute and space/space-time. The methods used in spatial data analysis can be broadly categorized into those concerned with visualizing data, those for exploratory data analysis and methods for the development of statistical models (Bailey and Gatrell 1995). Analyses can be hypothesis driven or they are used to trigger alarms if something unusual from a statistical perspective has been recorded. The first approach fits nicely into the classical framework of statistical analysis, whereas the second has resulted in controversy amongst scientists, as the risk of Type I errors can be high. Many studies will require post-hoc hypothesis formulation, and

involve multiple comparison analyses. In spatial analysis, effects have to be distinguished which result in long-range spatial trends (i.e. first order effects) and those which produce localised dependence (i.e. second order effects). Often both effects will be present, which complicates spatial analysis as most procedures make the assumption that only one of the two effects is present. First-order effects can be modelled relatively easily using regression models, whereas the second-order effect has to be specifically incorporated in the error terms (Bailey and Gatrell 1995). During most analyses, a combination of techniques will be used with the data first being displayed visually, followed by exploration of possible patterns and possibly modelling.

Analytical approaches can be divided into visualisation, exploration and modelling. Visualisation of the data should be the first step in a spatial data analysis. It involves showing the actual data values as two-, three or more-dimensional maps. Data is presented as points, coloured points or continuous surfaces/ lattices. It allows detection of data errors, as well as generation of hypotheses. Exploration of spatial data is aimed at description and quantification of spatial structure. During this phase, hypothesis testing is limited to detection of clusters and spatial dependence. Methods for quantifying spatial autocorrelation, variograms and specific tests for detection of spatial clustering are applied. Modelling is used to explain and predict spatial structure. Testing for cause-effect relationships is the main purpose of these techniques, which include statistical and simulation modelling, and also multi-criteria/multi-objective decision modelling

Visualisation

Visual analysis methods are extremely useful as they allow a process called visual thinking, and they eventually lead to visual communication through presentation of the data, for example, in map format. If the emphasis is purely on spatial occurrence such as whether a herd is infected or not, this can be shown using dot or polygon maps depending on whether point or polygon data has been used as the spatial reference. With dot maps any patterns become difficult to detect, as soon as the density of points increases. In this case, interpolation techniques can be used to generate continuous surfaces of the underlying point density. Kernel smoothing has become the standard method for these interpolations. The resulting surface represents the probability of the occurrence of a case, and is estimated using a bivariate probability density function (i.e. kernel), which is symmetric about the origin. The amount of smoothing is dependent on the chosen bandwidth, which can be fixed or adaptive. Fig. 1 shows kernel smoothed maps of the density of all dairy herds and those infected with enzootic bovine leucosis in New Zealand (Teekayuwat, 1999). The images improve presentation of point data, but the effect is strongly influenced by the parameters of the kernel estimator and its algorithm. It is also possible to estimate the ratio of kernel estimates, and thereby adjusting for differences in the population at risk (Bithell, 1990; Kelsall et al. 1995). With polygon data, differences in denominators can be taken into account through cartogram presentations or density equalized map projection (Merrill et al. 1996), where the size and shape of the polygons is basically re-scaled to represent differences in denominator values, such for example population density. Standardisation used to be the main technique for mapping of disease rates and ratios. It had the disadvantage that it could not take into account the increased uncertainty about true estimates resulting from small counts and denominators. Empirical or fully Bayesian estimates are more appropriate as they use the overall or local risk as a prior, with the effect

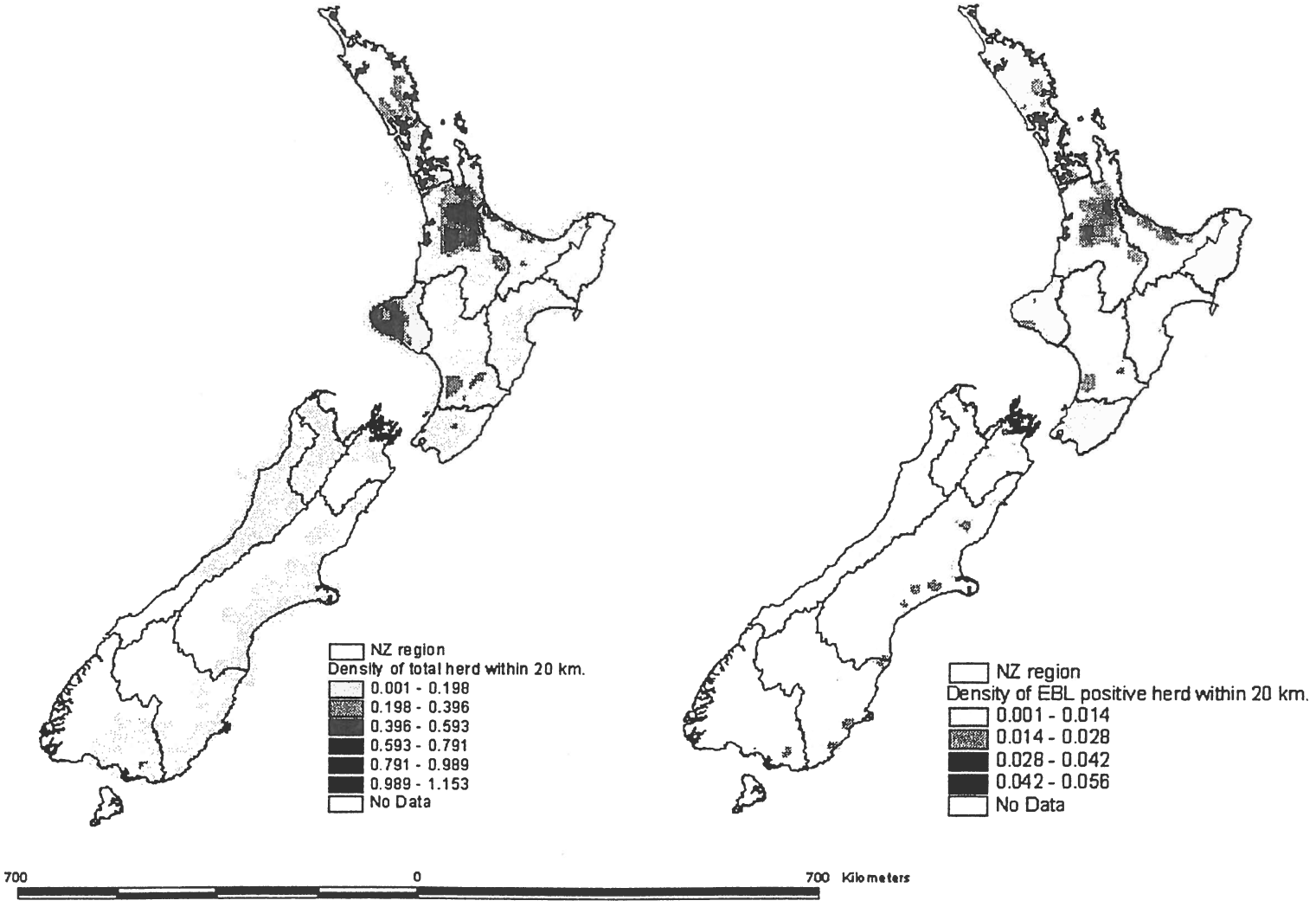

Fig. 1 Kernel density maps of all dairy herds and those infected with EBL in New Zealand

being that the local estimate is shrunken towards the overall or the neighbourhood mean risk particularly if it results from small numbers of observations in that particular location (Clayton et al. 1987; Langford, 1994).

Exploration

Exploratory analysis of spatial data is aimed at describing spatial patterns using inferential statistics, and it is used for the development of hypotheses. With the occurrence of disease it is mainly about whether diseases occur randomly in space or not. It is complicated by often having to take account of the spatially clustered distribution of the underlying population at risk. One effective method for dealing with this problem is the use of case-control data, where the cases of a particular disease are selected as usual and the controls are selected randomly from the non-diseased population, and therefore should represent the spatial distribution of the underlying population at risk. The controls could be matched to cases with respect to confounding factors other than spatial location (Lawson et al. 1996). This approach requires exact point locations for cases and controls to be available. If the data source is routine surveillance, it is likely to be aggregated at some administrative level such as veterinary district. As exploratory spatial data analysis involves statistical hypothesis testing, with the availability of fast computer technology bootstrap and permutation methods can be used to deal with the multiple testing problem (Kulldorff et al. 1995). In the statistical assessment of spatial clustering of point and polygon data global or local statistics can be generated. Global statistics will indicate if there is clustering somewhere in the area of investigation. Local statistics will also indicate where the likely clusters are and this will be particularly useful if the analysis is aimed at triggering an alarm.

Cuzick and Edwards (1990) developed a method which is based on nearest-neighbour distances. This aggregated test statistic compares the number of case-case pairs for a given number of nearest neighbors. Applying this technique to case-control data for New Zealand bovine tuberculosis breakdown herds, it suggests that there is significant clustering of cases compared with the control population according to the p-value for the Bonferroni statistic (see Fig. 2).

Other more recently developed techniques for analysing case-control point data for presence of spatial clustering are the K-function and the spatial scan statistic. The K-function produces an aggregated statistic. It expresses the mean number of cases with increasing distance from a given case scaled by the density of points in the area. The procedure is performed for cases and controls separately, and the difference between the two resulting K-functions can be plotted against distance. A random expectation statistic can then be generated by randomly permuting cases and controls at least 100 times and estimating K-functions and their differences for each permutation. If the observed curve extends beyond the simulation envelope, then significant clustering of cases relative to controls can be assumed (Bailey and Gatrell 1995; Jones et al. 1996). This technique assumes that only second-order effects are present and that these are isotropic (i.e. not dependent on direction). The example data presented in Fig. 3 represents locations of farms involved in the outbreak of an infectious animal disease (unpublished data). The shape of the difference of the kernel functions for case and control farms in relation to the simulation envelope indicates that there was significant clustering. The disadvantage of K-function is

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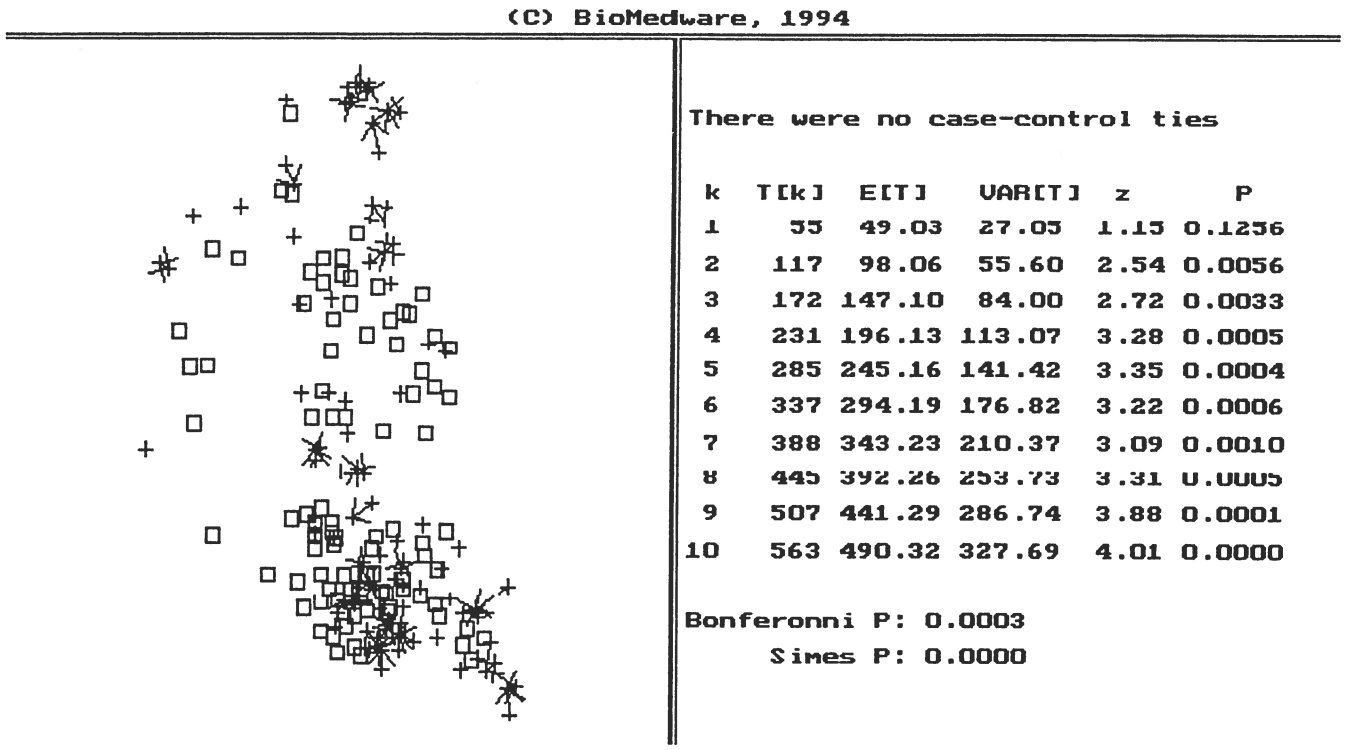
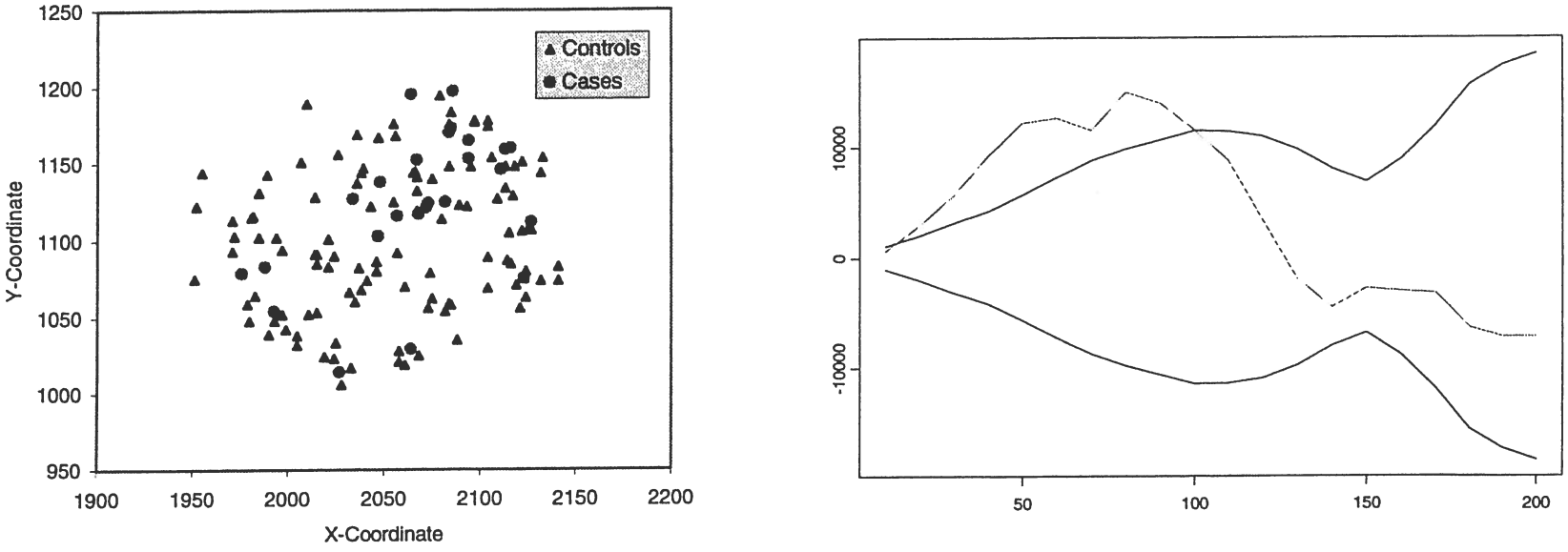


Fig. 2 Cuzick and Edwards' method applied to tuberculosis breakdown case control study data (+ = cases, • = controls, arrows identify nearest neighbours)



a b

Fig. 3 Map of case-control farm locations and difference K-function based on case-control data for animal disease outbreak

that it does not describe the location of the clustering. It is also quite common with animal diseases to have first-order effects present such as variation in climatic conditions as result of different levels of elevation.

In contrast, the spatial scan statistic is a local clustering statistic. The procedure involves generating ever-increasing circles around every point and calculation of relative risks based on disease risks within and outside the circle. A likelihood ratio test is calculated to assess for statistical significance, and the distribution of the test statistic is estimated using Monte-Carlo sampling (Kulldorff et al. 1995). Fig. 4 shows the map of trap locations used by tuberculous and non-tuberculous wild possums during a longitudinal field study in New Zealand (Pfeiffer, 1994). The spatial statistic identified a most likely cluster in one particular region of the study area. The associated relative risk was 2.1. A p value of 0.003 was estimated indicating that such a localised density of traps used by tuberculous possums only occurred 3 times in 1000 Monte-Carlo samples. The spatial scan statistic is one of the more robust techniques for exploratory analysis of spatial clustering.

For aggregate data such as disease rates, Moran's I, a global statistic, is used as an estimator of spatial autocorrelation. To allow a local description of spatial dependence for this type of data, the generic concept of Local Indicators of Spatial Association (LISA) has been developed recently (Anselin, 1995). It embraces Moran's I, Geary's C and the Getis-Ord G_i statistics under a single mathematical framework. This methodology can be complemented by visualisation of the resulting statistics as maps presenting spatial lag pies or bar charts (Anselin et al. 1993).

In dealing with infectious processes, testing for clustering in space and time may be of interest. This is usually done using the point locations of the cases and most statistics available will work on all possible pairs of time-space distances between the points. The Knox test (Knox, 1964) and Mantel method (Mantel, 1967) have been the classical techniques used for these analyses. More recently, the space-time scan statistic (Kulldorff et al. 1998) and the K-nearest neighbour test (Jacquez, 1996) have been developed. All four techniques are using permutation statistics. They make the assumption that population sizes do not change in time, but the statistics are not affected by spatially heterogeneous populations. The data from a longitudinal study of *Mycobacterium bovis* infection in a wild possum population in New Zealand already mentioned above will be used to demonstrate the usage of the two newer techniques. The *K*-nearest neighbour test of space-time interaction allows an assessment of the statistical significance of a potential space-time interaction process. The test statistic indicates the number of case pairs which are *K* nearest neighbours in time and space. The statistic is based on an approximate randomisation of the Mantel product statistic. Fig. 5 presents the results from applying the *K*-nearest neighbour method to the possum tuberculosis data. The map shows the locations of the traps where tuberculous possums had been caught and the arrows indicate k=2 nearest neighbours. The test statistic produced on the basis of 1000 random permutations suggests that only the cumulative statistic J_k is statistically significant, whereas ΔJ_k is not. The latter parameter measures the statistical significance resulting from increasing *K* by 1. The test statistic supports the presence of space-time interaction, and suggests that the first 5 nearest neighbours are probably involved in space-time interaction, and thereby provides an indication of cluster size, but not of its actual geographical dimensions. The result has to be

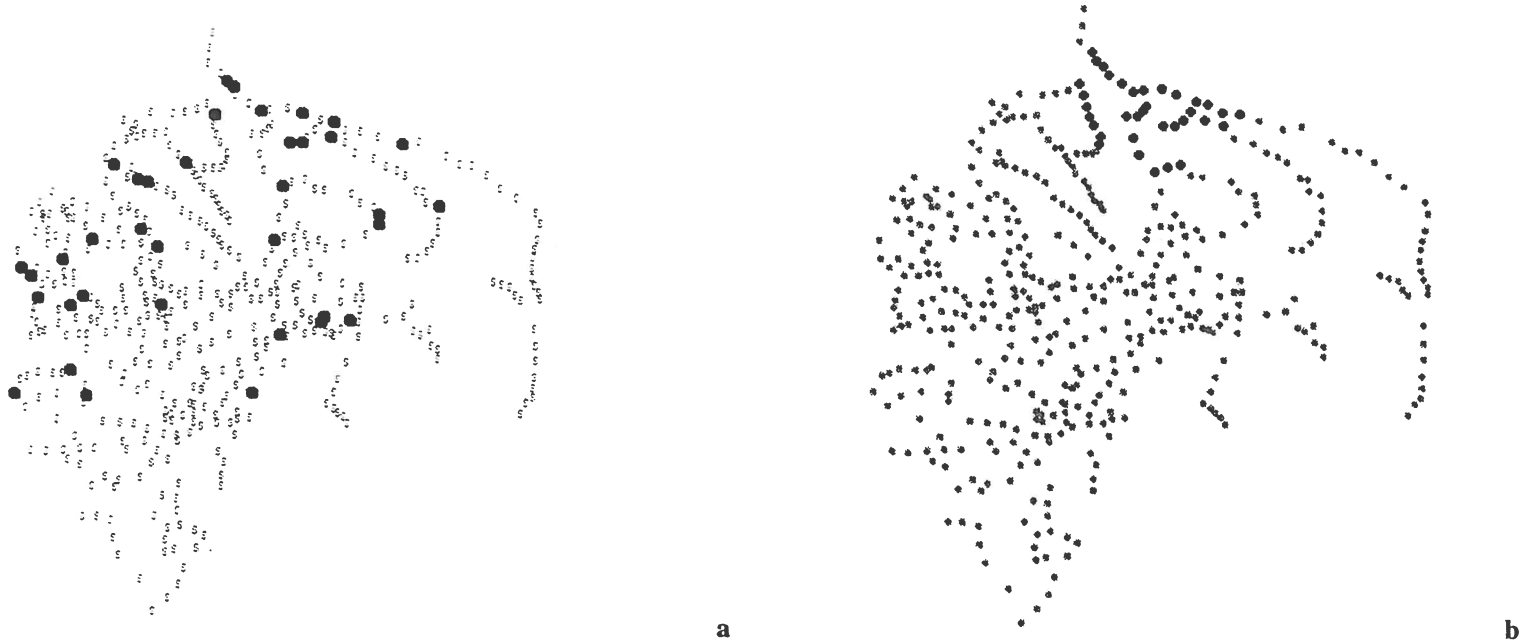


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Fig. 4 Locations of trap sites where possums with (circles) and without (triangles) tuberculosis had been caught and the location of the most likely cluster according to the spatial scan statistic

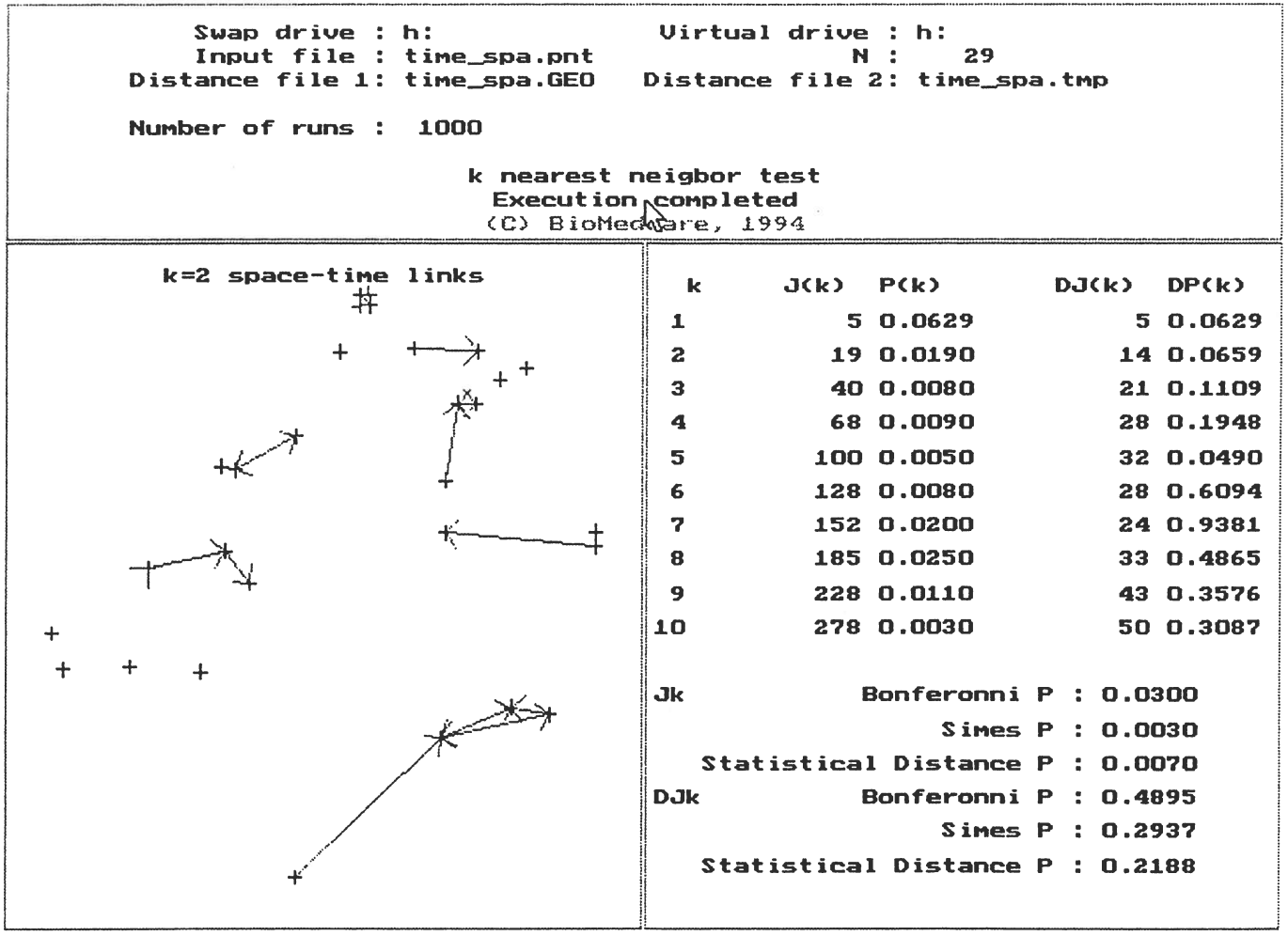


Fig. 5 Results from applying the K-nearest neighbour method to test for time-space interaction between cases of tuberculosis infection in wild possums

interpreted with caution though because none of the summary statistic for ΔJ_k is statistically significant.

An exploratory analysis of the spatial dependence of continuous type data involves the use of techniques such as the variogram method. This type of data is usually collected at sample point locations where some attribute such as density of disease vector populations is being measured. Spatial moving averages are used to represent first-order spatial effects, that is global trends in a particular geographical area, whereas variograms describe localised effects, i.e. second-order spatial effects. The presence of second order effects would result in positive covariance between observations a small distance apart and lower covariance or correlation if they are further apart. The covariogram describes the function of the covariance for varying distances h between sample points and the correlogram the corresponding correlation. The semi-variogram is a graphical representation of the variation between sampling points separated by a given distance and direction. For a stationary spatial process all three describe similar information. Estimates of the semi-variogram are considered to be robust to departures from stationarity represented as a general trend in the spatial process. A continuous process without spatial dependence will result in a horizontal line. A stationary process will reach an upper bound, referred to as the sill at a distance h called the range. Theoretically, the intercept with the y-axis should be at a value of 0 variation. In reality, sampling error and small scale variation will result in variability at small distances and the variogram will not meet the y-axis in the origin. This intercept with the y-axis is called the nugget effect. Variograms which do not reach an upper bound suggest non-stationarity in the data. Fig. 6 shows an isotropic sample semi-variogram for the proportion of tuberculous possums captured at trap sites during the above mentioned longitudinal study. The shape of the variogram suggests that the process is non-stationary, but given the relatively small nugget value there is also likely to be spatial dependence.

Modelling

Models derived from spatial data can be used to identify risk factors or they can remain within the spatial domain if these models are used for interpolation or smoothing. Particularly the latter are aimed at visual presentation. The most basic modelling techniques are based on map modelling (Bonham-Carter 1994). These involve overlaying different geographical layers of information using, for example, Boolean logic, whereas more advanced map modelling will apply fuzzy logic or Bayesian methods. A major area of continuing research development is the field of spatial risk factor modelling. The objective of these analyses in the context of epidemiology is to generate risk surfaces taking into account underlying geographical risk factor patterns. In epidemiology, parameters of interest are very often counts or proportions which should be modelled using generalised linear modelling techniques rather than ordinary least-squares regression. The most significant problem has been to determine mathematically sound techniques for taking into account the dependence in the spatial data structure. Bailey and Gatrell (1995) suggest introducing covariates into the regression model such as spatial coordinates or a variable representing broad regions to adjust for the effect of spatial dependence. Glass et al. (1995) developed a risk density map for Lyme disease based on a multiple logistic regression model, but they did not attempt to remove spatial dependence from the data. Williams et al. (1994) compared a number of different predictive modelling approaches for spatial data. They used linear and non-linear discriminant analysis, tree-based induction and neural

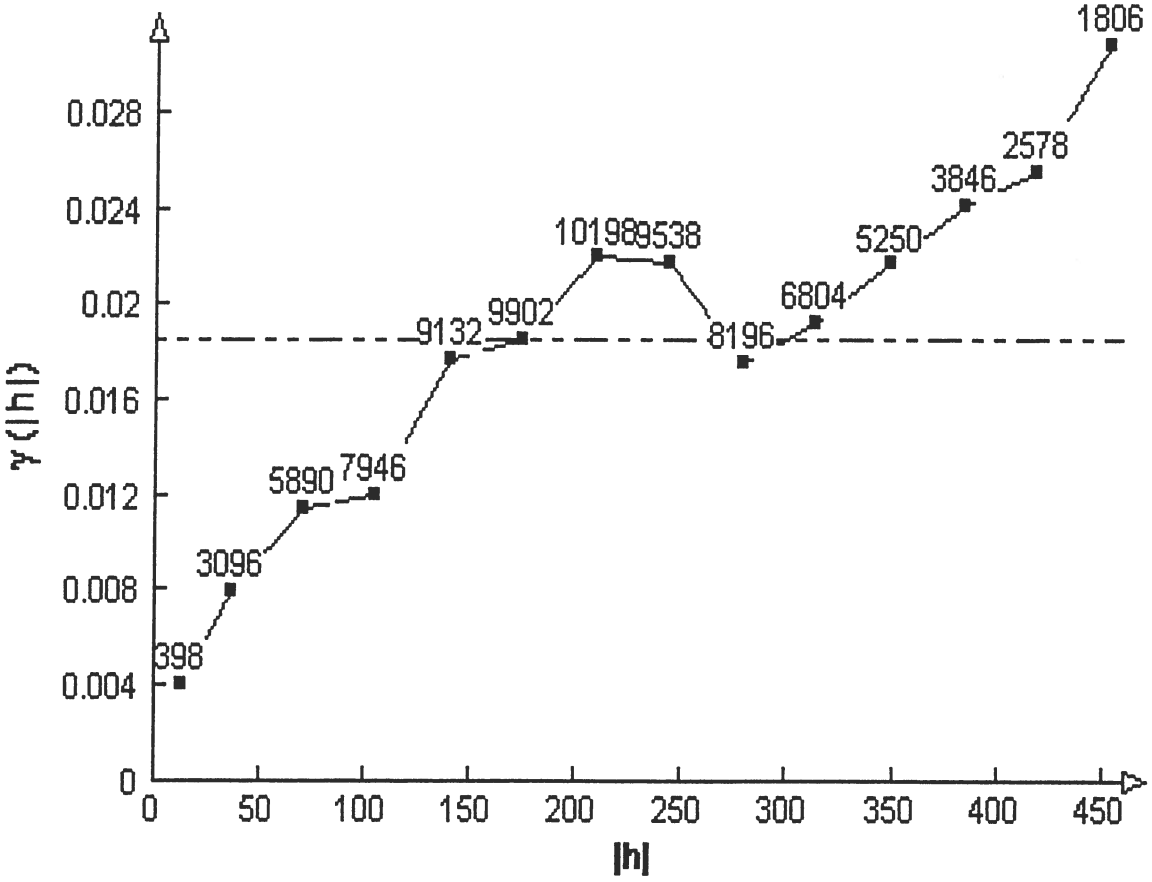


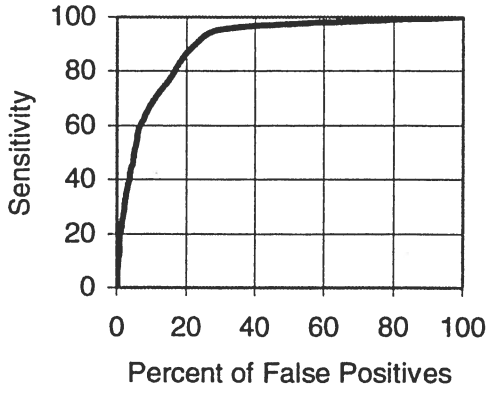
Fig. 6 Isotropic semi-variogram for the proportion of tuberculous possums captured at individual trap sites in the longitudinal study

networks to map tsetse distributions in Zimbabwe and concluded that while the simpler methods (linear discriminant analysis and tree-based induction) were less precise, they were easier to interpret. They also did not explicitly take account of spatial dependence. Fig. 7 presents results of a logistic regression analysis for prediction of *Theileria parva* presence in an African country (Pfeiffer et al. 1997). The regression model includes eight different environmental and land use variables and is based on information collected at random sample locations throughout the country. The model was used to generate a risk map representing the probability of *T.parva* presence at a particular location given a number of risk factors included in the model. This map is presented as a DTM and as a raster map. The receiver operating characteristic curve (ROC) characterizing the predictive accuracy of the model can be used to adjust the decision making cut-off for the prediction probability balancing sensitivity and specificity as required. In this analysis the possible presence of spatial dependence was taken into account using a categorical variable representing region as a random effect.

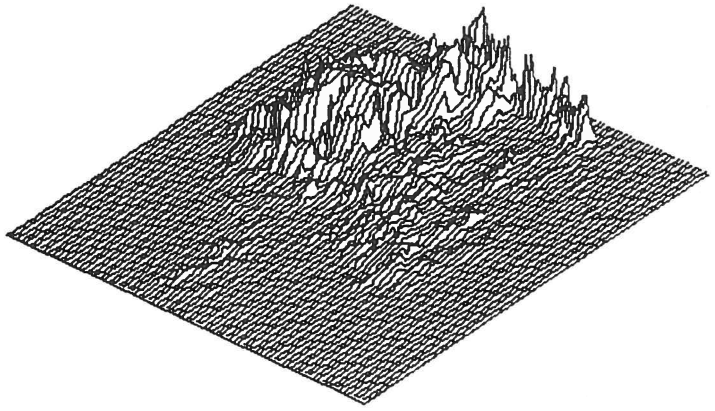
Three important developments have now provided more appropriate solutions to the problem of incorporating spatial dependence in regression models. Firstly, the advent of multi-level modelling provided a statistical framework for modelling spatial dependence in covariance structures (Langford et al. 1999). Secondly, the use of the prior in Bayesian statistics lends itself for representing the spatial dependence during the estimation of model parameters. Thirdly, the computation technique Markov chain Monte Carlo (MCMC) method using Gibbs sampling produces robust simulation- based estimates of the likelihood or the posterior distribution in the case of Bayesian inference (Lawson et al. 1996). One particular approach for representing spatial dependence with binary outcome variables involves the use of an autologistic term which was originally described by Besag (1974). This term can be used as a covariate additional to other risk factors in a logistic regression model to reflect the dependence of the local risk of disease on the level of disease in the neighbours (Gumpertz et al. 1997); (Augustin et al. 1998). These authors applied Gibbs sampling for parameter estimation. A Bayesian hierarchical spatial modelling was described by Xia et al (1997) who used a mixed prior, one conditionally autoregressive and the other unstructured. This approach of including non-spatial as well as spatial random effects in the models which was first suggested by Besag et al (1991) is now commonly adopted when performing spatial regression modelling (Wakefield et al. 1999); (Langford et al. 1999). The difficulty with applying both, spatial logistic regression and MCMC estimation techniques, is that they are not available as standard procedures in statistical analysis packages. This is likely to change in the near future, though. For example, a module called GeoBUGS for MCMC estimation of spatial generalised linear models will be released for the BUGS software (MRC Biostatistics Unit, Institute of Public Health, Cambridge, United Kingdom) in the first half of 2000.

A number of approaches can be used to model or predict spatially continuous data. For the *first-order* processes *trend surfaces* can be generated with ordinary polynomial least squares regression. Results have to be treated with caution, because the standard regression assumptions of independent random errors and heteroscedasticity are likely to be violated. Lessard et al. (1990) applied this type of approach when using an inverse distance-weighted mathematical algorithm to interpolate climatic measurements between sample points. Most trend surface models may be able to describe an overall trend, but are not useful for local prediction. In the presence of weak first order, but strong second order effects it is more appropriate to use models fitted to variograms. Such models can be defined 'by eye' and are

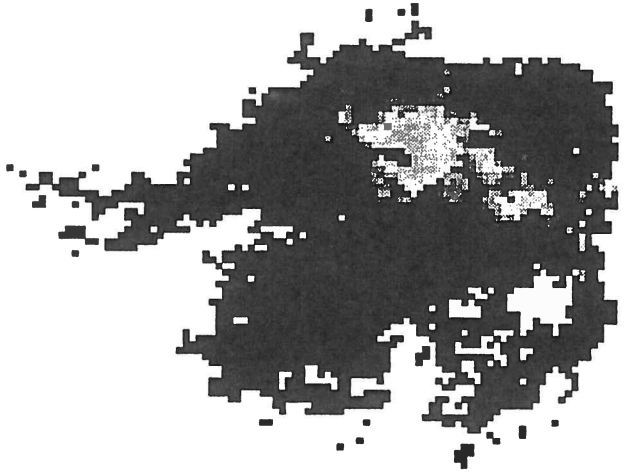
Sampling locations



ROC curve for logistic regression model



DTM of predicted probability of *Theileria parva* presence



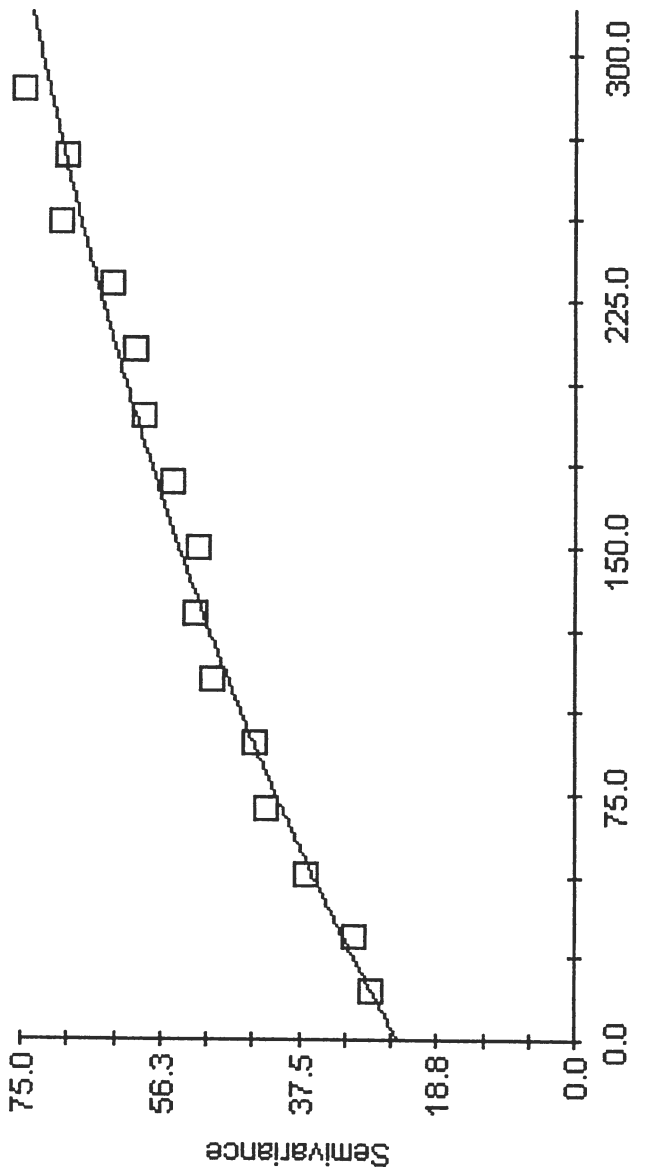
Raster map of predicted probability of *T.parva* presence

Fig. 7 Results of a multiple logistic regression analysis for prediction of *Theileria parva* presence in Zimbabwe

most commonly based on a spherical, exponential or gaussian model fitted to the variogram. The fit of a particular model can be assessed through cross-validation based on a comparison between the observed and interpolated values. Fig. 8 shows an isotropic exponential *variogram* model for the possum trap capture data from the longitudinal study discussed above. There is no upper bound to the variogram model indicating that the data is non-stationary. For this particular model to be a valid representation of the underlying data it would therefore be necessary to first remove the non-stationarity (i.e. first order spatial effect) through trend regression.

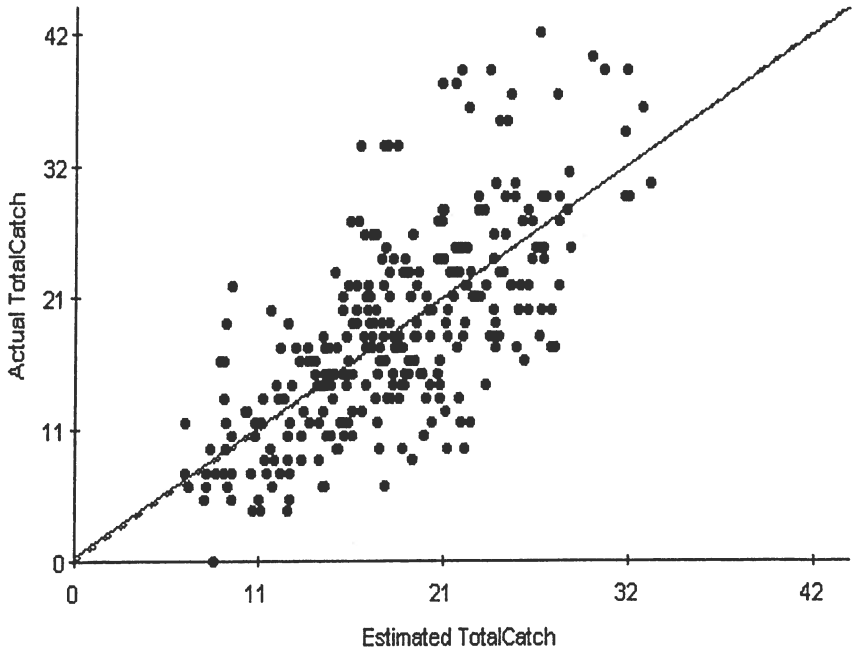
The variogram model itself does not allow prediction of values. This can be achieved with kriging. This is a weighted moving average technique for estimating the value of a spatially distributed variable from adjacent values while considering interdependence expressed in a variogram. It allows the interpolation error to be mapped and from a statistical viewpoint is considered to be an effective technique for interpolation of continuous type spatial data according to Oliver and Webster (1990). Webster et al (1994) used kriging to describe the risk of cancer in children for the West Midlands of England. The resulting maps showed that child cancer in this region clearly had a patchy distribution, in that areas of high risk were near to each other as was the case amongst those of low risk. The authors emphasise that with low incidence disease such as in their analysis it is important to have large amounts of observations available. They expressed concern about the validity of the confidence limits they had estimated for their binomial data, as the technique is more appropriate for prediction of continuous type variables. Carrat and Valleron (1992) applied kriging to generate a map of weekly influenza cases for France. They concluded that kriging had the advantage that it was not constrained by geographical boundaries and that it can be used to satisfactorily replace missing values. Pfeiffer (1994) used ordinary kriging to produce a surface of possum population density based on possum capture data at sample points (see Fig. 9). As discussed above, the isotropic variogram (see Fig. 8) suggests that this data is not stationary, but it also suggests strong spatial dependence. An exponential model was fitted and used as the basis for kriging. The crossvalidation scatterplot indicates that the model substantially under-estimates high trap catch. For regression modelling of continuous type dependent variables, spatial covariance structures can be specified as part of mixed models to estimate and adjust for spatial dependence (Littell et al. 1996).

A number of multivariate methods can be used for modelling of spatially continuous data. Principal components combine the information from multiple variables into a small number of components, each of them representing a particular combination of variables and explaining a particular proportion of the variation in the data. Eastman and Fulk (1993) used the technique to analyse the information contained in a time series of NDVI maps for Africa, thereby conducting a space-time analysis of continuous type variables. This technique could be used to assess the relative importance of spatial in comparison with temporal variation, for example the pattern of tick-borne disease incidence across a country could be separated into spatial and seasonal/cyclical variation. Cliff et al. (1995) discuss the application of multidimensional scaling (MDS) to spatial epidemiological data. They use the technique to map geographical information about measles mortality in Australia and New Zealand as a disease space where points with similar disease risks are closer to each other on the MDS map even though they are far removed geographically. Bailey and Gatrell (1995) discuss a range of other multivariate analysis techniques for spatially continuous data.

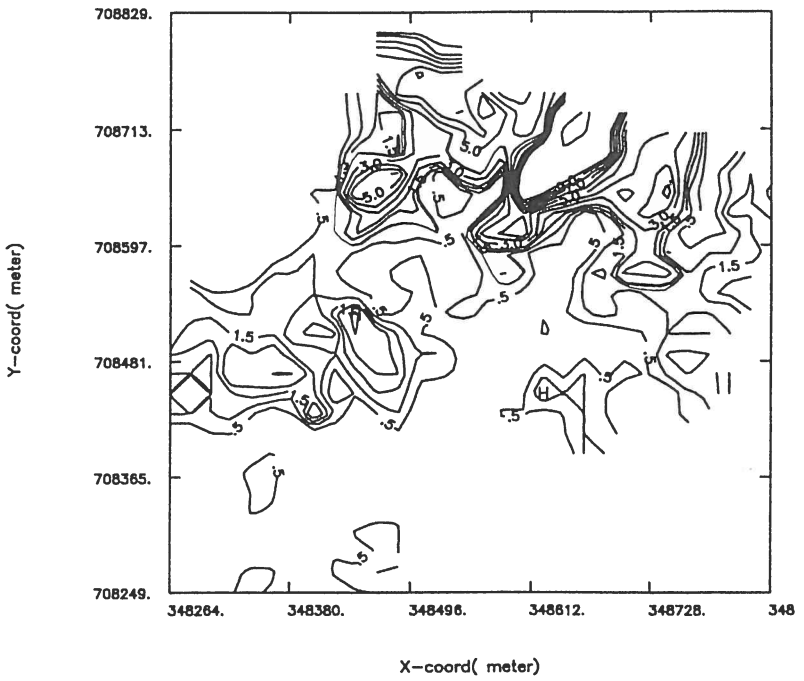


Exponential model (Co = 24.0000; Co + C = 102.2500; Ao = 314.10; r2 = 0.981;
RSS = 54.48)

Fig. 8 Isotropic exponential variogram model for possum trap capture data



Cross-validation of kriging values



Contour map based on kriging estimates

Fig. 9 Crossvalidation and contour map estimates for interpolated possum density in the longitudinal study on possum tuberculosis epidemiology

Recently, the use of spatial data for optimisation of resource allocation has been explored. Methods include multi-criteria and multi-objective evaluation techniques which have been adapted for spatial problems. They can take account of the uncertainty in the underlying input data as well as of the risk of making the wrong decision (Eastman et al. 1995). Systems are being developed which will take data from various spatial input data sets to, for example, define optimal types of animal production for specific geographical regions. With this methodology, it will be possible to define optimal disease control strategies given various spatially defined constraint variables.

CONCLUSION

Spatial data analysis provides a range of new techniques for descriptive epidemiological analysis and also explanatory or predictive investigations. In the context of the increasing number of geographically referenced databases, beyond the use of purely descriptive methods spatial analysis will allow hypothesis generation for example if used for triggering alarms for unusual disease occurrence. In the field of predictive modelling, the production of risk maps generated from observed disease risks as well as from predictions based on risk factor patterns provide a means for more effective resource allocation. New developments still have to be expected in the field of generalised linear modelling of spatial data, and the recent developments do point towards Bayesian techniques being the appropriate methodology for taking account of spatial dependence. In the new Millennium, spatial methods will become a standard component of the epidemiologist's tool chest of analysis techniques.

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OPEN SESSION

CASE CONTROL STUDY EXAMINING THE ROLE OF LIVESTOCK MARKETS IN THE TRANSMISSION OF BOVINE TUBERCULOSIS

D.A. ABERNETHY[1], D.U. PFEIFFER[2], S.D. NEILL[3]

Mycobacterium bovis infection in cattle is mainly a respiratory disease with airborne infection being the principal route of transmission (O'Reilly & Daborn, 1995). Infected cattle and the European badger (*Meles meles*) are the major sources of infection for cattle in Great Britain and Ireland, although the relative significance of each has not been clarified (Krebs, 1997). This study examined one potential factor in bovine-bovine spread, namely the movement patterns of cattle. The purpose was to assess if any significant difference could be detected between the movement frequency of cattle with confirmed tuberculosis (TB) and that of selected control animals. Lifetime movement history was assessed, in particular, movement through markets. The extent of contact between reactors within a market on the same day was calculated and compared to that of the control group.

Extensive use was made of the animal health database maintained by the Department of Agriculture and Rural Development in Northern Ireland. This was established in 1988 and holds animal and herd details of all cattle in the province as well as their TB-test and movement history. Department staff issue permits to authorise movement of cattle between herds or onto other premises, such as livestock shows and abattoirs. Computer terminals, based in markets and abattoirs, are used by Animal Health Inspectors to check the details and status of all incoming cattle and to issue outbound permits from markets to specified destinations. Such checks, together with the herd audit provided by annual TB testing of all herds, has resulted in a comprehensive database of animal movement history. Each animal can be traced from birth to death and the health status of each herd checked, including intermediate herds. Furthermore, forward and backward tracing of cattle from infected herds can be initiated immediately infection is disclosed, facilitating the rapid identification and testing of suspect cattle.

MATERIALS AND METHODS

Study design and selection of cases and controls

The sampling frame comprised all cattle herds from which reactors to the single intradermal comparative cervical test were removed between 1989 and 1997. To compare movement

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patterns, a matched case control study design was used. Cases consisted of confirmed (lesioned) reactors with no recorded discrepancies in their movement history. Controls were selected from the same birth herds as the cases, and shared the same birth-date, sex and breed. 1,544 cases and controls were matched 1:1 from 1,005 herds and a matched analysis was performed to compare their movements.

The movement history of 1,220 controls that had been slaughtered under routine farm management was first examined as an approximation of normal movement patterns. Movement history of reactors was inadequate for this purpose as they were slaughtered prematurely while it was incomplete for control cattle still alive at the time the data was collected.

To assess the extent of contact within markets, an unmatched analysis was performed that included all reactors and controls. If markets play a significant role in the spread of TB then one would expect a substantial number of reactors to have either acquired or transmitted infection to other cattle while in the market. If these cattle then reacted to a future TB test, the movement history would indicate they shared a common market and movement date in their past. All reactors, both non-lesioned and lesioned, identified at TB tests or at slaughter between 1989 and 1997, were included in this part of the analysis and the extent of their contact compared to that of the control group.

Data collection and analysis

All data for the study was obtained from the Animal Health database using proprietary data manipulation software (Mapper Manual Functions). Summary statistics were produced using Microsoft Excel (Microsoft Corp., 1997) and further analyses were performed with Statistix (Version 1, Analytical Software, 1996).

RESULTS

Movement pattern of controls: The mean number of lifetime moves for the slaughtered controls was 3.86. Twenty-one per cent of the sample moved directly from the herd of birth to slaughter while 1% had 12 or more moves recorded. Sixty-two per cent of all movement occurred through markets (Fig. 1).

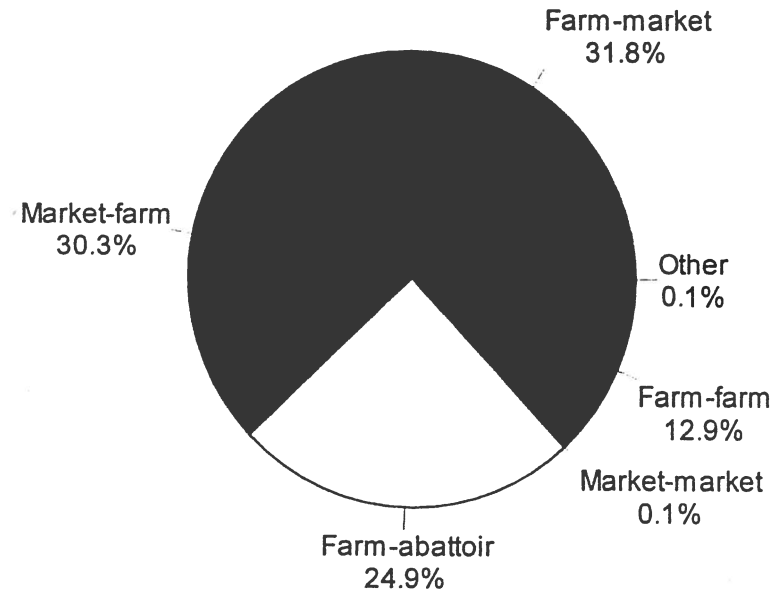

Fig. 1 Direction of movement of controls

Seventy-one per cent of the controls moved at least once through a market in their lifetime while 11% passed through 3 or more times (Fig. 2). Twenty-four per cent of all movement occurred when the cattle were less than 1 year and 29% at ages 1.5 to 2.5 years.

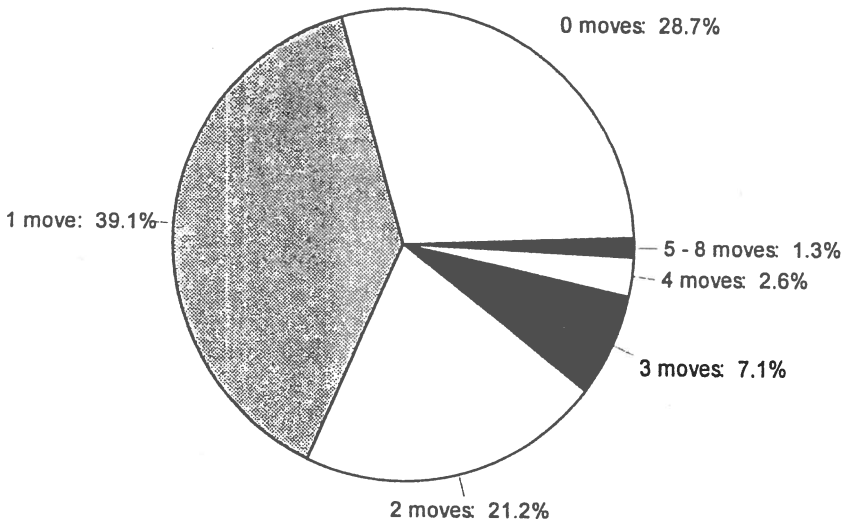

Fig. 2 Lifetime frequency of moves into a market

Comparison of reactors and controls

The routine movement history of reactors was compared to that of controls. The farm-to-abattoir moves for reactors were excluded as these were enforced moves specific to cases. Movement history of the controls was limited to that which occurred prior to the reactor removal date.

The mean number of moves was 2.20 for the cases and 2.13 for controls. To test if this difference was significant, the Wilcoxon signed rank test was used in a paired analysis. This returned a two-tailed p-value of 0.21.

The frequency of market movement was also compared between the 2 groups. The mean for cases was 1.30 and for controls, 1.28. The Wilcoxon signed rank test was used again, yielding a p-value of 0.22.

An unmatched analysis was used to assess if any association could be detected between increasing numbers of lifetime moves into markets and increasing disease prevalence. No statistically significant association was detected between the frequency of market moves and the risk of disease; analysis for trend failed to detect a significant association ($X_{trend} = 2.42$, $p = 0.12$).

In an analysis that included all TB-infected cattle between 1989 and 1997, reactors did not appear to have been presented in a market on the same day as another reactor any more than did the group of controls. The mean number of contacts was 0.69 for reactors and 0.71 . This was not significantly different ($p = 0.17$).

DISCUSSION

Northern Ireland has a national cattle herd of approximately 1.5 million, distributed among 36,000 registered herds. Livestock markets are a traditional and prominent feature of the industry and most cattle are traded through such premises. There are 34 that currently operate on a weekly basis and they vary in throughput from small yards selling 15 cattle in 1 auction per week, to large, dedicated premises that deal with 1,200 cattle in 3 or 4 sales over a similar period. It is common for cattle of various age or production groups to be auctioned on the same day and, although largely separated within the premises, segregation is usually insufficient to prevent inter-group contact. The potential exists, therefore, for infectious diseases to spread to cattle of a wide range of ages and types.

In Northern Ireland, tuberculosis in cattle is subclinical and infected animals may be presented in a market without the farmer being aware of the disease. It is also not unusual for farmers to sell cattle shortly before the annual TB test of their herd in order to reduce the numbers, and hence the inconvenience, for testing. In the reactor group in this study, 65% of the animals moved through a market at some time and 11% did so within 2 months of being slaughtered and 7% moved within 1 month of slaughter. It is, therefore, likely that some of these cattle were infected when passing through a market where they might have direct contact with a large number of susceptible stock.

Bovine tuberculosis has been greatly reduced in the province over the last 45 years, from an annual incidence of 20% to 0.03% (G.O. Denny, unpublished data). Total eradication may prove elusive however, until the potential risk from badgers is negated (Morris, R.S. & Pfeiffer, D.U, 1990; Denny & Wilesmith, 1999). Nevertheless, other bovine risk factors remain to be clarified and addressed. The significance of the infected bovine in spreading the disease is unclear for, although infection can be experimentally introduced and transmitted (Neill *et al*, 1988; Neill *et al*, 1989), transmission under field conditions is difficult to demonstrate (Griffin & Dolan, 1995, Wilesmith & Williams, 1986).

Should cattle-to-cattle transmission be significant, the nature of cattle farming in Northern Ireland is highly conducive to this means of spread. Farms in the province tend to be small, with fragmented grazing and a high dependence on leased summer pastures. In one extreme case, 29 farmers utilised 49 separate parcels of land within a $4km^2$ area (D. Abernethy, unpublished data). In a study involving 427 farms in Northern Ireland, Denny & Wilesmith (1999) reported that 79% of boundary fences could not prevent nose-to-nose contact of adjacent cattle, while no farms were noted where similar contact could be prevented along their entire boundary. The potential exists, therefore, for significant contact between cattle, enhanced further by winter confinement and an industry characterised by a high volume and frequency of inter-herd movement.

Control cattle in the study moved approximately 4 times during their lifetime but this underestimates the true amount of movement from Northern Ireland herds. Firstly, the control animals were selected from herds in which outward movement was restricted at least once due to tuberculosis. The period of restriction would have been at least 4 months and in many cases, would have exceeded 8 months. Secondly, only inter-herd movement is registered on the database. Informal intra-herd movement between the home-farm and separate holdings or rented pasture is not quantified, although all contact herds are evaluated in the event of a TB incident.

Almost three-quarters of the sample cattle moved through a market at least once in their lifetime, reflecting the dominant role of livestock markets in the cattle industry in Northern Ireland. Most movement occurred at 2 distinct ages: within the first year, often as young calves, and at 1.5 to 2.5 years, most likely as near-finished beef, pregnant heifers or freshly-calved young cows. The stress associated with this type of marketing should enhance excretion of the organism if an animal were in the infectious stage of a disease, and the extent of contact should facilitate spread to a large number of cattle, of different ages.

Cases and controls were closely matched on birth-herd, date of birth, sex and breed in order to reduce inter-subject variability and avoid possible confounding. In this way, any excess movement of reactors could be considered as a possible risk factor for tuberculosis. Failure to detect any significant difference suggests that frequency of movement may not be a significant factor. It does not, of course, negate movement of cattle *per se* as a risk factor. Various studies have concluded that purchase of infected cattle, for example, accounts for a significant proportion of breakdowns (Griffin & Hahesy, 1992; Griffin, 1993). A Northern Ireland study determined that the risk of a TB breakdown was twice as likely for farmers who purchased 100 cattle per annum than those of similar herd size and type but who did not buy in (G.O. Denny, unpublished data). This present study is the first though, to evaluate the frequency of movement as a potential factor.

The analysis also failed to detect any significant difference between cases and controls in the movement through a market. There was no statistically significant difference between the groups in the number of lifetime market visits, nor did frequency of visits appear to be a risk factor. Reactors did not share common market dates any more than controls. This would suggest that markets are possibly not significant in the epidemiology of tuberculosis, although the limitations of the study must prompt some caution in interpretation of the results. One limitation was the difficulty in identifying when reactors became infected. It was thus not possible to determine at which point this occurred in the movement history and this might have diluted any association, particularly in comparing inter-group contact within markets. However, it is unlikely that significant numbers moved undetected for an extended period, due to the high frequency of testing in the province and the efficiency of computerised tracing. A more serious limitation is the obvious inability to determine the infectious state of any reactors that passed through markets. The intermittent nature of *M. bovis* excretion and the difficulty in demonstrating between-cattle spread is well documented and has been extensively reviewed (O'Reilly & Daborn, 1995). This study used the presence of lesions as an indicator of infection but this says little about the excretory status of the animal when it last moved through a market. Nevertheless, failure to demonstrate any difference or increased risk associated with confirmed reactors in a sample of the size used in this study implies that the transient contact between cattle in a market is probably insufficient to allow significant spread of the disease. Further work is required to overcome these limitations, possibly by restricting cases to infected cattle which moved as a group through a market shortly before disease was confirmed, and assessing inter-herd spread associated with the purchase of reactors.

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STUDY ON THE SUSCEPTIBILITY AND DETECTABILITY OF BOVINE TRYPANOSOMOSIS UNDER NATURAL INFECTION CHALLENGE BY SURVIVAL ANALYSIS

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African bovine trypanosomosis (*Trypanosoma (T.) brucei, T.congolense, T.vivax*) transmitted by flies of the genus *Glossina* ("tsetse"), constitutes a major constraint to prosperous economic development of the agro-industrial sector in many sub-Saharan countries. Ecological and livestock management (including animal feeding) factors influence the degree to which domestic animals are exposed to tsetse and are important determinants of parasite and disease control (Connor, 1994). Certain autochtonous cattle breeds such as N'Dama (*Bos taurus*) have the capacity to limit the pathological effect of *Trypanosoma* infection – a phenomenon known as trypanotolerance (Murray et al., 1982; Dwinger et al., 1994).

Investigations into the time to first clinical, parasitological and serological detection of infection after challenge are of interest in the context of susceptibility and test validation studies. Requirements for such investigations are that the time of challenge is known and that infection follows natural routes. Mattioli et al. (1998) exposed previously uninfected N'Dama, Gobra zebu (considered more susceptible than N'Dama) and Gobra × N'Dama crossbred cattle to natural tsetse and tick challenge and studied the clinical response of the animals and the parasitological detectability of trypanosomes. In this paper we present the results of a supplementary analysis of the data obtained focusing on the incidence aspect of the events such as first occurrence of clinical signs and detectable trypanosomes and trypanosomal antigen. The objective of our study was to substantiate differences among the breeds with regard to time to first clinical signs and detection of parasites using survival analysis.

MATERIALS AND METHODS

Animals, clinical, parasitological and serological investigations

The experimental design of the study is described in detail by Mattioli et al. (1998). Briefly, 12 N'Dama, 12 Gobra zebu and 12 N'Dama × Gobra (F1) crossbred cattle (6 males and 6 females in each cohort) were raised in tsetse-free areas and treated with an anthelmintic (albendazole, 7.5 mg/kg body weight) and a trypanocidal drug (diminazene aceturate, 7 mg/kg body weight) about 4 weeks prior to exposure. In August 1995 the animals were exposed to natural moderate to high tsetse challenge in the Niamina East area in The Gambia.

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The animals were investigated clinically (packed cell volume, PCV), parasitologically (detection of trypanosomes by buffy coat technique) and serologically (detection of *T.brucei, T.congolense* and *T.vivax* antigen by ELISA) every other day during the first 3 months of the study and twice a week thereafter over a total period of 180 days. The PCV was measured using a micro-centrifuge. Subject-based (i.e. specific for individual animals) cut-off values for PCV were established as mean minus 2 standard deviations of all PCV measurements available for the animal up to and including day 10 post exposure. A PCV value below the individual cut-off value is denoted as "low" PCV value. Enzyme-linked immunosorbent assays based on monoclonal antibodies against *T.brucei, T.congolense* and *T.vivax* developed by Nantulya et al. (1987) and calibrated by the Joint FAO/IAEA Division were used for detection of trypanosomal antigen. The test protocol (TPR 1.2) was followed strictly. A cut-off value of 25 percent positivity was selected according to the test protocol.

Statistical analysis

Events of interest were the first relevant drop in PCV, the first parasitological detection of *T.brucei, T.congolense* or *T.vivax* and the first detection of *T.brucei, T.congolense* or *T.vivax* antigen. Time was measured in days post exposure. The time of first relevant drop in PCV was defined as the first time when the number of low PCV values among three subsequent measurements (the actual, the preceding and the following) exceeded 1. The incidence was defined clinically (I_c) based on PCV, parasitologically (I_p) and serologically (I_s). Recurrent events were not considered. The time difference (TD) between first parasitological and first serological detection was established as I_s time minus I_p time. The effect of breed and sex on the subject-based PCV cut-off and on TD was investigated using ANOVA. The effect of breed and confounding factors on "survival" time (i.e. time until event of interest occurs) was assessed by Kaplan-Meier survivor function plots and estimated using Cox regression (Stata, version 5.0; StataCorp., 1997). Hazard ratios for time-independent (breed, sex) and time-dependent (PCV, parasitological status) variables were obtained as exponentiated coefficients from the Cox regression and can be interpreted as relative risks (RRs) for the factor category compared to the reference category or as RR for a one unit increase in case of dichotomous or continuous explanatory factors, respectively (see e.g. Sahai and Kurshid, 1995). The fit of the models was assessed by a chi-square statistic.

RESULTS

Clinical incidence (packed cell volume)

The variability of the subject-based cut-off PCV values (mean cut-off values 22.1, 23.1, 21.2% for N'Dama, Gobra and crossbred cattle, respectively) was greater within than among breeds (ANOVA, $P>0.15$). Apparently, N'Dama responded later to exposure than Gobra or crossbred cattle (Fig. 1). The minimum I_c time (i.e. time to first relevant drop in PCV) was 24, 14 and 20 days in N'Dama, Gobra and crossbred cattle, respectively. A maximum I_c time of 156 days was observed in one male N'Dama. One crossbred cattle never responded clinically to exposure during the observation period according to the definition of I_c. However, this individual had the lowest cut-off value (16.2%) of all 36 animals.

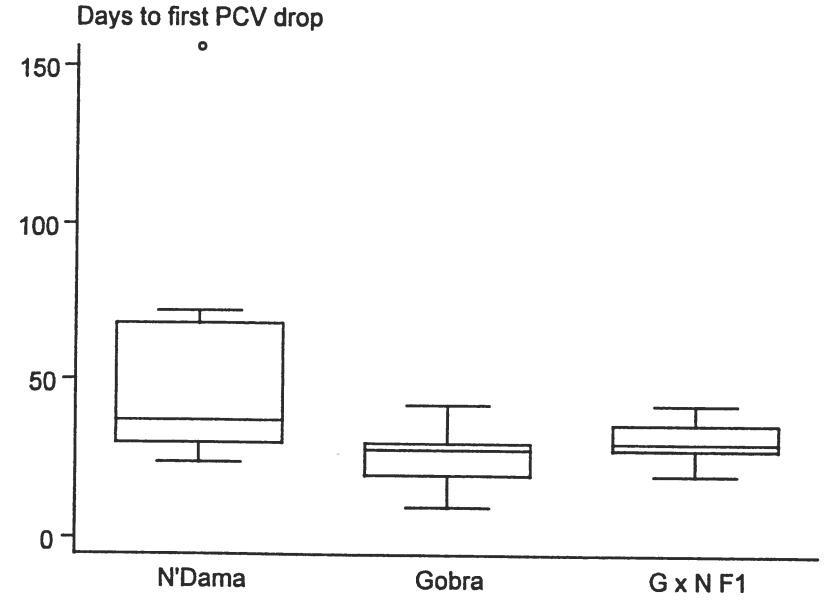

Fig. 1. Time to first PCV drop (in days) for N'Dama, Gobra and Gobra × N'Dama F1 crossbred cattle (n=12 each cohort) under natural tsetse challenge in The Gambia displayed as box plots. A box indicates the interquartile range (IR) and is divided by the median. Whiskers extend to the upper and lower adjacent value (i.e. highest or lowest value within 1.5 IR above and below the 75th and 25th percentile, respectively). Values beyond the adjacent values are plotted individually.

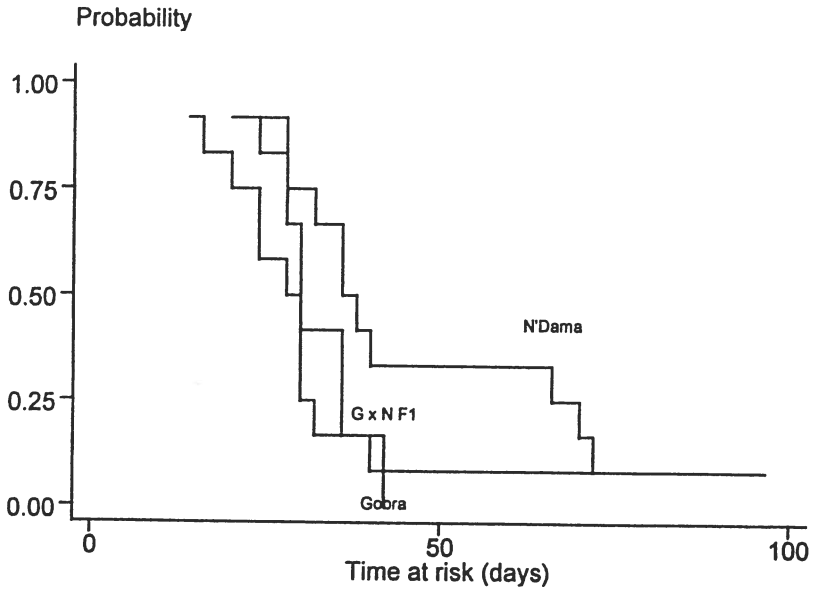

Fig. 2. Kaplan-Meier survivor functions for the clinical incidence (I_c) as defined by drop in PCV below subject-based cut-off value for N'Dama, Gobra and Gobra × N'Dama F1 crossbred cattle.

For the further analysis the effect of N'Dama versus Gobra and Gobra × N'Dama crossbred cattle was considered. The hazard ratio (95% confidence interval) for the factor breed, sex, detection of trypanosomes at a given time t and number of times trypanosomes were detected

up to (and excluding) t was 1.5 (0.7-3.3), 0.8 (0.4-1.7), 2.6 (1.2-5.7) and 1.1 (0.9-1.4), respectively, estimated by Cox regression (chi-square=10.6, df=4, P=0.03). Thus, the RR of a relevant drop in PCV was 1.5 in parasitologically positive compared to parasitologically negative cattle irrespective of breed and sex. The detection of trypanosomes at the respective time rather than the "history" of trypanosome detection for the given individual was an important factor.

Parasitological detection of trypanosomes

The time to first parasitological detection ranged from 14 (Gobra × N'Dama) to 74 (N'Dama) days (Fig. 3). Trypanosomes could be detected in all 36 animals. More time elapsed until trypanosomes were detected in N'Dama than in the other breeds (Fig. 3). Also the Kaplan-Meier survivor function estimate for N'Dama cattle was clearly different from the other breeds (Fig. 4). Using Cox regression (chi-square=13.9, df=4, P=0.008) we found a hazard ratio (95% confidence interval) of 4.8 (1.7-13.9), 2.0 (0.9-4.1), 1.0 (0.8-1.2) and 1.0 (0.9-1.2) for the factor breed (N'Dama vs the two other breeds), sex, PCV (in %) at time t and number of times up to (and excluding) t where low PCV values occurred, respectively. Thus, the estimated relative risk of parasitological detection of trypanosomes for Gobra or crossbred cattle versus pure N'Dama was 4.8. The RR estimate of 2.0 for male compared to female cattle failed statistical significance (P=0.07). The clinical status (PCV) was not related to the parasitological incidence.

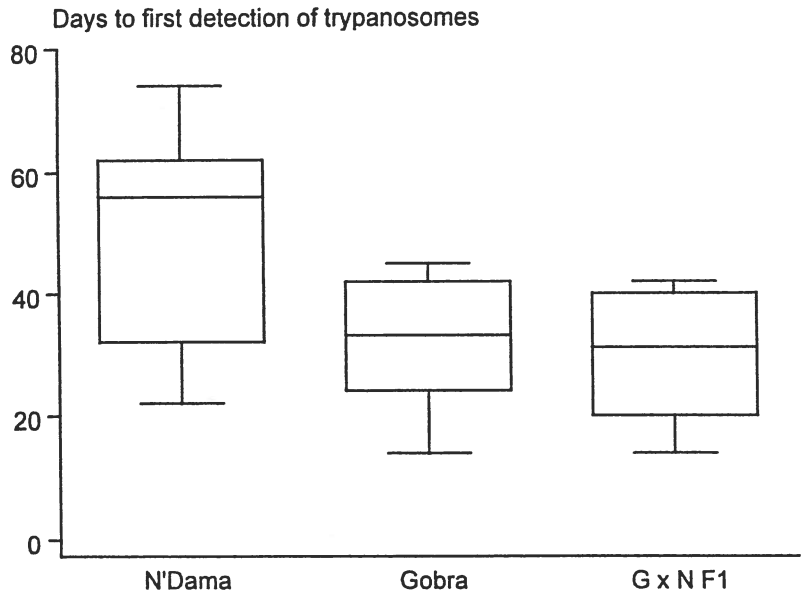

Fig. 3. Time to first parasitological detection of trypanosomes in three cattle breeds under natural tsetse challenge. See Fig. 1 for further explanations.

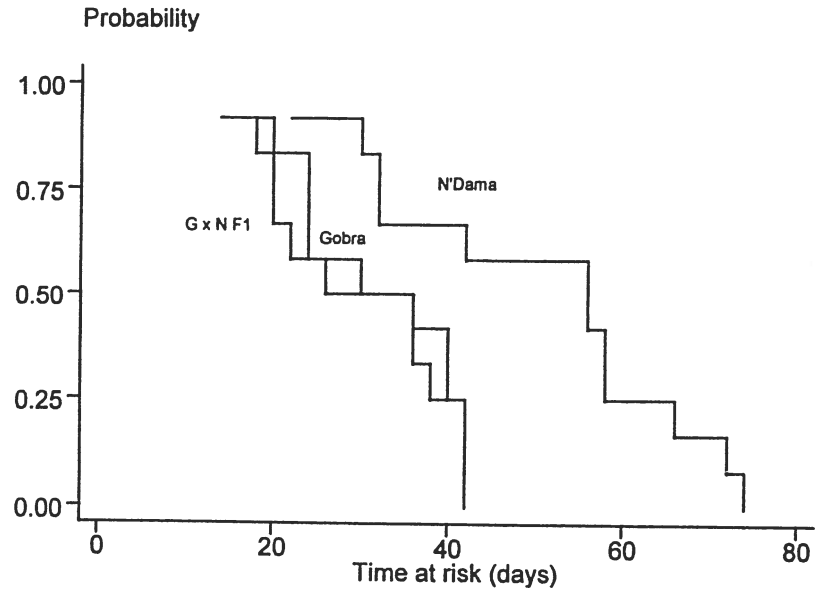

Fig. 4. Kaplan-Meier survivor functions for the parasitological incidence (I_p) for N'Dama, Gobra and Gobra × N'Dama F1 crossbred cattle.

Serological (antigen) detection of trypanosomes

Two N'Dama cattle remained serologically negative throughout the observation period. Time to first detection of trypanosomal antigen in all other animals ranged from 2 (1 N'Dama and 1 crossbred) to 159 (N'Dama) days (Fig. 5).

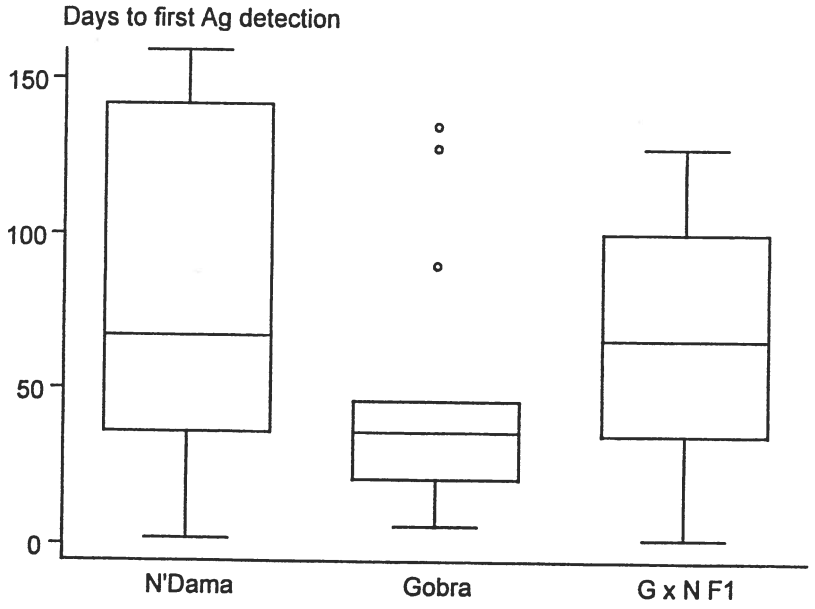

Fig. 5. Time to first serological (antigen (Ag) ELISA) detection of trypanosomes in three cattle breeds under natural tsetse challenge. See Fig. 1 for further explanations.

The Kaplan-Meier survivor function estimates for the three breeds have different shapes (Fig. 6). The hazard ratio (95% confidence interval) for breed (N'Dama vs the two other breeds), sex, detection of trypanosomes at time t and number of times trypanosomes were detected up to (and excluding) t estimated by Cox regression (chi-square=7.1, df=4, P=0.13) was 3.0 (1.2-7.3), 1.0 (0.5-2.0) and 0.7 (0.2-2.0) and 1.0 (0.9-1.2), respectively. A similar model (chi-square=4.3, df=4, P=0.37), in which only Gobra and Gobra × N'Dama crossbred cattle were included yielded hazard ratios (95% confidence intervals) of 0.8 (0.3-2.0), 1.7 (0.7-4.2), 0.3 (0.1-1.3) and 1.1 (0.9-1.3) for the factors breed, sex, detection of trypanosomes at t and cumulative detection rate, respectively. Both models were not significant according to a chi-square statistic. Thus, the relative risk of 3 for serological detection in N'Dama cattle compared to both other breeds must be interpreted with caution. Sex and the parasitological detection of trypanosomes had no explanatory value in the models.

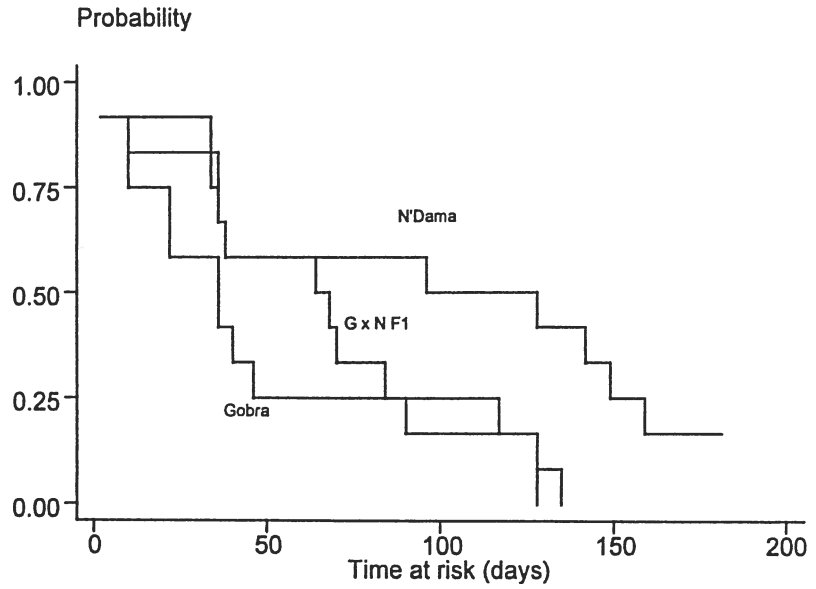

Fig. 6. Kaplan-Meier survivor functions for the serological (antigen ELISA) incidence (I_s) for N'Dama, Gobra and Gobra × N'Dama F1 crossbred cattle.

Serological versus parasitological detection

The time difference (TD) between first parasitological and first serological detection varied between -56 and 115 (mean 27.7). Negative values for TD arise if the serological detection precedes the parasitological detection. Mean (standard deviation) of TD for N'Dama, Gobra and Gobra × N'Dama was 32 (60), 17 (44) and 35 (45) days, respectively (Fig. 7). The number of animals with positive TD was 7/10, 6/12 and 9/12 for the three breeds, respectively. The TD of two N'Dama could not be established because these cattle remained serologically negative throughout the observation period. By ANOVA no significant differences in TD among breeds (P=0.64) and between sexes were found (P=0.79).

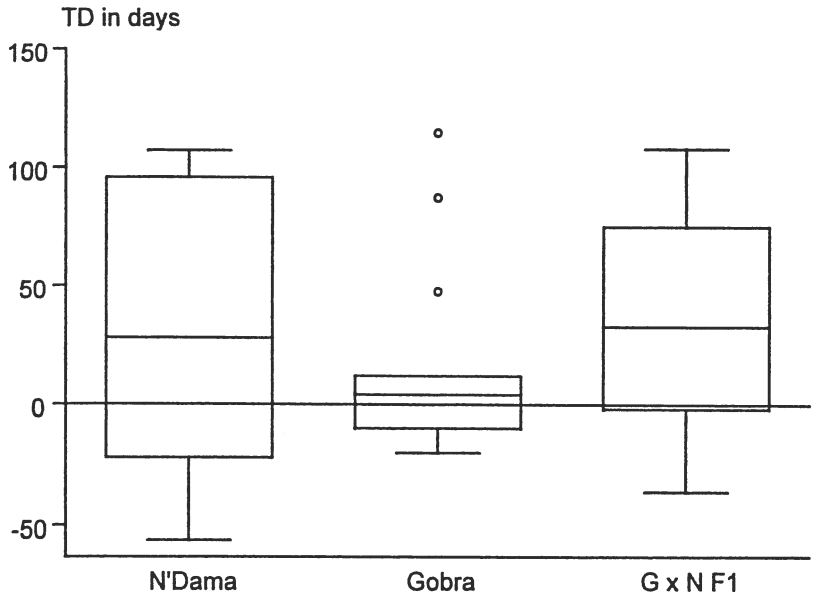

Fig. 7. Time difference (TD) in days between first parasitological and first serological (Ag-ELISA) detection of trypanosomes for N'Dama (n=12), Gobra (n=12) and Gobra × N'Dama (n=12) cattle displayed as box plots. A positive TD (above horizontal line) indicates that an animal was first diagnosed parasitologically and then serologically.

DISCUSSION

The PCV as well as the parasitological and serological detection of trypanosomes play a role as diagnostic and pathogenicity markers in research and control of animal trypanosomosis. Anaemia is one of the dominating pathological features. However, a proper quantification of the effect of *Trypanosoma* infection on the PCV value should take into consideration the individual "base line" PCV, which depends on the physiological status and other infectious or non-infectious diseases of the animal. Our study showed considerable variation of the base-line PCVs that were used to derive subject-based cut-off values for a clinical definition of incidence. Therefore, our definition of clinical incidence has a higher chance of reflecting trypanosomosis-related effects than the use of a common cut-off for all animals. The time to first drop in PCV was defined using a moving-average-like approach to rule out the use of irrelevant drops in PCV values. We denote all low (i.e. below cut-off) PCV values as "irrelevant drops" if they are not followed by at least one more low values within a time window of three consecutive measurements. Occasional low PCV values, as per our definition of the cut-off value, are expected to occur with 2.5% probability even during the pre-infection stage and are therefore regarded as irrelevant. Univariate analysis (Fig. 1) suggested that N'Dama cattle respond to tsetse challenge with a PCV drop later than the other two breeds. In a multivariate Cox regression, however, this effect could be attributed to the current infection state approximated by parasitological detection of trypanosomes.

Univariate (Fig. 3, 4) and multivariate (Cox regression) analysis clearly showed that the parasitological incidence time in N'Dama cattle was longer than in the two other breeds. However, in this study the physical act of infection was unobserved. We suppose that the cattle were all infected within a period of a few weeks, but true infection incidence times cannot be given. Therefore, the interpretation of the important finding of longer parasitological incidence

time in N'Dama can only be speculative. N'Dama were either infected later or, given a constant mean incidence time for all breeds, trypanosomes multiplied slower in N'Dama and detectable parasite densities in the blood circulation were reached later than in the other breeds. Both effects might concurrently occur. Clausen et al. (1989) found a similar difference in prepatent periods between Baoulé (*Bos taurus*) and Zebu (*Bos indicus*). However, there is evidence that after experimental inoculation of trypanosomes the time until infection becomes detectable is about the same in trypanotolerant (e.g. Baoulé) and other breeds (personal communication Dr. P.-H. Clausen). For reasons given above, the time period from infection to first parasitological detection is unobserved in our study. Generally, this period depends on the analytical sensitivity (i.e. lower detection limit) of the detection method used.

The serological results are difficult to interpret. Two animals (1 N'Dama and 1 Gobra × N'Dama) tested positive by antigen ELISA already at day 2 post exposure. Trypanosomes in these two animals were detected at days 58 and 38 post exposure, respectively. Two other N'Dama cattle never tested positive throughout the observation period. Trypanosomes were detected in these two animals at days 30 and 66, respectively. Thus, from a test validation point of view, the combined (parallel interpretation of separate tests for three trypanosome species) antigen ELISA showed a sensitivity of 0.83 (10/12) in N'Dama cattle. Given the "repeated measurement design" (i.e. test considered positive if at least one positive test result over the entire observation period was obtained) this must be considered a low value. An assessment of specificity is beyond the scope of this study. However, according to a report of a working group on "Ag-ELISA: current problems and potential solutions" it is recognised that both the sensitivity and specificity of the trypanosomosis antigen ELISA are lower than desired and highly variable (Mozaria et al., 1998). Therefore, the true infection status of the two animals with early serological diagnosis is obscure. Differences in the serological incidence among breeds revealed by univariate analysis (Fig. 5, 6) could not be substantiated by Cox regression because the latter did not reach overall significance. Since the cohort sizes were small, one should not rule out that there is an increased RR of serological response of the Gobra and Gobra × N'Dama compared to N'Dama. This interpretation would be in line with our parasitological findings described above.

The time difference (TD) between first serological and parasitological detection showed a considerable variability. In most cases, trypanosomes were first diagnosed parasitologically and then serologically (Fig. 7). We assume that in cases of great absolute time differences the diagnoses refer to different events of infection, presupposing that a positive serological response always reflects true infection.

CONCLUSIONS

Using data from a longitudinal study we could confirm important differences in the clinical and parasitological response to trypanosomosis challenge between N'Dama and other breeds. These differences were elaborated using survival analysis techniques. We suggest that incidence studies are more suitable to detect risk factors for animal trypanosomosis than prevalence-based (cross-sectional) studies because the latter often result in misinterpretation of factors that increase the survival time under infection as risk factors. From the practical viewpoint this study supports reservations against using the antigen ELISA tests as a single diagnostic tool.

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RISK OF ANIMAL MOVEMENTS FOR THE INTRODUCTION OF CONTAGIOUS ANIMAL DISEASES INTO DENSELY POPULATED LIVESTOCK AREAS OF THE EUROPEAN UNION

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Risk is defined as the measure of the likelihood and magnitude of an adverse event (Ahl et al., 1993). An important risk for the livestock production sector is the introduction of a contagious animal disease into an area hitherto free of the disease and its epidemiological and economic consequences. Classical swine fever (CSF) and foot-and-mouth disease (FMD) are contagious animal diseases which can spread rapidly under such circumstances and may have devastating effects not only for the infected farms, but also for other partners in the livestock chain and the national economy. The impact of these diseases is expected to be positively related with the density of farms and animals in the affected area.

The enormous epidemiological and economic consequences of an epidemic can be illustrated by recent outbreaks of CSF in the European Union (EU). In 1993/94 Germany and Belgium were hit by CSF epidemics that caused considerable losses, 217 farms being affected in Germany (Kramer et al., 1995; Pittler et al., 1995) and 55 affected farms in Belgium (Laevens et al., 1998; Koenen et al., 1996; Vanthemsche, 1996). Even more disastrous was the 1997/98 CSF epidemic in the Netherlands which affected 429 farms (Anon., 1998a). The total losses of this epidemic (direct costs and consequential losses for farms and related industries) amounted to US$ 2.3 billion (Horst et al., 1999a; Meuwissen et al., 1999).

Most of the outbreaks in the CSF epidemics mentioned above did occur in so-called densely populated livestock areas (DPLAs), that have a high concentration of livestock farms, cattle, pigs, sheep and goats. Economic factors such as the availability of feeding stuff, reasonably priced land, and local consumer demand have led to the development of these areas (Dijkhuizen & Davies, 1995). Economies of scale play an important part in maintaining these areas and attracting even more farms. The recent outbreaks have, however, shown the vulnerability of DPLAs to the introduction of highly contagious diseases.

Animal movements are ascribed a major role in the introduction of CSF and FMD virus into areas that were free of the disease. In an expert elicitation performed by Horst et al. (1998) import of livestock was considered to be the most important risk factor for the introduction of CSF and FMD into the Netherlands. More insight into the specific causes of virus introduction might help to reduce the risk of future epidemics. The aim of this paper is to explore the contribution of animal movements to CFS and FMD virus introduction, in particular for DPLAs in the EU. Two sources of information about historical outbreaks are considered: the Animal Disease Notification System of the EU and scientific literature. The predictive value of history for the future risk of virus introduction can, however, be questioned. Trade patterns have changed due to globalisation, liberalisation of trade and the opening up of (former) communistic countries, such as Eastern Europe and China.

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Furthermore, in 1992 the EU enforced a non-vaccination policy for CSF and FMD. As a consequence, the entire animal population is susceptible to these contagious diseases nowadays. Therefore, in this paper emphasis will be placed on primary CSF and FMD outbreaks since 1992.

DENSELY POPULATED LIVESTOCK AREAS IN THE EUROPEAN UNION

DPLAs are areas that have an extremely high concentration of cattle, pigs, sheep and goats in comparison with other areas in the EU. The definition of the minimum density criterion for a DPLA has been made on the basis of animal numbers per km^2, taking into account total land area. A distinction was made between CSF and FMD. In order to classify as a DPLA for CSF, a region should have more than 300 pigs per km^2. To classify as a DPLA for FMD, a region should have more than 300 pigs per km^2 or more than 450 animals[1] per km^2. Using these pragmatically chosen criteria, about five percent of the municipalities in Germany, Belgium, France, Italy and the Netherlands are classified as a DPLA[2].

In Table 1 the pig and animal densities and the total land area for DPLA regions in Germany, Belgium, France, Italy and the Netherlands are given. Although only few regions classify as a DPLA, differences among these regions are enormous, both in densities and total land area.

Table 1. Pig and animal densities and total land area of DPLA regions in Germany, Belgium, France, Italy and the Netherlands.

Country	Region	Pigs/km^2	Animals/km^2	Total land area (km^2)
Germany	Grafschaft Bentheim	375	496	981
	Borken	476	636	1417
	Cloppenburg	593	728	1418
	Coesfeld	593	669	1110
	Emsland	358	439	2880
	Minden-Luebbecke	341	395	1152
	Osnabrueck	359	435	2122
	Schwaebisch-hall	301	366	1484
	Steinfurt	428	525	1793
	Vechta	977	1117	812
	Warendorf	550	629	1316
Belgium	Antwerp	443	546	3028
	East-Flanders	576	724	3271
	West-Flanders	1340	1379	3293
France	Côtes du Nord	453	522	6881
	Finistère	383	431	6729
Italy	Lodi	428	592	782
	Mantova	341	545	2339
	Cremona[a]	266	455	1771
The Netherlands	East	467	659	6603
	South	1201	1369	7291

[a] Cremona classifies as a DPLA region for FMD only.

[1] The term animals includes the most important domestic animals that are susceptible to FMD: cattle, pigs, sheep and goats.

[2] Germany, Belgium, France, Italy and the Netherlands are the five countries that participate in the EU FAIR5-PL97-3566 research project on contagious animal diseases in DPLAs. Information on animal densities on region and community level was only available for these EU member states.

In order to enforce preventive measures that should reduce the risk of virus introduction into DPLAs, a DPLA region should be delineated with an administrative or geo-political border. Besides, adequate veterinary control should be possible in an area that is defined as DPLA. Therefore, the concept of zoning, as described in the code of the Office International des Epizooties (OIE) on zoning and regionalisation (OIE, 1998a) has been used to define DPLAs on a regional level (Table 1). The regions used for zoning in the EU are to a large extent the same regions that are used in the so-called Animal Disease Notification System (ADNS) (Laddomada, personal communication) and are mostly on province or county level. ADNS was started by the EU in order to inform its member states in time about outbreaks of list A diseases (OIE, 1998b) and some other highly contagious animal diseases in the EU. The member states are obliged to notify each primary outbreak on their territory within 24 hours to the EU by means of a notification form designed for ADNS. Secondary outbreaks have to be notified on a weekly basis. All notifications are stored within a computerised database.

PRIMARY CSF AND FMD OUTBREAKS IN THE EUROPEAN UNION

The introduction of CSF or FMD virus into an area free of the disease will in most cases result in a first infected farm, the so-called primary outbreak. Subsequent spread of the virus to other farms in the area might lead to an epidemic of the disease. To define a primary outbreak, we have used the definition given by Council Directive 82/894/EEC: 'an outbreak not epizootiologically linked with a previous outbreak in the same region of a member state, or the first outbreak in a different region of the same member state'. The regions used for this definition are defined in Article 2 of Council Directive 64/432/EEC and correspond to a large extent to the regions used in ADNS. By using this definition of a primary outbreak, it is thus possible that within one epidemic, more than one primary outbreak occurs, because the virus is spread over more than one region. This was, e.g., the case during the 1997/98 CSF epidemic in the Netherlands in which four primary outbreaks were registered, because four different regions were affected.

A dataset was obtained from ADNS with information on CSF and FMD outbreaks in the EU in the period January 1984 till April 1999. In Tables 2 and 3 an overview is given of the number of primary CSF and FMD outbreaks in the EU as recorded in this dataset. The number of primary FMD outbreaks is much smaller than the number of primary CSF outbreaks. Since the start of the non-vaccination policy for FMD (from January 1992 onwards; Council Directive 90/423/EEC) 30 primary outbreaks occurred, 11 in Italy in 1993 and 19 in Greece in 1994 and 1996. Primary CSF outbreaks occurred every year since the start of the non-vaccination policy (also from January 1992 onwards; Council Directive 91/685/EEC), as they did before. The EU as a whole has not been free of CSF yet.

The introduction of CSF and FMD virus is a continuing threat for the EU. The diseases are still present in countries neighbouring the EU. FMD, for instance, is still endemic in Turkey and CSF in some eastern European states (OIE, 1998b). Trade of live animals and animal products has increased tremendously, products coming from all over the world, whereas travelling times are too short now for animals incubating the disease to show clinical signs on arrival. Besides, there is a permanent CSF virus reservoir in the EU due to its presence in wild boar populations in Germany, France and Italy (Laddomada, 1999).

PRIMARY CSF AND FMD OUTBREAKS IN DPLAS

In Table 4 an overview is given of the number of primary CSF outbreaks in the DPLA regions of Belgium, France, Germany, Italy and the Netherlands for the period 1984-1999. In the Netherlands and Belgium, the majority of primary CSF outbreaks were in DPLAs, and also for Germany a

Table 2. Number of primary CSF outbreaks in the European Union from January 1984 until April 1999 (Source: ADNS).

	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	Total
Germany	328	21	14	12	3	12	8	2	10	37	30	19	4	12	8	0	520
France	18	2	13	5	1	0	4	1	1	1	0	0	0	0	0	0	46
Italy	13	8	8	2	1	0	0	3	6	7	4	4	2	10	4	2	74
The Netherlands	53	17	1	1	0	0	1	0	5	0	0	0	0	4	0	0	82
Belgium	4	8	11	3	2	1	0	0	0	1	1	0	0	1	0	0	32
Luxembourg	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
United Kingdom	0	0	6	1	0	0	0	0	0	0	0	0	0	0	0	0	7
Ireland	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Denmark	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Greece	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Spain[a]			0	0	0	0	0	0	0	0	0	0	0	3	6	0	9
Portugal[a]			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Austria[b]												2	1	0	0	0	4
Finland[b]												0	0	0	0	0	0
Sweden[b]												0	0	0	0	0	0
Total	418	57	53	25	7	13	13	6	22	46	36	25	7	30	18	2	778

[a] Member of EU since 1986. [b] Member of EU since 1995.

Table 3. Number of primary FMD outbreaks in the European Union from January 1984 until April 1999 (Source: ADNS).

	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	Total
Germany	2	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	6
France	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Italy	29	24	30	9	4	3	0	0	0	11	0	0	0	0	0	0	110
The Netherlands	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Belgium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Luxembourg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
United Kingdom	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ireland	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Denmark	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Greece	2	0	0	0	0	0	0	0	0	0	4	0	15	0	0	0	21
Spain[a]			1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Portugal[a]			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Austria[b]												0	0	0	0	0	0
Finland[b]												0	0	0	0	0	0
Sweden[b]												0	0	0	0	0	0
Total	35	24	31	11	6	3	0	0	0	11	4	0	15	0	0	0	140

[a] Member of EU since 1986. [b] Member of EU since 1995.

considerable number of primary CSF outbreaks were located in DPLA regions. On the contrary, only 6 out of 118 primary FMD outbreaks in these five member states occurred in DPLA regions.

Table 4. Number of primary CSF outbreaks in DPLA regions as compared to the number of primary CSF outbreaks in the whole country for the period 1984-1999 (Source: ADNS).

	No. of primary outbreaks in DPLA regions	No. of primary outbreaks in whole country	Percentage of primary outbreaks in DPLAs
Germany	174	520	33.5
France	3	46	6.5
Italy	0	74	0
The Netherlands	67	82	81.7
Belgium	20	32	62.5

In order to see whether DPLAs in the EU run a higher risk on CSF and FMD virus introduction, scatterplots have been made to show the relation of animal density to the number of primary outbreaks. Each dot in the scatterplots represents one region with its specific animal density and the number of primary outbreaks registered for that region. In total almost 700 regions are included in each scatterplot. A distinction has been made between the period before the start of the non-vaccination policy and afterwards. Before 1992 the number of primary CSF outbreaks was in general higher for DPLAs (> 300 pigs/km^2) than for other regions (Fig. 1). After the start of the non-vaccination policy, no difference in the number of primary CSF outbreaks can be observed between DPLAs and less densely populated regions (Fig. 2). For FMD no relation could be seen between animal density and the number of primary outbreaks, neither before nor after 1992.

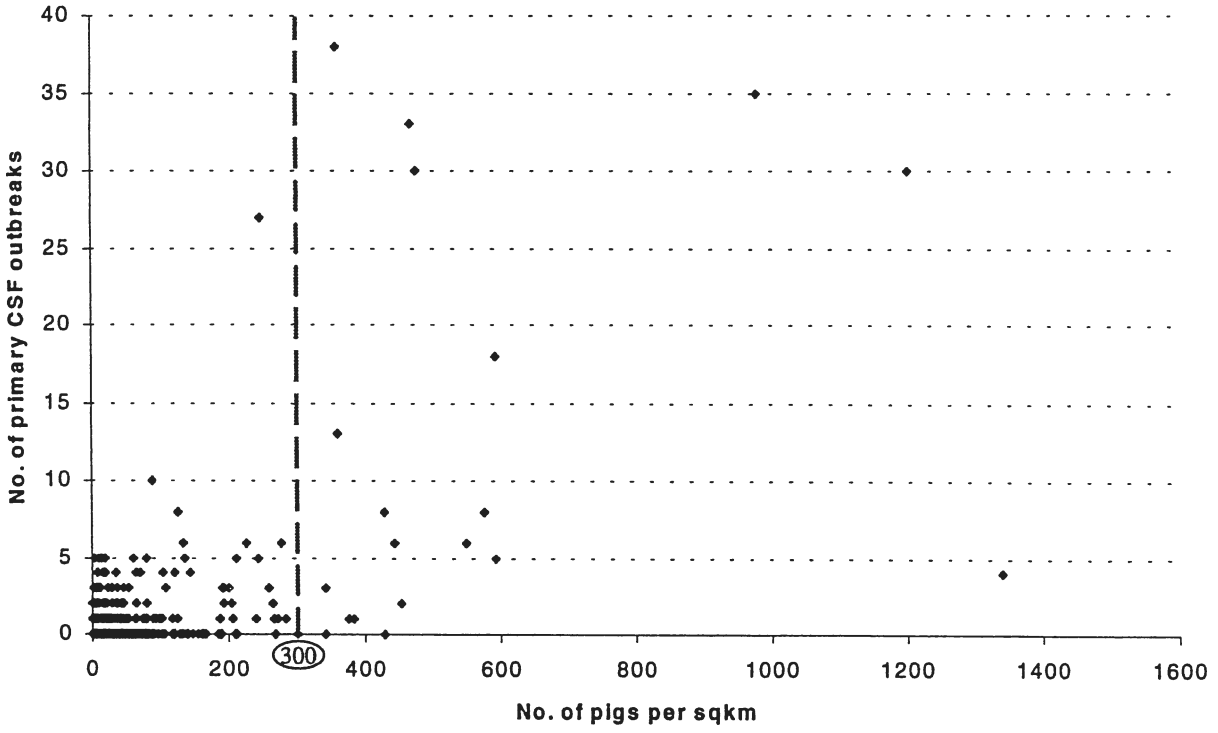

Fig. 1 Number of primary CSF outbreaks in the EU in relation to pig density before the start of the non-vaccination policy (1992).

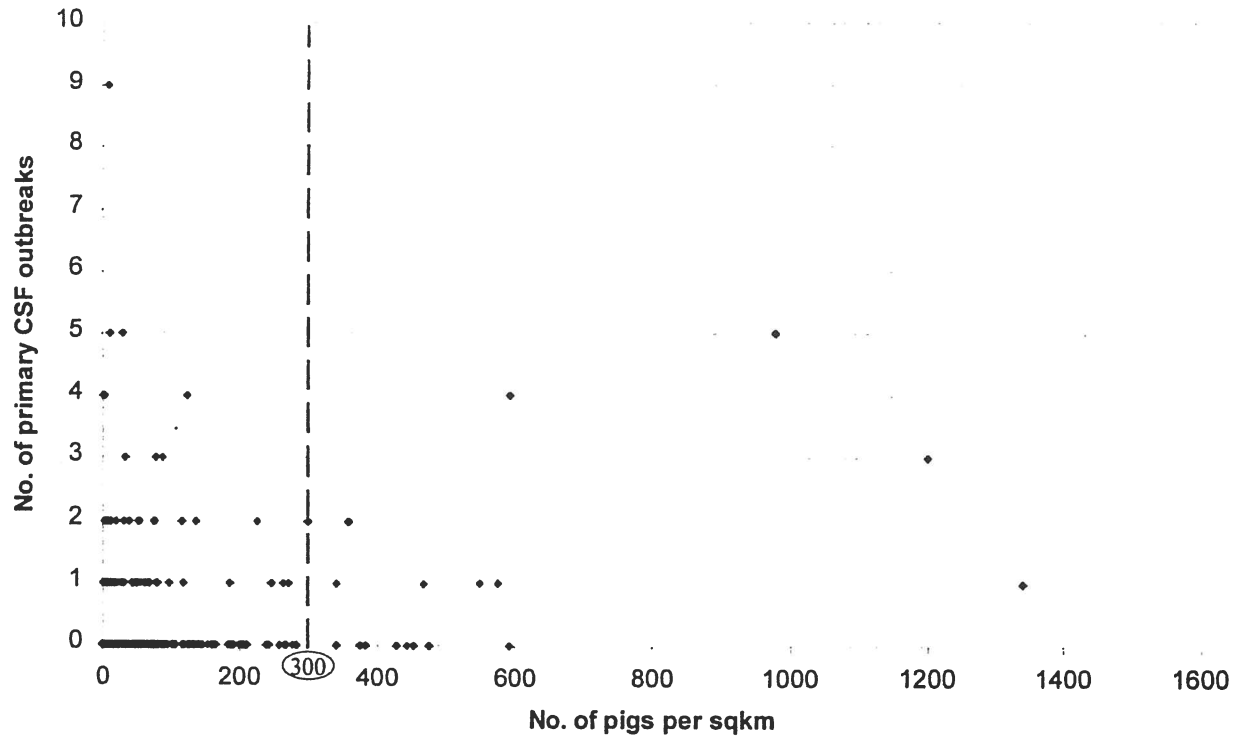

Fig. 2 Number of primary CSF outbreaks in the EU in relation to pig density after the start of the non-vaccination policy (1992).

The patterns observed in Fig. 1 and 2 have not been tested statistically. The plots show, however, that if any relation exists between animal density and the number of primary outbreaks, it will be for the period before the start of the non-vaccination policy. But the predictive value of information from this period is considered rather low. Besides, the animal density figures used are mainly from 1996 and 1997. Data for France were, however, from 1995 and for Italy from 1990. The primary outbreaks in ADNS are from the period 1984 till 1999. Animal densities will have changed considerably over these years. The general trend that has been observed in the EU is that dense areas have become more densely populated with livestock during the eighties and nineties, with the exception of Germany and Italy (Anon., 1998b). Despite changes in absolute density figures, the areas that nowadays are relatively densely populated with livestock in comparison to the rest of the EU are the same areas that were relatively densely populated in the eighties.

CAUSING RISK FACTORS

A risk factor is defined as a 'vehicle' with which virus is transported and disseminated to a susceptible animal. Most of the risk factors for a primary outbreak come from outside the region where the primary outbreak occurs. Examples are diseased animals or animals incubating the disease, infected animal products, not properly disinfected livestock trucks, air currents and people or materials carrying the virus. Risk factors can, however, also reside in the region affected, being, for instance, infected wildlife or laboratories working with the virus and/or related vaccines.

Animal movements are often mentioned as one of the most important risk factors that contribute to the spread of CSF and FMD virus (e.g. Mackay, 1997; Donaldson & Doel, 1992; Kitching, 1992; Terpstra, 1991; Edwards, 1989). When reviewing information from ADNS and the literature on the causing risk factors of recent primary CSF and FMD outbreaks in the EU, it appears that some other risk factors also play an important role in starting an epidemic. Animal movements are a major cause of dissemination of virus from one region to another.

Animal disease notification system

A lot of information should be provided by the notification form of ADNS that has to be filled in by the member states for each CSF and FMD outbreak. Many of the fields on this form are, however, not obligatory to fill in. An example of such a field is the so-called 'origin of disease', which indicates the causing risk factor of the outbreak. For many CSF and FMD outbreaks, the causing risk factor will only be known after tracing and investigation and can therefore not immediately be filled in on the notification form.

In Tables 5 and 6 the causes mentioned for primary CSF and FMD outbreaks in the EU between 1984 and 1999 are shown, both for the period before the start of the non-vaccination policy and afterwards. The main causes mentioned for CSF are purchase of animals, waste food feeding and spread by fomites. For about 75% of the primary CSF outbreaks, however, the causing risk factor is not known (unknown – investigation continuing, unknown – obscure after investigation and no cause mentioned). The main causes mentioned for FMD are purchase of animals, spread by humans and spread by fomites. Before starting the non-vaccination policy, only about 10% of the primary FMD outbreaks a causing risk factor is known. After the start of the non-vaccination policy this is about 65%. The relative contributions of the different causing risk factors for CSF virus introduction into DPLAs in the EU since 1992 are similar to those in Table 5. No primary FMD outbreaks were registered for DPLAs after starting the non-vaccination policy.

As for such a large proportion of primary outbreaks the causing risk factor is unknown, no conclusions can be drawn from these tables. Relative contributions of causing risk factors might completely change if, for all primary outbreaks, the causing risk factors were known.

Table 5. Causing risk factors for primary CSF outbreaks in the EU as recorded in ADNS for the period from January 1984 until April 1999.

	Before the start of non-vaccination policy	After the start of non-vaccination policy
Unknown – investigation continuing	426	129
No cause mentioned	39	4
Purchase of animals	32	15
Waste food feeding	73	13
Infection spread by fomites	4	14
Infection spread by human	2	1
Infection spread because of transport vehicle	1	3
Unknown – obscure after investigation	9	4
Infection via market contact	1	0
Animals for breeding – move for service	1	0
Infection via neighbouring contact	4	2
Other	0	1
Total	592	186

Table 6. Causing risk factors for primary FMD outbreaks in the EU as recorded in ADNS for the period from January 1984 until April 1999.

	Before the start of non-vaccination policy	After the start of non-vaccination policy
Unknown – investigation continuing	57	11
No cause mentioned	46	0
Purchase of animals	4	6
Waste food feeding	0	0
Infection spread by fomites	0	7
Infection spread by human	1	5
Infection spread because of transport vehicle	2	0
Unknown – obscure after investigation	0	0
Infection via market contact	0	0
Animals for breeding – move for service	0	0
Infection via neighbouring contact	0	0
Other	0	1
Total	110	30

Literature

Classical swine fever: Since the start of the non-vaccination policy in 1992, two large CSF epidemics have affected the EU. Both were initiated in Germany. The CSF epidemic of 1993/94, which affected Germany and Belgium, started on a farm in Rhineland Pfalz due to contact with infected wild boar (Kramer et al., 1995; Davies, 1994). Infected breeding stock from this farm was sold to Baden Württemberg. From there the disease spread to Lower Saxony due to movement of infected piglets (Teuffert et al., 1998; Kramer et al., 1995; Davies, 1994). Importation of infected piglets from Baden Württemberg also brought the virus to West-Flanders in Belgium (Teuffert et al., 1998; Vanthemsche, 1996; Kramer et al., 1995; Davies, 1994), from where it spread to East-Flanders. This was probably due to contact of the first infected farm with one of the outbreaks in West-Flanders (Vanthemsche, 1996). Three months after the last outbreak in East-Flanders, CSF was once more diagnosed on a farm in East-Flanders. Despite thorough epidemiological investigation the origin of infection remained obscure (Vanthemsche, 1996).

The most recent CSF epidemic started at the end of 1996 in the Paderborn area of Germany and hit five EU member states. The first primary outbreak resulted from illegal feeding of unheated swill (OIE, 1998b; Teuffert et al., 1998) and was detected in the first week of January 1997. On February 4, CSF was diagnosed on a mixed sow and finishing pig herd in the southern part of the Netherlands. The virus isolated was identical to the isolate in Paderborn. Introduction was most probably due to a transport lorry that had been in contact with infected pigs or infectious material in the Paderborn area (Elbers et al., 1999; Anon., 1997). Export from the Netherlands to Italy and Spain of a group of piglets that had illegally been mixed with infected piglets, led to subsequent CSF outbreaks in these countries (Elbers et al., 1999). In Italy only two primary outbreaks occurred, whereas in Spain 78 farms got infected in 1997 (OIE, 1998b) and the epidemic continued in 1998. In June 1997 CSF was diagnosed in a mixed sow-finishing herd in Bocholt in Belgium, which is in the border area with the Netherlands. This primary outbreak is ascribed to indirect contacts with the Netherlands (Elbers et al., 1999).

The remaining primary CSF outbreaks from 1992 onwards had less severe consequences and the virus could often be kept within the area where the first outbreak occurred. In 1992 a primary CSF outbreak occurred in the Netherlands due to importation of infected sows (Terpstra et al., 1992). In Germany many primary CSF outbreaks have occurred since 1992, most of them with only few or even without subsequent secondary outbreaks (Teuffert et al., 1998). According to Fritzemeier et al. (1999), more than fifty percent of the primary CSF outbreaks in Germany in the period 1993-1997

were due to direct or indirect contact to infected wild boar and wild boar meat. Many of the other primary outbreaks were due to swill feeding (Fritzemeier et al., 1999). The latter was for instance the case with the primary outbreak of a small CSF epidemic (13 outbreaks) in Bayern (Teuffert et al., 1998). According to Winkenwerder and Rassow (1998), ten primary CSF outbreaks occurred in the Regierungsbezirk of Weser-Ems (part of Lower Saxony) during the period 1993-1998. Almost half of the regions in this Regierungsbezirk classify as DPLA. Nine of these outbreaks were attributed to animal movements (fattening pigs from other German regions) and one to illegal feeding of swill. Contacts with wild boar have been a major cause of primary CSF outbreaks in Italy, mainly on the island of Sardinia but also in Tuscany (Laddomada, 1999; Laddomada, 1998; Laddomada et al., 1994).

It can thus be concluded that feeding of improperly heated swill and direct or indirect contact with wild boar have been two major sources of primary CSF outbreaks in the EU in recent years, and especially in Germany. Most of these primary outbreaks had little consequences, because further spread of the virus was limited. As soon as more or larger farms got involved, however, trade of livestock led to rapid and unbridled spread of the virus and could not easily be stopped at country borders.

Foot-and-mouth disease: Since the start of the non-vaccination policy in 1992, FMD virus was introduced into the EU three times. In 1993, the virus was introduced into the southern part of Italy by two consignments of cattle imported from eastern Europe (Maragon, 1995). These cattle had Croatian export certificates that were forged. The likely origin of the cattle was Czechoslovakia, although no FMD was reported from that area. It is possible that Czech cattle had been mixed with infected cattle illegally imported from the Middle East, but this could not be proven (Kitching, 1998). The virus subsequently spread from the southern part of Italy to Verona province in the Po-Valley by transport of beef cattle (Maragon et al., 1994). In 1994 and 1996 FMD virus was introduced into Greece. In 1994 the disease spread from the island of Lesbos, which is close to the Turkish mainland, to the mainland of Greece by transport of infected sheep. Illegal importation of live infected sheep from Turkey into Lesbos was the most likely explanation for the origin of the virus (Kitching, 1998). Besides, five outbreaks in the prefecture of Evros, which is close to the mainland border with Turkey, probably occurred as a result of mixed grazing by Turkish and Greek sheep (Kitching, 1998). In 1996 FMD virus was once again introduced into Greece from Turkey. The first group of outbreaks was attributed to illegal importation of a sheep by a farmer with close family connections in Turkey. The other primary outbreaks were close to the river Evros, which forms the border with Turkey. These were said to be due to illegal immigrants crossing over from Turkey (Kitching, 1998).

Only three FMD epidemics occurred in the EU since the start of the non-vaccination policy, although many more primary outbreaks were recorded. All three epidemics were initiated by illegal import of live animals (cattle and sheep) into the EU. Animal movements induced further primary outbreaks in other regions of the countries affected. Donaldson & Doel (1992) mentioned the illegal entry of animals as one of the major risks for FMD virus introduction into the EU after 1992, especially of sheep and goats, in which clinical signs are often mild or even subclinical, from the Mediterranean area. Recent history has confirmed this.

DISCUSSION AND CONCLUSION

ADNS is the most detailed computerised database with information on CSF and FMD outbreaks in the EU. Although the OIE also registers all outbreaks of list A diseases that are notified by its members (153 countries world-wide, including all 15 EU member states), they do not store detailed information about the outbreaks in a central database. For the years 1991-1998 the number of outbreaks reported to the OIE was compared with the number of outbreaks registered in ADNS. It appeared that these numbers were almost equal and therefore the information in ADNS about the number of CSF and FMD outbreaks per country per year was considered reliable.

The number of outbreaks registered per region per year was used to see if a relation exists between the number of primary outbreaks in a region and its animal density. Although before 1992 the number of primary CSF outbreaks seemed to be somewhat higher in DPLA regions than in other regions, from 1992 onwards no relation between animal density and the number of CSF and FMD virus introductions could be detected. However, if virus was introduced into a DPLA region and was not detected in time, the epidemiological and economic consequences would be tremendous.

Additional information provided to ADNS by the member states when notifying an outbreak is less useful for research purposes. The information about the 'origin of disease' was used to explore the main causing risk factors of virus introduction. Since this information is often unknown when notifying an outbreak, usually the classification 'unknown – investigation continuing' is given. Furthermore, it is not obligatory to provide this information. In comparing the information for an outbreak from ADNS with scientific literature, it appeared that sometimes the same risk factor was mentioned, but that in other cases the origin of disease in ADNS was indeed indicated by 'unknown – investigation continuing'. Although the causing risk factor had been identified after epidemiological investigation, the information in ADNS was not updated. No conclusion about the main causing risk factors of primary CSF and FMD outbreaks could thus be drawn on the basis of ADNS. Therefore, scientific literature was reviewed. On the basis of the information obtained from this source, it can be concluded that the majority of CSF epidemics originated from contact with infected wild boar and swill feeding. As soon as more or larger farms became involved, animal movements played a major role in further dissemination of the virus to other regions in the EU. All three FMD epidemics since 1992 were started by illegal importation of live animals to the EU. Further spread to other regions was mainly caused by animal movements.

Information on causing risk factors of primary CSF and FMD outbreaks from ADNS and scientific literature cannot easily be compared with each other. In this paper the definition of a primary outbreak given by the EU was used. This is also the definition used for classifying outbreaks in ADNS as being primary or secondary. In the literature reviewed for causes of primary outbreaks often no definition was given. It can be questioned whether all authors had the same definition in mind when describing primary outbreaks. Furthermore, the risk factors given are usually not explained. Purchase of animals in ADNS, for instance, might include both legal and illegal import of animals. Wild boar was not even included in the list of risk factors ('origin of disease') used in ADNS.

In order to make the information stored in ADNS more suitable for research purposes, it should be obligatory for the member states to provide the information and the information should be updated after epidemiological investigation. Furthermore, definitions of, e.g., all categories of risk factors ('origin of disease') used in ADNS should be clear and unambiguous. However, even if information from historical CSF and FMD outbreaks would be fully available and completely reliable, this would still not be sufficient to predict the future risk of virus introduction into DPLAs of the EU and the relative contribution of animal movements to this risk. Trade patterns keep on changing, the number of EU member states will most probably increase in the coming years and strategies to prevent virus introduction into the EU might be changed. Therefore, a simulation model is currently under development to predict the future risk of virus introduction for DPLAs. Simulation modelling has proven to be a useful tool for risk analysis on animal disease introduction (e.g. Horst et al., 1999b) and can be used as a decision support tool in setting priorities for preventive measures. For such a model, a lot of information is needed, e.g. on disease parameters and the major trade patterns between regions. In order to obtain information on parameters that is not readily available from existing databases and scientific literature, expert opinion might be used. Monte Carlo simulation will be used in order to incorporate the uncertainty and variability that is inherent to the input parameters (Vose, 1996). Only if a simulation model is built with correct calculation procedures and fed with reliable input parameters, will it provide valuable output. On the other hand, the modelling process itself already helps to obtain more insight into the complexity of disease introduction and risk factors contributing to it. Furthermore, sensitivity analyses with the model can show the possible

impact of missing and uncertain input data and therefore help to set priorities for further (empirical) research (Dijkhuizen & Morris, 1997).

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DISEASES IN HIGH PRODUCING DAIRY COWS FOLLOWING POST PARTURIENT NEGATIVE ENERGY BALANCE

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At least 80% of dairy cows experience a period of negative energy balance (NEB) as a consequence of insufficient energy intake relative to requirement (Villa-Godoy et al., 1988). The typical NEB period starts two weeks before calving (Bertics et al., 1992), reaches its lowest point (nadir) at about 2 weeks postpartum (Brand et al., 1996) and resumes to be positive at 6-8 weeks of lactation (De Vries et al., 1999). To compensate for metabolic demands dairy cows mobilise body reserves as a source of energy. Cows with severe NEB may loose substantial body weight, increase fat and decrease protein concentration in milk (Grieve et al., 1986), develop ketonemia and sub-clinical ketosis and be more susceptible for other health disorders, production decline or poor reproductive performance later in lactation (Goff & Horst, 1997).

The objective of the present study is to estimate to what extent NEB in early lactation may increase the incidence of production diseases in high yielding dairy cows.

MATERIALS AND METHODS

Cows

The study evaluated 608 lactations of 279 cows (parity 1 – 11) included in 9 feeding experiments during eleven years (1985-96) carried out at the research farm 'Institute of Animal Nutrition, De Schothorst', Lelystad, The Netherlands. Approximately 130 dairy cows are regularly housed in a free stall with access to a transponder operated concentrate dispenser and up to 96 of them can be simultaneously used for nutrition experiments. Premixed roughage, consisting of grass and maize silage at varying proportions, was fed twice a day.

Research at the farm aims at improving milk production through various combinations of ration components. The present data comes from controlled feeding experiments, all rations were formulated to meet requirements. Table 1 describes the experiments and Table 2 shows dry matter intake and ration composition.

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Data collection

Dairy personnel of the farm collected production and cow data. All measurements and calculated ration components were averaged by week of lactation and stored in a computerised database. For the purpose of the present study, the production and nutrition data were limited to the first 12 weeks postpartum. This resulted in 5916 weekly records from 608 lactations.

Parity was categorised as one (n = 129), two (n = 175), three (n = 123), or four and higher (n = 181). Milk production and nutrition data of week 1 and 2 postpartum were available from 115 and 181 lactations, respectively, while almost complete information was available for weeks 3 to 12. The amount of roughage intake was calculated for each cow from roughage offered minus roughage left over. Samples of roughage and concentrate were taken once a week and pooled for digestibility analysis and for check of water, ash, crude protein, fat, crude fibre, starch, sugar according to standard methods at the end of each experiment. The daily maintenance requirements were based on body weight taken twice a day after milking (6h a.m., 4.30h p.m.). Four milk tests (Monday and Wednesday evening, Tuesday and Thursday morning) determined the average daily requirement for milk production for each week. Energy requirements were calculated according to the Dutch standard for net energy lactation (NE_L) that includes maintenance, fat protein corrected milk (FPCM), and correction for level of production and growth (Van Es, 1975; Tamminga et al., 1994).

Table1. Description of feeding experiments

Trial (Year)	Cows/ heifers	Peak yield (FPCM, wk6)	Type of diet
2 (1992)	72 / 28	38.9 kg	different types of seed-oils (rape, sunflower,flax) or fish oil plus extra protein and/or extra starch vs. control
8 (1988)	28/18	35.8 kg	extra starch of low or high degradability vs. control
9 (1989)	59 / 0	43.0 kg	extra starch plus nicotinamide or yeast or high by-pass protein vs. extra starch
11 (1993)	72 / 23	40.3 kg	solubility (x2) and degradability (x3) of organic matter
16 (1987)	21 / 6	35.9 kg	extra starch (2 levels) x starch of high or low degradability vs. control
17 (1985)	19 / 0	36.3 kg	5 diets, effect of extra fat of differing source, and extra protein, all adjusted to meet requirements
19 (1994)	94 / 0	44.2 kg	4 levels of methionine vs. 'norm methionine'
21 (1995)	68 / 30	43.2 kg	4 levels of lysine x 2 levels of starch degradability vs. 'norm lysine' and same level of starch degradability
23 (1996)	46 / 24	40.4 kg	Compensation of poor roughage quality with more standard or more energy dense concentrates

The studied diseases were those clinical disorders detected by farm workers and/or veterinarians during regular or ad-hoc farm visits. A standard protocol with detailed disease codes was used for all recordings done immediately after a case had been diagnosed and treated. Health related data, along with date of occurrence and individual cow information (birth date, parity, calving date, dry off, next calving date, culling date and reason), were used to build a lactation record for each cow. The event and date of recurrence of mastitis, cystic ovary disease and endometritis were considered if 3 weeks or more had elapsed between two subsequent occurrences and of lameness if the interval was 4 or more weeks (Deluyker et al. 1991). Clinical mastitis was reported as udder and quarter cases. Mastitis occurring within 4 days after calving (PREMAST) was differentiated from later mastitis for which the risk period from 5 days postpartum to 14 days after dry off, or to culling, was considered. Culling for udder problems before 365 d postpartum was also included as mastitis. Lactation periods longer than 365 d were only considered until 365 d. Infection of the soft parts of the toe, mainly footrot and dermatitis inter-digitalis, were summarised as inter-digital lameness (infectious lameness) with a maximum risk period from calving to 365 days of lactation or to culling or to the next calving. Sole ulcer and laminitis were labelled 'digital lameness' and had the same risk period as inter-digital lameness. Culling for lameness before 365 days postpartum was also recorded as digital or inter-digital lameness depending on the type of lameness. Abnormal vaginal discharge occurring within 20 days postpartum was defined as puerperal endometritis and differentiated from later endometritis >20 days postpartum until conception.

Table 2. Dry matter intake (DMI) and ration composition from week 2 to week 12 of lactation

Items	Mean	SD	Min	Max
DMI (kg/cow/day)	22.23	3.60	10.60	32.23
Ash (g/kg)	85.77	6.90	70.48	107.33
CP (g/kg)	177.04	10.32	153.71	213.84
Fat (g/kg)	41.25	5.84	31.21	64.80
Crude fibre (g/kg)	186.67	16.04	145.52	224.43
Neutral detergent fibre (g/kg)	370.48	32.02	287.41	453.88
Starch (g/kg)	147.79	42.69	55.74	273.36
Sugar (g/kg)	65.63	14.36	36.15	101.11
Digestible organic matter (g/kg)	382.87	42.41	208.44	534.77
Energy for lactation (MJ NE_L/kg)	6.93	0.16	6.49	7.61
Digestible protein (DVE[a]/kg)	95.40	5.00	79.11	113.08
OEB[b]/kg	22.64	7.92	2.25	60.57
Body weight (kg)	609.40	69.48	422.00	834.00
Milk yield (kg/cow/day)	39.48	7.80	18.00	63.60
Milk protein (%)	3.29	0.24	2.44	4.16
Milk protein (g/cow/d)	129.46	24.16	63.92	198.43
Milk fat (%)	4.01	0.62	1.64	6.28
Milk fat (g/cow/day)	156.94	33.65	50.30	268.29

[a]True protein digested in the small intestine

[b]Degraded protein balance

Any sort of fluid-containing enlargement of the ovary detected by rectal palpation before conception and not beyond 365 days postpartum, was defined cystic ovary disease (COD). Cases of ketosis and displaced abomasum reported not later than 365 days postpartum, were both considered expressions of metabolic disorders (Gröhn et al., 1989). Placenta was considered retained (RP) if not released > 24 h after calving. Recumbency occurring within 2 days after calving and successfully treated with calcium was diagnosed as milk fever (MF); dystocia (DYS) was defined as assisted calving of any degree.

Definitions of low energy balance

Missing values of energy balance and body weight loss in week one (493) and week 2 (427) were estimated from the available data by polynomial regression (Lucy et al., 1991). The regression models included an intercept and linear, quadratic, and cubic terms for lactation week (Lucy et al., 1991; Østergaard & Gröhn, 1999; De Vries et al. 1999). Energy balance was then either calculated (= energy intake - requirement) or approximated as (Fig. 1):

- energy balance in the first week of lactation (EBwk1),
- minimum weekly average energy balance from week 1-12 (EBmin),
- accumulated negative weekly average energy balance from week 1 to 12 (AUC).
- accumulated weekly body weight loss of weeks 1-12 (BWL), and
- a maximum ratio of percent milk fat to percent milk protein greater than 1.5 (FP).

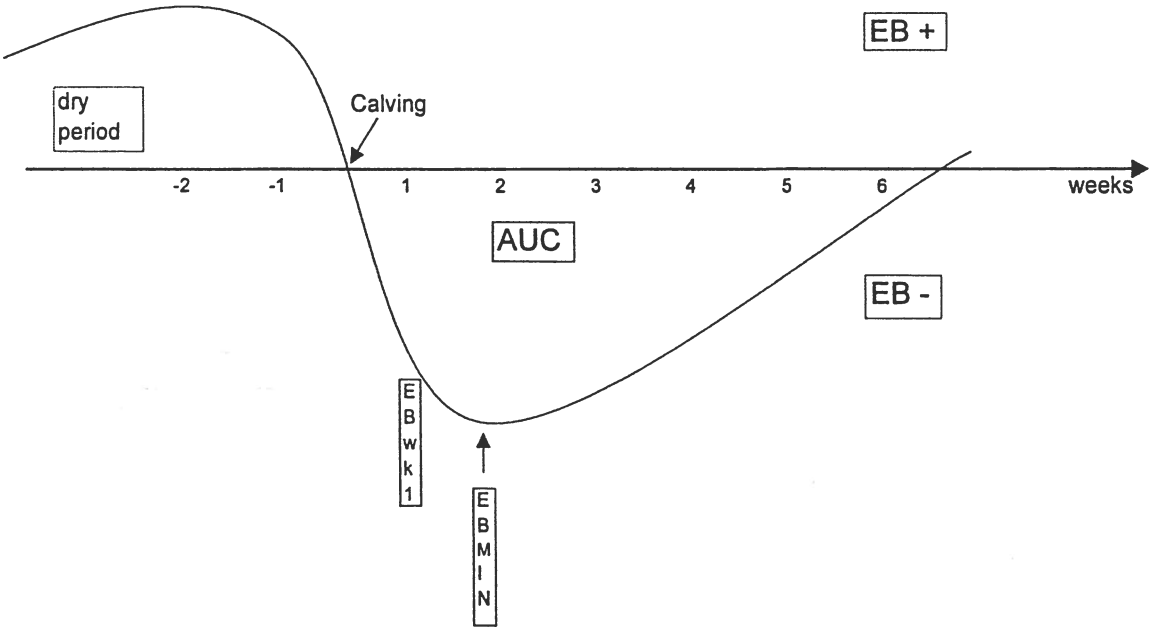

Fig. 1. Definition of energy balance

A lactation was considered in low energy balance (i.e. at risk for disease) if the energy balance parameter was either in lower 10% or in the lower 25% of the frequency distribution (Table 3).

Data analysis

Factors related to both energy balance and disease were considered to be potential confounders and included in all statistical models. It included parity, twin calving, feed experiments (fixed effects), dystocia, milk fever, and retained placenta (Dohoo & Martin, 1984; Goff & Horst, 1997).

Cumulative incidence was used for diseases with short risk periods (DYS, RP, MF, PREMAST) while incidence density was the number of new cases per cow-year at risk and used if the risk period was long (all other diseases). The cumulative (lactation-) incidence was defined as the number of lactations with at least one occurrence of the disease of interest divided by the total number of lactations.

Survival analyses: Time from calving to first case of disease was the outcome of this analysis. The cumulative survival functions were graphically and statistically compared (SAS^R, 1992). To consider multiple effects on time to disease, the Cox proportional hazards model was applied to the data (proc phreg, SAS^R, 1992). A lactation was censored if disease had not occurred until 365 days after calving, or until dry-off or culling. One model was calculated for each NEB parameter and each disease as follows:

$$h_i(t) = h_0(t)^* \exp(z_i \beta)$$

where $h_i(t)$ = hazard function assumed to describe the survival time of each observation, t = days in lactation, $h_0(t)$ = baseline hazard that is not specified because it is cancelled out in the Cox model, β = vector of unknown regression parameters associated with the explanatory variables, and z_i = matrix of measured explanatory variables for the individual i. Explanatory variables included in each model were one of the measures of NEB (Table 3), experiment (9 feeding experiments categorised with dummy variables), parity category, twin calving, DYS, RP, MF, and PREMAST. Statistical significance of the regression parameters was $P < 0.05$, $P < 0.1$ was considered a trend in the association between NEB and disease. The survival models assumed proportionality of the hazard over time (Østergaard & Gröhn, 1999).

Table 3. Definition of low energy balance

Variable	Coded	Exposure level	n	mean	SD
Total accumulated negative	...	control	445	-578.55	418.78
energy balance[a] (MJ NE_L)	AUC-25	lower 25%	163	-2401.24	1369.63
	AUC-10	lower 10%[b]	64	-3421.69	1718.98
Energy balance in first week	...	no	456	-5.84	35.37
of lactation[c] (MJ NE_L)	EBwk1-25	lower 25%	152	-77.18	40.41
	EBwk1-10	lower 10%	60	-109.88	48.02
Minimum energy	...	no	458	-14.92	10.35
balance[ac] (MJ NE_L)	EBmin-25	lower 25%	150	-47.20	12.96
	EBmin-10	lower 10%	61	-59.82	11.02
Total accumulated body	...	no	462	-17.14	12.23
weight loss[a] (Kg)	BWL-25	lower 25%	146	-57.18	15.81
	BWL-10	lower 10%	57	-70.84	17.75
Fat-protein ratio[ad]	...	<=1.5	472	1.53	0.60
	FP	>1.5	136	1.84	0.75

[a] from calving to 84 d postpartum

[b] lower 10% included in lower 25%

[c] weekly mean

[d] Maximum average milk fat to milk protein ratio from 4 weekly tests

Incidence models: Poisson regression was used to model the effect of NEB on the number of cases or the number of affected quarters (mastitis) as outcomes. The models used a log-link function and included the natural logarithm of days at risk as an offset (SAS[R], 1992):

$$Ln(a) = \alpha + EB + EXP + PAR + DYS + TWINS + RP + MF + Ln(DAR) + \epsilon$$

where Ln(a) = log-link of the number of cases (a) during the study period, α = intercept, EB = energy balance measure, EXP = feeding experiment, PAR = parity category, DYS = dystocia, TWINS = twin calving, RP = retained placenta, MF = milk fever, Ln (DAR) = the natural logarithm of number of cow days at risk, and , = residual random error (Poisson distributed). Models of cases or quarters of mastitis were adjusted for PREMAST.

RESULTS

Table 4 shows the number of cases, the incidence density or cumulative lactation incidence. Puerperal endometritis had the highest incidence rate followed by cystic ovary disease, mastitis and late endometritis.

Table 4. Incidence of the study diseases

Disease	Level	Cases	Cow-years	Incidence
Cystic ovary disease	cows	132	172.4	0.766
	cases	152	172.4	0.882
Mastitis < 5d	cases	30	...	0.049[a]
	quarters	38	...	0.016[a]
Mastitis >5d	cows	108	503.1	0.215
	cases	139	503.1	0.276
	quarters	147	503.1	0.292
Puerperial endometritis	cows	29	32.8	0.884
Endometritis	cows	35	145.1	0.241
	cases	41	145.1	0.283
Digital lameness	cows	28	595.4	0.047
	cases	30	595.4	0.050
Inter-digital lameness	cows	72	591.7	0.122
	cases	78	591.7	0.132
Metabolic diseases[b]	cows	14	988.8	0.014
Dystocia	cows	29	...	0.048[a]
Milk fever	cows	44	...	0.072[a]
Retained placenta	cows	34	...	0.056[a]

[a]Cumulative incidence over 608 lactations

[b]Clinical ketosis and displaced abomasum

Measures of association for each disease are shown in Tables 5 and 6. No interaction was observed between a significant risk factor and any of the possibly confounding variables in the model.

Mastitis: after adjusting for the confounding variables there was no effect of any of the measures of NEB on the proportional hazard of mastitis (Table 5). The hazard rates steadily increased with age; cows in parity2, 3 and 4+ had about 2, >2 and >4 times higher risk than cows in first parity ($p< 0.1$, <0.05 and <0.01, respectively). Early mastitis (before day 5) was significantly associated with the development of later mastitis (HR = 2.5, $p < 0.01$). Periparturient diseases and experiments were not related with the time to a first event of mastitis. The cases incidence of mastitis was higher in exposed than in control cows (IRR = 1.4, $p< 0.1$, Table 6) when the total accumulated NEB was moderate (AUC25). Almost the same incidence rate ratio (1.5) was found in cows exposed to FP > 1.5, both at udder and quarter level ($p< 0.05$). A two times higher risk of mastitis (udder and quarters) was observed for cows with early mastitis (PREMAST) compared to cows without early mastitis ($p< 0.05$). Parity significantly increased the incidence of mastitis.

Cystic ovary disease (COD): NEB (AUC10) significantly reduced the time to COD ($p < 0.05$, Table 5) independent of age, experiment or peri-parturient diseases. The crude survival curves of cows exposed and not exposed are depicted in Fig. 2. The occurrence of dystocia was associated with a lower hazard rate of COD (HR = 0.3, $p < 0.1$). Compared with parity one, the proportional hazard ratio of COD increased with parities 2 and 3, but was similar in parities 4+ (HR = 2.2, 2.4 and 1.9, respectively). The incidence rate of new cases of COD tended to be higher in cows within the 10% risk group of total accumulated NEB and in the 10% risk group of NEB in the first lactation week than in control cows (IRR = 1.5, $p < 0.1$, Table 6). First parity cows had about half the incidence of COD compared to cows with four and more lactations ($p < 0.01$), and cows with dystocia had a lower incidence of COD (IRR = 0.2, $p < 0.05$).

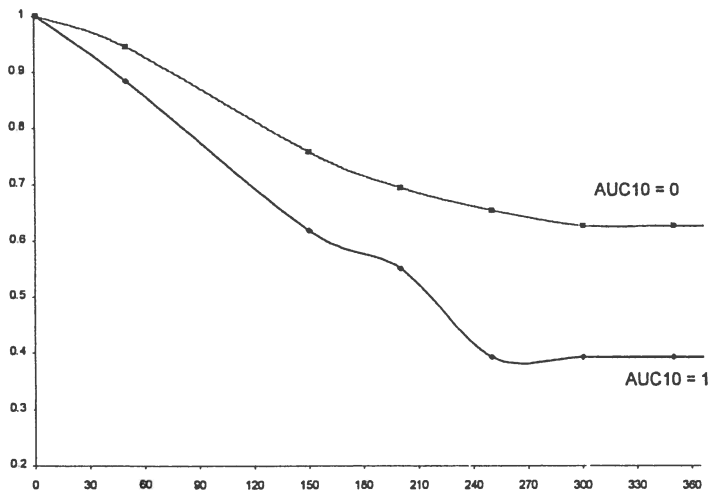

Fig. 2. Crude survival curve for cystic ovary disease

Lameness: A moderate accumulated NEB (AUC25) tended to be associated with a lower hazard of digital lameness (HR = 0.6, $p < 0.1$, Table 5). However, the hazard of time to inter-digital lameness (HR = 3.5, $p < 0.01$, Table 5) was clearly higher in cows losing substantial body weight (BWL10). Figure 3 shows the crude survival curve for inter-digital lameness in exposed and not exposed cows to severe body weight loss (BWL10). The incidence rate models with inter-digital lameness as outcome did not converge because only two recurrences occurred. Therefore, logistic models adjusted for time at risk were used to evaluate inter-digital lameness. The adjusted relative risk of the logistic regression models resulted in highly significant association with severe body weight loss (RR = 4.00, $p < 0.01$, Table 6).

Endometritis: The time to puerperal endometritis was significantly shorter in cows with moderately low energy balance in week one postpartum ($p < 0.05$, Table 5) and with fat-protein ratio >1.5 ($p < 0.01$). The proportional hazard of endometritis after d 20 was 2.1, 3.6 and 2.3 for cows in the NEB categories AUC25, BWL10 and BWL25 ($p < 0.1$, < 0.01 and $<$

0.05, respectively). The case incidence of late endometritis was twice as high (IRR = 2.00, p < 0.1, Table 6) in animals undergoing severe body weight loss during the first12 weeks of lactation (BWL10) compared to the controls.

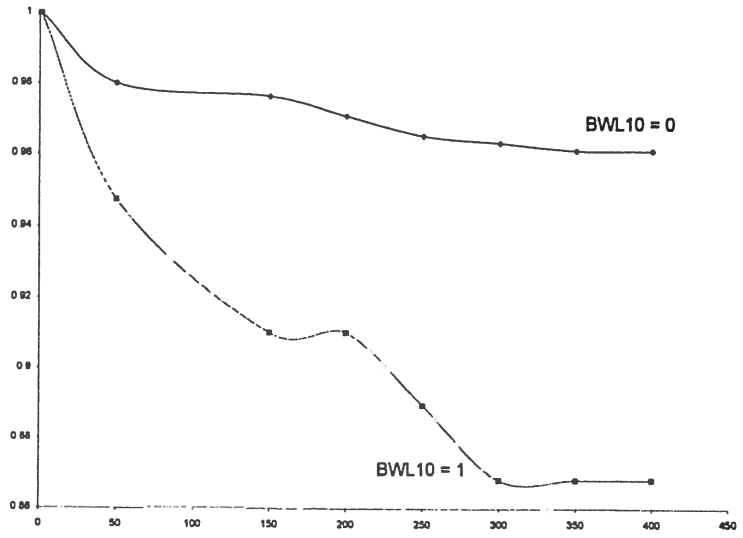

Fig. 3. Crude survival function for inter-digital (infectious) lameness

Table 5. Hazard rate ratio of recorded diseases and categorised risk factors

	Risk factor[a]								
Disease	Auc10	Auc25	EBmin10	EBmin25	EBwk1-10	EBwk1-25	FP > 1.5	BWL10	BWL25
Mastitis	1.37	1.38	1.09	1.06	1.29	1.16	1.38	1.07	1.14
Puerperal endometritis	0.59	1.37	0.68	0.27	4.13	4.68**	7.51***	0.68	1.62
Late endometritis	0.82	2.06*	0.81	0.99	0.79	1.19	1.39	3.62***	2.31**
Cistic Ovary Disease	1.69**	1.11	1.16	1.14	1.49	1.19	0.98	0.97	1.11
Inter-digital lameness	0.75	0.6*	0.73	1.2	0.73	0.87	0.88	1.37	1.07
Digital lameness	0.35	0.87	0.38	1.07	0.89	0.74	0.88	3.31***	1.59

[a]Hazard rate of lower 10% and lower 25%

*P < 0.1, **P < 0.05, ***P < 0.01

DISCUSSION

Energy balance starts becoming negative already before parturition continues to decrease after calving to reach the lowest point about 2 or 3 weeks postpartum and returns to be

positive at about weeks 6-8 of lactation. However, there are large variations between cows (Villa-Godoy et al., 1988; Veerkamp & Thompson, 1999). Our three approximations of negative energy balance calculated from the ratio aimed at differentiating extremes at one point in time (EBwk1 and EBmin) from a long term effect lasting several weeks (AUC). Additionally, the fat-protein ratio in milk has been suggested as a potential indicator of lack of energy supply through feed (Grieve et al., 1986) and body weight loss is an expression of fat mobilisation at time of NEB (Staples et al., 1990; Maltz et al., 1997). Our estimates of negative energy balance in the first lactation week and minimum energy balance at any time during weeks 1 – 12 were higher than reported by Villa-Godoy et al. (1988). The difference might have been caused by predicting missing week-one or week-two values by regression. To evaluate the fit, we compared 20 randomly selected curves and found that the third order polynomial regression curves fitted the observed data surprisingly well. However, some predictions might still have come out extreme. If some control cows had been misclassified as NEB-exposed and vice-versa, the hazard and incidence rate ratios would likely have been underestimated. Therefore, in the worst case our inferences on the effect of NEB on disease were biased towards nil.

Table 6. Incidence rate ratio of recorded diseases and categorised risk factors

	Risk factor[a]								
Disease	Auc10	Auc25	EBmin10	EBmin25	EBwk1-10	EBwk1-25	FP > 1.5	BWL10	BWL25
Mastitis (cases)	1.26	1.42*	1.19	1.04	1.00	0.94	1.48**	1.02	1.01
Mastitis (quarters)	1.19	1.34	1.21	1.00	0.92	0.94	1.52**	0.93	0.98
Late endometritis	0.68	1.31	0.67	0.95	0.58	0.95	0.89	2.00*	1.60
Cistic Ovary Disease	1.53*	1.10	0.88	1.11	1.56*	1.18	1.13	0.86	1.02
Inter-digital lameness[b]	0.34	0.93	0.37	0.96	0.92	0.73	0.80	4.00***	1.69
Digital lameness	0.86	0.69	0.85	1.11	0.78	0.95	1.05	1.45	1.13

[a]Incidence rate ratio of the lower 10% and lower 25%

[b]Relative risk

*P < 0.1, **P< 0.05, ***P< 0.01

Mastitis: NEB clearly affected the occurrence of mastitis. In two independent previous studies in commercial herds, we had observed more frequent mastitis after an increased fat-protein ratio > 1.5 (Heuer et al., 1999a; Suriyasathaporn et al., 1999b). The study of Suriyasathaporn et al. (1999b) suggested that fat-protein may be increased before, but not significantly and to a lesser degree after mastitis. Therefore, the association found in this study is probably based on a fat-protein ratio increase before mastitis, even though there was a time overlap of risk and mastitis as 36 of 52 mastitis cases occurred from day 5 to day 84 of lactation during which the highest fat-protein ratio was selected. The fat-protein ratio was described to correlate well with energy balance (Grieve et al., 1986; Heuer et al., 1999b). The relationship between accumulated NEB (AUC25) and mastitis underlined the conclusion that NEB is a contributing cause to clinical mastitis. A possible explanation is the adverse effect of ketone bodies on immune function (Van Werven et al., 1999; Kremer et al., 1993; Suriyasathaporn et al., 1999a).

Endometritis: The temporal direction of the clear positive association between a high fat-protein ratio and puerperal endometritis in the first 20 days of lactation was not clear in our study. A fat-protein ratio > 1.5 was followed as well as preceded by endometritis 1-16 days postpartum in another study (Suriyasathaporn et al., 1999b). However, the association with NEB in week one during which only 3 cases occurred (1 exposed, 2 not exposed) hinted at a causal relationship, i.e. peak NEB in week one increased endometritis during the puerperium, and this was independent of retained placenta. The immune system of the cow is depressed from 3 weeks before to 3 weeks after calving giving way for bacterial invasion (Mallard et al., 1998). Endometritis in the later course of lactation was associated with accumulated NEB especially with body weight loss. When cows are fat at calving they are more likely to loose body weight and develop fatty liver syndrome and ketonemia than cows in normal condition (Rukkwamsuk et al., 1998). Cows in such a condition may not be as immune-competent as normal cows (Van Werven et al., 1999; Kremer et al., 1993; & Suriyasathaporn et al., 1999a).

Cystic ovary disease: Peak NEB in week one as well as accumulated NEB until three months after calving predisposed cows for COD (Table 5). It was found in experimental studies that low energy balance in early lactation negatively affects oocyte quality (Kendrick et al., 1999; O'Callaghan & Boland, 1999) and that improving energy balance increases the conception rate at first service (Senatore et al., 1996). Our study suggested the hypothesis that NEB negatively affects follicular function. The effect of an increased fat-protein ratio on COD observed in commercial herds previously (Heuer et al., 1999a), was not significant in this study.

Lameness: NEB may be associated with rumen acidosis (Goff & Horst, 1997) which is considered a major risk factor of digital (non-infectious) lameness because rumen acidosis may cause metabolic acidosis and this predisposes cows for digital dermatitis (Mortensen, 1994; Brand et al., 1996). This study did not confirm this hypothesis, on the contrary, there was a trend of a lower risk of digital lameness with accumulated NEB (AUC25, $p < 0.1$) in the Cox model. However, the survival analysis and the logistic model resulted in relative risks of 3.5 – 4 ($p < 0.01$) suggesting a highly significant adverse effect of severe body weight loss (BWL10) on inter-digital (infectious) lameness (Table 5). The effect of ketone bodies on immune-function, discussed under mastitis above, is a possible explanation for this finding. Gearhart et al. (1990) reported that cows overweight at calving were more likely to develop foot problems after calving without, however, differentiating infectious and non-infectious types of lameness.

Metabolic disease: The non-significant relationship between metabolic diseases and any of the NEB parameters was probably due to the low number of cases (n = 14) resulting in large variances in the categorical analysis, too large to reveal significant effects in a relatively small sample of 608 lactations. The low incidence of clinical disease might be the result of the routine farm management where feed intake was checked twice a day and prompt corrective action undertaken whenever a problem emerged. Moreover, only 3 of those cases occurred within 15 days after calving, the other 11 later than 100 days postpartum. Hence, ketosis and displaced abomasum observed in this study appeared to be rather atypical cases. The effect of NEB on displaced abomasum and clinical or sub-clinical ketosis has been shown before (Cameron et al., 1998: Geishauser et al., 1997), but other nutritional factors may have a causal role alone or in combination with low NEB as well, e.g. forage to concentrate ratio pre-partum (Shaver, 1997).

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AGE AT FIRST CALVING AND CALVING INTERVAL IN BOVINE VIRUS DIARRHOEA VIRUS (BVDV) SERO-CONVERTED DAIRY HERDS – A LONGITUDINAL STUDY.

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Reproductive disorders including decreased non return rate, abortions and stillbirths have been reported as negative effects of bovine virus diarrhoea virus (BVDV) infections, in several case reports (e.g. Barber et al., 1985; Houe & Meyling, 1991a; David et al., 1994; Woodard, 1994; Aaby, 1995), but only a few epidemiological studies have been carried out (Houe, 1999). In one of these, Niskanen et al. (1995) found a prolonged calving interval in Swedish dairy herds going from a low to a high bulk tank milk antibody BVDV titre.

As a consequence of the focus on BVD as an infectious disease of significant economic impact, the Norwegian BVD control and eradication program - hereafter denoted the BVD program, was established in 1992 (Waage et al., 1996). Since January 1993 the entire Norwegian cattle herd population – dairy and beef herds – has been screened annually. The herd antibody prevalence for dairy herds, which in 1993 was about 30 % was less than 5 % in 1998 (Valle, in progress). A cost benefit evaluation of the BVD program yielded a positive net present (1993) value when assessed over the five-year period from 1993 throughout 1997 (Valle, in progress).

The present study was carried out to provide input information to the above mentioned cost benefit evaluation. We aimed, both, at investigating for the presence of an effect of BVDV on the herd reproductive performance as well as for the size of this potential effect. The latter being necessary for assessing the economic impact. Factors believed to be associated with or affecting the main reproductive parameters – the number of breeding services and culling patterns, respectively, were also investigated.

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MATERIALS AND METHODS

Data from 1993 to 1997, about 1620 dairy herds in the Møre and Romsdal county were used in the study. Information regarding herd BVDV serological status and herd reproductive performance was merged from two data sources - the BVD database, and the Norwegian National Board of Animal Production Records (NNBAPR).

Information regarding herd BVDV infection status

Dairy herds were defined as sero-converted (cases) – a surrogate measure for infection – or free (controls) based on the annual bulk tank milk (BTM) screening which was carried out using an antibody ELISA test kit (SVANOVA, Biotech) (Niskanen et al., 1989). Information from two additional ELISA tests being carried out as a part of the BVD program 1) the first calver's milk (FCM) test, and 2) the young stock (YS) blood test (animals 8-12 months old), was also used in the study. Due to the test scheme design the FCM and YS tests were only available for two decreasingly smaller sub sets of the BTM tested herds.

The BTM test yielded a continuous result- the sample to positive (S/P) ratio, and only herds with a BTM S/P ratio < 0.05 in 1993 – herds regarded as BVDV free – were included. A dairy herd was regarded as sero-converted within a period, if the BTM test showed an increase to a S/P ratio ≥ 0.1. The case (sero-converted) herds were assigned with a set of three indicator variables (coded zero or one) denoting the time period relative to the year of sero-conversion (SC) as follows:

1) SC_minus_1 = the year before SC,
2) SC = the year of SC, and
3) SC_plus_1 = the year after SC.

The indicator variable SC_minus_1 was defined based on the herd having a value of 1 for the SC indicator in the following year. The indicator variables SC_plus_1 were set to 1 as long as the BTM S/P ratio was above 0.1. Indicator variables were also created for herds testing FCM or YS positive.

There were two comparison (control) groups for these categories of case herds. One consisted of the herds remaining below the BTM S/P ratio of 0.1 for the entire period (1993–1997) – the BVDV free controls. The second consisted of the case herds themselves either before being enrolled as a case herd or after their test results came down below the S/P ratio of 0.1 with concomitant negative or no FCM or YS test results – the case controls. These two control groups were separated by an indicator variable which was set to one for all herd observations from herds remaining BVD free for the entire study period.

Herd reproduction status information

Information regarding age at first calving (date of first calving minus date of birth) and calving interval (date of the most calving minus the date of the previous calving) were the outcome variables of main interest. Data on the number of artificial inseminations (AI),

reported natural services and the culling frequency were also obtained. With regards to culling we investigated the total culling frequency and the culling frequency below and above two years of age. We, also, investigated for the frequency of animals being reported lost or died – a reported reason for culling.

The individual animal data provided by the NNBAPR was aggregated to herd average values for periods equal to the periods between two subsequent BTM tests, before being merged with the BVD herd level information (by herd and period) using the common herd identifier for the two databases.

Descriptive data analysis

Descriptive statistics for the main outcomes (age at first calving and calving interval) and the additional dependent variables, were calculated using the PROC UNIVARIATE (SAS). Descriptive statistics were also calculated for the BTM S/P ratios by the different BVD case categories. The outcome variables were investigated for spatial clustering by community separations and herd by a variance component analysis in PROC VARCOMP (SAS). The variance estimates of the factors were compared to the total variation in the outcome variables as a proportion yielding an estimate for the intra class correlation (ICC) coefficient (ρ).

Multivariable regression models

The potential association between the main outcome variables and the BVD case categories (indicator variables) were modelled using two different approaches and software to account for the potential herd temporal effect and spatial clustering. The first method was a population average or marginal approach - the generalized estimating equation (GEE) (Liang & Zeger, 1986) ran in PROC GENMOD (SAS). The second was a random effects or multilevel approach, run within the special purpose multilevel statistics program MLwiN (University of London) (Prosser et al., 1991; Rasbash et al., 1995).

The multivariable regression models applied had a common set of explanatory variable combinations as shown in Eq. (1):

$$\text{Outcome variable} = \text{BVD\_FREE} + \text{SC\_minus\_1} + \text{SC} + \text{SC\_plus\_1} + \text{FCM status} + \text{YS status} + \text{period\_2} + \ldots + \text{period\_5} + \text{"herd size"} \quad (1)$$

The variables SC_minus_1, SC and SC_plus_1 together with the FCM and YS indicators were the explanatory variables of interest in this study. The BVD_FREE indicator was included to investigate for a basic difference in the reference level for the two control groups. The period indicators were included to control for temporal trends contrasted with the first period. The number of animals which the herd average was based on, was included as a control variable in the models as a surrogate measure for the herd's size. Due to the small number of case herds in the FCM and YS sub sets and a potential for over-parameterization of the model we decided not to include interaction terms.

Herd nested within the community was defined as the level of clustering for the GEE models - being a one level model only. In addition to the selected unstructured correlation structure used, the effect of using an exchangeable correlation structure was investigated. The multilevel model became a three level model based on the hierarchical structure: time periods, within herds, within communities at levels one, two and three, respectively.

From PROC GENMOD (SAS) both the initial or unadjusted regression parameter estimates and the GEE estimates were recorded. The likelihood ratio (profile likelihood) confidence intervals (CI) were recorded for the initial parameter estimates and both the model based and the empirically based CI were recorded for the GEE method. The levels for the CI's were set to 0.05. The multilevel model was estimated by a Bayesian approach using the Markov Chain Monte Carlo (MCMC) simulation by Gibbs sampling (Zeger & Karim, 1991; Gilks et al., 1993; Goldstein, 1995). The mean, 2.5 and 97.5 percentiles of the simulated estimate distribution (corresponding to the CI above), was recorded.

The breeding and culling information was investigated for a potential effect of BVDV using the population average method (GEE, in PROC GENMOD), only.

RESULTS

The descriptive analysis showed that more than 50 % of the SC herds were below a BTM S/P ratio of 0.1 one year after sero-converting (Table 1). There was a reasonable number of observations (n=209) from herds in the period of sero-conversion, while the number of case herds that were YS positive was relatively small (n=39). There appeared to be a tendency towards increased age at first calving in BVDV sero-converted herds, but no apparent effect on calving interval except for an apparent small increase in the FCM sub set (data not displayed). With regard to age at first calving the effect tended to be largest for the YS positive herds. On average there were 5.0 heifers calving in each period (std. 3.1) with a range of 1 to 40, and there were on average 8.5 cows calving (std. 4.4) with a range from 1 to 70.

The BTM S/P ratios (Table 2) showed a clear increase for herds sero-converting as well as from this first year to the next. Furthermore we observed that the FCM and YS positive herds have had an increasingly high level of antibodies in the BTM.

Table 1. Age at first calving in dairy herds in M&R county, Norway, 1993-97, separated into the BVD categories.

	Age at first calving				
BVD Categories	N	Mean	Std Dev	Q1-Q3	min-max
SC_minus_1	258	784.9	75.8	735 – 805	655 – 1090
SC	209	791.4	77.9	742 – 826	650 – 1232
SC_plus_1	90	813.0	109.4	751 – 861	617 – 1409
FCM positive	80	808.2	98.5	758 – 847	687 – 1409
YS positive	39	819.5	69.0	766 – 852	689 – 998
Case controls	387	779.8	68.3	735 – 807	627 – 1104
BVD free controls	4890	781.7	76.0	734 – 813	582 – 1600

Table 2. BTM S/P ratios in dairy herds in M&R county, Norway, 1993-97, separated into the BVD categories.

BVD Categories	BTM S/P ratio			
	mean	median	Q1-Q3	min-max
SC_minus_1	0.01	0.0004	0 – 0.017	-0.192 – 0.098
SC	0.305	0.248	0.146 – 0.391	0.102 – 1.174
SC_plus_1	0.422	0.351	0.230 – 0.590	0.108 – 1.304
FCM positive	0.503	0.438	0.3 – 0.661	-0.008 – 1.304
YS positive	0.643	0.644	0.513 – 0.837	0.007 – 1.304
Case controls	0.009	0.001	0 – 0.008	-0.041 – 0.099
BVD free controls	0.004	0	0 - 0.004	-0.071 – 0.098

The variance component analysis showed a strong clustering of the above two outcomes, by herd (n = 1620), especially so for age at first calving with an estimated ρ of 0.51. The clustering by herd was less prominent for the calving interval variable, but still present with a ρ of 0.32. The contribution to the variation in the data by the spatial factor - community (n=37), was not large, but relatively stronger for age at first calving - ρ of 0.04 as compared to ρ of 0.008 for calving interval.

There was little evidence of any effect of BVDV sero-conversion on breeding. With respect to the culling information, once again, there was little evidence of an increased culling due to BVDV; in fact there appeared to be a tendency towards reduced total culling in the SC herds. With respect to animals recorded as lost (died) it appeared to be more frequent in the BVDV SC herds, and highest in the FCM and the YS positive herds. Clustering by community and herd was again noted - ρ's ranging from 0.01 to 0.04 for community and ranging from 0.06 to 0.48 for herd - being 0.48 for animals lost/died.

Multivariable analysis

The indicator variable BVD_FREE only showed up to be of importance when modelling the reason for culling – animals lost/died, and was excluded from the other models. The data included herds with missing observations, but the two groups of herds – case and control (remaining free) herds – showed similar patterns. Less than ten percent of the observations came from herds with an incomplete set of observations. There appeared to be a marked difference between the unadjusted regression estimates (drop in point estimates) as well as their CI's (enlarged CI), when compared to the two methods where the dependency in the outcome variables was accounted for (Table 3).

Table 3. Multivariable model initial (unadjusted), GEE and multilevel/random effect (Gibbs) regression estimates for BVD categories and their association with age at first calving in dairy herds in Møre and Romsdal county, 1993-1997.

BVD	PROC GENMOD (SAS)				MCMC (MlwiN)	
Categories	Initial	CI[a]	GEE	CI[b]	Gibbs	CI[c]
Intercept	781.5	779.5 – 783.6	782.6	779.4 – 785.8	782.7	775.8 – 788.9
SC_minus_1	3.2	-6.3 – 12.7	-1.7	-10.3 – 6.9	-2.0	-9.7 – 5.9
SC	7.4	-3.5 – 18.2	2.1	-7.2 – 11.6	1.7	-7 – 10.3
SC_plus_1	28.6	12.4 – 44.7	16.1	1.5 – 31.2	14.2	1.4 – 27.1
YS positive	23.5	-1.8 – 48.7	18.2	-2.2 – 38.6	18.2	-.7 – 37.4

[a] unadjusted $\alpha = .05$

[b] model based $\alpha = .05$

[c] 2.5 and 97.5 percentiles

Overall, the model for age at first calving showed an impact of BVDV in the year after SC; and an additional effect in YS positive herds. The correlation matrix available from GEE showed a correlation of 0.53-0.58 for two subsequent herd observations, and a correlation of .42 for herd observations being three years apart. From the multilevel model we observed the spatial random effect to be significant (α=0.05), and the herd effect to be both significant and large in size. The GEE method appeared to give consistently higher point estimates (except for the YS coefficient estimates being the same) when compared to the multilevel approach. The empirically based and the model based GEE CI's showed some variation – the empirically based being slightly narrower. The 2.5 - 97.5% range from the Gibbs simulation gave narrower CI when compared to the models based GEE CI's. The FCM indicator was insignificant (p-value of 0.42) and was removed from the model. When modelling with an exchangeable correlation structure in GEE the SC_plus_1 estimate dropped to 15.3 (1.1–29.4) while the YS estimate increased to 19.6 (-1.4–40.6). The model based standard error and therefore also the CI's showed a slight increase. The constant correlation estimate between the herd observations was 0.49.

There appeared to be no effect associated with the SC indicators with respect to calving interval, and only a very weak tendency towards an increased calving interval in FCM positive herds (GEE point estimate = 3.9, 95 % CI -2.1 – 9.9). In the GEE models of breeding for heifers (which relates to age at first calving) there was no clear associations with the SC indicators nor with regards to the FCM or YS indicators. For breeding in earlier calved animals (related to calving interval) there was, likewise, no increased number of breeding.

There tended to be a higher odds (odds ratio (OR) = 1.07 (.99 – 1.16)) for total culling in herds in the year of SC, and for animals older than two years of age there tended to be higher culling both in the year of SC as well as in the year after (OR's 1.05 (.99 – 1.13) and 1.09 (.98 – 1.22), respectively. The odds of animals being reported lost or died showed a tendency of an effect in the year before SC as well in the year of SC (when modelled without the FCM and YS indicators), OR's being 1.27 (.97 – 1.67) and 1.31 (.99 – 1.75), respectively. When

including the FCM it had an estimated OR of 1.38 (.92 – 2.09). If only keeping the YS indicator together with the SC indicator the estimated OR were 1.4 (.97 – 2.1).

DISCUSSION

Before discussing our results with regards to the reproductive effects of the bovine virus diarrhoea virus sero-conversion, we would like to address a few points concerning the materials and methods used in this study.

The BVD data

Clearly there are uncertainties embedded in using sero-conversion data. The BTM test is not 100% sensitive. In an evaluation of the BTM test a 90-95% sensitivity was estimated for detecting an active infection (as represented by having positive young stock) at the applied cut-off for sero-conversion (S/P ratio $\geq$ 0.1). At the same time there are specificity considerations - herds being misclassified as case herds. This could occur if an antibody positive pregnant heifer had been purchased, as discussed by Fredriksen (1998a). The specificity of the BTM screening test with regards to having positive young stock was about 80% in the above mentioned BTM evaluation.

We assume that a continued infection would, mainly due to the presence of a PI animal, within a short time cause the young stock to test positive for antibodies (Houe & Meyling, 1991; Wentink et al., 1991) as well as the calving heifers. Our concern regarding false positives was therefore mainly related to herds having only BTM information. Based on the imperfect identification process and also the expected more massive spread of the infection within a herd, we expected an increasing impact of the infection from the SC herds, to those testing FCM positive, up to those testing YS positive. This was also reflected in the observed increasing level of BTM S/P ratio for these BVDV serological categories.

The NNBAPR data

The NNBAPR have been collecting data in the current form since 1989, and about 95% of the farms in M&R county are members of this system. Every animal in a member herd gets an individual identification number on an ear tag just after birth, and all events related to reproduction, health and production are recorded in the national NNBAPR data base against that tag number. Date of birth and calving are recorded with a high degree of precision.

With respect to reported breeding, more than 90% were associated with AI services, and this information was reported to be precise (Norwegian Cattle) as well. Natural breeding was, however, less accurately recorded, and there has been a tendency for farmers having problems with getting their females pregnant to use their own bull.

The culling information used in this study was collected for general purposes and not designed to pick up special cases such as the culling of PI animals. It is, therefore, a less precise tool with regards to evaluating the special effects of the BVDV infection. Also, we believe that the main effect of BVD would be reflected in a shift in the "reasons" for culling,

and not so much in the overall or total culling frequency. We believe that the majority of PI animals developing MD and which eventually died, would be reported under this category.

The analytical methods

Because we measured the outcome variables repeatedly, within the same herd, a dependency in the outcome variable was expected. A measure for this homogeneity is the intra class correlation coefficient, which we observed was high for most of the studied outcomes. Adjusting for the dependency was necessary to avoid underestimated variance, but, also, since the estimation of the fixed effects are linked to the variance components, taking the variance components into account was a necessary prerequisite for sensible inference on fixed effects (Davidian and Giltinan, 1995), as observed.

Several methods have been described to account for the violation of independence in the outcome variable when performing statistical tests on such data, see McDermott et al. (1994a) for a review. The theoretical development of the generalizing estimating equation (GEE) (Liang & Zeger, 1986), generalized linear models (McCullagh & Nelder, 1989), Gibbs sampling (Zeger & Karim, 1991; Gilks et al., 1993) and multilevel models (Goldstein, 1995) together with software becoming commercially available (e.g. Proc Mixed (Littel et al., 1996), Proc Genmod (SAS Institute Inc. 1997) and MlwiN (Goldstein, 1995)), have made the analysis of such data more feasible. In this study we applied two of the more frequently used methods - the GEE procedure and random effects or multilevel model. The last by a Bayesian estimation procedure using the Gibbs sampling simulation procedure.

Gröhn et al. (1999) recommend a careful application of these methods especially when there are few data points (repeats), as in this study. Both the choice of correlation structure (McDermott et al., 1994b) and the estimation method are important. Under the GEE method we choose an unstructured correlation, but tested for the effect of choosing an exchangeable structure. Effects, though in this case not large, were observed. We believed it would not be reasonable to expect the same correlation for two repeats being one, two or three years apart (i.e. exchangeable). We, also, observed a drop in correlation by increasing time between the measurements. However, no information was available with regards to the auto-correlation structure of the data. When applying the unstructured option the correlation between repeated measures was not constrained by any specific patterns but was estimated based on the available data. We observed that the empirical based CI's (Z-robust (Liang & Zeger, 1986)) and model based CI's differed to some degree, but since we in this case, based the correlation structure on data from close to the entire study population, we found it reasonable to put more weight on the model based CI's. The multilevel model as presented may be regarded as an exchangeable correlation structure model, but the difference from the GEE model above the fixed effects are taken into account when estimating the model variance.

Depending on the question of interest a population average model or a subject specific model may be the appropriate choice. In our case, aiming at finding an average effect of sero-conversion in the population to use for later economic estimates, the population average was sufficient. Therefore, we did not expand the multilevel/random effect models by testing for random slopes (variation in the effect by farm).

Some differences in results may be expected since the two modelling methods are quite different with regards to estimation procedures. Also the GEE model is a one level model, only, while the multilevel model also takes the spatial clustering into account. The latter, however, was small in this case. In general, there was reasonably good agreement between the two different estimation procedures. It has been claimed that the GEE procedure is a conservative approach (larger standard errors/CI) as opposed to the random effects model (Liang & Zeger, 1986), as observed. However, when looking at the size of the effect explained by the independent variables one may argue that the random effect models appears to be the more conservative ones. Which is in contrast to what in general, is expected from population-averaged estimates (Breslow & Clayton, 1993).

Effect of BVD on reproduction

Based on finding the BVD free indicator of importance only for the animals lost/died outcome variable we conclude that the BVD case herds, when BVD free, and the control herds which remained free throughout the period, were comparable with respect to the other outcomes. However, separating the two control groups when modelling animals lost/died was in fact crucial for discovering an effect of sero-conversion in this study.

The study supports the view that BVD infection has a negative effect on the reproductive performance in dairy herds. However, we only observed an effect among the heifers as reflected by the impact on age at first calving. The fact that the effect is larger in YS positive herds is reasonable since the YS test is taken from young stock close to breeding age causing a close contact between the infectious agent and the susceptible animals. Also, among young stock there will always be susceptible animals as soon as the maternal antibodies have been depleted. Therefore, herds having a persistent or continued BVDV infection (as indicated by being YS positive) would experience continued losses among young stock, but to a lesser degree among adult animals since a majority of these would likely be immune.

The calving intervals among primiparous and older cattle were found not to be different from the reference population. This was in accordance with a study in 32 recently BVDV infected dairy herds carried out by Fredriksen (1998b). When viewing this result together with another study based on the same herds showing an increased risk of abortion in the SC herds (Valle, in progress) this was a somehow unexpected result although we did not separate between abortion in heifers and adult cows. However, we believe that the effects of the referred findings, which were in accordance with earlier reports on both early and late abortions, may have been camouflaged by the culling of animals experiencing these disorders. According to the advisory service (Norwegian Dairies) animals aborting (either early or late) or showing problems with conception are at high risk of being culled.

We found no effect on the number of AI services or reported natural breeding in accordance with the earlier mentioned study carried out by Fredriksen (1998b) as well as the study by Niskanen et al. (1995). However, the potential reproductive problems caused by the BVD infection and the need for additional repeated breeding may have caused a higher degree of underreporting of breeding in BVD infected than in non-infected herds associated with the use of a farm bull. Also, the culling of non-performing animals (e.g. animals returning in heat) may be a possible explanation.

One would expect an increased number of breeding in heifers related to a reduced conception rate, which would be a possible explanation for the increased age at first calving. A reduced conception rate has been reported by Houe et al. (1993) in Danish dairy herds. The observed effect among young stock may on the other hand have different explanations such as a reduced growth rate or a prolonged time before reaching reproductive age (delayed maturity) associated with e.g. the reported weak calf effect of BVDV (Woodard, 1994) or immunological stress (Howard, 1990) induced by the continued presence of the agent as in YS positive herds.

The expected effect of a BVDV infection giving rise to the birth of PI animals which later may develop MD and die, we believe was reflected by a higher risk of animals being lost or dying in sero-converted herds. The increase in this effect going from only sero-converted to those also testing YS positive would be in accordance with the degree of animals affected within the herd as judged by the size of the BTM S/P ratio. However, the effect tended to be present in all BVDV stages and classes. Due to the fact that the herds were enrolled in the BVD program some of the PI animals would be detected before developing MD, and slaughtered. Hence, the increased risk of animals lost is likely to underestimate the risk of PI animals in the herd.

Although it may seem unusual to use two different analytical methods, we believe that it is a useful approach. The models, and the data structure, are complex and because the methods use two different approaches to estimate the same parameters, if both approaches indicate the same underlying associations, it provides support for the inferences made. Hence, the fact that both modelling methods agreed quite well with regards to testing for an association with BVDV sero-conversion and in estimating the effect of BVDV sero-conversion on age at first calving and calving interval, gives us confidence in our results. The results were to some extent in agreement with earlier reports, showing that there truly was a marked negative effect, but restricted to the young stock. The discrepancy may be related to differences in the cattle industry from where the study populations have been selected.

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AN ECONOMIC MODEL TO ESTIMATE FARM-SPECIFIC LOSSES DUE TO BOVINE RESPIRATORY DISEASES IN DAIRY HEIFERS

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Bovine respiratory disease (BRD) is a broad term that embraces a range of respiratory diseases, caused by a wide variety of micro organisms (essentially viruses and bacteria). The disease leads to considerable losses among cattle, especially in younger animals. These losses include the cost of treatments (medicines and veterinary visits) and losses associated with the effects of the disease on the current and future performance of the animals affected ('performance effects'). The performance effects following BRD in dairy heifers include an increased probability of death and culling, depressed growth rates, an increased age at first calving and (possibly) a decreased milk production in first lactation (Correa et al., 1988; Donovan et al., 1998; Virtala et al., 1996; Waltner-Toews et al., 1986; Warnick et al., 1995). Information on the presence and, in particular, on the impact of these effects is scarce and, if available, often contrary. This makes it hard to calculate the total losses due to BRD in dairy heifers. Despite this lack of knowledge, farmers together with their veterinarians frequently have to make decisions with respect to the on-farm control and prevention of BRD. In order to make better and economically sound decisions they need to have more insight into the economic impact of the disease on their farm. To provide this insight a computer model is developed to estimate the farm-specific losses due to clinical BRD in dairy heifers in the Netherlands. In this paper the outline of the model as well as some preliminary results are presented.

MODEL DESCRIPTION

The model calculates the economic consequences of BRD in Black and White dairy heifers raised on a particular farm, relative to the same farm not having BRD among these animals ('reference situation'). Generally, the disease affects only a relatively small set of farm variables and, therefore, the partial-budgeting technique is applied. Partial budgets are used to estimate the difference in farm profit that will occur due to a relatively small change in the farm situation by considering only those items of returns and costs that actually change (Dijkhuizen & Morris, 1997). As a consequence, fixed cost, e.g. cost for labour of the farmer and housing cost, are not included in the model.

The model can be divided into three major parts, being 1) input, 2) simulation of BRD, and 3) calculation of the losses. The three steps are illustrated in Figure 1, and explained in more detail in the following three sections.

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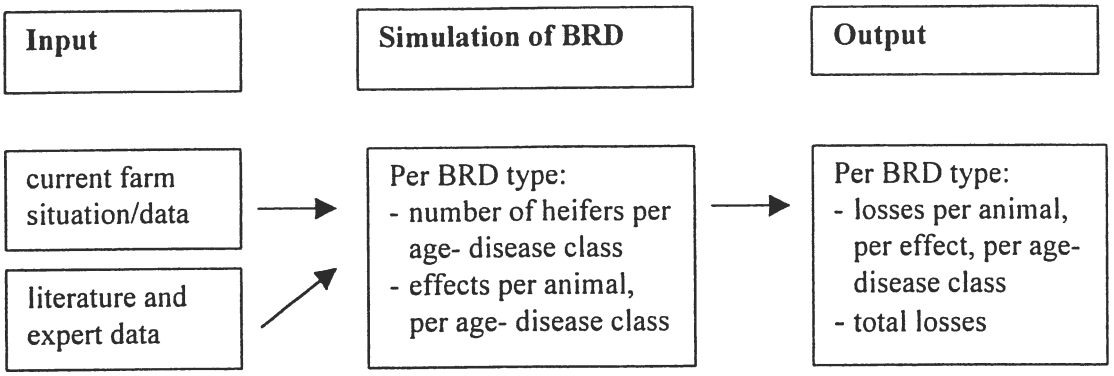

Fig. 1 Outline of the model

Model input

The input of the model includes farm-specific data, general data such as prices, and data on the incidence of BRD as well as performance effects of the disease. Farm-specific data includes, among others, number of cows in the herd, replacement rate and calving interval. Prices used in the model represent the current Dutch prices (KWIN-V, 1999). Information on the performance effects is retrieved from literature completed with expert opinion. Experts' estimates on these effects were obtained by an extensive expert consultation, which was held for this purpose (Van der Fels-Klerx et al., 1999). All quantitative information found with respect to the performance parameters is stored into a PC based database, that was developed using Access. The various tables of this database are directly linked with the economic model. For each of the performance effects, all information available in the database, as received from various sources, is weighted against each other in order to get one overall value for the particular parameter. Weights were given, assigning credits on scale 0 to 10, for the relevance and reliability of the particular information source. This is done for both BRD types considered by the model (see next section), separately. The weighted parameter values thus obtained are used as default values (for the various performance effects) in the model. Also, the model uses default values for input related to farm-specific data, i.e. values for a typical Dutch dairy farm, and general data (KWIN-V, 1999; NRS, 1998; PR 1997), but not on incidences. All default values are reported to the user using Excel worksheets, and, as no (intermediate) calculations are made in the program's worksheet cells, these values can easily be modified. The model itself is built using Visual Basic language, and the output is reported to Excel worksheets.

Simulation of BRD

In accordance with the opinion of the consulted experts (Van der Fels-Klerx et al., 1999), the model distinguishes two types of clinical BRD, i.e. pneumonia and outbreaks. The losses are calculated for each of the two types, separately. In short, an outbreak is assumed to occur in the winter season with heifers of all ages possibly affected whereas pneumonia can occur all year round, but in the younger animals only. In both types, three classes with regard to severity of (clinical) disease, being mild, severe and chronic disease, are. Classes considered and definitions used equalled those set by the experts distinguished (Van der Fels-Klerx et al., 1999), and are presented in Appendix 1 for BRD in general, both BRD types, and the three disease classes. The simulation of BRD as well as the calculation of the losses is comparable for the two types, and, therefore, is outlined in the following sections for BRD (in general) with derivations from this general approach indicated.

The simulation of BRD is based on the calculation of numbers (fractions) of heifers present in each of the various combinations of classes. Besides the disease classes, the heifers (birth -2 years) present on the dairy farm are subdivided into eight age classes of three months each. Furthermore, a year is divided into four quarters of three months each, with the winter season defined from November 1st to January 31st. The model starts with the specific farm situation, which, among others, is characterised by the number of heifers present in each age group, in the winter season. The model's assumptions with regard to the heifers that are at risk for BRD follow the definition of the particular BRD type (see appendix 1). In case of pneumonia, these heifers are 0-3 months old, and are either present on the farm in the starting situation or born in one of the three following quarters. With an outbreak, heifers in each age class at the starting situation are at risk, and heifers up to the (user-defined) maximum age might get affected.

Calculation of losses

The economic losses following BRD are calculated and expressed for the disease during a one-year period (four quarters) in case of pneumonia, and for one outbreak occurring during the winter season in case of an outbreak. The model takes into account the (current and future) performance effects of BRD occurring during the total rearing period. The (resulting) total time span considered equals the period until the last heifer that was present in the starting situation has calved.

Besides treatment cost, the model considers the losses due to the following performance effects:

- increased death and culling
- decreased growth rates in various stages at/after the disease episode
- decreased fertility: increased risk of return into oestrus after first insemination, foetal losses, and abortion
- decreased milk production in first lactation

Culling refers to involuntary culling (directly) after the disease episode and to voluntary culling of (the surplus of) down calving heifers. The culling of heifers in the period between the disease episode and calving was not considered, because this is not regularly seen on Dutch dairy farms.

First, for each performance effect, the losses are calculated on the animal level for all combinations of age and disease class, separately. Total losses per effect as well as the overall losses (per type) follow from the calculated losses (per heifer) in each combination of age and disease class and the numbers of heifers present in each of these combinations. The calculation of the losses due to the various performance effects is described below.

Treatment: All heifers affected receive a given number of treatments (per head) against a certain cost per treatment, both specified per disease class. In case of an outbreak, the veterinarian is called one or more times for the total herd of heifers, whereas with pneumonia the veterinarian visits (or consults) each individual animal a certain number of times. Medication is assumed to be given by the veterinarian during visits and consults. Follow up doses, if applicable, are administered by the farmer (no extra cost).

Death and culling: Losses due to both death and culling include the total rearing cost from birth until the moment (day) of occurrence of these events. Total rearing cost are composed of the cost for feeding, general veterinary cost (medicines and veterinary visits) and some general cost (e.g. for straw). The total cost for feeding in the particular raising period is retrieved by

summation of the feed cost per month. Monthly feed costs are based on the animal's energy needs for maintenance, growth and pregnancy, and are calculated using the animal's body weight, average daily gain and pregnancy status in each particular month. The resulting total energy needed, together with the season of birth and the feed contents of the particular feed stuffs fed in the particular season define the feed cost, based on least cost ration, for each month.

Growth and fertility disorders: In the model, the values for both the depression in growth and the occurrence (probability) of each of the three fertility disorders are related to the disease classes. As heifers are assumed to be inseminated when they have reached both a specified age and body weight, a decrease in growth indirectly results in a higher age at first calving. Return into oestrus after first insemination and foetal losses directly lead to an increased calving age, and heifers that abort are assumed to do so at a certain stage of their pregnancy. Losses from the depression in growth with or without return into oestrus (after first insemination) or foetal losses include the extra rearing cost due to the delayed first calving, and cost for extra inseminations in case of both the fertility disorders. A heifer that aborts is assumed to be culled from the farm, and the resulting losses include the rearing cost until moment of abortion, corrected for the (low) revenues of the culled heifer. The rearing cost until calving or abortion are calculated for one heifer in each of the various combinations of classes, and include the feed cost, veterinary cost and some minor cost. Total losses for the increased rearing period due to BRD follow from the total rearing cost for all heifers in the situation with BRD, relative to this total in the reference situation.

Number of heifers available: The previous mentioned performance effects of BRD, i.e. deaths, (direct) culling, and reduced growth and fertility, lead to a decreased total number of down calving heifers at the farm and/or an increased calving age of these heifers, when compared with the reference situation. Also, the number of down calving heifers present on the farm might be changed in various periods of the total time span. The resulting losses are calculated by comparing, in both the situation with BRD and the reference situation, the number of down calving heifers available with the number needed in each three months time step of the total time span. In each time step and in both the situation with BRD and without BRD, down calving heifers (the shortage) are bought at the farm in case the number of down calving heifers needed is higher than the particular number available at the farm, and the surplus is sold otherwise. Total losses follow from the difference in both situations between total purchase cost and total selling revenues.

Milk production: Losses (or revenues) due to milk production following BRD in the rearing period result from the total difference in first lactation milk production of (all) heifers in the situation with BRD, relative to the reference situation. It is assumed that first lactation milk production depends on body weight at calving, and, therefore, the difference in milk production is related to the heifer's body weight at first calving.

Uncertainty and output: Uncertainty is recognised in calculating the losses of BRD. The model estimates the losses for deviation from the average (median). The 5^{th} and 95^{th} percentiles of each of the three input parameters (see below) that turned out to have the highest influence on the (average) losses are calculated (Sørensen, 1999). These three key variables are disease incidence, death and (direct) culling after disease. Besides the average losses, both the best and worst case scenario losses are calculated by taking into account, respectively, the 5^{th} and 95^{th} percentiles of these three parameters. The underlying distribution assumed is a normal distribution for the incidence parameters, and a beta distribution for death and culling variables

(Sørensen, 1999). The parameters of the distributions are established using information from literature and experts on spread of the outcome of the particular variable. The total losses for BRD as well as the contributions of the various performance effects to this total are presented to the user for each of the three possible outcome scenarios (best, average and worst). In addition, a detailed presentation is given for the losses following each of the various performance effects in the average scenario.

Preliminary calculations were done to estimate the losses due to BRD (per type) on a so-called typical Dutch dairy farm using the model's default values. For both types, the total proportion of heifers affected was set to be 50 %, with 30 % being mild, 15 % being severe and 5 % being chronic diseased. In case of the outbreak, the maximum age of the heifers that might get affected was assumed to be 18 months.

RESULTS AND DISCUSSION

The losses following clinical BRD in heifers (50 % affected) on a typical Dutch dairy farm are shown in Table 1 for pneumonia and in Table 2 for a BRD outbreak. In both cases, the total losses as well as the contributions of each of the various effects to these totals are given for each of the three scenarios considered.

Table 1. Losses due to pneumonia in heifers (50 % affected) on a typical Dutch dairy farm, estimated for each of three scenarios (expressed in Dutch guilders)

Performance effect	Scenario		
	Best	Average	Worst
Treatment	68	156	206
Rearing cost[a]	46	-104	-1391
Number of heifers	8	336	2392
Milk production	97	217	271
Total: per year	219	605	1478

[a] cost due to deaths and (direct) culling as well as due to decreased growth and fertility

Table 2. Losses due to a BRD outbreak in heifers (50 % affected) on a typical Dutch dairy farm, estimated for each of three scenarios (expressed in Dutch guilders)

Performance effect	Scenario		
	Best	Average	Worst
Treatment	348	496	595
Rearing cost[a]	222	-17	-1046
Number of heifers	72	840	3714
Milk production	44	81	98
Total: per outbreak	686	1400	3362

[a] cost due to deaths and (direct) culling as well as due to decreased growth and fertility

The total losses following pneumonia on a typical Dutch dairy farm (Table 1) can be nearly as high as the variable rearing cost (housing and labour not considered) for one heifer, estimated to be Dlf 1535 (Boxem et al., 1991), on this farm. The total losses due to a BRD

outbreak (Table 2) on average almost equal to the (variable) rearing cost per heifer and in the worst case are even more than twice this cost.

In each scenario, the total losses due to an outbreak of BRD on a typical Dutch dairy farm are more than twice as high as the losses following pneumonia (Tables 1 and 2). It must be reminded that the losses due to pneumonia are calculated for the disease occurring during a one-year period whereas the losses following an outbreak are estimated for the total outbreak (occurring in the winter season). Depending on the frequency of occurrence of outbreaks over the succeeding winter periods, the losses due to one or more consecutive outbreaks can also be expressed per year. If an outbreak is observed on the farm every year, heifers affected are assumed to be, at the highest, one year old. This is because animals older than one year have experienced the outbreak in their first rearing year, and, therefore, will not get clinically affected in their second rearing year. With an outbreak occurring every second year or less frequent, all heifers (0-2 years) present on the farm might get clinically diseased. In the current situation, it was assumed that the outbreak was not seen in the previous year and heifers up to 18 months were affected. Except for the best case scenario, the losses per year, for two consecutive years, due to this outbreak almost equal the yearly losses following pneumonia. Should the outbreak occur every year (with heifers affected aged up to 12 months at the maximum), the total losses average Dfl 913, and range from 517 and 1881 in the best and worst case, respectively. Comparing these losses to those following pneumonia, it can be seen that the total average losses per year are 1.5 times higher for the BRD outbreak, but that its variation is comparable between both BRD types.

Extra rearing cost (Tables 1 and 2) comprise the total difference in rearing cost for the situation with and without BRD. With BRD, rearing cost per year are lower because of the shortened rearing period on the farm of the heifers that die, are (directly) culled or abort due to the disease. On the other hand, these cost might be higher due to the delay in first calving, resulting from depressed growth and fertility, for affected heifers that actually reach calving age. This explains the revenues (negative losses) of the extra rearing cost in the average and worst case scenario of both BRD types. Because of heifers that die or are culled, the number of down calving heifers present on the farm will be lower. The resulting losses are very much related to losses following differences in rearing cost, and for an accurate interpretation of the contribution of the two effects to the total losses, they should be considered at the same time.

Milk production in first lactation is related to the heifer's body weight at calving and, therefore, might either be increased or decreased following BRD during the rearing period, depending on the effects of the disease on growth and fertility. The (net) milk production losses following pneumonia are much higher compared to an outbreak (Tables 1 and 2), so apparently the former BRD type has a greater impact on the heifer's body weight at calving than the latter, at least on average.

CONCLUDING REMARKS

The model is made flexible in the sense that weights given in the underlying database can be changed, and new information available can easily be added to the database. With a new run of the model the outcome automatically will be updated (with the latest information available). Default values used in the model can also be changed by the user, which maximises the user's confidence.

The model presented could be used as an interactive tool by farmers together with their veterinarians to provide more insight into the farm-specific losses due to BRD. The results from this model can help them to decide on the control and prevention of BRD. From an economic point of view, the maximum amount of money that should be spent on improving farm management with regard to BRD should not exceed the total losses of the disease. Depending on the farmer's attitude with regard to risk, losses estimated from the best or worst case scenario, or any value in between, should be chosen as the threshold value.

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APPENDIX 1. DEFINITIONS AND CLASSES OF BRD

BRD in dairy heifers (birth – 2 years of age): A disease of the respiratory tract caused by a viral, bacterial or mycoplasmal infection (parasites excluded) or a combination of these infections.

BRD types:

1. *Calf pneumonia:* BRD cases seen one after the other, periodically or whole year round, mostly occurring in the first three months of life. These cases can be caused by a variety of primary pathogens, but commonly are caused by bacteria, mainly *Pasteurella spp.*, and preceded by an infection with respiratory viruses.
2. *BRD outbreak:* A certain number of heifers in a group suddenly shows clinical signs. Outbreaks of BRD mostly occur during the housing period, but are also regularly seen during the pasture period. Dairy heifers affected are mainly older than 3 months, but younger heifers might also be affected. Outbreak cases are mostly caused by a primary viral infection, mainly with *Bovine Respiratory Syncytical Virus*, with or without a secondary bacterial infection.

BRD disease classes:

Definition of each of the three disease classes of clinical BRD by common and specific signs present at the animal level without treatment[a]

Mild clinical BRD	*Severe clinical BRD*	*Chronic BRD*
Duration/start		
Duration < 14 days	Duration < 14 days	Starts > 14 days after onset (mild or severe) of clinical disease
Common signs		
2 or more of:	*1 or more of:*	*1 or more of:*
Nasal discharge	Nasal discharge	Nasal discharge
Coughing	Coughing	Coughing
Respiratory rate increased	Respiratory rate increased	Respiratory rate increased
Body temperature increased		
Specific signs		
1 or more of:	*1 or more of:*	*1 or more of:*
Vital	Body temp. ≥ 40 ° (fever)	Body temp. ≥ 40 ° (fever)
	'Harsh' breath sounds	'Harsh' breath sounds
	Abdominal breathing	Abdominal breathing

[a] As it was not the intention to give a complete description of all signs that can be seen in each of the three disease classes, only those symptoms that are most characteristic and distinguishing for the particular disease class are given. The signs are divided into common and additional signs with common signs seen in each of the three disease classes and additional signs only seen in the particular disease class.

THE APPLICATION OF GIS AND REMOTE SENSING BASED MODELLING TECHNIQUES, FOR USE IN THE ECONOMIC AND EPIDEMIOLOGICAL ASSESSMENT OF DISEASE CONTROL INTERVENTIONS, AT A REGIONAL OR NATIONAL LEVEL

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The quantitative economic assessment of interventions in livestock production systems requires the analysis of the outputs and resource requirements of all the production systems within the target area. Such analyses are not possible without accurate data describing both livestock and human populations, and the production systems within which the livestock are managed. When, as is often the case, such information is unobtainable, this missing data poses a significant constraint to planned livestock development. The approach described, demonstrates a cost-effective solution for augmenting existing, conventionally gathered field data, using techniques appropriate to the needs and resources of developing countries.

Novel techniques based on geographical information system and remote sensing technology are used to classify livestock farming systems, agro-ecological zones, and predict animal populations at a high level of resolution, using a combination of highly objective, remotely sensed satellite and eco-climatic data, combined with existing animal population information. This results in the significant ability to predict the distribution of livestock, and relate them to the production parameters of the farming systems within which they are managed, over a very large geographical area.

A major assumption of this work is that all the animals of one species that are predicted to lie within each of the 0.05° pixels (6 km x 6 km) are managed in the same way e.g. as small-scale, intensive, East African, coastal, dairy cattle, and thus have a recognisable set of production parameters that can be attributed to them. In the context of developing countries, where intensive, housed farming systems are comparatively rare, this is not an unreasonable assumption

The techniques described have the potential for application at two levels:

- At the national and sub-national level – in this case the main advantage gained is due to the increased resolution (i.e. small unit area) of the predicted data, and the freedom from analytical constraints imposed on data gathered using administrative boundaries (political and administrative boundaries rarely have any relationship to the eco-climatic determinants of human cattle cropping and farming practices within them). This freedom permits novel analyses on the basis of time, space, ecological, physical and other features. This flexibility in analysis allows more accurate and meaningful assessment of disease control strategies, and other types of interventions in production systems, such as: improvements in infrastructure, provision of marketing resources, changes in restocking policies and breed improvement.
- At the regional and continental level – on this larger scale, the advantage is due to the ability to predict missing field data over large geographical areas, where gathering

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representative data is either financially or logistically impractical, and existing national census data is either outdated or inaccurate.

As described above, logistical and other problems are often responsible for inaccuracies in conventional census data describing animal populations, and although often the best that is available, census studies are by no means a gold standard in the context of the developing world. The underlying data layers and models used in this system complement existing data as they can be retrained using new sets of observed data derived from conventional micro-survey techniques that are well developed and understood for use in the field, cheap to operate and known to be highly accurate in the conditions that prevail in developing countries. Thus, areas of potentially unreliable data can be identified and targeted using the system, ensuring increased accuracy of the underlying population predictions at a greatly reduced cost.

It is envisaged that the final version of this tool will be used primarily at the national level. Examples of the use of this technique could be to estimate production losses due to movement control during disease eradication, and changes in production and resource requirements due to changes in milk-marketing opportunities resulting from new technology e.g. the lacto-peroxidase system, that may be used to improve the keeping quality of milk in tropical countries.

MATERIALS AND METHODS

Components of the system

The system is comprised of five major components (Fig. 1):

- The Project Against African Animal Trypanosmiasis Information System (PAAT-IS) provides the predictions of farming systems and livestock populations within each 0.05° pixel
- A custom written database of production parameters, and supporting literature that can be matched to the farming system characteristics of each pixel and provides appropriate production parameters for each farming system in the area of interest
- An "event" database that allows the entry of interventions and "events", described in terms of the resulting effects on production parameters (as percentage change)
- Various livestock production system model(s) as appropriate to the issue under investigation
- A set of custom written software modules produced in Visual Basic 6.0® (Microsoft) and Avenue® (ESRI) that serve to link and integrate the other components

The PAAT-IS system

The underlying predictions of animal populations and farming systems are based on work carried out for the Project Against African Animal Trypanosomiasis Information System (PAAT-IS), by the Environmental Research Group Oxford (ERGO), on behalf of the Animal Production and Health Division (AGA) of the Food and Agriculture Organisation of the United Nations.

The predictions are made using regression analyses performed on data grouped into ecozones, produced in turn by using clustering of the results of principal components analysis of a subset of satellite imagery using the non-hierarchical clustering module of the ADDAPIX software package. A second clustering identifies farming systems, based on information on human population density, cropping intensity and predicted cattle population density.

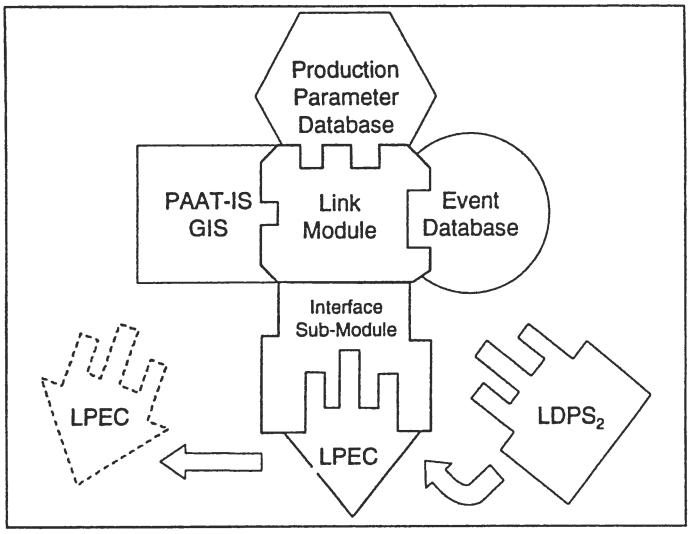

Fig. 1 Relationship of the constituent modules of the system, illustrating the interchangeability of the modelling systems component (in this case LPEC and $LDPS_2$)

Derivation of the predicted animal populations

The cattle populations are based on observed cattle population density data produced from national records at national administrative boundary level two and below. The increase in resolution of the animal production and farming system data resulting from the modelling techniques can be seen in figures 2 and 3, illustrating the difference between observed and predicted cattle population data. The principle of the raster GIS layer as a two-dimensional matrix of cattle population data is illustrated in figure 4. In the system, it appears as a thematically shaded image enabling the distribution of the population to be visualised, and correlated with other relevant factors.

Derivation of the predicted farming systems.

The derived farming systems are categorised based on a geographical classification of farming practices, human population density, eco-physical factors and objective measures of eco-climate and agricultural intensity rather than the largely qualitative, conventional descriptions based on management practices e.g. pastoral systems have been shown to be reliably identified as those with little cultivation, and relatively high cattle densities relative to the human population density.

This classification of farming systems correspond closely with both the ecozones identified and the standard agro-ecological zonation based on length of growing period. This method of classification was used to ensure that farming systems are easily identified in the field, and thus can be directly matched to production parameters in the literature.

The predictions were made using regression analyses performed according to two separate zonations produced by clustering the results of principal components analysis using ADDAPIX.

- the first zonation is into "ecozones" using a subset of the available contemporary satellite imagery
- the second into farming systems' using the available human population, cattle density and cropping intensity information

The full protocol for the predictions and zonation procedure are described in FAO, 1996a, 1996b, 1996c, 1997, 1998 & 1999.

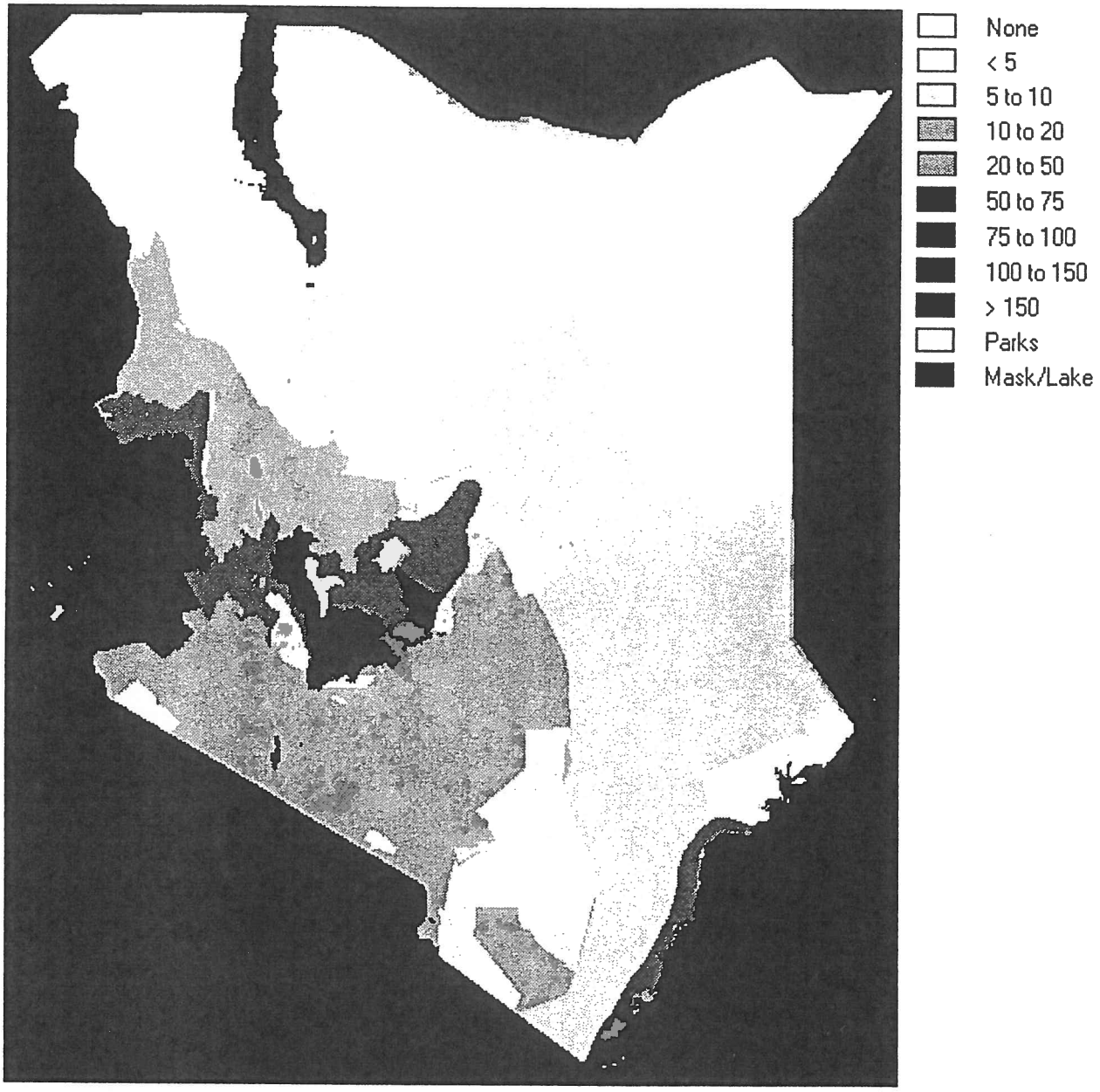

Figure 2: Cattle population density, based on observed data from national administrative level two census data

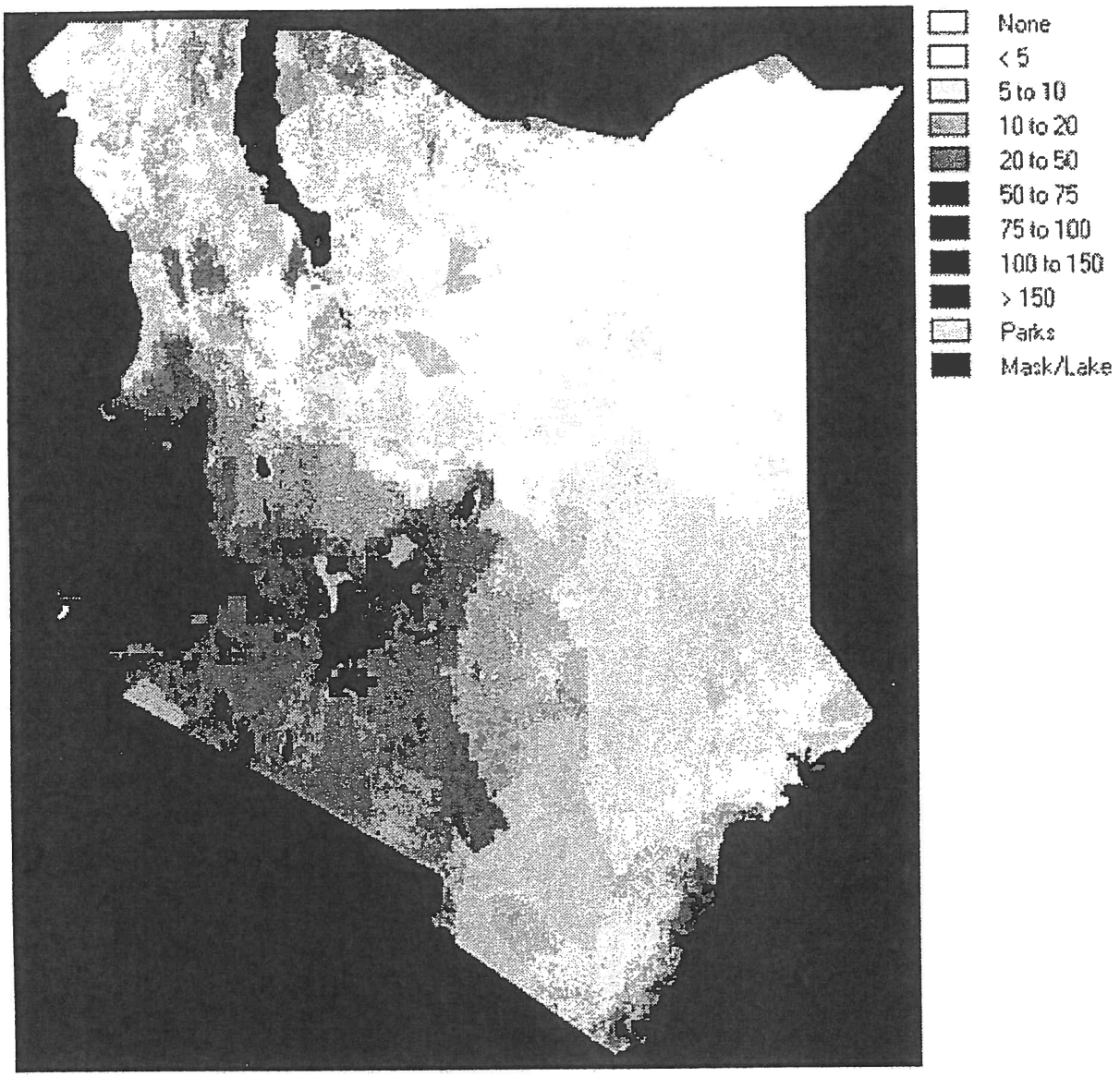

Fig. 3 Predicted distribution of cattle density showing the increased resolution achieved by the modelling techniques

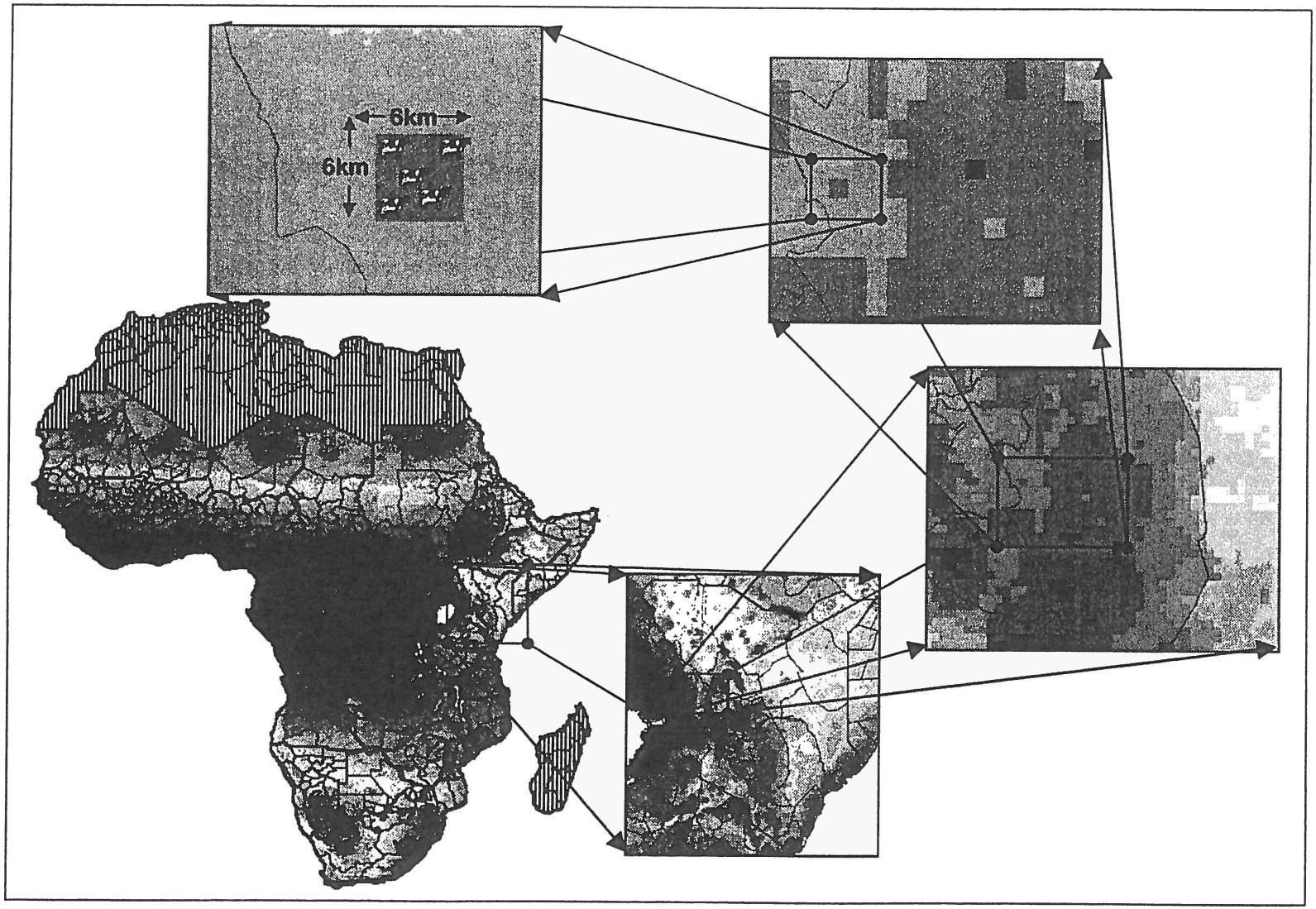

Fig. 4 Demonstration of the principle of the predicted cattle population densities data-layer as a huge two-dimensional matrix, displayed as a false colour image

Prediction of ecozones

Eco-climatic information is essential for the derivation of the predicted farming systems. The study used the following satellite-derived measures of land-surface and atmospheric characteristics to predict the set of eco-zones used:

a) the Normalised Difference Vegetation Index (NDVI)

b) a measure of ground surface temperature, derived from one of the thermal infra-red channels (Channel 4) of the same Advanced Very High Resolution Radiometer (AVHRR) instrument that also produces the NDVI data; and

c) a measure of surface rainfall, the Cold Cloud Duration (CCD), derived from the METEOSAT satellite

Background to the zonation process

The agro-ecological zonation that is widely used as a standard internationally and by FAO, consists of six classes (ranging from desert to humid, and highland) and is based on historical data. Recent work within the Trypanosomiasis and Land-use in Africa Research Group (TALA) at Oxford, UK has shown how a collection of thermal (Price 1984 split-window and AVHRR Channel 3) and NDVI or other vegetation index data can give remarkably good pattern matches to White's (1983) vegetation map of Africa, using unsupervised classification methods. It was therefore decided to attempt to define two separate zonations using the higher resolution data - the first into 'ecozones' using a subset of the available contemporary satellite imagery; the second into 'farming systems' using the assumed human population, cattle density and cropping intensity information.

Zones were defined using the ADDAPIX package to cluster the predictor or observed variables prior to regression. These clustering techniques are widely used in many areas of ecology and biology, where the underlying need is to identify regions of similarity in multi-variate data sets. ADDAPIX adopts the principal components analysis, followed-by-clustering approach.

Zonation is of substantial intrinsic interest, as it represents an objective geographical categorisation of farming system practices, based on reliable measures of agricultural intensity rather than qualitative descriptions of management styles. Relationships between regional ecozones are complex and have yet to be explored.

Production system parameters

The production system parameters used to drive the models were amassed from an extensive, on-going review of the literature. The results were catalogued and stored in a custom written database that permits an automated ranked, best-fit matching to the farming systems found within the area of interest. An important feature of the database is the ability of the user to examine data and supporting references from similar production systems, to determine those production systems which best fit those under consideration, and make their own intuitive modifications.

The "event" database

The event database stores profiles of parameters that are changed in the system under study. These may be management events such as an improvements in inter-service interval, changes in culling policy, weight gain or calf mortality; alternatively, these events may be disease effects manifested as e.g. increased mortality and decreased fertility, or effects on infection rate due to vaccination. The effect of disease control policies such as buffer vaccination, and culling may also be represented using this approach. The profiles are stored in the form of percentage increase and decrease of values above default levels.

Software

The regression analyses were carried out using SPSS® (SPSS Inc., Chicago, USA), the principal components analyses were performed with ADDAPIX® (FAO, Rome, Italy), Image analysis was performed using IDRISI® (Clark Labs., Massachusetts, USA), the geo-referencing of livestock numbers and farming system by PAAT-IS (FAO, Rome, Italy). General GIS functionality was provided by ArcView® (ESRI, Redlands, USA), programming for spatial analysis and modelling using Avenue® (ESRI, Redlands, USA) and database programming using Visual Basic® (Microsoft, USA) .

Sequence of events when using the system (figure 5)

Stage 1: Selection of the area of interest: The area of interest is selected using one of a number of standard geographic information system (GIS) tools to enclose the areas within either a user-defined polygon, or a regular geometric shape. Alternatively the area may be defined in other ways such as the physical distance from a point, as being above a certain altitude, or inside a buffer zone within *x* kms of e.g. a road, border, crushpen or village. If a time surface is available in the GIS (one that contains a measure of the speed of travel over the surface), then it is possible to establish a temporal distance from a point e.g. three hours on foot. The area may also be selected on a simple administrative boundary basis e.g. at sub-district, national, regional and potentially even continental level. Individual production systems or agro-ecological zones within a region may also be identified and selected i.e. only small-scale intensive dairy farmers, or all animals within the semi-arid agro-ecological zone.

Stage 2: Determination of the farming systems within the area of interest: The farming systems are identified and stored as unique integer values for each pixel within the area of interest, and then stored in a table for processing.

Stage 3: Determination of the predicted number of livestock within each farming system: The numbers of each species within each pixel are identified, cross-tabulated with farming system and stored within a table for processing.

Stage 4: Determination of the most appropriate production parameters for each species within each farming system: For each farming system and species identified, the production parameter database supplies the closest matching data held on record (ranked in descending order from the closest match), and the user is then given the opportunity to modify parameters using "expert" knowledge if an exact match is not available. The user is also able to "drill-down" through the records to examine the source of the reference that was the basis for that information.

Stage 5: Entry of species numbers and production parameters into the model, for each pixel within the area of interest: The model is run once for each farming system, using the production parameters identified in the database, and the species population within each system

Stage 6: Determination of the output and resource requirements of the area of interest, depending on the type of model used: Depending on the model used, the result is an estimate of e.g. the

population dynamics, system output and/or resource requirements. The models used in this work were $LDPS_2$ (FAO, Rome, Italy) and LPEC (VEERU, Reading, UK).

Stage 7: Entry of the "after" event production parameter data at stage 6: The information from the event profile database can be used to re-run the simulation with a second series of parameters to allow comparison of the result of an intervention.

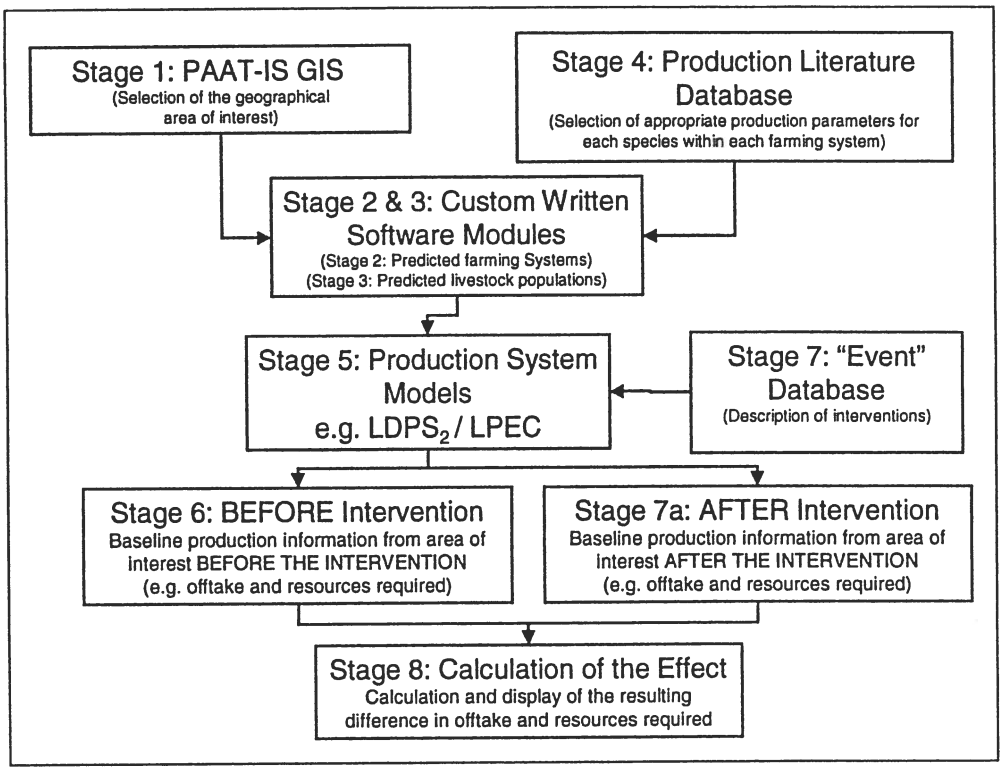

Fig. 5 System flowchart

DISCUSSION AND CONCLUSIONS

The role of this technique is seen as that of a planning tool, to allow the evaluation of animal health and production interventions on a large geographic scale, from sub-national to regional level. The initial field application is likely to be to identify base population data of doubtful quality, and fill in those missing data that exist for many areas in Africa, and for which there is little hope of gathering the information in the near future using conventional methods. The major analytical strength of this approach is that it allows selection of target populations using novel methods, free of the constraints of administrative boundaries. It is complimentary to existing census techniques. Missing data can be updated relatively cheaply by targeting pre-identified characteristic areas, and following-up with focussed micro-surveys on the ground. The farming system classification used is objective, and is easily related to the situation on the ground. Weaknesses in the models used are easily visualised as thematically shaded data layers, and the input of users with expert knowledge has proved extremely valuable in identifying areas for focussed data collection.

Before the system will be ready for use in a live evaluation exercise, the animal population and farming system predictions will have to be validated using ground truthing in the field. Currently, only predicted livestock numbers for cattle are available, which restricts the use in

mixed farming system situations, however, the prediction protocol followed appears to be amenable to small ruminants and these should be added to the model shortly. Other work remains to be undertaken to adapt the system to peri-urban agriculture, and to take account of seasonal animal movement in pastoral systems. In the near future, the increased resolution of the animal population and the production system data, coupled with features available in most modern desktop GIS systems will permit these new types of analyses to become a tool used by non-GIS specialists from other disciplines. It is intended that the system will be extended to include cropping prediction from remotely sensed data, which is already well developed. This will allow evaluation of mixed farming situations (taking account of both cropping and livestock) to be carried out. It will be possible to take more realistic account of seasonal effects if forage availability is taken into consideration. Increasingly higher resolutions will become possible and do produce distributions, which have been shown to be more realistic using qualitative assessment with detailed local knowledge. However, with the present level of resources it is unlikely to be justifiable to decrease the unit area of interest to less than 0.01° (approximately 1 km at the equator) as below this area, one would be operating at a level less than the area of most single farm units.

The ease of use of the system is dependent on the body of literature that the production parameter database refers to. Failure to amass and maintain sufficient up to date literature in the database will reduce the usefulness of this as a tool.

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THE BASIC REPRODUCTION NUMBER FOR SCRAPIE IN A FLOCK OF CHEVIOT SHEEP

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Of fundamental importance in understanding the dynamics of infectious disease outbreaks is the basic reproductive number, R_0, the average number of secondary infections produced by one infected individual introduced into a fully susceptible population. The parameter R_0, therefore, defines threshold behaviour: for R_0 less than unity, each infection on average fails to reproduce itself and the number of infected individuals is expected to decline towards zero; if, however, R_0 is greater than unity, each infection, on average, more than replaces itself and the outbreak, at least initially, will escalate (Anderson & May 1991). The concept of R_0 is, therefore, a valuable one in disease control: if the system can be manipulated so as to reduce R_0 below unity major outbreaks can be prevented. Thus, this quantity can be used to both devise and assess the efficacy of disease control measures.

Scrapie is a naturally occurring disease of sheep which causes deterioration in neurological function, loss of condition and death. It is a transmissible spongiform encephalopathy (TSE), a category which includes BSE in cattle and nvCJD in humans, and is associated with an abnormal form of the prion protein (PrP) (Caughey & Chesebro 1997). The disease has been known to exist in the UK for over two hundred years. The epidemiology of scrapie has recently been reviewed by Hoinville (1996). Scrapie can be transmitted horizontally within a flock and vertically, from ewe to lamb, though the mechanisms involved remain uncertain. A feature of this disease is its long (of the order of two years) incubation period. Experimental studies in mice (Bruce et al. 1991) demonstrate that the incubation period is dose dependent and that levels of abnormal PrP in the tissues increase from the time of infection until the appearance of clinical signs. Available evidence suggests that genetic susceptibility to scrapie is governed by alleles present at the PrP locus with resistance alleles being dominant or partially dominant (Hunter et al. 1996 & Dawson et al. 1998).

In a previous paper (Matthews et al. 1999) we derived an expression for R_0 based on a recently developed model for the spread of scrapie through a flock of sheep (see Stringer et al. 1998 & Woolhouse et al. 1998 for a detailed discussion) and explored its sensitivity to a range of epidemiologically important parameters. The model incorporated genetic susceptibility to scrapie, allowing us to explore, within the context of a one locus two allele system, the consequences of genetic alteration and inbreeding within the flock.

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Here, we use this approach to determine R_0 for an outbreak of natural scrapie in a flock of Cheviot sheep (Dickinson 1974 & Hunter et al. 1996) We assess the sensitivity of this value to horizontal and vertical transmission rates and consider also the role of culling preclinically infected animals. The model incorporates genetic susceptibility to scrapie, a detailed genetic profile of the flock and a breeding program based on positive and negative selection lines for scrapie susceptibility, allowing investigation of the importance of the genetic composition of the flock and the role of the relative susceptibility of the partially susceptible heterozygotes. We characterise the level of inbreeding in the flock produced by this breeding program and assess the sensitivity of R_0 to this parameter.

The relationship between host population genetics and R_0 has been of theoretical interest for some time (e.g. Roberts & Heesterbeek 1995). But, as far as we are aware, this is the first time that host population genetics have been incorporated in an expression for R_0 for any infectious disease on the basis of empirical observation. This is relevant not only to scrapie but for other infectious diseases where breeding programmes are seen as an important method of disease control.

THE EPIDEMIC MODEL

The model comprises a set of partial differential equations in which the population is stratified by age and, for the infected individuals, by infection load. The model incorporates horizontal and vertical routes of transmission and an initial infection load which varies according to a gamma distribution. The infectiousness of an individual is assumed to be proportional to its infection load which increases exponentially with time until it reaches a maximum level at which the individual shows clinical signs and dies.

In this system susceptibility to scrapie is assumed to be governed by a single locus with the four alleles VRQ, ARQ, AHQ and ARR giving a total of 10 genotypes, all of which are represented in the flock. Natural scrapie has only been observed in two genotypes in this flock: VRQ/VRQ and VRQ/ARQ. It is assumed, therefore, that AHQ and ARR are both dominant for resistance (and so do not need to be distinguished in numerical analyses). ARQ is partially dominant for resistance. Therefore, VRQ/VRQ homozygotes are fully susceptible, VRQ/ARQ heterozygotes are partially susceptible and all other genotypes are resistant.

The model also incorporates a breeding program based on positive and negative selection lines for scrapie susceptibility. Mating is assortative: 40% of matings are random amongst sheep without the VRQ allele (the resistant line); 60% of matings are random amongst the remaining sheep (the susceptible line).

Full details of the model and its application are given elsewhere (Stringer et al. 1998, Woolhouse et al. 1998 & Woolhouse et al. 1999).

EXPRESSION FOR R_0

For simple epidemiological models with a single class of susceptibles and infecteds the parameter R_0 is straightforward to calculate – it is the initial number of susceptibles multiplied

by per capita transmission rate multiplied by the mean lifetime of an infected individual, which, for an SIR model (Anderson & May 1991) is simply the initial number of susceptibles multiplied by the ratio of the per capita transmission rate and the recovery rate.

Now consider a model in which the infected class is stratified by age and infection load. The per capita transmission rate of individuals of age, a, and infection load, θ, at the time of infection is calculated at all future times and integrated over the lifetime of the infecteds. R_0 is then obtained by integrating over all initial values of age and infection load and multiplying by the number of susceptibles.

For a model with several classes of infecteds the above calculation must be performed for each class. A matrix R is obtained whose elements, R_{ij}, give the average number of secondary infections in class i produced by one infection in class j. Standard theory (Heesterbeek & Dietz 1996) tells us that the required value of R_0 is given by the leading eigen value of this matrix.

If we formulate the expression for the general case, horizontal and vertical transmission between all six genotypes, we obtain a 12x12 matrix for R. Since only two genotypes in this system are susceptible to natural scrapie R reduces to a 4x4 matrix. We can regard the matrix R as being composed of four blocks, each a 2x2 matrix, which we shall denote A, B, C and D as below;

$$\boldsymbol{R} = \begin{bmatrix} A & B \\ C & D \end{bmatrix}.$$

The components A_{ij} determine the level of horizontal transmission from horizontally infected animals, the components D_{ij} the level of vertical transmission from vertically infected animals, the components B_{ij} the level of horizontal transmission from vertically infected animals and the components C_{ij} the level of vertical transmission from horizontally infected animals.

We denote the density of newly horizontally infected animals of genotype j, age a and infection load θ_0 by $I_h(j,a,\theta_0)$, and the density of newly vertically infected animals by $I_v(j,\theta_0)$. We shall consider initially the case of horizontal transmission from horizontally infected animals.

Horizontal Infections

A time τ after infection, animals with initial age a and infection load θ_0 will have age a_τ and infection load θ_τ where

$$a_\tau = a + \tau \qquad \text{and} \qquad \theta_\tau = \theta_0 e^{c\tau}.$$

Thus, neglecting mortality, at time τ, the density distribution of infecteds in terms of a_τ and θ_τ is given by

$$I_h(j, a_\tau - \tau, \theta_\tau e^{-c\tau}) e^{-c\tau},$$

where the scaling factor, e^{-ct}, is required to preserve the total number of infecteds obtained by integrating the expression over a_τ and θ_τ.

Allowing for natural mortality, the proportion of animals alive at $t=0$ still alive at $t=\tau$ is given by

$$\frac{S(a_\tau)}{S(a_\tau-\tau)}$$

where $S(a)$ is the survivorship function.

Thus, taking into account natural mortality and noting that the model assumes that all animals die when they reach either $a_\tau=a_{max}$ or $\theta_\tau=\theta_{max}$, the total number of animals remaining alive at time τ is given by

$$\int_{\tau}^{a_{max}}\int_{0}^{\theta_{max}} I_h(j,a_\tau-\tau,\theta_\tau e^{-c\tau})e^{-c\tau}\frac{S(a_\tau)}{S(a_\tau-\tau)}d\theta_\tau da_\tau,$$

or, equivalently,

$$\int_{0}^{a_{max}-\tau}\int_{0}^{\theta_{max}} I_h(j,a,\theta_\tau e^{-c\tau})e^{-c\tau}\frac{S(a+\tau)}{S(a)}d\theta_\tau da.$$

The rate of horizontal transmission from an animal with infection load θ is given by $k_{ij}^H\theta$ where

$$k_{ij}^H = kW_{ij}$$

and W_{ij} gives the relative susceptibility of a genotype i animal to infection by a genotype j animal.

Therefore, the rate of appearance of new horizontal infections at time $t=\tau$ per sheep horizontally infected at time $t=0$ is given by

$$N_i k_{ij}^H\int_{0}^{a_{max}-\tau}\int_{0}^{\theta_{max}} I_h(j,a,\theta_\tau e^{-c\tau})\theta_\tau e^{-c\tau}\frac{S(a+\tau)}{S(a)}d\theta_\tau da.$$

where N_i is the number of susceptible animals of genotype i. The elements A_{ij} are, therefore, obtained by integrating this expression over the maximum length of time for which an animal may live and dividing by $\bar{I}_h(j)$, the total number of animals of genotype j infected horizontally at $t=0$, given by

$$\bar{I}_h(j) = \int_0^{a_{max}} \int_0^{\theta_{max}} I_h(j,a,\theta) d\theta da.$$

Thus, the elements A_{ij}, which represent horizontal infections arising from horizontally infected animals, are given by

$$A_{ij} = N_i k_{ij}^H \int_0^{\tau_{max}} \int_0^{a_{max}-\tau} \int_0^{\theta_{max}} \frac{I_h(j,a,\theta_\tau e^{-c\tau})\theta_\tau e^{-c\tau}}{\bar{I}_h(j)} \frac{S(a+\tau)}{S(a)} d\theta_\tau da\, d\tau$$

where $\tau_{max}=a_{max}$. Similarly, the elements B_{ij}, which represent horizontal infections arising from vertically infected animals, are given by

$$B_{ij} = N_i k_{ij}^H \int_0^{\tau_{max}} \int_0^{\theta_{max}} \frac{I_v(j,\theta_\tau e^{-c\tau})\theta_\tau e^{-c\tau}}{\bar{I}_v(j)} \frac{S(\tau)}{S(0)} d\theta_\tau\, d\tau\,.$$

Note that in this case there is no integral over the age variable because, by definition, all vertically infected animals become infected at age zero.

Vertical Infections

For vertical infections the per capita rate of infection from animals of genotype j, age a and infection load θ to offspring of genotype i is given by

$$\gamma_{ij}^V(a,\theta)\, G_{ij}\, b(a)\,.$$

γ_{ij}^V is the fraction of births from infected animals of age a and infection load θ which result in vertical transmission and is given by $\gamma_{ij}^V = k_{ij}^V \theta$ where

$$k_{ij}^V = \gamma W_{ij}\,,$$

γ is a constant and W_{ij} is the susceptibility matrix as above. G_{ij} gives the proportion of offspring of genotype i born to genotype j mothers. The function $b(a)$ is the birth rate and set to zero for $a<2$ since animals are assumed not to give birth before the age of two years. Thus

$$b(a) = \begin{cases} 0 \text{ for } a < 2 \\ b \text{ for } 2 \le a \le a_{max} \end{cases}$$

where b is a constant.

The elements C_{ij} and D_{ij}, corresponding to vertical transmission from horizontally and vertically infected animals respectively are, therefore, given by

$$C_{ij} = k_{ij}^{V} G_{ij} \int_0^{\tau_{max}} \int_0^{a_{max}-\tau} \int_0^{\theta_{max}} b(a+\tau) \frac{I_h(j,a,\theta_\tau e^{-c\tau})\theta_\tau e^{-c\tau}}{\bar{I}_h(j)} \frac{S(a+\tau)}{S(a)} d\theta_\tau da\, d\tau$$

and

$$D_{ij} = k_{ij}^{V} G_{ij} \int_0^{\tau_{max}} \int_0^{\theta_{max}} b(\tau) \frac{I_v(j,\theta_\tau e^{-c\tau})\theta_\tau e^{-c\tau}}{\bar{I}_v(j)} \frac{S(\tau)}{S(0)} d\theta_\tau\, d\tau.$$

METHODS

Reference value

The reference value of R_0 is calculated for the set of parameters below as detailed in Woolhouse et al. (1999).

Demography is described by a truncated Weibull distribution with a maximum and mean lifespan of 12 years and 3.46 years respectively. As in the previous model, infection load has a mean initial level of 10% of the maximum and increases exponentially with time. The rate of exponential increase is set to give a mean incubation period of 1.9 years. The rate of horizontal transmission is 0.09 new infections per infected sheep per year and vertical transmission is assumed to occur at a level of 40%. The birth rate is taken to be the value calculated at the outset of the model run.

The relative susceptibility of the VRQ/ARQ heterozygotes compared to the VRQ/VRQ homozygotes is set at 0.28.

Mating is assortative: 40% of matings are random amongst sheep without the VRQ allele (the resistant line); 60% of matings are random amongst the remaining sheep (the susceptible line). It is assumed that initially 60% of the sheep i.e. all the susceptible line, carry at least one VRQ allele. The initial frequencies of the other alleles are estimated from their relative frequencies in other UK Cheviot flocks (Hunter et al. 1997). The initial frequency of the VRQ allele is estimated as 0.37, the upper limit given the above assumptions.

The genotype frequencies at the start of the outbreak are estimated in the following way: The founding population at the time of closure of the flock was assumed to be in Hardy-Weinberg equilibrium. The population was then bred, in the absence of scrapie, in accordance with the assortative mating system for a period of three years, producing the genetic composition of the naïve flock into which scrapie is introduced.

This breeding program results in a population which, whilst not satisfying the formal definition of an inbred population (see Appendix), can, for our purposes, be regarded as inbred and characterised by an inbreeding coefficient in the manner outlined below.

Inbreeding

Dividing the founding population into selection lines (as described above) results in a positive selection line population which is no longer in Hardy-Weinberg equilibrium but which instead contains a 'surplus' of VRQ heterozygotes. The effect of the breeding program is then to convert these heterozygotes into VRQ/VRQ homozygotes and ARQ/ARQ, ARQ/ARR & ARR/ARR genotypes. Thus, if we do not distinguish ARQ and ARR alleles (and denote them ARX) we can regard the whole population as a formally inbred population of VRQ/VRQ, VRQ/ARX and ARX/ARX genotypes, with an associated inbreeding coefficient. The level of inbreeding continues up to maximal level at which the three 'genotypes' in the positive selection line are again in Hardy-Weinberg equilibrium. The total ARQ/ARQ, ARQ/ARR & ARR/ARR population increases but remains in Hardy-Weinberg equilibrium.

The inbreeding coefficient at the end of the three year period was 0.26, with the maximal value being 0.4.

Sensitivity analysis

We explore the sensitivity of R_0 to changes in horizontal and vertical transmission rates and mean lifespan. In practice vertical transmission can be reduced by culling lambs born to infected ewes and horizontal transmission reduced by changes in husbandry. We also investigate the effect on R_0 of culling preclinically infected sheep, now possible due to recently developed diagnostic tests (Schreuder et al. 1996, 1998).

We explore the effect of a reduction in the VRQ allele frequency for different values of the relative susceptibility of the susceptible heterozygotes, and also examine the role of inbreeding in the flock.

RESULTS

The sensitivity of R_0 to variation in horizontal and vertical transmission rates and to removal of individuals before they reach the maximum infection load is shown in Fig. 1. In the absence of vertical transmission (not shown), R_0 declines linearly to zero with the reduction in the horizontal transmission parameter, k. The effect of including a small rate of vertical transmission is to introduce small nonlinearities into this relationship which are most noticeable at low horizontal transmission rates. The relatively small reduction in R_0 to be gained from reducing vertical transmission is clearly illustrated.

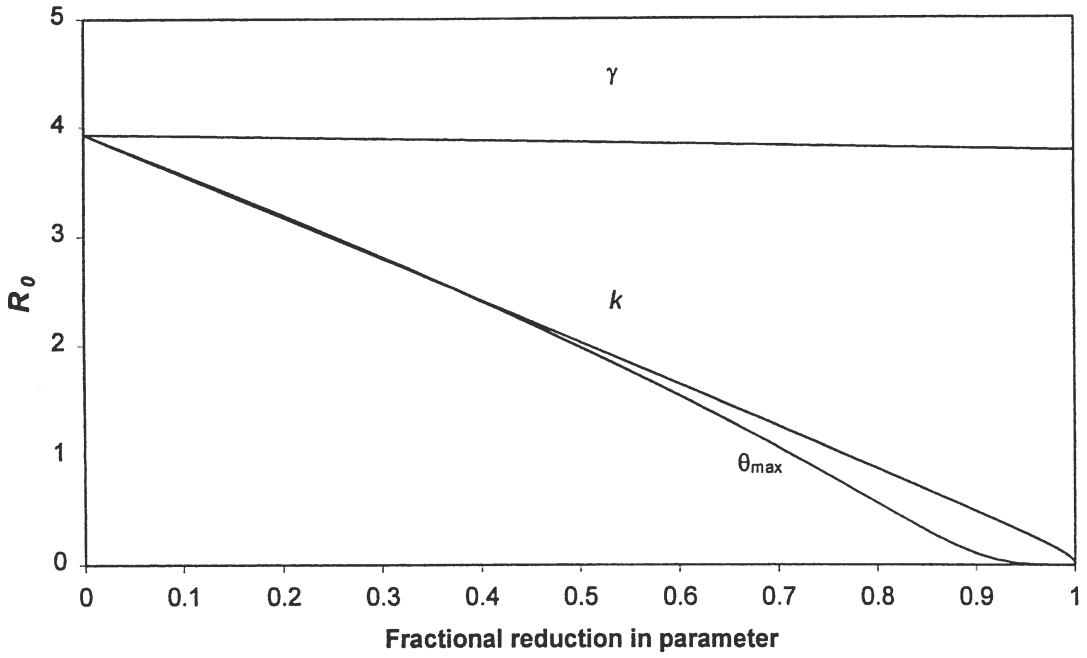
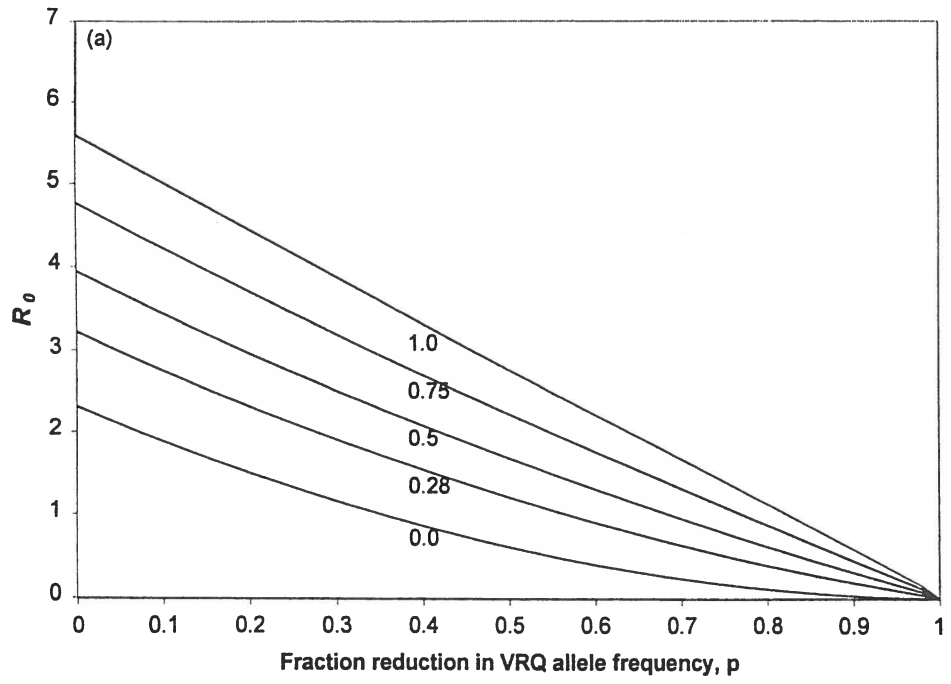


Fig. 1 Sensitivity of R_0 to reduction in the horizontal transmission rate k, the vertical transmission rate γ and the maximum infection load θ_{max}.

The effect of removing infected individuals before they reach the maximum infection load is also shown in Fig. 1. R_0 approaches zero rapidly when the infection load at which individuals are removed falls below the mean infection load of newly infected individuals. Under these circumstances most newly infected individuals are removed instantly and thus prevented from causing further infections.



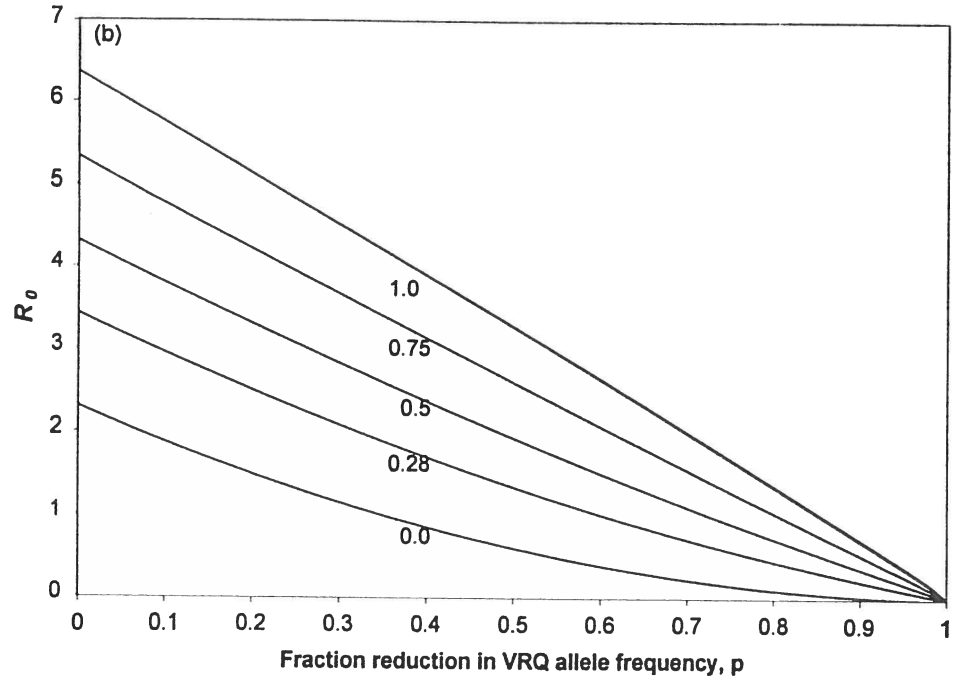
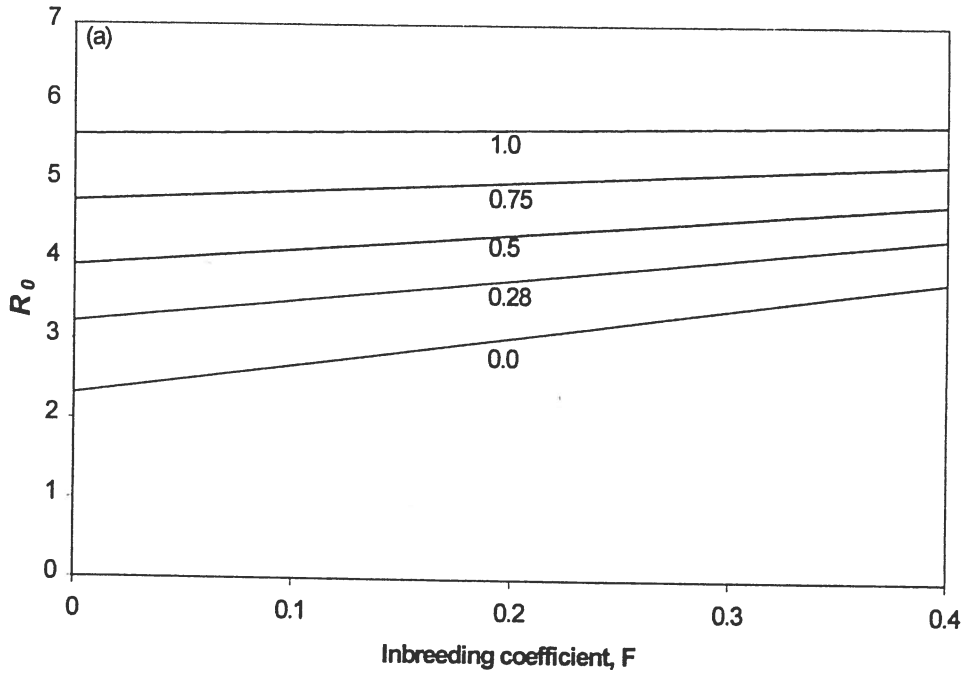


Fig. 2 Sensitivity of R_0 to a reduction (from an initial value of 0.37) in the frequency of the VRQ allele for a relative susceptibilities of the susceptible heterozygotes of 0.0, 0.28, 0.5, 0.75 and 1.0 for (a) a ratio of ARQ to ARR alleles of 0.81 and (b) a ratio of ARQ to ARR alleles of 1.23.

Figure 2 shows the sensitivity of R_0 to changes in the frequency of the VRQ allele as it falls from its maximal value (given the criteria for specifying the genetic composition of the flock)



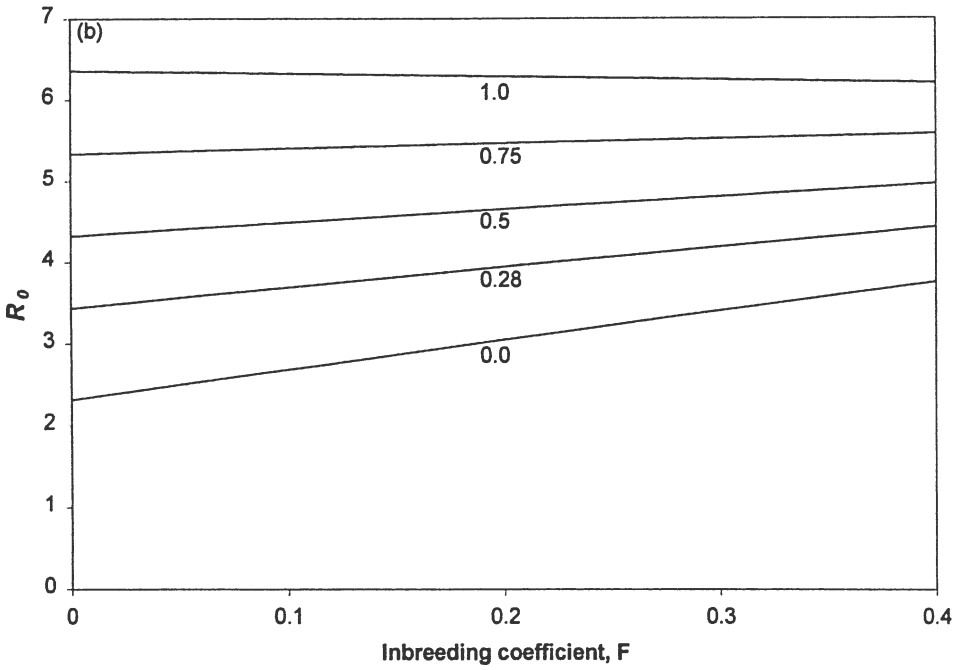


Fig. 3 Sensitivity of R_0 to variation in the level of inbreeding for relative susceptibilities of the susceptible heterozygotes of 1.0, 0.75, 0.5, 0.28 and 0.0 for a ratio of ARQ to ARR alleles of 0.81 and (b) a ratio of ARQ to ARR alleles of 1.23.

of 0.37 to zero. For clarity of argument we shall first consider the situation in the absence of vertical transmission (not shown). In the absence of vertical transmission, variation in R_0 due to changes in allele frequency depends only on the numbers of susceptibles available and their relative susceptibilities to infection. If the ratio of ARQ to ARR alleles is one, then any VRQ allele has an equal probability of being in either a VRQ/ARQ or VRQ/ARR individual. If in addition, the relative susceptibility of the VRQ/ARQ genotype is one, then the VRQ heterozygotes can be regarded overall as being 50% as susceptible as the VRQ homozygotes. Therefore, the overall susceptibility of the population is proportional to the frequency of the VRQ allele and a plot of R_0 against fractional reduction in VRQ allele frequency would be a straight line. If the ratio of ARQ to ARR alleles is greater than one then the straight line plot occurs for some critical value of the VRQ/ARQ susceptibility which is less than one. Above the critical value a greater reduction in VRQ allele frequency is required to reduce to R_0 to a given level, and below the critical value a smaller reduction is required. If the ratio of ARQ to ARR alleles is below one, the linear case can not be achieved. When vertical transmission is included (see Fig. 2) a small correction is introduced which is most noticeable at low values of R_0. Figure 2a shows the variation in R_0 for the reference value of the ratio of ARQ to ARR alleles, and Fig. 2b shows the variation in R_0 for the reciprocal of this value. In Fig. 2a, any decrease in VRQ frequency is more effective at reducing R_0 than the linear case would be. In Fig. 2b, we can see that we would obtain a linear plot at some value of the relative susceptibility between 0.5 and 1.0. Above this value a greater reduction in VRQ allele frequency is required to reduce to R_0 to a given level, and below this value a smaller reduction is required.

Figure 3 shows the sensitivity of R_0 to the level of inbreeding in the population. We will again, for simplicity, consider initially the situation in the absence of vertical transmission (not shown). The effect of the inbreeding is to convert VRQ heterozygotes into VRQ homozygotes and ARQ/ARQ, ARQ/ARR & ARR/ARR genotypes. Applying similar arguments to above, if the ratio of ARQ to ARR alleles is one (i.e. there are equal nos. of VRQ/ARQ and VRQ/ARR genotypes) and if in addition the VRQ/ARQ genotype has a relative susceptibility of one, then the overall susceptibility of the population is proportional to the frequency of the VRQ allele and the value of R_0 is unaffected by the inbreeding (our graph is a horizontal line). If the ratio of ARQ to ARR alleles is greater than one, the horizontal line is obtained for a value of the relative susceptibility below one. Above this critical value, R_0 falls as the level of inbreeding increases and below the critical value R_0 rises as the level of inbreeding increases. If the ratio of ARQ to ARR alleles is below one, R_0 must always increase as the inbreeding level increases. Note that when vertical transmission is included the horizontal line will become slightly sloped due to an enhanced possibility of vertical transmission in an inbred population. Figures 3a and 3b show the sensitivity of R_0 to the level of inbreeding for values of the ratio of ARQ to ARR alleles of 0.81 (the reference value) and 1.23. As can clearly be seen, for a ratio of 0.81 R_0 always increases as the inbreeding level increases, whereas for a ratio of 1.23 R_0 may increase or decrease as the inbreeding level increases.

DISCUSSION

This paper provides an expression for R_0 which allows us to assess its sensitivity to a range of epidemiologically important parameters. Furthermore, the expression explicitly incorporates genetic susceptibility to scrapie, allowing us to explore the effect of inbreeding within a flock and assess the impact on R_0 of reducing the frequency of susceptibility alleles. Thus, a formal expression for R_0 allows us to assess the impact of potential control measures. A similar study has been conducted for the transmission of BSE (Ferguson et al., 1999) but the model in that case involved different routes of transmission and no genetic susceptibility to infection.

The results from our exploration of the sensitivity of R_0 to transmission rates and to the effect of culling preclinically infected animals reiterate those found in Matthews et al. (1999). A reduction in horizontal transmission rates is an effective control measure, though implementation would be difficult because the mechanisms underlying this transmission route are poorly understood (though pasture decontamination has been attempted (Sigurdarson 1991)). In contrast, a reduction in vertical transmission is relatively ineffective because most cases arise through the horizontal transmission route.

The current development of diagnostic tests for scrapie (Schreuder et. al. 1996, 1998) makes the slaughter of preclinically infected individuals a viable option and our results show this to be an effective way of reducing R_0 provided the diagnostics are sensitive enough. However, this conclusion is crucially dependent on how infectiousness (especially by horizontal transmission routes) varies during the incubation period and there is still limited data on this.

Where our results show some differences from those presented in Matthews et al. (1999) is in the analysis of the efficacy of genetic improvement of the flock, achieved by selective breeding or culling of undesirable genotypes, as a potential control measure. Here, we have

incorporated a more complex genetic profile of the flock, the most important feature of which is the existence of a resistant genotype which contains the scrapie susceptibility allele. This genotype acts as a reservoir for the VRQ allele so that inbreeding in the population is more likely to lead to an increase in R_0 than a decrease, and thus increase the chances of an outbreak. Our results also demonstrate that a reduction in the frequency of the VRQ allele is more effective the less susceptible the VRQ/ARQ animals are, and that this effect is enhanced by increasing the numbers of resistant VRQ heterozygotes compared to the numbers of susceptible VRQ homozygotes. However, phenotypic selection against an allele is easiest to achieve when the allele is dominant. The work presented here provides a powerful analytical tool for the quantitative assessment of the potential impact of genetic control measures for scrapie.

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APPENDIX

Let the frequencies of the VRQ, ARQ and ARR alleles be denoted p, q, and r respectively. The genotype frequencies of a population in Hardy-Weinberg equilibrium are given by

$$f_{VRQ/VRQ} = p^2$$
$$f_{VRQ/ARQ} = 2pq$$
$$f_{VRQ/ARR} = 2pr$$
$$f_{ARQ/ARQ} = q^2$$
$$f_{ARQ/ARR} = 2qr$$
$$f_{ARR/ARR} = r^2$$

where $f_{X/X}$ denotes the frequency of the X/X genotype. If we do not distinguish the ARQ and ARR alleles, denoting them both ARX, then we can regard this as a system of three genotypes, VRQ/VRQ, VRQ/ARX & ARX/ARX, in Hardy-Weinberg equilibrium genotype frequencies given by

$$f_{VRQ/VRQ} = P^2$$
$$f_{VRQ/ARX} = 2PQ$$
$$f_{ARX/ARX} = Q^2$$

where $P=p$ and $Q=q+r$.

The construction of the selection lines is such that at any given moment only a randomly selected proportion of the ARX/ARX population is allowed to breed with the VRQ allele containing population. Thus, the positive selection line, which constitutes a proportion f of the total population and contains all the individuals with a VRQ allele and a proportion of the ARX/ARX population will no longer be in Hardy-Weinberg equilibrium. The consequence of random mating within the positive selection is to convert VRQ heterozygotes into VRQ homozygotes and ARX homozygotes. If we denote the converted fraction of the VRQ/ARQ population by F then our genotype frequencies are given by

$$f_{VRQ/VRQ} = P^2 + FPQ$$
$$f_{VRQ/ARX} = 2PQ(1-F)$$
$$f_{ARX/ARX} = Q^2 + FPQ$$

which, since $Q=1-P$ may be rewritten

$$f_{VRQ/VRQ} = P^2(1-F) + PF$$
$$f_{VRQ/ARX} = 2PQ(1-F)$$
$$f_{ARX/ARX} = Q^2(1-F) + QF\,.$$

Thus, our three genotype population may be regarded as an inbred population with inbreeding coefficient F (Crow & Kimura 1970).

Since the ARQ and ARR alleles in the VRQ heterozygotes are in the ratio $q : r$, the genotypes ARQ/ARQ, ARQ/ARR & ARR/ARR added to the ARX/ARX population must be in the proportion $q^2 : 2qr : r^2$, and the new genotype frequencies are given by

$$f_{ARQ/ARQ} = q^2 + FPQ\frac{q^2}{1-f} = \tilde{q}^2$$
$$f_{ARQ/ARR} = 2qr + FPQ\frac{2qr}{1-f} = 2\tilde{q}\tilde{r}$$
$$f_{ARR/ARR} = r^2 + FPQ\frac{r^2}{1-f} = \tilde{q}^2$$

where $\tilde{q} = \lambda q$, $\tilde{r} = \lambda r$ and $\lambda = \sqrt{1 + \frac{FPQ}{1-f}}$. The ARQ/ARQ, ARQ/ARR & ARR/ARR population has therefore remained in Hardy-Weinberg equilibrium. The inbreeding level will rise until the positive selection line is again in Hardy-Weinberg equilibrium. If the VRQ allele frequency is p, then its frequency within the positive selection line is *p/f*. The maximum level of inbreeding, F_{max}, is, therefore, given by

$$\left(\frac{p}{f}\right)^2 = \frac{p^2(1 - F_{max}) + pF_{max}}{f}$$

$$\Rightarrow \qquad F_{max} = \frac{(1-f)p}{(1-p)f}$$

THE NEED FOR AN ACTIVE (TARGETED) SURVEILLANCE SYSTEM FOR BSE AND SCRAPIE IN ADDITION TO THE MANDATORY REPORTING OF CLINICAL SUSPECT CASES

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In Switzerland, the first Scrapie case was reported in 1982 and the first case of bovine spongiform encephalopathy (BSE) at the end of 1990 (Fankhauser et al., 1982; Cachin et al., 1991). Only six additional Scrapie cases were diagnosed between 1990, the start of the mandatory reporting requirement for clinical suspects, and June of 1999. Reporting of clinical BSE suspects also became mandatory in 1990. The BSE epidemic peaked in 1995 and then started to decline, with a total of 282 clinical cases reported until December 31, 1998 (Table 1). Due to the lack of a reliable and fast diagnostic screening test, surveillance for both diseases, as in most other countries world-wide, was based on the mandatory reporting and examination of animals with clinical neurological symptoms (clinical suspects) alone. The effectiveness of a passive surveillance system, however, is very dependent on a variety of factors including the disease awareness of the farmers and veterinary professionals, the consequences of reporting diseased animals such as losses of the animal or the entire herd, the compensation paid for those losses, and the quality of the diagnostic laboratories involved. It is well established for a variety of (rare) diseases in animals and humans that passive surveillance systems typically underestimate the incidence of clinical cases (Kelsey et al., 1986; Martin et al., 1987; Toma et al., 1999) and it is likely that the degree of underreporting will vary between countries or regions. Meaningful comparison of disease frequency information, like incidence or prevalence estimates from different countries, therefore is almost impossible. The need for an active surveillance component that either allows to derive estimates on the degree of underreporting within the passive surveillance, or collects sufficient data to replace the passive system, becomes obvious.

Between 1991 and 1998, over 600 cattle and 68 sheep and goats where BSE and Scrapie respectively could not be excluded on clinical grounds, were examined in the Institute

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of Animal Neurology of the Bern University (Reference Laboratory) (Table 1). Confirmation of the disease was based on the histological and immunohistochemical examination of brain segments. This procedure, however, takes several days and is too labour-intensive for screening purposes. A new diagnostic test system sufficient to screen larger numbers of brain homogenates for the infections prion protein (PrP^{Sc}) became available at the end of 1998, making the implementation of an active surveillance component for BSE possible (Schaller et al., 1999, Moynagh & Schimmel, 1999). In parallel, efforts to increase disease awareness for BSE and Scrapie and thereby the reporting of clinical suspects were renewed. The objective of this paper is to briefly describe the active surveillance components for BSE and Scrapie implemented in Switzerland in 1999, and to present and discuss first results.

Table 1. Examined cattle, sheep and goats where BSE and Scrapie respectively could not be excluded on clinical grounds, and the number of confirmed cases in Switzerland

Category	1990	1991	1992	1993	1994	1995	1996	1997	1998
BSE suspects	n/a*	113	57	78	124	111	95	69	53
Confirmed cases	1	9	15	29	63	68	45	38	14
Scrapie suspects	n/a*	7	11	14	7	17	4	2	6
Confirmed cases	-	1	-	3	-	1	-	-	-

* not recorded since reporting of clinical suspects became mandatory at the end of 1990

MATERIALS AND METHODS

BSE surveillance

The total Swiss cattle population is composed of approx. 1.7 Million animals, 0.9 Million of them adult female cattle designated for breeding and dairy production (Doherr et al., 1999a). Over 10000 adult cows per year are subject to emergency slaughter or fallen stock. Between January and March of 1999 a targeted BSE surveillance system was established on three categories of adult cattle: (a) fallen stock, (b) cattle subject to emergency (sick) slaughter and (c) routinely slaughtered cattle. It was assumed that the first two categories would contain a considerable proportion of the detectable BSE-infected animals, therefore all animals in those two categories were examined in the Prionics Western Blot (PBW; Prionics AG, Zürich-CH). In addition, over 7000 of the 200000 annually slaughtered adult cattle were tested. All PBW–positive samples were submitted to the Swiss Reference Laboratory for Animal TSEs for confirmation by histopathology (TSE-specific lesions) and immunohistochemistry (PrP^{Sc} accumulation).

All official BSE suspect cases have to be reported to the veterinary authorities. The examination is free of charge, and the owners are compensated for at least 90% of the market

value of the animals. Several articles and announcements in the bi-weekly official bulletin of the Swiss Federal Veterinary Office (FVO) as well as two continued education workshops for large animal practitioners on neurological diseases in ruminants stressed the issue of identifying and reporting clinical BSE suspects.

Scrapie surveillance

An anonymous postal survey was performed on almost 50% of the Swiss sheep and goat owners with at least five breeding animals to derive data on the observed prevalence of small ruminants with neurological disease including Scrapie. An estimated 400-500 adult sheep and goats are rendered annually (total population 0.42 Million sheep and 0.06 Million goats). Since June 1999, approximately five small ruminant brain samples per week were collected at the single rendering plant designated for fallen stock and examined with the PWB. In addition, several publications in the FVO bulletin and two articles in a farmer's journal for small ruminants (Doherr & Baumgarten, 1999; Baumgarten & Doherr, 1999) informed about the ongoing Scrapie research.

All official Scrapie suspect cases have to be reported to the veterinary authorities. Examination is free of charge, and the owners are compensated for at least 90% of the market value of the animals. Within this campaign in addition, it was offered to examine all small ruminants with signs of neurological disease at one of the two veterinary institutions to exclude Scrapie. These services were free of charge.

RESULTS

Between January 1 and December 31, 1999, 25 BSE cases were captured through the passive surveillance (mandatory reporting of BSE suspects). In addition, a total of 25 BSE cases were diagnosed by the active surveillance (fallen stock: 16; emergency slaughter: 6; in sample of the 200000 routinely slaughtered adult cows: 3). Follow-up on the targeted surveillance cases revealed that most of these animals had some clinical signs of disease that – in retrospect – could have pointed towards BSE but at the time of slaughter or culling were not classified as BSE-specific.

In the same period, three clinical Scrapie suspects were reported to the veterinary authorities, and one of those was confirmed as a Scrapie case. In addition, 24 small ruminants for which Scrapie could not be excluded on clinical grounds collected within the project were examined for differential diagnosis. Of the approximately 200 [check numbers] small ruminant brains sampled from the fallen stock (adult sheep and goats), none showed any detectable accumulation of PrP^{Sc}.

DISCUSSION

With the data it can be documented that with the passive BSE surveillance – in Switzerland and for the year 1999 – only 50% of the detectable BSE cases, i.e. infected animals close to or actually showing clinical signs of disease, were captured. This, in combination with the

additional data on age distribution collected, was consistent with modelling results, i.e. expected number of cases assuming an underreporting level of 50%, of the Swiss BSE epidemic (Doherr et al., 1999a). The results of the targeted (active) surveillance in 1999 indicate that – for cattle – the fallen stock and emergency slaughter groups have a considerably higher proportion of BSE cases than the general population estimates derived from mandatory reporting of clinical suspects would indicate (by factors 85 and 54 higher, respectively). The BSE cases are accumulating in those cohorts, making them good populations for a targeted BSE screening (Doherr et al., 1999b). The annual costs for testing close to 17000 cattle for the presence of PrP^{Sc} accumulation (including sampling and transport) with the PWB were approximately 1 Million Euro (Heim et al., 1999). A main cost-driving factor was the need to process all samples from emergency and routine slaughter animals with the PWB overnight to both confiscate and destroy (positive result) or release the carcass for further meat processing (negative result). Despite the high costs of the screening programme it was decided to continue with the targeted surveillance in the year 2000 since the data from this surveillance is essential to derive a clear picture of the future course of the BSE epidemic and to evaluate the effectiveness of preventive measures. Unfortunately, in public mainly the fact is recognised that the total number of BSE cases has increased from 1998 to 1999, and it is ignored that this increase is exclusively the result of a change in the effectiveness of case detection (improved surveillance).

A more detailed description of the anonymous mail survey with first results is presented as a poster by Baumgarten et al. at this SVEPM meeting. The history of Scrapie in Switzerland, with a total of seven Scrapie cases confirmed between 1982 and 1999, seems to indicate that the disease is not a major problem within the small ruminant population. Results of the postal survey show that in 1998, a year without any Scrapie suspect cases reported to the veterinary authorities, a certain number of small ruminant owners had observed adult sheep and goats with clinical neurological signs (see poster). Since – in theory - all those animals should have been reported as Scrapie suspects, the level of underreporting in 1998 was high. In a recent postal survey performed in the United Kingdom, a country in which Scrapie is endemic and considered to be a problem, underreporting levels at the farm level were estimated at 87%, i.e. only 13% of the farms expected to have reported Scrapie cases officially did (Hoinville et al., 1999).

Good knowledge on the true BSE and Scrapie incidence is essential to understand the disease epidemiology, is required to make sound predictions on the future course of the epidemic (including the level of exposure of other species), and is important for comparison between countries or regions. Within the OIE and the EU, work is ongoing to define requirements for targeted surveillance activities within their respective BSE and Scrapie surveillance regulations. The results especially of the Swiss BSE surveillance have considerable implications for the design of those new surveillance systems for BSE and Scrapie; they confirm that passive surveillance will result in a considerable underestimation of the true disease prevalence and is likely to be insufficient to substantiate freedom from the two diseases. Active surveillance components, when scientifically designed, seem to be less biased in their results and – at the same time – provide valuable information on the functionality of the passive surveillance. It therefore is necessary to develop surveillance approaches for BSE and Scrapie that combine passive and active elements and will provide more reliable overall

estimates for the disease situation in the region or country - and at the same time fulfil the requirements of the OIE and the European Commission regulations. Several factors including the route of introduction and transmission of the infectious agent, the exposed population and the incubation time of the disease need to be taken into consideration when designing – or changing – BSE and Scrapie surveillance systems.

The "disadvantage" of improving an existing surveillance system, if the disease is present, certainly is the likely increase in the total number of cases detected in a given country or region. This might put considerable political and public pressure onto the researchers and veterinary authorities involved in those developments. This, however, is more than compensated by improvement of the quality of information that is available on the current status of the disease in a given country or region. For BSE and Scrapie, the combination of passive and active surveillance will very likely be the only approach to, at some point in time, reliably assess and categorise countries with respect to their disease status, and it certainly is better to implement active elements as early as possible.

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AN EPIDEMIOLOGICAL ANALYSIS OF A CANINE BIOPSIES DATABASE COMPILED BY A DIAGNOSTIC HISTOPATHOLOGY SERVICE

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There has been considerable interest in canine neoplasia over the years, particularly with the growth and development of veterinary practice in the small animal sector. Research in the field has advanced, both to increase knowledge of the disease process and meet the increasing demand for better therapy options. In the more developed parts of the world, the emergence of better diagnostic facilities at veterinary schools and the willingness of owners in more affluent societies to pay for the necessary costs of diagnosis have resulted in substantial accumulation of accurate data pertaining to canine neoplasia.

As well as directly benefiting the veterinary profession, research of epidemiological aspects of canine neoplasia may have a role to play in medical oncology, given that dogs live in close association with man and therefore share environmental exposures (Hahn et al., 1994; Knapp & Waters, 1997). They exhibit spontaneous tumour development like man and some of these tumours, such as osteosarcoma and prostatic carcinoma, have similar biological behaviour and histological characteristics to the equivalent human neoplasms (Knapp & Waters, 1997). The members of the canine pet population are also likely to live a natural lifespan, unlike many other species used in oncology research.

There are many clinical papers describing the several types of cancer in the dog from the diagnostic and therapeutic aspects, as well as epidemiological studies of potential risk factors, such as whether there is an association between metallic implants and bone cancer (Li et al., 1993), insecticides and bladder cancer (Glickman et al., 1989), herbicides and lymphoma (Hayes et al., 1991, Hayes et al., 1995), or electromagnetic fields and lymphoma (Reif et al., 1995).

Multivariable techniques such as multiple logistic regression are widely recognised in veterinary epidemiology, and have been used in studies investigating possible risk factors for certain types of neoplasia in particular sites, such as bone cancer (Ru et al., 1998), bladder cancer (Glickman et al., 1989) and mammary cancer (Sonnenschein, 1991). However, multiple logistic regression has not been used as a method for assessing risk of neoplasia being diagnosed in a biopsy sample associated with the classic potential confounders of age, sex and breed of dog, as well as site of biopsy.

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Most epidemiological studies of canine neoplasia in recent years have been performed using populations of dogs derived from veterinary referral teaching hospitals. The most common data source is the Veterinary Medical Data Program (VMDP), which contains data on all domestic animals seen at 24 participating veterinary hospitals in the United States and Canada. Although used rarely, data derived from first opinion practices was used to create the California and Tulsa Animal Neoplasm Registries (Dorn et al., 1968; MacVean et al., 1978). Age, sex, breed and site specific incidence rates for many types of neoplasia were derived from these data and are still quoted widely to provide these figures in the veterinary literature, although it is now over 30 years since some of the data was collected. Undoubtedly, these incidence rates have changed during this time due to the advancements in methods of detection and diagnosis in the field.

For this study we have utilised data collected from a histopathological service which serves first opinion veterinary practices, to remove the selection bias associated with the use of a teaching hospital canine population, such as is present in studies using VMDP data. The aim of this study was to provide histopathologists with a system for assessing the likelihood of an outcome of neoplasia in a biopsy from a dog, while controlling for the host factors of age, sex and breed, and site of biopsy.

MATERIALS AND METHODS

Data collection

The study was in the form of a retrospective analysis of canine biopsy samples submitted to the external veterinary histopathology service provided by the Department of Veterinary Pathology at the University of Glasgow Veterinary School (GUVS), from 1/1/86 to 30/6/98. A total of 21,371 biopsies were submitted from 545 privately owned and charity veterinary practices located throughout the United Kingdom during this time period.

Based on previous studies, the variables selected for investigation were age, sex and breed of dog from which a biopsy was taken, and site of biopsy. Only biopsies with a confirmed histopathological diagnosis were considered for this study, and dogs with multiple biopsy records were excluded. Criteria for a biopsy to be selected as a case were recorded age, sex, breed of dog, recorded biopsy site, and a histopathological confirmation of neoplasia. The same criteria as for the cases, though with a histopathological confirmation of a non-neoplastic diagnosis, were used for the selection of a control population from the study population.

Statistical analysis

The categorical independent variables, sex, breed and site, were subdivided for further analysis. Breed was grouped according to the top eight represented breeds and crossbreeds, with all other breeds being placed into a separate category. Site was categorised according to the top eight represented biopsy sites, with all biopsies from other sites being placed into a separate category. With neoplasia as the dependent variable, an initial screening was

performed to identify those variables that had little or no association with the outcome. Student's t-test was used for the continuous variable, age, and chi-square analysis and univariable logistic regression were calculated for the categorical variables. All variables significant at $P \leq 0.25$ were considered eligible for inclusion in the multivariable analysis (Hosmer & Lemeshow, 1989).

Dummy variables were generated for any categorical variable with more than two levels. Beginning with the full model of variables significant at $P \leq 0.25$, a backward elimination procedure was used to refine the multivariable model. The level of significance for a variable or factor to remain in the final model was set at 10%.

The goodness-of-fit of the final model was assessed by the Hosmer-Lemeshow statistic (Lemeshow & Hosmer, 1982), and outliers were assessed by inspection of the square of the standardised residuals, the leverage and the predicted probability. Outliers with the most influential covariate patterns were then removed and the significance of the variables included in the model re-evaluated. The criteria adopted for intervention was a change of greater than 20% in any of the variable coefficients and/or evidence of statistical instability.

Due to the heterogeneous distribution of covariate patterns within the multidimensional covariate space, a stratified approach was then adopted. For each of the top eight represented sites individually, univariable analyses as already described were carried out to assess the significance of age, sex and breed on the outcome of neoplasia in a biopsy from that site. Where appropriate, multivariable models were then created and assessed in the same manner as for the main effects model. All statistical analyses were performed using Minitab Release 12.21.

RESULTS

Of the total number of 21,371 biopsies submitted to the external histopathology service at GUVS during 1/1/86 to 30/6/98, 9969 neoplastic biopsies were eligible as cases and 7026 controls were selected for further analysis. There were 4376 biopsies excluded from the analysis due to unknown histopathological diagnosis, or missing data relevant to age, sex, breed of dog, or site of biopsy.

Univariable analysis

The results of the univariable screening of independent variables showed that age, sex and breed of dog and site of biopsy were all significantly associated with a histopathological diagnosis of neoplasia. The summary statistics for the cases and controls by age and by subcategory of the categorical independent variables identified for investigation are presented in Table 1.

Multivariable analysis

The final model is shown in Table 2. The Hosmer-Lemeshow statistic of the final model was 8.77 with 8 degrees of freedom (P = 0.36).

Results of the site-specific models are represented in Table 3, showing the direction of the differences in odds ratios to give an estimate of risk for a biopsy submitted from a certain breed from a particular site, relative to the Labrador Retriever breed.

DISCUSSION

Results of the final model show that age, the Boxer breed and four of the selected sites, *viz* mouth/pharynx, mammary gland, lymphatic system, and the male reproductive system, were significantly associated with increased risk of a biopsy sample having a neoplastic diagnosis. The increasing risk of neoplasia with increasing age was also observed in the site-specific analyses. The finding of an increasing risk of neoplasia in the dog with increasing age is in agreement with other studies (Priester & Mantel, 1971; Moulton, 1990).

Given that the referent category for sex was male, there was a marginal decrease in risk of neoplasia in samples submitted from neutered females, when controlling for age, breed and site. There were however, significant differences in breed risk of neoplasia. A biopsy from the Boxer breed was shown to have 1.3 times increased risk, compared to the risk in the Labrador Retriever, the referent breed. All other breeds selected for more detailed analysis showed no or decreased risk of neoplasia in a biopsy. The Boxer breed has been shown by various authors to have an increased risk of neoplasia (Priester & Mantel, 1971, Howard & Neilson, 1965, Nordstoga, 1997) and has been suggested as a good candidate for further genetic study (Misdorp, 1996). Interestingly, our study found the Boxer to have no increase in risk of neoplasia in biopsies of the male reproductive system, (Table 3).

Table 1. Summary statistics for case-control data for canine neoplasia, 1/1/1986 – 30/6/1998

	Controls		Cases	
Number	7026	(100%)	9969	(100 %)
Age (years)				
1st quartile	3.5		6.1	
Median	7		9	
3rd quartile	9.5		11	
Sex				
Male	3326	(47.3%)	4014	40.3%)
Male neutered	259	(3.7%)	306	3.1%)
Female	2558	(36.4%)	4437	44.5%)
Female neutered	883	(12.6%)	1212	12.2%)

Table 1. Continued

	Controls		Cases	
Breed				
Labrador Retriever	724	(10.3%)	1268	12.7%)
German Shepherd	606	(8.6%)	634	6.4%)
English Springer Spaniel	333	(4.7%)	493	4.9%)
Boxer	263	(3.7%)	417	4.2%)
Cocker Spaniel	226	(3.2%)	411	4.1%)
Jack Russell Terrier	234	(3.3%)	354	3.6%)
Doberman	274	(3.9%)	216	2.2%)
Crossbreed	1354	(19.3%)	2415	24.2%)
Other	209	(3.0%)	3761	37.7%)
Site of biopsy				
Skin[a]	5824	(82.9%)	5761	(57.8%)
Oropharynx	326	(4.6%)	641	(6.4%)
Mammary gland	255	(3.6%)	2014	(20.2%)
Lymphatic system	199	(2.8%)	447	(4.5%)
Liver	165	(2.3%)	33	(0.3%)
Spleen	104	(1.5%)	98	(1.0%)
Male reproductive system	129	(1.8%)	363	(3.6%)
Small Intestine	81	(1.2%)	47	(0.5%)
Other	483	(6.9%)	655	(6.6%)

[a]Includes biopsies of epithelial and mesenchymal cell origin

The study by Dorn et al. (1968), identified the German Shepherd breed as having an increased risk of neoplasia of the mouth and pharynx, compared to other purebreeds examined in the study. We found the German Shepherd to have a decreased risk, with the Doberman breed having the highest risk of cancer in this site, when compared to the Labrador. An important factor contributing to this apparent disagreement in breed susceptibility to oral-pharyngeal neoplasia may be that only malignant neoplasms were included in the California counties survey, whereas both benign and malignant tumours were eligible as cases in our analysis.

For site, skin, including biopsies of epithelial and mesenchymal cell origin, was chosen as the referent site. Biopsies from the mammary gland were six times as likely to be neoplastic than those from the skin, and there were also significant increases in risk of neoplasia in biopsies from the oropharynx, lymphatic system and male reproductive system, when controlled for sex, breed and site of biopsy. Over 80% of biopsies included in the study population originated from the skin or mammary gland, which is likely to reflect the relative ease of detection of abnormalities and subsequent sampling of tissues from these superficial sites. The high proportion of biopsies submitted from these sites was similar to datasets presented by Dorn et al. (1968) and MacVean et al. (1978).

Table 2. Coefficients, odds ratios and 95% confidence intervals from the multiple logistic regression model containing the main effects for factors associated with the risk of neoplasia being diagnosed from a canine biopsy submitted to a diagnostic histopathology service

Variable	Coefficient	Odds Ratio	95%CI	
			Lower	Upper
Constant	-0.62			
Age	0.13	1.14	1.13	1.15
Sex				
Male	0.00	1		
Male neutered	-0.07	0.93	0.78	1.11
Female	0.02	1.02	0.94	1.1
Female neutered	-0.16	0.85	0.77	0.95
Breed				
Labrador Retriever	0.00	1		
German Shepherd	-0.38	0.69	0.59	0.80
English Springer Spaniel	-0.24	0.79	0.66	0.94
Boxer	0.26	1.29	1.07	1.56
Cocker Spaniel	0.06	1.07	0.87	1.30
Jack Russell Terrier	-0.34	0.71	0.58	0.87
Doberman	-0.59	0.55	0.45	0.69
Crossbreed	-0.11	0.90	0.80	1.01
Other	-0.37	0.69	0.62	0.77
Site of biopsy				
Skin[a]	0.00	1		
Mouth/pharynx	0.35	1.42	1.23	1.64
Mammary gland	1.81	6.13	5.31	7.07
Lymphatic system	0.70	2.02	1.69	2.40
Liver	-1.87	0.15	0.11	0.23
Spleen	-0.40	0.67	0.50	0.89
Male reproductive system	0.73	2.07	1.67	2.56
Small intestine	-0.46	0.63	0.44	0.92
Other	0.09	1.09	0.96	1.24

[a]Includes biopsies of epithelial and mesenchymal cell origin

As well as methodological differences between this study and others examining risk factors for canine neoplasia, our study utilised a coding scheme for classification of primary site of neoplasms developed by members of the Department of Veterinary Pathology at GUVS. Most epidemiological studies in the field of canine neoplasia have used *A Standard Nomenclature of Veterinary Diseases and Operations*[2a] (NVDO) as the standard coding system. The California counties study used both the NVDO and the

[2a]A Standard Nomenclature of Veterinary Diseases and Operations, ed. 1. Bethesda, MD, National Cancer Institute, 1966; [2b]Manual of the International Statistical Classification of Diseases, Injuries, and Causes of Death, ed. 6. Geneva, Switzerland, World Health Organization, 1948.

Manual of the International Statistical Classification of Diseases, Injuries, and Causes of Death[2b]. Without a global standard, it is likely that there will be differences in the classification of tumour sites which prevents accurate direct comparison between studies. Unlike the two coding systems mentioned, the coding scheme for the GUVS database also does not include a specific coding for whether a tumour is benign or malignant, thus the ratio of benign to malignant neoplasms has not been derived for the current study.

Table 3. Comparison of odds ratios ("relative risk") for site-specific analyses

Breed [a]	German Shepherd	English Springer Spaniel	Boxer	Cocker Spaniel	Jack Russell Terrier	Doberman	Crossbreed
Site of biopsy							
Skin[b]	↓	-	↑	-	↓	↓	-
Mouth/pharynx	↓	↓	↑	↑	↓	↑↑	↓
Mammary gland	↑	↑	↑	↑	↑	↓	-
Male reproductive system	↓	-	-	-	↑	-	↑↑

[a] Referent breed is Labrador Retriever

[b] Includes biopsies of epithelial and mesenchymal cell origin

↑↑ High
↑ Increased
- No change
↓ Decreased

Heterogenous distribution of covariate patterns within the covariate space resulted in statistical instability when the interaction of site and breed was fitted to the model. For this reason, in the current study, biologically meaningful interaction terms among the independent variables were not assessed.

The source of the biopsy population were dogs attending first opinion veterinary practices. This removed the selection bias associated with data derived from dogs seen at referral establishments, such as the data from the VMDP. However, the use of a first opinion source still has many associated biases. These include the influence of an individual veterinary surgeon's approach to abnormalities detected by the owner or on clinical examination. The veterinary advice is of paramount importance in the owner's decision regarding management of their pet. Owners may be more ready to investigate abnormalities detected in the younger animal, and highly suspicious lesions may be not be investigated in the older animal due to the owner's unwillingness to subject their animal to

a surgical procedure, particularly if general anaesthesia is required. It is the interaction between the veterinary surgeon and owner that will lead to biopsy in these cases. A survey of veterinarian's attitudes and choice of treatment for selected neoplasms has been carried out in Australia (Peaston & Watson, 1995) however, the veterinarians involved in the survey were not asked specifically about the decision-making process that led to the diagnosis of the cancers on which comments regarding treatment were assessed. A survey of this aspect of the collection of data pertaining to neoplasia is warranted.

In conclusion, a multivariable approach has been applied to a dataset derived from a population of canine biopsies. The findings confirm many of the perceived risks for neoplasia based on previous clinical descriptive and univariable investigations. The results may provide a means for prioritising biopsy examination protocols in busy histopathology laboratories.

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THE ORIGIN, GENERATION, AND PERSISTENCE OF GENETIC VARIATION IN FOOT-AND-MOUTH DISEASE VIRUS

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Foot-and-Mouth Disease (FMD) is a highly infectious disease of even-toed ungulates, caused by a single positive stranded picornavirus in the genus *Aphthovirus*. Susceptible animals exposed to infectious doses of FMDV usually develop an intense viraemia within 3-5 days of exposure, and clinical signs that include initial temperature and lesions that usually last 1-2 weeks. Virus is opsonized by antibody and cleared mostly through macrophage action (McCullough et al. 1992; Brown 1995). Adult animals usually make a full recovery within 4 weeks, but a fraction go on to become 'carriers' (in cattle this fraction may be as high as 50%). Virus may be recovered from probang samples of carriers for up to 3 years post-infection.

FMD genomes are 8.4 kb in size. Virus capsids are small isocahedral structures, composed of 60 copies of each of 4 proteins, VP1-VP4. Only VP1-3 are exposed on the capsid surface. Each of the proteins VP1-3 take the form of similarly structured anti-parallel β-barrels, the 3 genes encoding these proteins constitute about 25% of the genome (~1950 nucleotides). FMDV exhibits substantial genetic variability in the field, expressed particularly in the capsid proteins. Various parts of the capsid proteins are recognized by the host immune system, exact details of which vary between serotypes. All serotypes share a principle antigenic site on a large loop connecting the G and H β-strands of the barrel, encoded by the VP1 gene, on the end of which is located the host cell receptor binding motif. Much of the antibody response to FMDV may be directed at this immunodominant G-H epitope (Mateu, 1995), but at least 4 other antigenic sites are thought to be implicated in the full response (Kitson et al. 1990; Crowther et al. 1993).

Because RNA viruses are thought to lack error-correction mechanisms they incur very high mutation rates. Commonly cited mutation rates for RNA viruses lie in the range 10^{-5} to 10^{-4} mutations per nucleotide site per genome replication (Domingo and Holland, 1988; Holland et al. 1982). At these rates of mutation, every replicated FMDV genome would differ from its parental strand at between 0.1 and 10 base positions. Such high error rates have lead to the development of the quasi-species concept (as reviewed by Eigen, 1993, Nowak, 1992) to describe viral genetic heterogeneity within a host. Foot-and-mouth disease is a notoriously variable virus. Genetic variants accumulate rapidly in the field (Martinez et al. 1992; Samuel et al. 1997, 1999; Pattnaik et al. 1998), and co-circulate together. Much of this genetic variation is expressed in the capsid genes and consequently results in antigenic variation. Antigenic variation accumulates through time (and has been shown to do so in cell culture even in the absence of antibody - Diez et al. 1989), however the adaptive value of this variation remains unclear. Antigenic variation might be of adaptive value

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for two reasons: first, antigenic variation generated over the course of a single viraemia might act to extend or intensify a single infection, thereby resulting in greater transmission potential from infected animals. Second, sufficiently distinct strains might be capable of more rapid re-infection of hosts with some previous experience of related antigen, thereby effectively increasing the susceptible host population size. However positive Darwinian selection for amino acid variation in these genes has yet to be demonstrated (Haydon et al. 1998). The occurrence of antigenic variation requires that vaccine strains be periodically updated (Feigelstock, 1996).

The relationship between viral genetic variation at different spatial and temporal scales remains poorly understood. Is it possible to quantitatively relate the rapid long-term rate of evolution in the field to the error-rate of the virus? Because mutations to the virus obviously occur within infected hosts this requires extrapolating up from the viral dynamics within a single infected individual to the larger scale epidemiology of the disease. In what follows we attempt to sketch out a very rough quantitative link between replication-mutation dynamics within the host and the broader scale changes observed through long term monitoring of FMDV epidemiology.

RATES OF FMDV EVOLUTION IN THE FIELD

Rates of nucleotide substitution in capsid genes in the field are consistently in the range of 0.5 - 1.5×10^{-3} nucleotide substitutions per site per year (this corresponds to 0.5 - 1.5 % of nucleotides changing per year). Field sub-types diverge from progenitor strains in a remarkably linear fashion, at least over periods of a few decades, providing a steady and reliable 'molecular clock' (see Fig. 1). Such incremental change in capsid proteins will result in antigenic changes that may accumulate slowly or quickly depending on exactly where nucleotide substitutions occur (Martinez et al. 1991).

RATES OF FMDV EVOLUTION IN INFECTED INDIVIDUALS

In order to consider the possible rates of virus evolution within an infected individual information is required on i) the mutation rate; ii) the proportion of mutations that are tolerated and give rise to viable virus; iii) the population dynamics of the virus in the host.

The mutation rate

The best data on error rates in picornaviruses comes from studies of mutation in Polio- and Rhinoviruses. Monoclonal antibody escape mutants (bearing a defined single mutation after a single round of viral replication) arise at frequencies of between $10^{-3.0} - 10^{-6.0}$ per round of viral replication (mean for 25 different Mabs: $10^{-3.56}$), and these are commonly cited mutation rates for RNA viruses (Drake, 1993; Smith and Inglis, 1987). This mean mutation rate corresponds to an average of two nucleotide point mutations occurring every time the genome is replicated. If the number of mutations that occurs is described by a Poisson process with a mean of two, it suggests that about 10% of virus would be replicated without mutation, 50% will incur either one or two mutations, 20% will incur 3, and 10% will incur 4 or more nucleotide changes. An error rate of $10^{-3.56}$ is high compared to cited figures for other RNA viruses which are more often reported in the range $10^{-5.0} - 10^{-4.0}$.

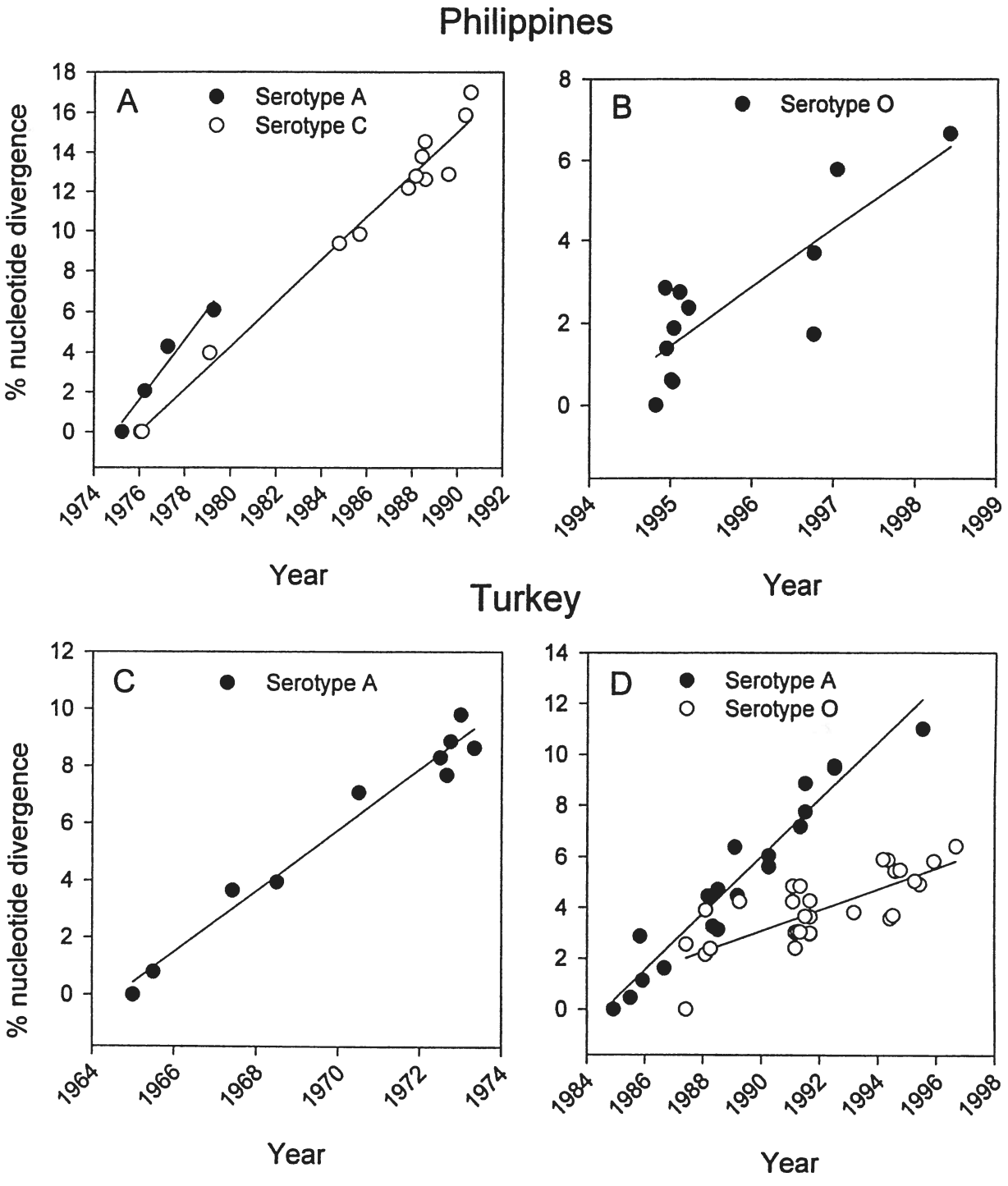

Fig. 1. Rates of FMDV evolution in the field. Divergence is measured relative to the earliest identified isolate of the introduced sub-type. Rates are A) 1.51 and 1.07 % per yr; B) 1.43% per year; C) 1.07 % per yr; D) 1.12 and 0.41% per yr.

The proportion of mutations tolerated

Mutations may be classified into two types, those that result in a change in amino-acid use (non-synonymous), and those that do not (synonymous). There are various reasons to suppose that not all synonymous mutations can be regarded as selectively neutral because such changes might influence the secondary structure and/or stability of the folded RNA genome. There is also a possibility that there might be some fitness implications of differential codon use. However, it may be safe to say that these fitness changes are probably small and that to a first approximation, synonymous mutations, which comprise about 25% of all possible mutations, might be regarded as neutral.

The extent to which non-synonymous mutations are likely to be tolerated depends a great deal on where in the genome they occur. Some genes are a lot more conserved than others. Genes encoding FMDV capsid proteins are highly polymorphic and it is evident that a significant fraction of non-synonymous changes to these genes are tolerated. A crude estimate of this fraction may be obtained by comparing the ratio of synonymous to non-synonymous nucleotide substitutions in sequences of FMDV capsid genes. Complete sequences of capsid genes VP1-3 for serotypes O, A, and C indicate ratios of synonymous to non-synonymous substitutions of about 3:1. This means that for every 3 synonymous changes, 1 non-synonymous change will be observed. Note that there are roughly three times as many possible non-synonymous as synonymous changes and assume synonymous changes are selectively neutral. For every 3 synonymous changes observed, it can be conservatively assumed that approximately 3 times this number of non-synonymous mutations were attempted, and thus that about 1/9 of non-synonymous mutations are sufficiently non-deleterious to be tolerated. Add this fraction to the quarter of all mutations that might be expected to be synonymous, and it suggests that, in all, about 30% of mutations to the capsid genes might to tolerated.

Within host viral population dynamics

Consider a stretch of the viral genome of length L nucleotides, and denote the error rate per nucleotide per replication event by ε. Consider a highly simplified discrete time model of within-host viral dynamics wherein a susceptible animal becomes infected with a genetically homogenous dose of $n_{w,0}$ viral particles, each of which gives rise directly to λ daughter viruses. In the first instance consider two kinds of virus, progenitor virus after t generations of replication, denoted $n_{w,t}$, that is identical to the initial infecting virus with respect to the L nucleotides under consideration, and virus after t generations that differs by at least one nucleotide substitution along the L nucleotides, denoted $n_{m,t}$. Suppose that new mutations are either neutral and tolerated, or highly deleterious and removed, and thus that all viable mutants have identical replication rates. Denote the probability that any mutant is neutral by f. Let mutations occur along the genome according to a Poisson process. The probability that the stretch of L nucleotides will be replicated without error is $q = e^{-\varepsilon L}$, and the probability of one error, $p = 1-q$. For current purposes we will assume the probability of two mutations occurring in the same stretch L during the same replication event to be zero and ignore back mutation.

The dynamics of unmutated virus can thus be written recursively as:

$$n_{w,t+1} = q\lambda n_{w,t} \qquad (1)$$

and thus $n_{w,t} = (q\lambda)^t n_{w,0}$. There are two sources of mutant virus, new mutations arising from $n_{w,t}$, produced at rate $pf\lambda$, and replication of existing mutant virus, at rate $\lambda(q+fp)$, thus the recursive dynamics of mutant virus may be written as:

$$n_{m,t+1} = n_{m,t}\lambda(q + fp) + n_{w,t} pf\lambda \tag{2}$$

This can be written non-recursively as:

$$n_{m,t} = pf\lambda n_{w,0} \sum_{k=1}^{t} [\lambda(q + fp)]^{k-1} (q\lambda)^{t-k} \tag{3}$$

The expression for the ratio of mutant to progenitor virus after t generations of replication is:

$$\frac{n_{m,t}}{n_{w,t}} = \left(\frac{q + fp}{q} \right)^t - 1 \tag{4}$$

Considering mutations to the 3 major capsid genes and assuming an error rate of 10^{-4}, with 30% of mutation tolerated, this formula suggests that mutant virus reaches a 50:50 mix with progenitor virus after about 10 viral generations, and is twice as abundant as progenitor virus after just 18 generations. However, as Fig. 2 shows, if the mutation rate is much less than 10^{-5}, less than 10% of virus may be mutant.

While a simple exponential model is clearly unrealistic over the whole viraemia, it may be an adequate description of the early dynamics. Furthermore, there is no particular reason to suppose that mutant virus would be cleared earlier than progenitor virus, - indeed, if mutant virus is antigenically distinct, it may be cleared less rapidly, so this assumption does not necessarily render the model predictions seriously erroneous. How many replication cycles does a virus goes through during the course of a single infection? The generation time for Poliovirus *in vitro* has been estimated to be 7 hours (Fields and Knipe, 1990), if this figure is at all close then it seems reasonable to assume that the virus goes through at least 20 viral 'generations' during the course of a single infection; it is not easy to see how the massive viral amplification exhibited within infected animals could be achieved by many fewer replication cycles. Thus assuming plausible parameter combinations and assuming that all viable virus is infectious and excreted in proportion to its abundance, it is likely that most of the virus excreted will be of a different genotype with respect to capsid genes, to that which originally infected the animal.

If mutant virus differs from the original infecting genotype by a single mutation it will be referred to as 1-step mutant. If it differs by two mutations it will be referred to as 2-step mutant. The basic model can be elaborated to predict the proportion of virus that is 1-step, 2-step, ... n-step mutant with respect to the progenitor strain. Under the stated assumptions the frequency of the i^{th}-step mutant will be:

$$\text{Frequency of the } i\text{th-step mutant} = \frac{\binom{t}{i} (pf)^i q^{t-i}}{(q + fp)^t} \tag{5}$$

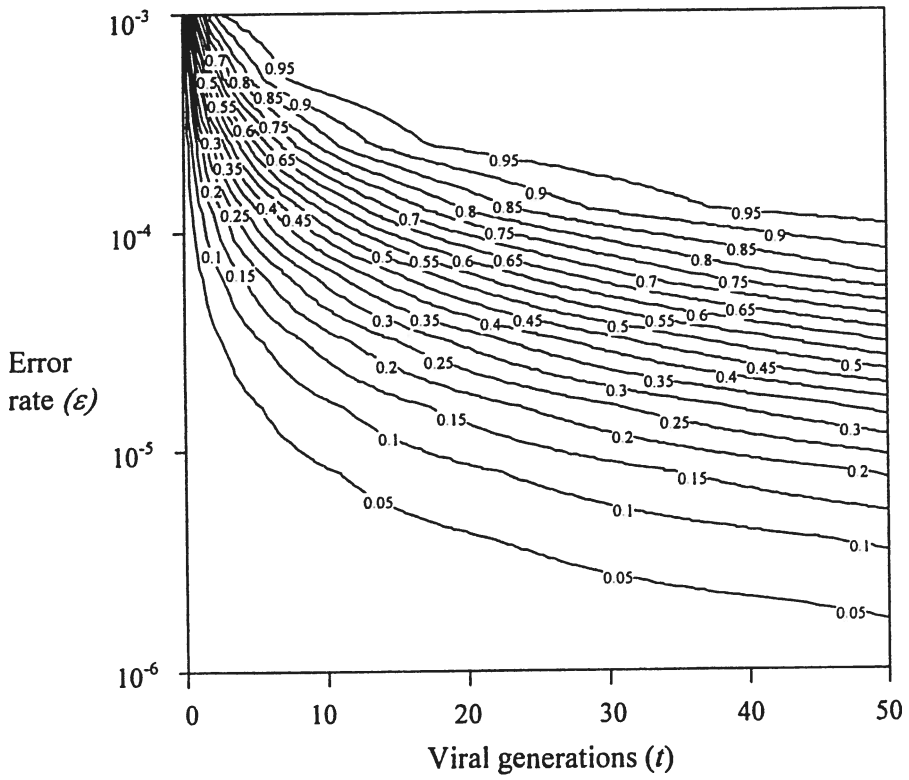

Fig. 2. Contours indicate the fraction of mutant virus in an infected individual after t generations of replication for different error rates (ε). L = 1952 (the combined length of the VP1-3 genes), and f = 0.3.

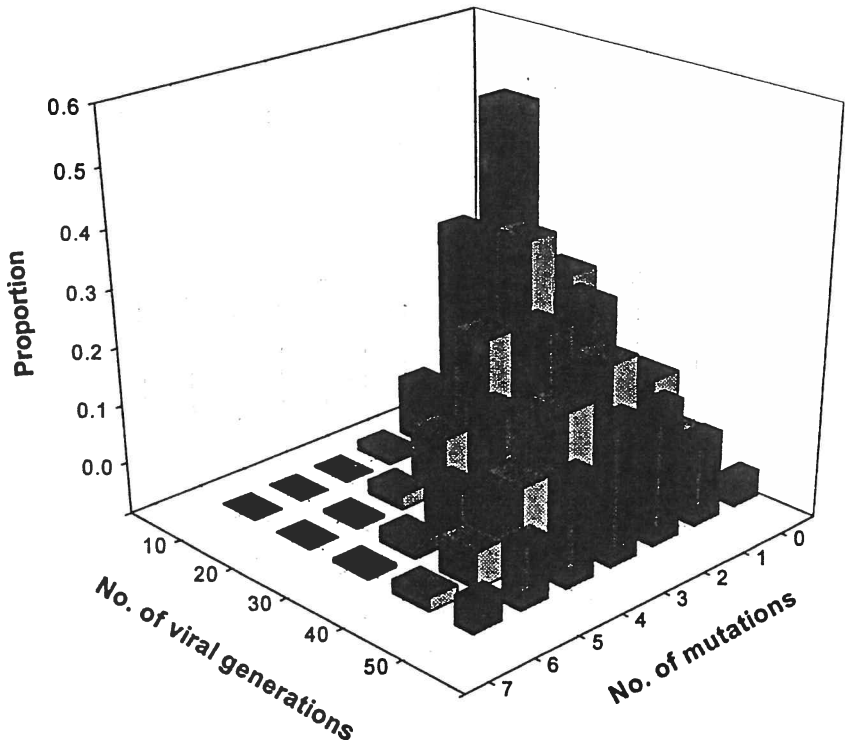

Fig. 3. The proportion of the viral load predicted by Eq. (5) to be comprised of virus that is different numbers of mutations away from the original infecting viral type (f = 0.3, $\varepsilon = 10^{-4}$, L = 1952).

Fig. 3 illustrates the distribution of *i*th-step mutant virus for a plausible error rate.

Using Eq. (5) it is straightforward to calculate the average number of mutation changes that a virus will have experienced after *t* generations of replication. This average is very sensitive to the assumed error rate, as indicated in Fig. 4. However, for reasonable parameter combinations these models suggest that it is likely that most excreted virus will differ by at least one point mutation in the capsid genes, relative to the genotype of the infecting virus.

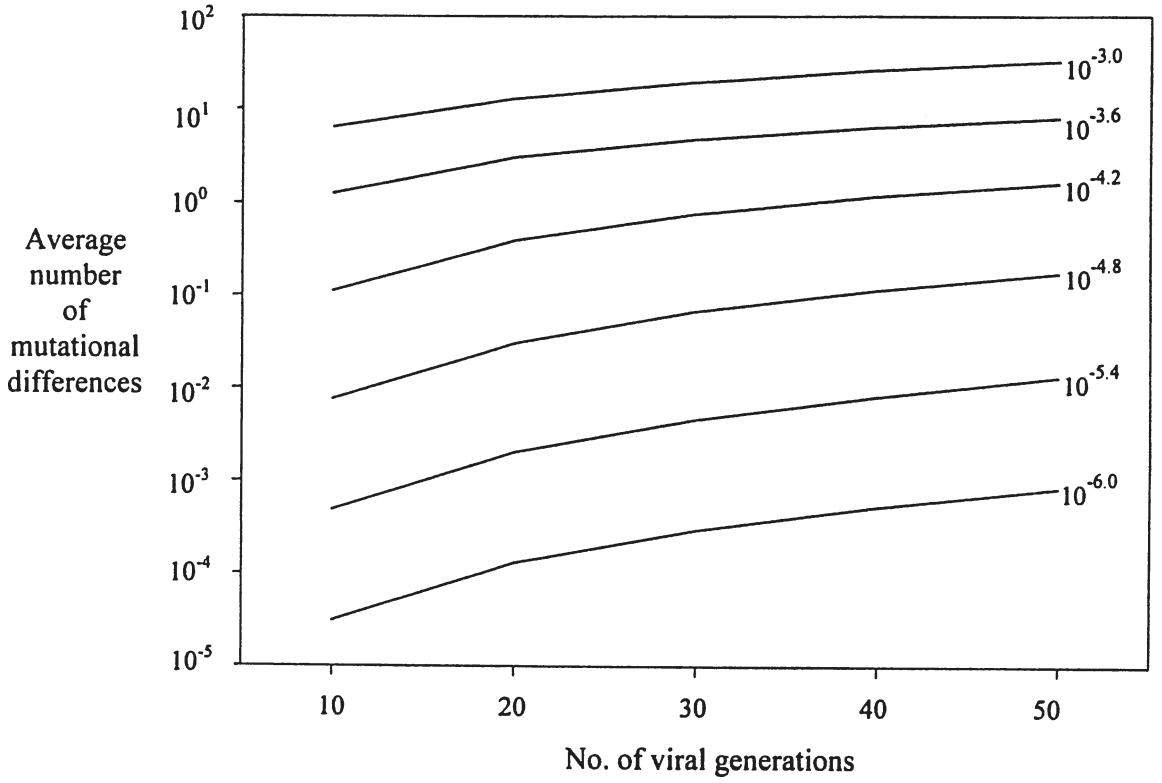

Fig. 4. The average number of mutations incurred after different numbers of viral generations (*t*) for different error rates (indicated on figure) relative to the infecting virus. Other parameters as for Fig. 3.

Genetic diversity

A genome of 8.4 kb can clearly incur mutation at all 8,400 nucleotide sites, each in 3 different ways, giving a total of 25,200 1-step mutants. By similar arguments, there can be shown to be roughly 635 million possible 2-step mutants. However even these large numbers are dwarfed by the inevitable massive scale of viral replication. Yields from cultures of various picornaviruses suggests that 100 – 100,000 virions per cell is a plausible range for the average 'burst size', and the number of cells lysing per day during an intense infection is very conservatively estimated to be in the range $10^{4} - 10^{6}$. Taking the products of the extremes of these estimates suggests that viral replication per day could easily be in the range $10^{6} - 10^{11}$. Combined with error rates of between 10^{-4} and 10^{-5} per site per replication cycle leads to the conclusion that every possible single step mutant arises independently many times daily. Table 1 shows that it is entirely possible that every single possible

1-step mutation is attempted independently thousands of time every day during infection. It is also likely that a substantial fraction of all possible double mutants will arise through the simultaneous occurrence of multiple mutations (see Table 1). Thus the viraemia that develops within a single individual is likely to be a rich soup of genetic variability. If this variation is selectively neutral, then much of this variation is likely to be observable through genetic sampling. If some of this variation is of adaptive value, one might expect dramatic selective sweeps to coarse through the viral population – even within an individual.

Table 1. The number of times that every possible single and double-step mutant might arise independently every day under various assumptions regarding error rate and viral replication. Calculations were performed assuming that single and double mutations arose according to a Poisson process.

Error Rate	Product of burst size and no. of cells lysed per day	
	10^6 - 10^8	**10^9 - 10^{11}**
10^{-5}	Each Single mutant: 3 - 300	Each Single mutant: 3000 – 300,000
	Each Double mutant: $5x10^{-6}$ – $5x10^{-4}$	Each Double mutant: 0.005 – 0.5
10^{-4}	Each Single mutant: 15 - 1500	Each Single mutant: 15,000 – 1.5 million
	Each Double mutant: $3x10^{-4}$ – 0.03	Each Double mutant: 0.3 – 30

DISCUSSION

It is entirely plausible that the bulk of virus excreted by an infected animal might differ by at least one point mutation to its capsid genes from that with which it was infected. Observed rates of evolution in the field are between 0.5 – 1.5% of sites changing per year - corresponding to 10-30 nucleotide changes in the capsid genes every year. Some of these changes could be back-mutations, but at divergences of less than 10%, most will not be. If we are correct to anticipate approximately one nucleotide change per infection, then the observed rates of capsid evolution in the field could be expected to arise from sequential chains of infection involving as few as 10-30 infected individuals. Given the short incubation period of FMDV, and its highly infectious nature, it is perhaps surprising that the virus does not actually evolve somewhat faster than 0.5 – 1.5% per year. None-the-less, many assumptions have been made in our analysis and there are no shortage of possible explanations as to why the virus may evolve a good deal slower than might be anticipated.

First, if the mutation rate is much less than 10^{-5} per site per genome replication, then genetic changes accumulate much more slowly during an infection. Fig. 1 suggests that with such slow mutation rates less than 10% of virus may be mutant, even after 20-30 viral generations. If this were the case, it would be hard to explain the observed rates of long-term evolution of FMDV without recourse to strong selective arguments. Not all studies have supported the existence of high

mutation rates. Indeed one of the most compelling studies of mutation rate in which mutations were screened directly through gene sequencing concluded that error rates may have been overestimated. Parvin et al. (1986) sequenced almost 100,000 nucleotides from 105 VP1 genes of Poliovirus type 1 after a small number of replication rounds, and found no mutations at all, they estimated the mutation rate to be no greater than 2.1×10^{-6}. If this figure is anywhere close to being correct it would have profound implications for evolutionary virology. Alternatively, if mutation rates were much greater than 10^{-4} then the majority of excreted virus is likely to be at least 2-step mutant, and it becomes harder to explain why FMDV evolves as slowly as it appears to do so. One possibility is that purifying selection is much stronger than has been anticipated. Synonymous changes may not be as neutral as we have assumed, perhaps non-synonymous changes are really only tolerated at very few positions – however the available data suggests that this is unlikely to be the case (Haydon et al. 1998a,b).

One further possibility is that the majority of mutant virus may be viable within the host, but is not transmitted, either because it is uninfectious for some reason, or because it is simply trapped and unable to exit the animal, or because it is mostly generated after the individual ceases to transmit infection. Various scenarios are summarized in Fig. 5.

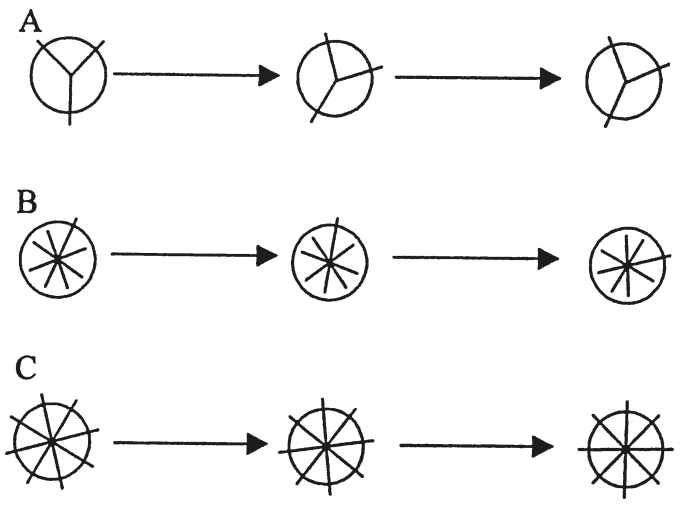

Fig. 5. FMDV transmission scenarios. Circles represent individuals; arrows, transmission events; asterisks indicate the extent of viral genetic radiations. A) Mutation rates are low, and limited diversity is produced by each infection. B) Mutation rates are high, but the bulk of mutant virus is either uninfectious, or cannot be transmitted. C) Mutation rates are high, and all is transmissible.

One possible reason that the long term rate of evolution of FMDV might be overestimated is that virus might persist in the field through different epidemiological processes. If the virus can only persist through active epidemiological activity akin to sequential serial passage, then it must undergo dozens of such passages every year. Under these circumstances, rates of evolution would be maximal. However if the virus can persist in less active states, characterized by slower average replication rates, then the number of serial passages per year might be much reduced, and rates of evolution would be correspondingly lower (Fig. 6). Possible mechanisms for such processes might be the existence of occasionally infectious carrier animals in which levels of viral replication are much reduced relative to active infections, or the storage of virus perhaps in preserved food stuffs. The role of carrier infections in facilitating FMDV persistence is largely unknown (Salt, 1993), and

conflicting evidence exists documenting the extent to which carriers may be regarded as sources of genetic variation (Salt et al. 1996; Gebauer et al. 1988; Malirat et al. 1994; Vosloo et al. 1996)

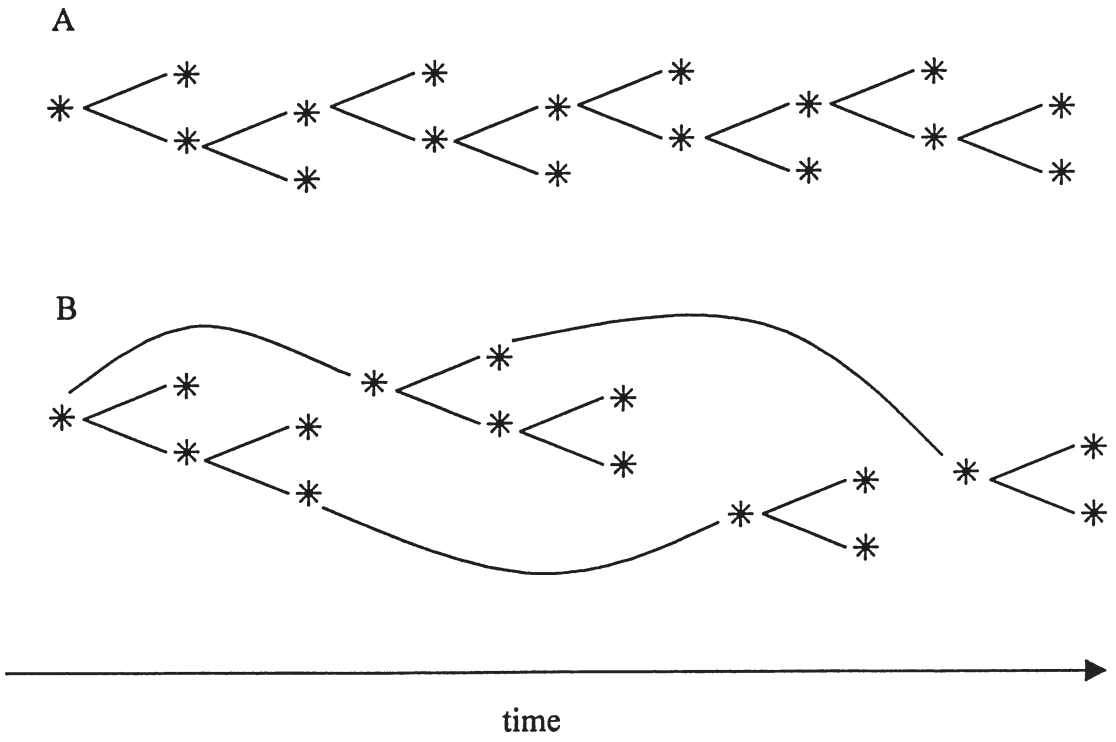

Fig. 6. Different patterns of FMDV transmission. Asterisks indicate viral genetic radiations that take place during infection of individuals or herds, lines indicate transmission events. A) Virus persistence maintained solely by continual serial step-wise infection. Long-term rates of virus evolution will be maximal under these circumstances. B) Virus can persist in a largely non-replicating state, thereby reducing the number of serial infections necessary for virus persistence. Note that under both schemes, much of the genetic heterogeneity does not persist in the viral population, but that under scenario B cumulative genetic divergence may be less than in scenario A.

The parameters that determine the within host demographic dynamics of viraemia remain to be satisfactorily quantified. Per cell virus yield, average duration of cellular infection, rate of viral turnover and number of viral 'generations' over the course of an infection might all in principle be estimated with appropriate experimentation. The genetic plasticity of capsid genes is well documented, data already exists to determine the selective status of synonymous changes. Uncertainty in the error-rate spans a critical range of values and more accurate estimates of FMDV genome wide mutation rates are required. Elucidating the mechanism by which micro-replication and mutation of virus within a host relates to the accumulation of genetic variability in circulating FMDV populations is likely to prove a complex task, mediated as it is by the within and between herd disease transmission dynamics. However the high mutation rates in, and apparent plasticity of viral capsid genes renders them a sensitive tracer of disease pathways and provides epidemiologists with a powerful tool with which to study the dynamics of disease transmission.

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AGRO-ECOLOGICAL DATABASES FOR SPATIAL CORRELATION STUDIES: METHODOLOGICAL ISSUES

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In epidemiology, **ecological studies** are defined as those in which the unit of analysis is populations or groups of animals or herds, rather than individuals (Last, 1995). These studies are possibly best known for the problem of interpretation they present, the so-called ecological fallacy. Briefly stated this is the problem presented by associations that occur at the group level but do not necessarily apply to individuals within that group (Greenland & Robins, 1994). In the definition of an ecological study, no particular methodology is defined, and in fact a number of case control studies reported in the veterinary literature are ecological studies, having been conducted at a herd or flock level.

A particular type of ecological study is the **spatial correlation study**. For these studies geographical variation in disease incidence or prevalence is associated with explanatory variables measured at an areal unit level. Such an approach is often implicit in disease mapping, in that most people – epidemiologists and the general public alike– seeing a map which has a geographical trend in disease incidence will attempt to explain it in terms of the their interpretation of the environment. For example, if a map were to be produced showing high levels of birth deformities of calves in south-west Cumbria, many would immediately "explain" this as being due to exposure to radiation emanating from the Sellafield nuclear complex.

For such a hypothesis to advance towards being proved causative, it would be helpful to have a map of radiation measurements to overlay those of the incidence of calf deformities. Similarly this applies to observations of increased incidence of disease if the animal's environment is at high altitude, is close to rivers, is on heavy clay soil or is co-habitated by a wildlife species which may be a reservoir host for the particular disease. What is needed in

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each instance is an **agro-ecological database** in which each of these variables is mapped and can be overlaid and analysed statistically for an association with the disease incidence. Nevertheless, no such "off the shelf" database exists for any country, and while simple in concept, spatial correlation studies are rare in the medical literature, and almost unknown in veterinary epidemiology. In this paper we will discuss some of the methodological issues that need to be addressed if such databases are to be constructed and usefully employed in ecological studies in veterinary epidemiology.

METHODOLOGICAL ISSUES

Variable selection

The selection of variables is a critical decision which will itself depend upon a number of factors, the most important being the **purpose of the study**. If it is to screen for a number of environmental variables which are hypothesised to affect the incidence of the disease, then a much larger number of variables is required than if the purpose is to undertake predictive modelling using well established co-variates. For example, in Great Britain (GB) many environmental variables have been hypothesised to affect the incidence of bovine tuberculosis at the herd level, including climate and landscape features (Krebs et al., 1997). For other diseases in which there is less uncertainty as to the role of an environmental determinant, then the purpose may be to use that variable to predict the distribution and prevalence of the disease. Accordingly, acute fasciolosis in sheep in England and Wales was reasonably predicted using a minimum data set of summer/autumn rainfall and potential evapo-transpiration (Ollerenshaw, 1966). Nevertheless, it was recognised that information on other variables such as type of soil, topography and stocking rates, would improve the accuracy of the forecasting.

Therefore, general advice would be to subdivide the possible variables affecting the spatial incidence of a disease into an essential group which are strongly suggested determinants, and a subsidiary group which should be collated if justified by the existence of funding, manpower and availability. A list of ecological and agricultural variables potentially useful for veterinary spatial correlation studies is provided in Tables 1 and 2.

Spatial resolution, scale and accuracy

In the geographical information sciences, **spatial resolution** is one of the most important parameters, and refers to the smallest distance over which it is possible to record change. For data sets in which a raster (grid) model is used, resolution generally equates to the grid size. For data that uses a vector model, resolution has a more complex meaning, measured objectively – as on paper maps – by the minimum line width detectable by the human eye. However resolution is intimately related to the cartographic **scale** – the relationship between distance on the vector map and the real world – in that a data-set at scale 1:10 000 and a minimum line width of 0.5 mm cannot represent phenomena smaller than 5 m (Jones, 1997). For both raster and vector data, spatial resolution must be differentiated from **accuracy**,

Table 1. Ecological variables

Variable group	*Minimum variables*	*Other variables*	*Spatial resolution*[a]	*Temporal resolution*	*Cost and availability*
Physical geography	Rivers Altitude	Streams Slope Aspect	1:10,000 to 1:50,000 (vector data)	Dependent upon rate of landscape change. Updates every 5-10 years generally necessary	Good, except for altitude which requires a DTM and is relatively expensive
Climate	Temperature Rainfall	Relative humidity Evapo-transpiration Solar radiation Wind speed & direction	10-km^2 to 100-km^2	Long term (30 year) averages vs. annual summaries, dependent upon requirement	Good availability at coarse resolution; generally poorer availability at fine resolution
Geology and soils	Rock stratum Soil classification Soil geochemistry	Soil texture Soil pH Available water capacity Stream sediment geochemistry	Because of considerable spatial heterogeneity, 1-km^2 is preferable	For some elements with significant input from air-borne pollution (e.g. S), may need updates every 10 years	Generally good for geology and soil as most countries; however resolution is often coarser than optimum
Vegetation	Land cover	Vegetation classes	If vegetation maps remotely sensed, may be as high as 25-m^2	At a minimum, mapping needs to be updated every 10 years	Currently few countries have complete vegetation maps at high resolution – however will become increasingly available in the coming years
Wildlife populations	Distribution (presence/absence) of disease vectors or reservoir hosts	Abundance (density) estimates	Dependent upon wildlife. 10-25-km^2 reasonable for larger mammals	At a minimum, estimates should be available for 10 year periods	Generally poor, unless specific surveys have been undertaken

Table 2. Agricultural variables

Variable group	*Minimum variables*	*Other variables*	*Spatial resolution*[a]	*Temporal resolution*	*Cost and availability*
Human Geography	Roads Cities & towns Administrative boundaries	Country lanes Livestock markets Slaughterhouses Import and export ports	1:10,000 to 1:50,000 (vector data)	Dependent upon rate of landscape change. Updates every 5-10 years generally necessary	Digitised historical civil parish boundaries for England & Wales have until recently not been available
Farm details	Farm location Farm area	Farm boundaries	For farms of size 100 ha need maps at 1:10,000 scale	Dependent upon rate of landscape change. Updates every 5-10 years generally necessary	Farm boundary information not available in GB
Pastures	Grassland area	Area of leys, permanent pasture etc. Species	If vegetation maps remotely sensed, may be as high as 25-m^2	At a minimum, mapping needs to be updated every 10 years	May be available through census collection, but variable quality and may be restrictions on disclosure
Livestock	Stock numbers Predominant breed	Stratification of livestock by breed Management (housing, culling etc.)	Dependent upon enterprise and confidentiality requirements.	Many countries with agricultural support policies have annual census	May be available through census collection, but variable quality and may be restrictions on disclosure

[a] These recommendations strictly apply only to our experience of the heterogeneous and complex landscape of England and Wales. In more homogenous landscapes - such as the central European plain or the mid west of the USA - a coarser resolution is probably acceptable

which is the difference between the value recorded for the data-set and the true value. This discrimination is especially important with spatial data-sets as a small grid size and large scale often imply an accuracy that does not reflect the original quality of measured data.

Ideally, every recorded measurement value should have an associated value of **uncertainty** that expresses the range within which the true value lies. This measurement uncertainty can be used to take a probabilistic approach to mapping risk in the environment (Ramsey & Argyraki, 1997). At present, this is rarely achievable.

The spatial resolution required for a study is generally **scale dependent**, with scale used in this sense to refer to the size of the study area. In general small-scale studies need a higher resolution than large-scale national or regional studies. Therefore a study investigating the beneficial effects of hedges on lamb survival would need climatic variables to be measured at metres, while one comparing survival rates in lowland and upland England might satisfactorily be undertaken with a data-set at a resolution of 10-25 km^2.

Data-set harmonisation, aggregation and interpolation

When data-sets at different resolutions are required to be used together, the issue of **harmonisation** arises. A critical step is to decide which common resolution to use. Ideally this would be determined by the minimum resolution of one of the key data-sets, and the remainder aggregated upwards. However, this in turn introduces a potential bias in the form of the "modifiable areal unit problem", which is the variable effect introduced when data is aggregation across zones (Openshaw, 1984). Therefore, in practice, a compromise needs to be adopted where some fine resolution data-sets are aggregated and other coarse resolution ones are reformulated at a lower resolution.

Aggregation presents few technical problems, as the larger unit is simply the sum of the component smaller units averaged over the larger area. In practise aggregation is most likely to be encountered with remotely sensed data, which can be obtained at very high resolutions. For example, the Land Cover Map of Great Britain (Fuller et al., 1994), is available at two levels of resolution – 25 m^2 at which the data was captured and processed – and 1.0 km^2. The latter is simply aggregated from the 25 m^2 dataset and is available for a fraction of the cost of the finer resolution data-set. Another situation requiring aggregation is to preserve census anonymity. Consequently in GB, returns from the annual Agricultural Census are generally aggregated if the areal unit – the civil parish – contains so few farms that identification of individuals becomes a possibility.

By contrast to aggregation, reformulation of a coarse data-set to a finer one is more difficult and cannot be accomplished by a simple assignment of the value of the larger unit to all the component parts, as this ignores the spatial autocorrelation between these smaller units. This problem is best solved by estimating the values of the smaller grid areas by one of various methods of **spatial interpolation** (Burroughs & McDonnell, 1998). If the original data is available and is at a sufficient sampling density, then sophisticated geostatistical techniques such as kriging may be used, which offer an additional advantage in allowing some random components of uncertainty to be assessed. However, if only the data of the

coarse grid is available, interpolation is restricted to simpler techniques such as inverse distance weighting.

Availability, cost and contractual restrictions

Since the advent of desktop GIS software packages such as Idrisi, MapInfo and ArcView, there has been increasing demand for data-sets. The predominant ones of commercial interest are the high resolution geographical, census and land ownership (cadastral) data, and these are being collated and sold as strictly commercial transactions either by national mapping agencies or specialist resellers. For scientific data, the sale or lease of data-sets is increasingly being written into projects as an essential part of cost recovery.

Few data-sets are actually sold outright and instead are leased with specific restrictions imposed in a legally binding contract. The principal limitation is the passing of the data-sets onto third parties. Furthermore, the use of the data-sets is frequently restricted to a specific named project, and require payment of annual renewal fees to allow continued use.

THE VLA AGRO-ECOLOGICAL DATABASE ("VET-BASE")

Bovine tuberculosis in England and Wales – the problem is uncommon in Scotland – is a disease with a strong spatial component. It occurs predominantly in the Southwest, and within this region, the disease occurs in clusters. The occurrence of the disease is related to persistence of chronic tuberculosis in the Eurasian badger (*Meles meles*). Nevertheless the situation is complex, as locally infected badgers do not always result in infections in cattle herds, and similarly herd breakdowns occur in areas without infected badgers (Cheeseman et al., 1989). This complexity has even led to the hypothesis that the badger may not even be the primary cause of the persistence of the disease (Hancox, 1999).

The essential spatial nature of bovine tuberculosis led to the early exploration by the Epidemiology Department of the then Central Veterinary Laboratory – now VLA (Weybridge) – of the use of GIS to understand the disease (Clifton-Hadley, 1993). Although GIS was successfully used to represent the disease, and enabled the important finding of spatial clustering of the *M. bovis* spoligotypes (Clifton-Hadley et al., 1998), it has been utilised only to a limited extent for explicitly spatial epidemiological studies.

The greatest limitation to a more adventurous use of the GIS technology has been the lack of spatially referenced data-bases. Many of the factors that have been hypothesised to affect the incidence of herd breakdowns in GB are explicitly ecological. These include climate (King et al., 1999); micro-nutrient deficiencies; linear features in the landscape (White et al., 1993), badger density (Anderson & Trewhella, 1985) and stocking rates. Once data-sets of these variables are collated, then it is possible to undertake an "exploratory spatial data analysis" using the range of spatial tools modern GIS software provide such as theme overlays, buffering, transformation and spatial filtering. Following this step, single or

combinations of variables can then be used for statistical modelling by, for example, spatial regression techniques (Clayton et al., 1993).

In 1999, the Epidemiology Department in collaboration with Imperial College, London began a MAFF funded project to undertake such a spatial analysis of bovine tuberculosis in GB. This three-year project recognised the importance of good input data, and over half of the time and resources involved the accumulation and construction of databases relevant to the disease.

For each of the variables listed in Table 1 and 2, various problems were encountered. Some data-sets were readily available at an appropriate resolution (e.g. soil geology), but relatively expensive with a high annual leasing cost. For others (e.g long term climatic averages), a low cost data-set was available, but at a resolution considered too coarse for the analysis required. For the vegetation data, based upon remotely sensed land cover, resolution was extremely high, but with considerable problems of accuracy. For the soil map of England and Wales, the problem was interpreting the relevance, for the purposes of the study, of the classification of the 67 dominant soil groups and their associated subgroups.

Not all the data-sets listed are available for purchase, and this has required that some be specifically constructed. For example there is no existing data-base for soil and herbage micro-nutrients, and for copper and cobalt this has required specific research to collate existing data sources and expert opinion, and thereby achieve maps of best estimates. Two examples of the type of map produced by VET-BASE are provided in Figures 1 and 2.

CONCLUSIONS

The main purpose of accumulating environmental data-sets is either to undertake predictive modelling for diseases, principally parasitic ones, or alternatively to screen for spatial correlations to assess their role as possible determinants. While desktop GIS are becoming increasingly popular for veterinary epidemiologists (Staubach et al., 1998; Robinson & Hopkins, 1999), the absence of data-sets severely restricts their utility. To date the only national example mentioned in the veterinary literature is that by Duchateau et al. (1997) who constructed a climatic, vegetation and land use data-base for Zimbabwe to predict the distribution of theileriosis. The compilation of the VET-BASE agro-ecological data-base extends this pioneering work by including a much wider range of variables at a finer resolution. Accordingly it should permit enhanced GIS-based research into animal disease in England and Wales by the VLA, as well as providing the basis for extensive collaboration with other research teams.

ACKNOWLEDGEMENTS

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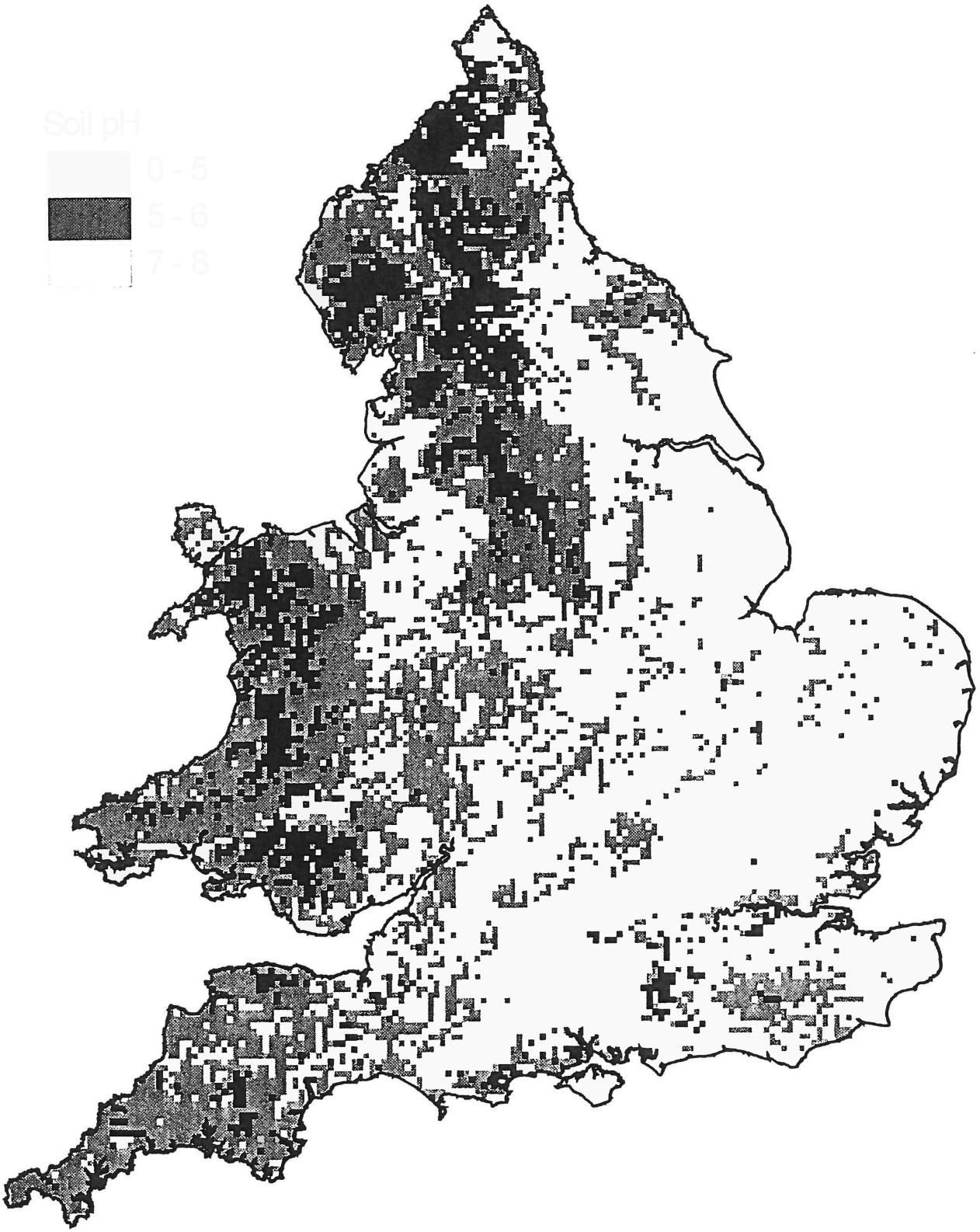
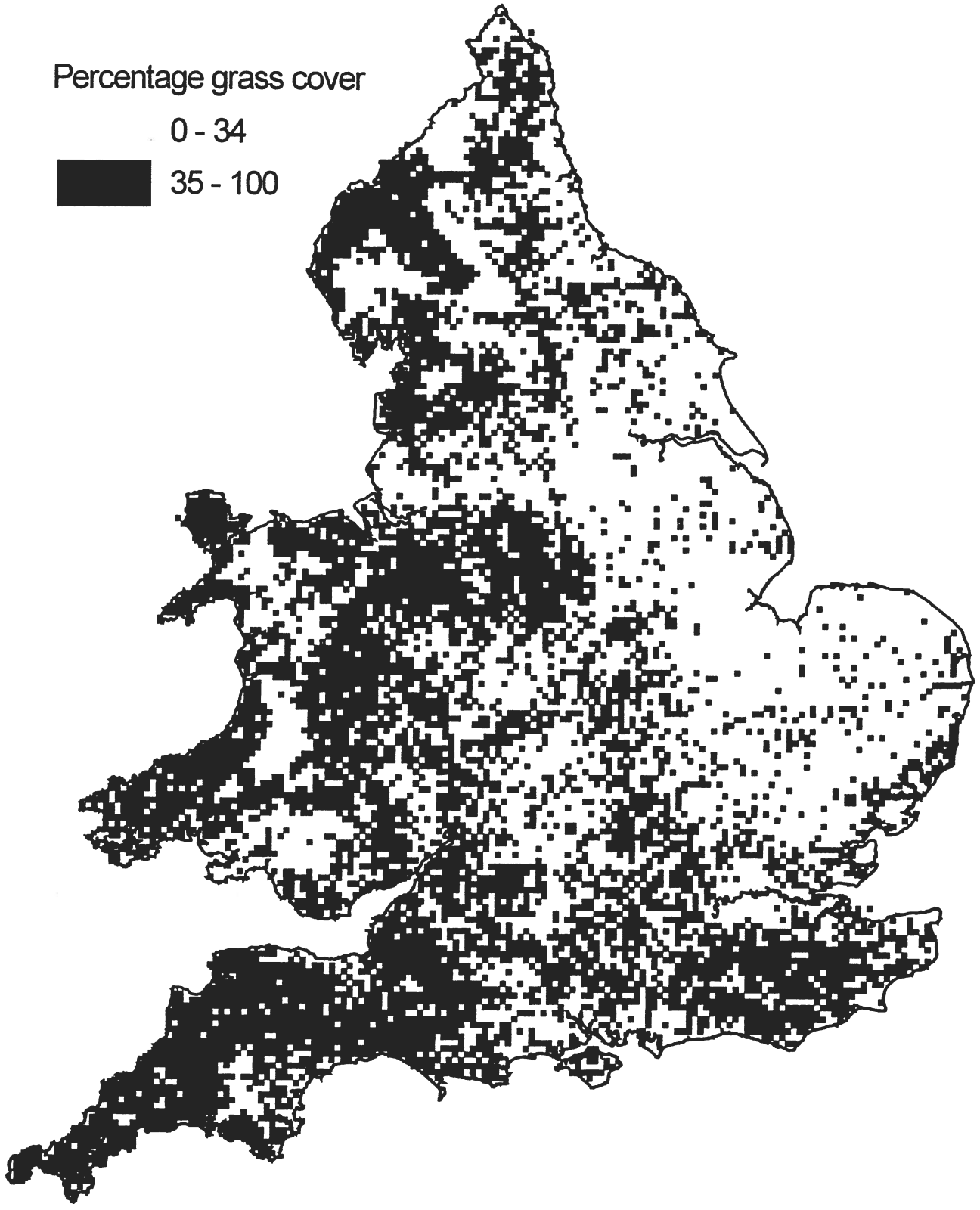
Fig 1. Topsoil pH for England and Wales



Source data: SSLRC

Fig 2. 1990 grass cover for England and Wales



Source data: ITE

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HEALTH CONTROL COSTS IN DAIRY GOAT HERDS IN WESTERN FRANCE

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Dairy-goat farming systems in the region of Pays de la Loire (western France) are characterised by a high milk production level, and large herd size. Most of herds are fed in zero-grazing systems using maize silage, hay and dehydrated alfalfa complemented with high levels of concentrates. Despite high levels of mortality (Malher *et al.*, 1996), health economics are poorly documented in such farming systems.

The objectives of this study were (1) to describe health control costs (HCCs) at herd level in dairy-goat herds in the region of Pays de la Loire, involved in the national milk recording scheme and economic follow-up programmes, and (2) to relate these costs to the herd economic results.

MATERIALS AND METHODS

The target population was mainly located in the Vendée and Maine et Loire departments where, in the herds involved in the national milk recording scheme, productivity averages 843 kg/lactation and herd size 160 lactating goats (Institut de l'Elevage, 1997). In this area, goat diets are mainly made up of maize silage with concentrate (about 350 kg /year/goat [ypg]) or hay with concentrates (about 420 kg/ypg).

A retrospective survey was proposed to a group of 51 farmers involved in an economic follow-up service in Vendée and Maine-et-Loire, conducted by the local milk recording service. This survey was based on retrospective examination of invoices dealing with expenses related to health control during 1997.

Forty farmers (mean size: 175 ypg, mean productivity: 880 l/ypg, Alpine or Saanen breed) agreed to participate to the study. A file was kept on each expenditure based on the invoices of the year in order to identify the target category of animal (adults, kids, goat kids, herd), the target category of health disorder (9 categories), the category of expenses (preventive drugs, curative drugs, laboratory assays, fees), the type of supplier (veterinarian, pharmacy, cooperative, private company), and category of drugs (7 categories).

Expenditures linked to artificial control of reproduction were removed. Prices were expressed per ypg. Expenditures for the common use of the different categories of animal were assigned, in a second step, to the different categories of animals according to the

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distribution of the other expenditures in the categories. Whatever their route of administration, hepatoprotective agents and vitamins were taken into account each time these products were bought separately from food and targeted on a specific health control indication by the farmer; these were called "health complements". This point made a difference between the health control costs evaluated by the survey and those routinely gathered by the extension service under the name of "veterinary costs", where these products may have been registered as "feed additives".

Two herds presented peculiar profiles, therefore they were designated to be outliers, and were not included in every calculation and set of figures (when, therefore, n = 38, instead of 40) but were presented in the results. Statistical methods were descriptive statistics, anova and linear regression.

RESULTS

Global costs

The average health control cost was 46.0 French Francs (FF) per ypg (sd = 3.7 FF, minimum = 8.6 FF, maximum = 99.7 FF, n = 38). This cost is equivalent to the sale of 15 litres of milk per goat, or 3.3 % of the total variable costs. Relative to profitability, HCCs averaged 3.2% of the gross margin (sd = 1.8%, n = 38), and relative to production, HCCs averaged 5.5 FF/ 100 litres (sd = 2.5 FF, n = 38).

The two outliers had the highest average yields per goat (1293 kg/ypg and 1434 kg/ypg, respectively), associated with a high percentage of prolonged lactations or no drying-off before kidding. Those herds presented very high HCCs (280 FF and 147 FF / ypg, respectively) mainly due to a very high consumption of hepatoprotective agents and vitamins (217 FF/ypg and 117 FF/pyg, respectively).

According to its distribution for other herds (Fig. 1), a threshold of 50 FF/goat was proposed to separate high HCCs from low HCCs. Herd with high level HCCs had higher productivity than herds with low levels (+ 120 kg/present goat, Anova, $p<0.05$).

HCCs were mainly devoted to adults (79.3%) then to milk fed kids (14.1%) and weaned goat-kids (6.6%). The average distribution of expenses according to indication in adults is displayed in Fig. 2.

In adults

The metabolic and digestive disorders accounted for the first group of indications for health control costs. Nevertheless, 5 herds had no expenses in this group. Special preventive use of hepatoprotective agents and vitamins in some herds was supposed to protect animals exposed to "at risk" diets, leading to rather high levels of expenses (11 herds had over 10 FF/ypg).

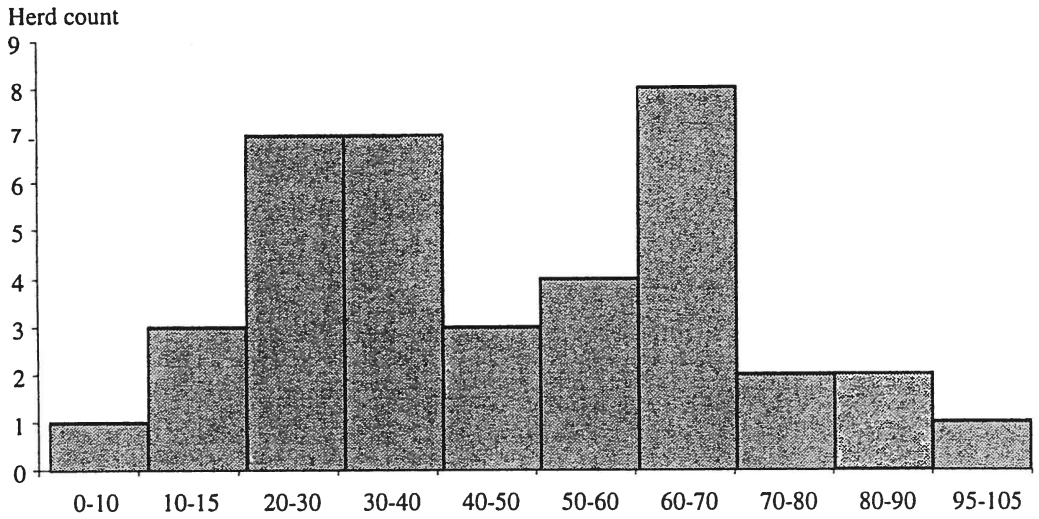

Fig. 1 Distribution of health control costs per goat (FF)

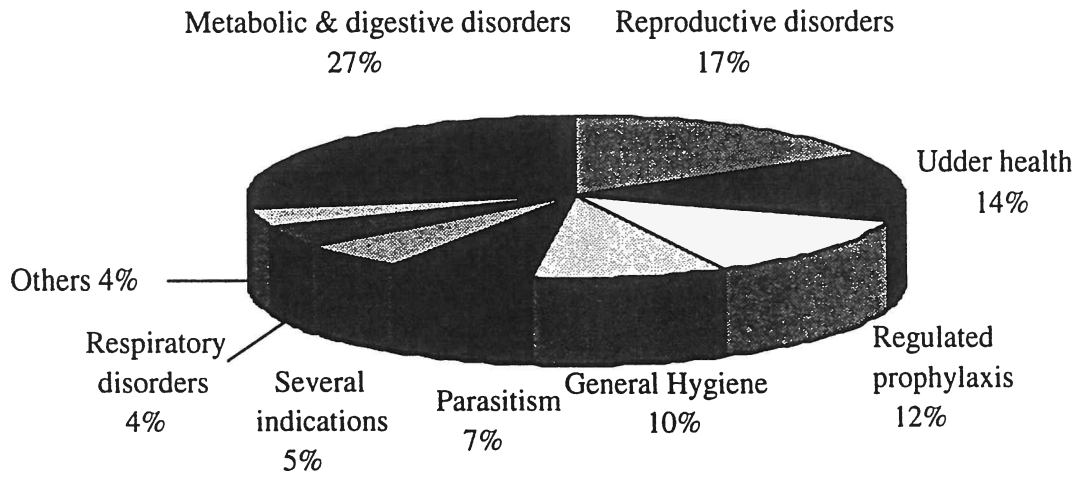

Fig. 2 Average composition of health control costs according to indication in adults (n = 38)

Costs for reproductive disorders were mainly attributed to kidding disorders and periparturient infectious complications, as well as to vitamin cures which were supposed to improve fertility at mating. All but one herd involved this group of indications.

Udder health control is mainly effected by antibiotic therapy for the treatment or prevention of mastitis. Systematic preventive treatment at drying off was found in 53% of the herds, and a selective one (related to previous mastitis or high somatic cell count) in 10%. Sixty-five percent used antibiotics for curative purposes, and 20% did not buy any antibiotics for udder health.

Regulated prophylaxis in goats is compulsory for brucellosis and elective for caprine arthritis-encephalitis virus (CAEV). All herds but one had at least one invoice for regulated prophylaxis. General hygiene included the costs of good practices, such as the use of disposable needles and pesticides. Anthelminthic drugs were limited in connection with "zero-grazing" diets.

In kids and goat-kids

A large range of variation in costs was observed in the 40 herds. The average costs in both categories of animal are given in Table 1.

Digestive disorders were the first group of indications for HCCs in milk-fed kids, and also the most common (66% of the herds). Respiratory diseases were the first reason for HCCs in goat-kids, but were found in only 40% of the herds. Anticoccidiostatic drugs were commonly use around weaning (75% of herds). Pooling kids and goat-kid treatments, the average cost was 3 FF/ypg.

Drugs and services

The average composition of health control costs in 38 herds according to drugs and services is given in Fig. 3.

Regarding drug purchases, 62% percent were made for preventive purposes, whereas 38% were made for curative purpose. The suppliers were the veterinarian (66.9%), the chemist (5.4%), a co-operative (6.3%) and private companies (11.4%). At herd level, the veterinarian may be the only provider (9 herds), but most herds have 2 types of provider (12 herds) or 3 (18 herds).

Biological assays were conducted in 15 herds (1 to 3 times in each). The main indications were respiratory diseases (5 herds), parasitism in young animals (5 herds), breeding diseases (4 herds) and trade (2 herds).

Herd economic results

Technico-economic follow-up allowed connections between the HCCs and the profitability in the herds as measured by the gross margin/ypg. No statistical association was found between the two parameters as shown in Fig. 4.

Table 1. Average composition of health control costs in non-weaned kids and goat-kids.

Health category	Non-weaned kids (%)	Weaned goat kids (%)
Digestive parasitism	22	22
Other digestive disorders	32	11
Respiratory diseases	14	37
Nervous diseases	5	5
Other health disorders	14	15
Several health disorders	7	6
Hygiene	6	4

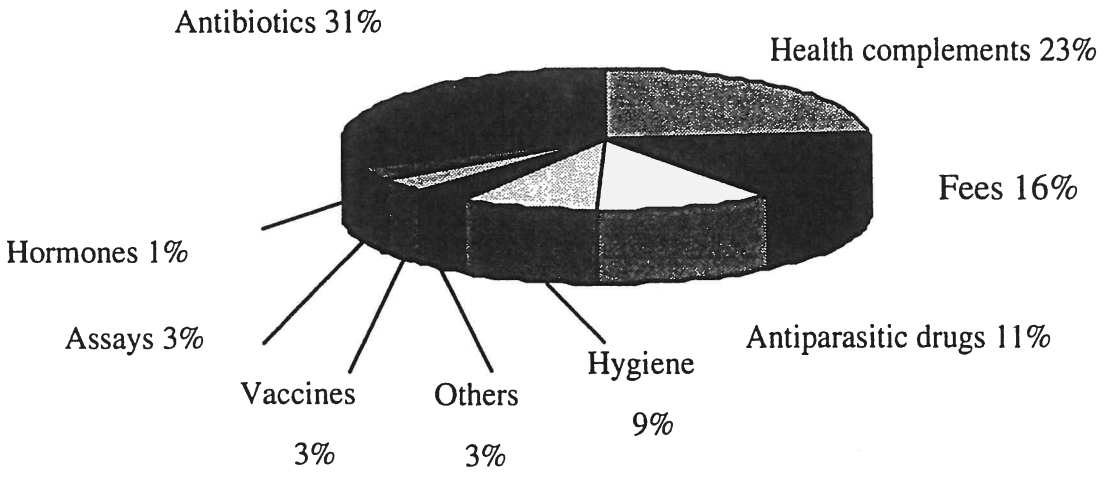

Fig. 3 Average composition of health control costs according to drug and service category (n = 38)

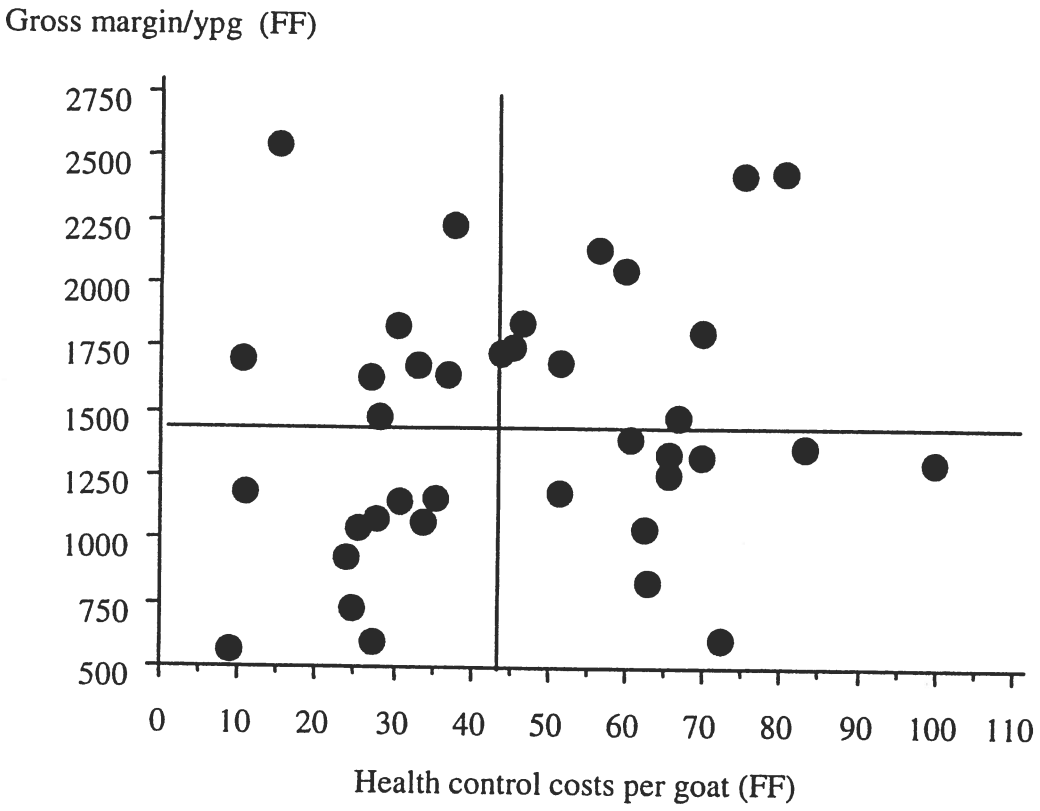

Fig. 4 Relationship between health control costs and profitability in the herds (n=38)
(The lines represent the median value for each variable)

DISCUSSION

In this study, HCCs in dairy goat herds exhibited a large variation with two modes: 20 to 40 FF/ypg and 60 to 70 FF/ypg. The main indications for HCCs in the population were digestive and metabolic diseases and reproductive problems in adults, digestive diseases in kids, and respiratory diseases in goat-kids. Curative and preventive drugs and products were mainly purchased from the veterinarian.

The target population was herds using an extension service in a defined area. This choice was made to improve response to questions, to increase access to invoices, and to facilitate farmers' comprehension of the results and conclusions. However, it limited the number of herds to be investigated. Nevertheless, the size of the sample appeared large enough to provide evidence of variation in the health control costs in the herds, and to allow description of some major trends. Eleven herds did not participate in the survey. However, it was noticed that the 'veterinary costs', as evaluated by the extension service scheme, presented a similar range of variation for non-participants, but there was a higher proportion of non-participant herds with a low level of 'veterinary costs' than in the study group.

Average HCCs appeared to be rather low when compared with those in the dairy cow herds in the same area : 5.5 FF/100 litres in goat versus 7 FF/100 litres in cows (Seegers *et al.*, 1997) or 3.3 % of the variable costs in goat versus 5% in cows (Seegers *et al.*, 1991).

Conversely, mortality rates in dairy goat herds were high (mean = 5.9 %) and variable (s.d. = 5.2) (Malher *et al.*, 1996) compared with the overall level of mortality in dairy cattle (1.9 %; Seegers *et al.*, 1998).

Considering the indication groups for HCCs in adults and mortality rates associated with various health disorders described in a previous survey in the same area (Malher & Boerlen, 1995), metabolic and digestive diseases appeared to be of prime importance in both studies. The second group of diseases associated with death in the previous study comprised nervous disorders, mainly related to listeriosis. However, little HCCs were directed at them in the herds in the study. Peri- and post-parturient disorders appeared to be of importance in both studies. The main indication group for HCCs in non-weaned kids and weaned goat kids are in accordance with previous epidemiological descriptions in the area (Polack *et al.*, 1989). Mastitis control costs could soon increase because penalties for high somatic cell count (SCC) have been introduced recently by dairy plants.

In production medicine, the dispersion of herds according to their profitability and HCCs may suggest that some veterinary input could be relevant to some herds having low profitability and low HCCs. This should give the opportunity for promoting profitable services offered by the veterinarian, such as health-data recording, health and production data analysis in relation to economic results, and goal-oriented visits with the setting of objectives and action lists (Malher & Seegers, 1996).

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CONSTITUTION AND RULES

NAME

1. The society will be named the Society for Veterinary Epidemiology and Preventive Medicine.

OBJECTS

2. The objects of the Society will be to promote veterinary epidemiology and preventive medicine.

MEMBERSHIP

3. Membership will be open to persons either actively engaged or interested in veterinary epidemiology and preventive medicine.

4. Membership is conditional on the return to the Secretary of a completed application form and subscription equivalent to the rate for one calendar year. Subsequent subscriptions fall due on the first day of May each year.

5. Non-payment of subscription for six months will be interpreted as resignation from the Society.

OFFICERS OF THE SOCIETY

6. The Officers of the Society will be President, Senior Vice-President, Junior Vice-President, Honorary Secretary and Honorary Treasurer. Officers will be elected annually at the Annual General Meeting, with the exception of the President and Senior Vice-President who will assume office. No officer can continue in the same office for longer than six years.

COMMITTEE

7. The Executive Committee of the Society normally will comprise the officers of the Society and not more than four ordinary elected members. However, the Committee will have powers of co-option.

ELECTION

8. The election of office bearers and ordinary committee members will take place at the Annual General Meeting. Ordinary members of the Executive Committee will be elected for a period of three years. Retiring members of the Executive Committee will be

eligible for re-election. Members will receive nomination forms with notification of the Annual General Meeting. Completed nomination forms, including the signatures of a proposer, seconder, and the nominee, will be returned to the Secretary at least 21 days before the date of the Annual General Meeting. Unless a nomination is unopposed, election will be by secret ballot at the Annual General Meeting. Only in the event of there being no nomination for any vacant post will the Chairman take nominations at the Annual General Meeting. Tellers will be appointed by unanimous agreement of the Annual General Meeting.

FINANCE

9. An annual subscription will be paid by each member in advance on the first day of May each year. The amount will be decided at the annual general meeting and will be decided by a simple majority vote of members present at the Annual General Meeting.

10. The Honorary 'Treasurer will receive, for the use of the Society, all monies payable to it and from such monies will pay all sums payable by the Society. He will keep account of all such receipts and payments in a manner directed by the Executive Committee. All monies received by the Society will be paid into such a bank as may be decided by the Executive Committee of the Society and in the name of the Society. All cheques will be signed by either the Honorary Treasurer or the Honorary Secretary.

11. Two auditors will be appointed annually by members at the Annual General Meeting. The audited accounts and balance sheet will be circulated to members with the notice concerning the Annual General Meeting and will be presented to the meeting.

MEETINGS

12. Ordinary general meetings of the Society will be held at such a time as the Executive Committee may decide on the recommendations of members. The Annual General Meeting will be held in conjunction with an ordinary general meeting.

GUESTS

13. Members may invite non-members to ordinary general meetings.

PUBLICATION

14. The proceedings of the meetings of the Society will not he reported either in part or in whole without the written permission of the Executive Committee.

15. The Society may produce publications at the discretion of the Executive Committee.

GENERAL

16. All meetings will be convened by notice at least 21 days before the meeting.

17. The President will preside at all general and executive meetings or, in his absence, the Senior Vice-President or, in his absence, the Junior Vice-President or, in his absence, the Honorary Secretary or, in his absence, the Honorary Treasurer. Failing any of these, the members present will elect one of their number to preside as Chairman.

18. The conduct of all business transacted will be under the control of the Chairman, to whom all remarks must be addressed and whose ruling on a point of order, or on the admissibility of an explanation, will be final and will not be open to discussion at the meeting at which it is delivered. However, this rule will not preclude any member from raising any question upon the ruling of the chair by notice of motion.

19. In case of an equal division of votes, the Chairman of the meeting will have a second and casting vote.

20. All members on election will be supplied with a copy of this constitution.

21. No alteration will be made to these rules except by a two-thirds majority of those members voting at an annual general meeting of the Society, and then only if notice of intention to alter the constitution concerned will have appeared in the notice convening the meeting. A quorum will constitute twenty per cent of members.

22. Any matter not provided for in this constitution will be dealt with at the discretion of the Executive Committee.

April, 1982
Revised March, 1985; April, 1988; November 1994
Corrected January 1997